Fingermarks, more than just a ridge pattern
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CHAPTER 6
IMMUNOLABELING OF FINGERMARKS LEFT ON FORENSIC RELEVANT SURFACES, INCLUDING THERMAL PAPER

A. van Dam, K.A. van Nes, M.C.G. Aalders, T.G. van Leeuwen, S.A.G. Lambrechts, Immunolabeling of fingermarks left on forensic relevant surfaces, including thermal paper. Analytical Methods 2014, 6, 1051-1058
ABSTRACT

The chemical composition of a fingermark contains donor profiling information. Immunolabeling is a technique that can be used to retrieve this chemical information from fingermarks. Additionally, immunolabeling can be used to (re)develop fingermarks. To be of interest in the forensic field, the applicability of immunolabeling should be highly diverse. Therefore, in this study we investigated the applicability of one method of immunolabeling of fingermarks left on non-porous (aluminum foil, stainless steel keys, plastic sheets, different colored garbage bags, sandwich bags, Ziploc bags), semi-porous (tiles, laminated chipboard), and porous surfaces (thermal and copy paper). Successful immunolabeling of specific components in fingermarks was possible on all surfaces tested, except for laminated chipboards and copy paper. Additionally, high quality images could be obtained from the immunolabeled fingermarks. Surprisingly, fingermarks left on thermal paper showed improved visibility when developed with the immunolabeling method. In conclusion, one intrinsically similar immunolabeling method can visualize fingermarks left on non-porous, semi-porous and porous surfaces.
1. INTRODUCTION

Fingermarks found at crime scenes can be used to identify individuals using the ridge pattern. Currently, the identification is mainly restricted to the morphological aspects of the fingermark, but more donor information such as gender [1], smoking habits [2-3], contact with drugs [4-5] and diet information [6] may be hidden in its chemical composition and can thus be helpful for creating a donor profile. Several analytical techniques exist for retrieving chemical information from fingermarks [1, 3-4]. Immunolabeling is one such method and recent technological developments have sparked the renewed interest in immunolabeling in the forensic field [2, 7-8]. Besides retrieving information about the donor, immunolabeling of fingermarks can also be used to (re)develop fingermarks [4, 7-15]. Recently, we found that immunolabeling can still be applied to the fingermark after visualization by common fingerprint visualization techniques, powder dusting and ninhydrin staining, which demonstrates the possibilities of redeveloping a fingermark using immunolabeling [8, 13]. By specific detection of substances present in the fingermark, other components than those targeted with the standard visualization methods, may lead to the enhancement or potentially to the (re)development of a non-identifiable fingermark to an identifiable one.

Fingermarks left at crime scenes can be found on a wide variety of surfaces, varying from non-porous, semi-porous to porous surfaces. It has already been shown that immunolabeling of dermcidin, keratin, cathepsin and albumin in fingermarks was possible on different porous surfaces [9, 11, 16]. Different immunogenic techniques, which can be applied to fingermarks, are described in literature [2, 4, 7, 10-11, 13-14]. Blood group typing of fingermarks was performed using an immunogenic technique that is based on agglutination occurring between blood group antigens, antibodies and indicator cells [13-14]. Other research groups used micro- or nanoparticles functionalized antibodies to increase the visibility of the fingermarks [2, 4, 10]. However, all these techniques have their own distinctive protocol, making comparison of the efficacy of the different methods on the various surfaces impossible. Furthermore, to this day, none of these immunolabeling methods are implemented in the forensic field. Yet, we are convinced that immunolabeling of fingermarks has a great forensic future. However, a complicating factor is the nature of the surface, which is known to influence the effectiveness of fingermark visualization methods, like the smoothness, roughness and porosity of the surface. Thus, a large number of surfaces occurring at crime scenes need to be tested, ideally, resulting in one immunolabeling method that can be used for the detection of specific components in fingermarks left on all possible surfaces.

The aim of this work therefore is to evaluate one single method of immunolabeling of fingermarks left on several common non-porous, semi-porous as well as porous surfaces, chosen based on their forensic relevance. Thermal paper was of special interest as the development of fingermarks on this particular surface is challenging [17-19]. Dermcidin as antigen is a good candidate for testing the immunolabeling method on different
surfaces [8-9]. Dermcidin is an antimicrobial peptide that serves a protective role to the skin and is one of the most abundant proteins in eccrine sweat [9, 20].

