Fingermarks, more than just a ridge pattern
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CHAPTER 5
IMMUNOLABELING AND THE COMPATIBILITY WITH A VARIETY OF FINGERMARK DEVELOPMENT TECHNIQUES

ABSTRACT

Much information can be obtained from the chemical composition of a fingermark, which can be helpful in crime scene investigation. Immunolabeling can be used to extract information about the donor of the fingermark and it can also act as a fingermark development tool in sequence with the standard fingermark development techniques. However, before immunolabeling can be used in forensic practice more information on the possibilities and limitations of this technique is required. In this study, our aim was to investigate if immunolabeling is compatible with standard development protocols (indanedione-zinc, indanedione-zinc followed by ninhydrin spraying, physical developer, cyanoacrylate fuming, cyanoacrylate followed by basic yellow staining, lumicyanoacrylate fuming and polycyanoacrylate fuming). Immunolabeling was carried out successfully on all developed fingermarks, whereby dermcidin was selected as antigen of interest. We can conclude that immunolabeling is compatible with a wide variety of different fingermark developers. This finding in combination with previous findings, makes immunolabeling an interesting technique, which can be of great value in the forensic field.
1. INTRODUCTION

Fingermarks play a key role in crime scene investigations because their friction ridge pattern can be used for identification purposes [1-2]. Fingermarks found at a crime scene are invisible in most cases and need development before the ridge pattern can be recognized and can be used for identification purposes. The substrate on which the fingermark is left, the presence of contaminants and environmental factors is of influence in determining the most suitable method for the development of latent fingermarks [3].

The most common used techniques to develop fingermarks are: powder dusting, ninhydrin spraying and/or cyanoacrylate fuming. However, a recovered fingermark cannot always be used for the identification of the donor, because it can be poorly developed, smudged or distorted [4]. Another limiting factor is the current availability of fingerprints registered in the databases.

In case of an unsuitable fingermark pattern, donor profiling information from its chemical composition can be used to reduce the possible donors of the fingermark. A method to retrieve donor profiling, such as blood group type and drug usage, is the application of immunolabeling [5-11]. Immunolabeling can also be used to redevelop fingermarks to increase image quality for identification purposes [10, 12-13]. Recently, we have shown that simultaneous and multiple immunolabeling of more than one antigen is possible in single fingermarks [14]. We also described the compatibility of immunolabeling with powder dusting and ninhydrin spraying [12]. To increase the usability of this immunolabeling technique, it's compatibility with other commonly used fingerprint development techniques has to be investigated.

Therefore, the aim of this study was to investigate whether immunolabeling is compatible with conventional fingerprint development techniques, including indanedione-zinc chloride (IND-ZnCl), IND-ZnCl followed by ninhydrin spraying (IND-NIN), physical developer (PD), cyanoacrylate fuming (CA), CA followed by basic yellow staining (CA-BY), lumicyanoacrylate fuming (Lumi-CA) and polycyanoacrylate fuming (Poly-CA) with immunolabeling. To demonstrate the compatibility of the immunolabeling technique with these methods, we choose to work with two earlier investigated surfaces; the porous surface nitrocellulose membrane (NCM) and the non-porous glass slides [12]. Dermcidin was selected as antigen of interest, dermcidin is an antimicrobial peptide secreted via pores present in the skin. Prior investigation demonstrated that dermcidin is a good target to investigate the possibilities and limitations of the immunolabeling technique [12, 14-15].

2. MATERIALS AND METHODS

2.1 Fingermark collection

Natural fingerprints were placed on nitrocellulose membranes or glass slides in a de-
pletion series of eight, which means that donors were asked to place the same finger more once on the same material, but on a different site, in order to provide poorer versions of the same finger. For the control experiments, volunteers were asked to place two extra fingermarks on both substrates, which were placed by other fingers, than the once used in the depletion series. One day after placement, fingermarks were transported to the Netherlands Forensic Institute (NFI) for development.

Materials and instruments used in our experiments are listed in table 1.

Table 1. Materials and instruments.

