



## Immunolabeling and the compatibility with a variety of fingerprint development techniques



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### ABSTRACT

Much information can be obtained from the chemical composition of a fingerprint, which can be helpful in crime scene investigation. Immunolabeling can be used to extract information about the donor of the fingerprint and it can also act as a fingerprint development tool in sequence with the standard fingerprint 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 fingerprints, whereby dermcidin was selected as antigen of interest. We can conclude that immunolabeling is compatible with a wide variety of different fingerprint developers. This finding in combination with previous findings, makes immunolabeling an interesting technique, which can be of great value in the forensic field.

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### 1. Introduction

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

The most common used techniques to develop fingerprints are: powder dusting, ninhydrin spraying and cyanoacrylate fuming. However, a recovered fingerprint 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 unsuitable fingerprint pattern, donor profiling information from its chemical composition can be used to reduce the possible donors of the fingerprint. A method to retrieve a donor profile, such as

blood group type and drug usage, is the application of immunolabeling [5–11]. Immunolabeling can also be used to redevelop fingerprints 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 fingerprints [14]. We also described the compatibility of immunolabeling with powder dusting and ninhydrin spraying [12]. To increase the usability of this immunolabeling technique, its 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). 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 the 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].

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## 2. Materials and methods

### 2.1. Fingerprint collection

Natural fingerprints were placed on nitrocellulose membranes and glass slides in a depletion series of eight. Donors were asked to place one of their fingers eight times on the same material, but on a different site of the material, in order to provide poorer versions of the same finger. For the control experiments, volunteers were asked to place two extra fingerprints, from other fingers, on both substrates. One day after placement, the fingerprints were transported to the Netherlands Forensic Institute (NFI) for development.

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

### 2.2. Fingerprint development

Details about the numbers of donors and amount of fingerprints used in each experiment are described in Table 2.

#### 2.2.1. IND-ZnCl development

Fingerprints were developed three days after placement. A working solution of IND-ZnCl, was prepared by mixing, ZnCl stock solution (8.0 ml) with IND stock solution (100 ml), which resulted 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 2 min. The material was placed in a fingerprint development cabinet (FDC) and left to develop for 20 min at 100 °C. After development the fingerprints were visualized and recorded.

#### 2.2.2. IND-NIN development

Fingerprints were developed three days after placement. Firstly, fingerprints were developed using the IND-ZnCl treatment as described in Section 2.2.1. Fingerprints 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 2 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 the fingerprints were visualized and recorded.

#### 2.2.3. PD-development

Fingerprints were developed three days after placement. Specifications about the stock and working solutions of PD-development are described in Table 3. The PD working solution 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 1 l demi-water) for approximately 10 min or until no more CO<sub>2</sub> 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 fingerprints were visualized and recorded.

#### 2.2.4. CA, Lumi-CA or Poly-CA development

Fingerprints 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 fingerprints were observed in white light.

#### 2.2.5. BY development

0.1% of BY was dissolved in 1 l ethanol. Fingerprints 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 fingerprints

#### 2.3.1. Immunolabeling of fingerprints on porous surfaces

Labeling was performed according to our protocol for porous surfaces [12,14]. Developed fingerprints were incubated for 30 min with a 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 the blocking

**Table 1**

Materials and instruments.

Material	Supplier	Address information
Nitrocellulose membrane, Tween-20, MilliQ-water	Millipore, Merck KGaA	Darmstadt, Germany
Glass slides	Superfrost plus, Gerhard Menzel GmbH	Braunschweig, Germany
Fingerprint development cabinet (FDC)	Gallenkamp	Loughborough, UK
Cyanoacrylate fuming cabinet (Mason vactron MVC1000), UV Crime-lite® 2 torch, blue Crime-lite® 2 torch, green Crime-lite® 2 torch, clear filter (GG420), yellow filter (GG495) and red filter (OG590), polycyano UV	Foster and Freeman	Worcestershire, UK
Nikon Eclipse E600 microscope and Nikon DS-Fi2 camera	Nikon	Tokyo, Japan
Zinc chloride (ZnCl <sub>2</sub> , >99%), ethanol (absolute, >99%), ethyl acetate (>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	Sigma Aldrich	Zwijndrecht, The Netherlands
Hydrofluoroether (HFE)	3 M	St. Paul, USA
1,2-Indanedione (99%), ninhydrin and cyanoacrylate	BVDA	Haarlem, The Netherlands
Synperonic N	VWR	Amsterdam, The Netherlands
Lumicyano	Global Forensics	Coventry, UK
PBS	Biowhittaker, Lonza Cologne GmbH	Köln, Germany
Anti-dermcidin	Santa Cruz Biotechnology, INC	Santa Cruz, USA
Goat anti-mouse horseradish peroxidase (HRP)	Abcam	Cambridge, UK
Goat anti-mouse Cy3	Jackson Lab	Brunschwig, Switzerland
Vector SG peroxidase kit	Vector labs Brunschwig	Amsterdam, The Netherlands
Dako pen, Dako fluorescent mounting media	Dako	Glostrup, Denmark
Fixogum	Marabu	Tamm, Germany

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