2. MATERIALS AND METHODS

2.1 Fingermark collection and surfaces

Fingermarks were deposited during working hours on non-porous, semi-porous and porous surfaces, type of surface and supplier are listed in table 1. No special instructions were given to the donors. Every experiment included fingermarks of at least six different donors, both female and male and was performed in duplicate or triplicate. A total of twelve to eighteen fingermarks were immunolabeled per experiment. Fingermarks left on non-porous surfaces were treated one hour after deposition; fingermarks left on porous surfaces were treated after one day after deposition to give the material time to adsorb the fingermark.

All experiments were performed at room temperature, unless mentioned otherwise.

Table 1. Overview of type of surfaces used including the supplier’s name.

<table>
<thead>
<tr>
<th>Type of surface</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-porous surfaces</td>
<td></td>
</tr>
<tr>
<td>Aluminum foil</td>
<td>ProsmartChoice, Niewegein, the Netherlands</td>
</tr>
<tr>
<td>Stainless steel keys</td>
<td>Euro Locks, Bastogne, Belgium</td>
</tr>
<tr>
<td>Plastic sheets (Polypropylene Carbonate (PPC))</td>
<td>Purio, Lidl, Woerden, the Netherlands</td>
</tr>
<tr>
<td>Black garbage bags (Low Density Polyethylene (LDPE))</td>
<td>Depa, Beuningen, the Netherlands</td>
</tr>
<tr>
<td>Ziploc bags (High Density Polyethylene (HDPE))</td>
<td>Albert Heijn, Woerden, the Netherlands</td>
</tr>
<tr>
<td>Sandwich bags (HDPE)</td>
<td>Albert Heijn, Woerden, the Netherlands</td>
</tr>
<tr>
<td>Semi-porous surfaces</td>
<td></td>
</tr>
<tr>
<td>White tile (matt, unglazed)</td>
<td>Praxis, Amsterdam, the Netherlands</td>
</tr>
<tr>
<td>White laminated chipboard</td>
<td>Praxis, Amsterdam, the Netherlands</td>
</tr>
<tr>
<td>Porous surfaces</td>
<td></td>
</tr>
<tr>
<td>White copy paper (80 g/m², Océ Black Label Zero)</td>
<td>Canon, ’s Hertogenbosch, the Netherlands</td>
</tr>
<tr>
<td>Thermal paper</td>
<td>Office palace, Etten-leur, the Netherlands</td>
</tr>
</tbody>
</table>

2.2 Immunolabeling of fingermarks left on non-porous and semi-porous surfaces

The location of the fingermark deposition was known. Labeling was performed according to our protocol [8]. In short, fingermarks left on a non-porous surface were fixed in methanol (Sigma Aldrich, Munich, Germany) for twenty minutes at -20°C. Next, the samples were washed three times for 2 minutes with MilliQ water (Millipore, Merck KGaA, Darmstadt, Germany). Samples were air-dried for fifteen minutes. A layer of fixogum (Marabu, Tamm, Germany) was applied around the fingermark and left to dry for thirty minutes. The fixogum serves as a water-repellent barrier that keeps staining reagent localized on the fingermark sections. After drying, the section was covered with MilliQ
for 5 minutes. Next, the sections were covered for 5 minutes with phosphate buffered saline (PBS) (Biowhittaker, Lonza Cologne GmbH, Köln, Germany). The samples were incubated with blocking buffer 1: PBS + 3% bovine serum albumin (BSA) (Sigma Aldrich, Munich, Germany), for at least one hour. Mouse monoclonal IgM anti-dermcidin (Santa Cruz Biotechnology, INC, Santa Cruz, USA) was diluted in blocking buffer 1, 1:20 (v/v) and 100 µl was applied on the sample and left overnight in a wet chamber. The next day, samples were three times washed in PBS for two minutes. The samples were incubated with 100 µl secondary antibody, goat anti-mouse Fluorescein Isothiocyanate (FITC) (Jackson Lab, Brunschwig, Switzerland) diluted in blocking buffer 1, 1:100 (v/v) or goat anti-mouse Cy3 (Jackson Lab, Brunschwig, Switzerland) diluted in blocking buffer 1, 1:250 (v/v), for one hour in a dark room. Both fluorescent tags were tested separately on all non-porous and semi-porous surfaces to examine which fluorescent tag gave the best contrast with the particular surface carrier. The samples were washed three times for two minutes in PBS. The fixogum was removed and the samples were air-dried for 20 minutes before imaging. Adaptation of the protocol for labeling on soft plastics (garbage bags, sandwich bag, Ziploc bag) required the exclusion of fixogum, as fixogum reacts with the plastic.