<table>
<thead>
<tr>
<th>Material</th>
<th>Supplier</th>
<th>Address information</th>
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<tbody>
<tr>
<td>Nitrocellulose membrane, Tween-20, MilliQ-water</td>
<td>Millipore, Merck KGaA</td>
<td>Darmstadt, Germany</td>
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<td>Glass slides</td>
<td>Superfrost plus, Gerhard Menzel GmbH</td>
<td>Braunschweig, Germany</td>
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<tr>
<td>Fingerprint development cabinet (FDC)</td>
<td>Gallenkamp</td>
<td>Loughborough, UK</td>
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<tr>
<td>Cyanoacrylate fuming cabinet (Mason vactron MVC1000), blue Crime-lite® 2 torch, UV Crime-lite® 2 torch, green Crime-lite® 2 torch, clear filter (GG420), yellow filter (GG495) and red filter (OG590), Polycyano UV</td>
<td>Foster and Freeman</td>
<td>Worcestershire, UK</td>
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<tr>
<td>Nikon Eclipse E600 microscope and Nikon DS-Fi2 camera</td>
<td>Nikon</td>
<td>Tokyo, Japan</td>
</tr>
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<td>Zinc chloride (ZnCl2, &gt;99%), ethanol (absolute, &gt;99%), ethyl acetate (&gt;98%), acetic acid, dodecylamine acetate, silver nitrate, iron nitrate, ammonium ferric sulfate, citric acid, maleic acid, basic yellow, skim milk powder (SMP), methanol, bovine serum albumin (BSA), and solvents</td>
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<td>Zwijndrecht, the Netherlands</td>
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<td>Hydrofluoroether (HFE )</td>
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<td>St. Paul, USA</td>
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<td>1,2- indanedione (99%), ninhydrin and cyanoacrylate</td>
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<tr>
<td>Goat anti-mouse Horse Radish Peroxidase (HRP)</td>
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<td>Goat anti-mouse Cy3</td>
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<td>Vector SG peroxidase kit</td>
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<td>Dako pen, Dako fluorescent mounting media</td>
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<td>Fixogum</td>
<td>Marabu</td>
<td>Tamm, Germany</td>
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2.2 Fingermark development
Details about the numbers of donors and amount of fingermarks used in each experiment is described in table 2.

Table 2. Details about the number of donors and amount of fingermarks used in the described experiments

<table>
<thead>
<tr>
<th>Technique</th>
<th>Donors</th>
<th>Depletion series</th>
<th>Controls</th>
<th>Total number of fingermarks</th>
<th>Development after (x) days</th>
<th>Immunolabeling after (x) days</th>
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<td>2</td>
<td>50</td>
<td>3</td>
<td>4-5</td>
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<tr>
<td>IND-NIN</td>
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<td>8</td>
<td>2</td>
<td>50</td>
<td>3</td>
<td>4-5</td>
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<td>8</td>
<td>2</td>
<td>20</td>
<td>3</td>
<td>4-5</td>
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<tr>
<td>Non-porous surface</td>
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<td></td>
<td></td>
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<tr>
<td>CA</td>
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<td>8</td>
<td>2</td>
<td>50</td>
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<td>2</td>
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<td>CA-BY</td>
<td>5</td>
<td>8</td>
<td>2</td>
<td>50</td>
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<td>3</td>
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<td>Lumi-CA</td>
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<td>8</td>
<td>2</td>
<td>40</td>
<td>1</td>
<td>3</td>
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<tr>
<td>Poly-CA</td>
<td>5</td>
<td>8</td>
<td>2</td>
<td>40</td>
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</tbody>
</table>

2.2.1 IND-ZNCL development
Fingermarks were developed three days after placement. A working solution of IND-ZnC was prepared by mixing ZnCl stock solution (8.0 ml) with IND stock solution (100 ml), which results in a IND-ZnCl working solution of 7.4 % (v/v), for specifications of the used stock and working solutions, see table 3. The working solution was poured in a flat dish. The porous material to be examined was immersed for no more than 5 s in the solution using a pair of tweezers. After immersion, the material was left to dry for two min. The material was placed in a fingerprint development cabinet (FDC) and left to develop for 20 min at 100°C. After development fingermarks were visualized and recorded.

2.2.2 IND-NIN development
Fingermarks were developed three days after placement. Firstly, fingermarks were developed using the IND-ZnCl treatment as described in 2.2.1. Fingermarks were further developed with NIN, specifications about the NIN stock and working solutions are described in table 3. The working solution was poured in a flat dish. The material to be examined was immersed in the solution using a pair of tweezers. After immersion, the material was left to dry for two min. The material was placed in a FDC and left to develop for at least 10 min at 80°C ± 3 °C and 65 ± 3 % relative humidity. After development fingermarks were visualized and recorded.

2.2.3 PD-development
Fingermarks were developed three days after placement. Specifications about the stock and working solutions of PD-development are described in table 3. The PD working so-
olution was poured into a flat glass dish and placed on a shaker. The material to be examined was immersed in 2.5% maleic acid solution (25 g maleic acid in 1L demi-water) for approximately 10 min or until no more CO₂ bubbles were formed. The material was then rinsed in distilled water and left in the working solution, until marks became visible, but before blackening of the carrier material occurs. The working solution was placed on the shaker on a slow rocking motion. The material was immersed in three more bowls of water for approximately 5 min in each bowl. The material was left on filter paper to dry at room temperature. After drying the developed fingermarks were visualized and recorded.

2.2.4 CA, LUMI-CA or POLY-CA development

Fingermarks were developed one day after placement. The glass slides were placed in a cyanoacrylate fuming cabinet and CA (0.5 g), Lumi-CA (0.5 g) or Poly-CA (0.5 g) was added to the container in the cabinet. The cabinet was activated as described in the manufacturer’s manual and ran through a full automated cycle for 20 min. The glass slides were removed from the cabinet and the developed fingermarks were observed in white light.

Table 3. Preparation of stock solutions and working solutions.
2.2.5 BY development
0.1% of BY was dissolved in 1 L ethanol. Fingermarks were developed one day after placement with cyanoacrylate, as described above and then treated with the BY solution. The treatment involved the spraying of the glass slides with the BY solution, followed after only 10 s by rinsing with copious amounts of tap water.

2.3 Immunolabeling of fingermarks
2.3.1 Immunolabeling of fingermarks porous surfaces
Labeling was performed according to our protocol for porous surfaces [12, 14]. Developed fingermarks were incubated for 30 min with blocking buffer (Phosphate buffer saline (PBS)+5% skim milk powder (SMP)). Directly after the blocking step, samples were incubated with 100 µl primary antibody anti-dermcidin diluted in blocking buffer 1:20 (v/v) for 1 h. After incubation the samples were washed three times with a washing buffer (PBS + 0.1% Tween-20) for 2 min. The samples were incubated with 100 µL secondary antibody, (i) pre-diluted goat anti-mouse horseradish peroxidase (HRP) and diluted 1:1 (v/v) in blocking buffer 2 or (ii) goat anti-mouse Cy3 diluted 1:250 (v/v) in blocking buffer, for 0.5 h. After incubation, the samples were three times washed with washing buffer. Samples incubated with the HRP conjugated antibody were incubated for 5-10 min with a SG peroxidase mixture prepared according to the manufacturer’s protocol to activate the HRP. A second washing was performed on the HRP activated samples (i) and were left to dry before imaging.

2.3.2 Immunolabeling of fingermarks non-porous surfaces
Labeling was performed according to our protocol for non-porous surfaces [12, 14]. In short, developed fingermarks left on a non-porous surface were fixed in methanol for 20 min at -20°C. The samples were washed with MilliQ water and left to dry for 15 min. A hydrophobic layer was drawn around the fingermarks with a Dako Pen or a layer of fixogum was applied around the fingermark and left to dry for 30 min. The Dako Pen and fixogum serve as a water-repellent barrier that keeps staining reagent localized on the fingermark sections. After drying, the section was covered with MilliQ for 5 min. The sections were incubated with phosphate buffered saline (PBS) for 5 min. The blocking buffer (PBS + 3% BSA) was applied to the fingermarks and fingermarks were incubated for at least 1 h. After, the blocking step, fingermarks were incubated with the 100 µl primary antibody; mouse monoclonal IgM anti-dermcidin diluted in blocking buffer, 1:20 (v/v) and then left overnight in a wet chamber. The next day, samples were three times washed in PBS for 2 min each time. The samples were then incubated with 100 µl secondary antibody, goat anti-mouse Cy3 diluted in blocking buffer 1, 1:250 (v/v) for 1 h in a dark room. The samples were washed three times for 2 min in PBS. After washing the fixogum was removed and a drop of DAKO fluorescent mounting media was applied to every glass slide, after which the cover slips were put on. Samples were left to dry for 20 min before imaging.
2.4 Imaging