2.3 Immunolabeling of fingermarks left on porous surfaces

Due to the specific characteristics of porous surfaces, compared to the non-porous surfaces, some protocol adaptations were made: exclusion of fixation step and fixogum layer. First, fingermarks left on a porous surface were incubated for thirty minutes with blocking buffer 2 (PBS + 0.1% Tween-20 + 5% skim milk powder (SMP) (Tween-20, Merck KGaA, Darmstadt, Germany, SMP, Aldrich, Munich, Germany)). Next, the samples were incubated with anti-dermcidin diluted in blocking buffer 2 1:20 (v/v) for one hour. Upon incubation the samples were washed three times with washing buffer: PBS + 0.1% Tween-20, for two minutes. Then, the samples were incubated with 100 µl secondary antibody, pre-diluted goat anti-mouse Horse Radish Peroxidase (HRP) (Abcam, Cambridge, UK), and diluted 1:1 (v/v) in blocking buffer 2 for half an hour. After incubation, the samples were three times washed with washing buffer. To activate the HRP, 3,3’-Di-aminobenzidine (DAB) (Immunologic, Duiven, the Netherlands) was added to the samples and incubated for two to four minutes in a DAB solution (prepared according to the manufacturer’s protocol). Last, the samples were washed three times for two minutes in washing buffer.

To improve the visualization of fingermarks left on thermal paper, a total of three fingermarks of three different donors were incubated with a mixture of anti-dermcidin 1:20 (v/v), mouse anti-human serum albumin 1:100 (v/v) (Sigma, Munich, Germany) and mouse anti-keratin Pan ab-1 1:100 (v/v) (Thermo-scientific, New Hampshire, USA). Also, single labeling of human serum albumin and keratins was performed on three fingermarks of different donors.
To assess the potential of using immunolabeling to redevelop fingermarks, fingermarks left on thermal paper were firstly visualized with ninhydrin NIN-Print Aerosol Spray (BVDA International bv, Haarlem, The Netherlands). After imaging, fingermarks were redeveloped with a mixture of anti-dermcidin (1:20), anti-albumin (1:100) and anti-keratin Pan (1:100). The experiment was performed on the coated as well as on the uncoated side of the thermal paper.

2.4 Control experiments
Negative controls included; the isotype control (monoclonal mouse IgM FITC (BD Biosciences, Breda, the Netherlands), the exclusion of the primary ABs and the exclusion of both primary and secondary ABs. Positive controls were performed in triplicate. Within each series four spots were applied to the surface containing diluted dermcidin (1 mg/ml) (Dermcidin, DCD-1L, Bio-connect, Huissen, the Netherlands), after drying, spots were immunolabeled.

2.5 Imaging
After the samples were dried, overall images were taken using a Nikon D40X digital camera (Nikon, Tokyo, Japan). FITC was excited using a blue/green Crime-lite® 2 torch (480 nm, 10% band width 460 – 510 nm) and its fluorescence was detected in combination with an orange filter (OG550, Foster and Freeman, Worcestershire, UK) placed in front of the lens. Cy3 was 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 (G590, Foster and Freeman, Worcestershire, UK) in front of the camera. Microscopic imaging was performed using a Nikon Eclipse E600 microscope (Tokyo, Japan) 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. Images shown in figure 6 were obtained with a Canon EOS 40D and a Canon Macro Lens EF 100mm f/2.8 USM.

3. RESULTS
3.1 Immunolabeling of fingermarks left on non-porous and semi-porous surfaces
Successful detection of dermcidin was possible in all fingermarks placed on the investigated non-porous surfaces using a secondary antibody conjugated with FITC or Cy3. Figure 1 shows an example of the detection of dermcidin with a FITC tagged secondary antibody in fingermarks left on aluminum foil and a stainless steel key. Dermcidin could also be detected on different types of plastics, as depicted in figure 2. However, due to the low fluorescence intensity, no overall images could be obtained from these immunolabeled fingermarks using FITC as detection dye. Instead, the use of Cy3 resulted in a good contrast between the surface carrier and the detected dermcidin, as depicted in figure 2. Figure 2-F shows a magnified image of a dermcidin immunolabeled fingerprint left on a black garbage bag with pore positions that can easily be identified.
Using FITC as secondary antibody, dermcidin could successfully be detected on white tile, as depicted in figure 3, but not on white laminated chipboard. Also, no successful immunolabeling could be obtained from fingermarks left on white laminated chipboard using Cy3 as fluorescent dye.