Overall images of the fingermarks were obtained using a Canon EOS 40D and a Canon Macro Lens EF 100mm f/2.8 USM. CA-BY was excited using a blue Crime-lite® 2 torch (445 nm, 10% band width 420-470 nm) and its fluorescence was detected in combination with an yellow filter placed in front of the lens of the camera. Lumi-CA and Poly-CA developed fingermarks were excited using the UV Crime-lite® 2 torch (365 nm, 10% band width 350–380 nm) and a clear filter placed in front of the lens of the camera. IND and Cy3 were excited using a green Crime-lite® 2 torch (530 nm, 10% band width 500-560 nm) and fluorescence was detected in combination with a red filter placed in front of the lens of the camera.

Microscopic images were obtained using a Nikon Eclipse E600 microscope using either a FITC filter: excitation filter 465-495 nm, dichroic mirror 505 nm, barrier filter 515-555 nm, or a cy3 filter: excitation filter 510-560 nm, dichroic mirror 575 nm, barrier filter 590 nm, and a Nikon DS-Fi2 camera.

3. RESULTS

3.1 Compatibility of IND-ZnCl with immunolabeling

Figure 1 shows the development of a fingermark left on NCM porous surface with IND-ZnCl (fig. 1-A), followed by immunolabeling (fig. 1-B). After development with IND-ZnCl, a decrease in fluorescent signal could be observed along the depletion series of eight fingermarks. Additionally, immunolabeling was performed successfully on the IND-ZnCl developed fingermarks and dermcidin could be detected in all eight fingermarks of the depletion series, with a noticeable decrease of the detectable amount along the series. Since the product of the reaction of amino acids with IND-ZnCl has fluorescent properties, a secondary antibody with an HRP conjugate was used as visual enhancer when applying the immunolabeling method.

Figure 1 Immunolabeling of a fingermark (first in depletion series) left on NCM developed with IND. A: Fluorescence image of an IND developed fingermark. B: Bright field images of a fingermark after immunolabeling with anti-dermcidin and HRP-tagged secondary antibody. C: Overlay of image A and B. Scale bar is 0.5 cm.
3.2 Compatibility of IND-NIN with immunolabeling
Fingermarks were also developed with IND followed by NIN. After both treatments fingermarks were successfully immunolabeled. In figure 2, a depletion series is shown, in which the IND development is presented in the first row, followed by NIN development (second row) and finally, immunolabeling was performed on the IND-NIN developed fingermarks (third row). A HRP tagged secondary antibody was used as a detection tag of dermcidin. From preliminary studies we know that the reaction products of ninhydrin with the amino acids are washed away during the washing steps [12]. Therefore, no ninhydrin reaction products could be observed after immunolabeling has been performed on the fingermarks. In some fingermarks, more information from the ridge pattern could be obtained from the fingermarks after both applying IND-NIN development and immunolabeling. Specific targeting of dermcidin resulted in improved ridge details and detailed pore information (fig. 2, fingermark 2, 4 and 5). Some parts of the fingermarks presented in figure 2 are out of focus due to the wrinkling of the substrate. IND and NIN development involves a heating step. This heating step causes wrinkling of the membrane. Since, we did not want contamination of the membrane, as the membrane has a high affinity to bind proteins and other amine-containing compounds, no attempts were made to unwrinkle the membrane.

3.3 Compatibility of PD with immunolabeling
Fingermarks left on NC were developed successfully with PD. In all depletion series only partially or fully developed fingermarks were visible in the first four fingermarks of the depletion series of a total of eight fingermarks. Brightfield images were obtained from the developed fingermarks (fig. 3-A). Ridge details could be observed in the PD develop-

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Figure 2. Immunolabeling of fingermarks left on NCM developed with IND followed by NIN. First row: Fluorescence images of fingermarks developed with IND. Second row: Brightfield images of fingermarks subsequently developed with NIN. Third row: Brightfield images of fingermarks after immunolabeling with anti-dermcidin and a HRP–tagged secondary antibody.
Figure 3. Immunolabeling of fingermark (first in depletion series) left on NCM developed with PD. A: Brightfield image of fingermark developed with PD. B: Fluorescence image of a fingermark after immunolabeling with anti-dermcidin and a Cy3–tagged secondary antibody. White arrows indicate details present in the fingermark. C: Overlay of image A and B. Scale bar is 0.5 cm.