3.2 Immunolabeling of fingermarks left on porous surfaces
Due to the high background fluorescence, we used an HRP conjugate instead of fluorophores for detection of dermcidin in fingermarks left on copy paper and thermal paper. First, in fingermarks left on copy paper, no detection of dermcidin could be observed. In some cases background staining could be observed in fingermarks and the copy paper, which did not resemble specific binding either by pattern or by intensity. An explanation is the ability of cellulose to bind to amino acids and probably also to HRP. In most of the immunolabeled fingermarks left on copy paper, no color reaction was visible at all. Thus, our protocol did not allow immunolabeling with dermcidin on copy paper. Second, in contrast, thermal paper seems to be a suitable surface for our immunolabeling protocol. Fingermarks were easily visualized with dermcidin as target (Fig. 4), albeit that only in some cases the ridge pattern and second level details are visible (Fig 4-A), whereas in other cases only the pore sites and overall pattern of the fingermark are visible (Fig 4-B). These differences can be caused by the amount of dermcidin secreted or the total amount of sweat on the fingertip resulting in a more dispersed spread of dermcidin. Because immunolabeling of dermcidin in fingermarks left on thermal paper shows promise as development method, we tried to improve the visualization of these fingermarks.

Figure 1. Immunolabeling of dermcidin in fingermarks left on non-porous surfaces using anti-dermcidin and a FITC tagged secondary antibody: A: Aluminum foil. B: Stainless steel key. Scale bar represents 0.5 cm.
Fingermarks were incubated with three different antibodies; anti-dermcidin, anti-human serum albumin and anti-keratin, as depicted in figure 5-A, figure 5-B and figure 5-C, respectively. However, these labeling resulted in less well-developed fingermarks.

Figure 2. Immunolabeling of dermcidin on non-porous plastics using anti-dermcidin and a secondary antibody tagged with Cy3. A: Blue garbage bag. B: Black garbage bag. C: Ziploc bag. D: Plastic sheet. E: Sandwich bag. Scale bar is 0.5 cm. F: Magnified image of immunolabeled fingermark left on black garbage bag.

Figure 3. Immunolabeling of dermcidin in a fingermark left on a white tile. Anti-dermcidin and a secondary antibody tagged with FITC were used to detect dermcidin. Scale bar is 0.5 cm.
The fingermark presented in figure 5-A, incubated with anti-dermcidin only, shows less contrast and more background staining, compared to the other developed fingermarks. Almost no labeling was present in fingermarks incubated with anti-keratin (fig-5C). Fortunately, a mixture of the three antibodies results in perfectly developed fingermarks, as depicted in figure 5-D.

Figure 6 shows the immunolabeling of fingermarks left on thermal paper, after they had been visualized using ninhydrin spraying. Most of the fingermarks developed with ninhydrin resulted in clear developed fingermarks. Successful immunolabeling was obtained on fingermarks left on the coated and uncoated side of thermal paper (figure 6). After immunolabeling, no coloration of the reaction products of ninhydrin spraying present in the fingermark can be observed anymore. The purple color is completely washed away [8-9]. Thus, immunolabeling can led to the redevelopment of fingermarks left on thermal paper.
In all cases, control experiments were negative. The results of the controls are available on request.

4. DISCUSSION

We have demonstrated that our method of immunolabeling of fingermarks is compatible with most surfaces, which are typically encountered in forensic practice, including porous, semi-porous and non-porous materials. In this study, one intrinsically similar immunolabeling method was tested on various surfaces, which made a consistent comparison of the method possible. We have chosen dermcidin as target antigen, because this protein is a secretion product and is released by the sweat glands in the friction ridge skin in healthy individuals, as demonstrated in previous studies [8-9]. Using our protocol, immunolabeling was compatible with aluminum foil, stainless steel keys, plastic sheets, different colored garbage bags, sandwich bags, Ziploc bags, white tiles, and thermal paper. No specific detection of dermcidin was possible on fingermarks left on laminated chipboard and on copy paper using the method described in this study. Immunolabeling of fingermarks left on the normally problematic thermal paper turned out to be a surprisingly good manner to visualize latent fingermarks and is therefore an interesting method to use as an additional tool for the development of fingermarks on thermal paper.

Next to the porosity, we also observed differences in the intensity and visibility of the
two used fluorophores (FITC and Cy3) between the different surfaces. In the case of autofluorescence by the surface material, other staining techniques, like HRP, have to be considered.

The porosity and hydrophobic character of the surface can affect the applicability of immunolabeling of fingermarks. The pore size of the carrier surface influences the absorbance of the fingermark deposit and thus affects the detection of the antigens. The contrasting outcomes between the two tested semi-porous surfaces, the tile and laminated chipboard, can possibly be explained by the different absorption properties. Possibly, the covered layer on the laminated chipboard is able to absorb the fingermark components partially or fully, this layer is thus hampering the detection of the antigens by the antibodies. Also, the texture of surface can be of influence for the detection of the antigen using immunolabeling.