Figure 4. Immunolabeling of fingermark (first in depletion series) left on a glass slide developed with CA. A: Brightfield image of fingermark developed with CA. B: Fluorescence image of a fingermark after immunolabeling with anti-dermcidin and a Cy3–tagged secondary antibody. C: Overlay of image A and B. Scale bar is 0.5 cm.

Figure 5. Immunolabeling of fingermarks left on glass slides developed with CA-BY. First row: Fluorescence image of fingermark developed with CA-BY. Second row: Fluorescence image of a fingermark after immunolabeling with anti-dermcidin and a Cy3–tagged secondary antibody.
Immunolabeling was performed successfully on all developed fingermarks with PD. A decrease of the detected amount of dermcidin could be observed proportional to the depletion series. A secondary antibody tagged with a fluorescent dye, Cy3, was used to allow discrimination between the PD and the detection of dermcidin. Detection of dermcidin resulted in an improved visualization of second and third level details, as shown in figure 3. Details are indicated with white arrows.

3.4 Compatibility of CA with immunolabeling
Four different strategies of CA fuming in combination with immunolabeling were investigated. Figure 4-A shows the development of fingermarks with the standard CA. All developed fingermarks in the depletion series were of excellent quality. Immunolabeling of CA developed fingermarks resulted in the specific detection of dermcidin (fig. 4-B). Dermcidin could be detected in all eight fingermarks of the depletion series, however the detected amount of dermcidin decreases along the depletion series.

3.5 Compatibility CA-BY with immunolabeling
Fluorescence images of high quality could be obtained from fingermarks developed with CA followed by BY. After excitation with a blue crime-lite® torch in combination with an yellow filter, a green fluorescent signal was observed. As depicted in figure 5, (first row) all eight fingermarks of the depletion series resulted in strongly developed fingermarks, showing good ridge detail. Immunolabeling was possible in all eight fingermarks of the depletion series (second row), already developed with CA followed by BY. The decrease of the intensities of the fluorescence of the secondary antibody (Cy3) proportional to the depletion series, implies that the amount of detected dermcidin decreases in the same fashion.

3.6 Compatibility of Lumi-CA with immunolabeling
Development of fingermarks left on glass slides with Lumi-CA resulted in good quality fluorescent and brightfield images. Lumi-CA is a fluorescent cyanoacryle and can be used on fingermarks left on backgrounds that are hard to develop with the standard CA. We found that Lumi-CA affects the fingermark to such a degree that the detection of dermcidin could only be observed with a magnification of 4 times or higher. Since the immunolabeling result was very minimal, it was not possible to locate the same area of the fingermark for both the visualization of the developed fingermarks and to show the detection of dermcidin. Therefore, a magnified image of developed fingermarks with Lumi-CA is shown in figure 6-A and B. After excitation with a UV crime-lite® torch a blue fluorescent signal could be observed. No fluorescence other than the autofluorescence could be observed in the green and red control channels (fig. 6-C and D). Immunolabeling was performed successfully on the Lumi-CA developed fingermarks, however most of the fingermark components and reaction products of the polymerization were washed away or removed after the immunolabeling method, resulting in a poor detection of dermcidin in all developed fingermarks. Dermcidin could be detected in the finger-
marks as visualized in figure 6-H, but low fluorescence intensities could be observed. Detection of the presence of dermcidin was only possible using a microscope and a 4× magnification. The fingermarks shown in figure 6 are from the same fingermark, however images obtained from the developed fingermarks and of the immunolabeled fingermarks are from different locations in the fingermark.

3.7 Compatibility of Poly-CA with immunolabeling
Poly-CA treatment of the latent fingermarks resulted in well-developed fingermarks. Brightfield (fig. 7-A) and fluorescent images (fig. 7-B-D) could be obtained from these developed fingermarks. Poly-CA displays a blue fluorescence when excited with an UV Crime-lite® torch, as shown in figure 7-B. All eight fingermarks in the depletion series

![Figure 6. Immunolabeling of fingermark (first in depletion series) left on glass slides developed with Lumi-CA. Brightfield image (A) and fluorescent images (B-D) of fingermark developed with Lumi-CA. Brightfield image (E) and fluorescent images (F-H) of fingermark after immunolabeling with anti-dermcidin and a Cy3–tagged secondary antibody. Scale bar is 500 µm.](image)

![Figure 7. Immunolabeling of fingermark (first in depletion series) left on glass slide developed with Poly-CA. Brightfield image (A) and fluorescent images (B-D) of fingermark developed with Poly-CA. Brightfield image (E) and fluorescent images (F-H) of fingermark after immunolabeling with anti-dermcidin and a Cy3–tagged secondary antibody. Scale bar is 500 µm.](image)
could easily be developed with Poly-CA. Like Lumi-CA, Poly-CA development also affected the fingerprint, which resulted in an inferior detection of dermcidin compared with the other five development techniques. Detection of dermcidin was possible, but in low amounts and with low intensities. Therefore, only microscopic imaging at 4 times magnification or higher could detect dermcidin, as depicted in figure 7-H. Almost no Poly-CA polymers could be observed in the brightfield and blue channel after immunolabeling was performed on the fingerprints (fig. 7-E and F). The fingerprints shown in figure 7 are from the same fingerprint, however images obtained from the developed fingerprints and of the immunolabeled fingerprints are from different locations in the fingerprint.

4. DISCUSSION

Immunolabeling can be used to i) extract donor profiling information from fingerprints, like blood group typing, drug usage and diet habits and ii) to redevelop fingerprints to increase image quality for identification purposes [9-10, 12-15]. However, before this technique can be used in practice more information on the possibilities and limitations of the technique is required. In this article we have shown that immunolabeling is compatible with a wide variety of fingerprint developers; IND, IND-NIN, PD, CA, CA-BY, Lumi-Ca and Poly-CA.

Fingermarks left on porous surfaces were developed with IND, IND-NIN or PD and sequentially processed with immunolabeling resulting in a good detection of dermcidin. None of these three fingerprint development techniques had a notable influence on the immunolabeling of fingerprints. A combination of both the fingerprint development technique and the immunolabeling technique can provide more details about the ridge pattern or pore position when detecting dermcidin. The standard development of fingerprints with IND and IND-NIN involves a heating step, with temperatures of 80 and 100°C. Surprisingly, the heating step did not influence the detection of dermcidin. Increased temperatures normally lead to the destruction and formation of proteins, suggesting that the detection of dermcidin should have been more difficult.

The immunolabeling of fingermarks left on the non-porous surfaces and developed with different types of CA gave various results. Dermcidin could be detected successfully in all developed fingerprints. However, only a minimal amount of dermcidin in fingerprints developed with Lumi-CA and Poly-CA could be detected. The intensity of the observed fluorescence was much lower than in the other CA developed and immunolabeled samples. An explanation for this finding may be found in differences in physical structure of the standard cyanoacrylate and the Lumi-CA and Poly-CA. Lumi-CA and Poly-CA are a mixture of a fluorescent precursor and polycyanoacrylate. Both end products have different molecular weights. Groeneveld et al. observed a difference in development when comparing developed fingerprints with CA, Lumi-CA and Poly-CA using SEM [16]. Fingermarks developed with Lumi-CA resulted in more dendritic nanofibers
compared to the standard CA and this could have influenced the detection of dermcidin. Therefore, in the case of Lumi-CA and Poly-CA development it is not recommended to use immunolabeling to improve the visibility of the fingerprints. But if additional information about the donor is required, immunolabeling can be used to obtain this information, as only a minimal amount of the antigen of interest is necessary for positive detection. As can be most clearly seen in figure 3 and 7, labeling for dermcidin specifically visualizes the pores in a fingerprint. Pores are not yet used for identification purposes, but the inclusion of this third level detail into the identification process is under discussion [17-18].

5. CONCLUSION

In conclusion, immunolabeling is compatible with IND, IND-NIN, PD, CA, CA-BY, Lumi-CA and Poly-CA. In all investigated fingerprints dermcidin could be detected successfully. However, detection of dermcidin in fingerprints developed with Lumi-CA and Poly-CA is minimal. The results presented in this article bring us one step closer to the implementation of fingerprint immunolabeling techniques in the forensic field.

REFERENCES

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