In the tested porous surfaces, successful immunolabeling was only possible in fingermarks left on thermal paper on the coated as well as the uncoated side. As detection tag, HRP was used, instead of FITC or Cy3, because of the fluorescence properties of thermal paper and copy paper. HRP is an enzyme and in the presence of DAB an enzymatic reaction takes place which lead to a color reaction that can be easily visualized [21]. In general, the development and visualization of fingermarks left on thermal paper is more complex than the development of fingermarks left on standard papers. Thermal paper is composed of several layers, including an active coating, making this material different from traditional porous surfaces [17, 22]. The active layer is sensitive to heat and polar agents and if exposed a dark turnover of the paper can be observed, causing irreversible damage, which affects the visualization and quality of the fingermark [18-19]. We observed that immunolabeling did not introduce such a reaction to the thermal paper. Almost all immunolabeled fingermarks left on thermal paper could be imaged with high contrast, except for the fingermarks of which we assume that they did not contain much dermcidin. In those cases, a significantly enhanced visualization of fingermarks occurred when fingermarks were incubated with a mixture of antibodies. Simultaneous detection of dermcidin, albumin and keratin resulted in a fingermarks of high quality. However, when activating the HRP, care should be taken. When fingermarks are incubated too long with DAB, high background staining can be observed, as shown in fig 5-A, resulting in decreased contrast between the background and the fingermark. Both sides of the thermal paper were investigated, the coated and uncoated side, on both sides immunolabeling was successful. To investigate whether immunolabeling could be used as an additional tool to redevelop fingermarks, fingermarks were first developed with ninhydrin and after imaging immunolabeling was performed on the fingermark. In some cases immunolabeling led to the enhancement of the fingermark, whereas in other cases no added value was observed (figure 6). From these results, we can conclude that immunolabeling should not be used as a primary visualization method, but as a method to redevelop the visualization of fingermarks. Future research should focus
on the identification of other targets that will improve the visualization of fingermarks.

In contrast to two other studies, we were not able to detect dermcidin in fingermarks left on copy paper using our method [9, 16]. An explanation for this finding is uncertain, but probably, the pore size, brand of paper and/or other characteristics of the copy paper used, influences the binding capacity of the primary and secondary antibody to the antigen present on the paper. In one sample we observed background staining on copy paper, which could have been caused by binding of HRP to the cellulose present in the paper. The labeled fingermarks and controls were further all negative (total of 20 fingermarks). Probably, improperly blocking could have caused this non-specific binding. However, we only investigated one type of copy paper.

Immunolabeling can be used to (re)develop fingermarks [4, 7-14]. As previous studies have shown, immunolabeling is compatible with magnetic fingerprint powdering and ninhydrin staining, thus after visualizing and recording of the fingermarks, immunolabeling can be applied, either to retrieve donor profiling information or to redevelop fingermarks [8, 13]. Indeed, fingermarks sometimes showed great contrast when developed with our immunolabeling method. First, second and third level details could be obtained from fingermarks of individuals who secreted a lot of dermcidin or who have a high sweat rate, as shown in figure 2-A and B, figure 3 and figure 4-A. These fingermarks contain enough minutiae and can therefore be used for dactyloscopic identification. However, not all individuals secrete this high amount of dermcidin. In these other individuals dermcidin is mainly located at the pore sites. Therefore, we suggest, when using immunodetection to visualize fingermarks, to target multiple general antigens in the fingermark. Possible other targets, described in previous studies, can be cathepsin D, keratins, human serum albumin and amino acids [8-10, 16]. As depicted in figure 5-D, multiple detection can lead to high quality developed fingermarks. Single detection of keratins resulted in low quality images in all investigated fingermarks, therefore we suggest that multiple detection of dermcidin and albumin is already appropriate to obtain high contrast images on thermal paper.

We used three different visualization enhancers: HRP, FITC and Cy3. The selection of the (fluorescent) dye is of importance, because it affects the visibility and contrast of the detection. Some surfaces, like copy paper and thermal paper, displayed a considerable amount of autofluorescence, which interferes with the fluorescent signal obtained from the immunolabeling. In these cases, the use of fluorescent tags is not the best choice. Instead, HRP or alkaline phosphatase tagged antibodies can be used; these dyes give a color reaction when applying a chemical reagent. The two fluorescent dyes used during our study were FITC and Cy3. FITC is a green fluorescent dye and Cy3 is an orange fluorescent dye. In general, we observed that Cy3 gave more contrast and showed less bleaching than FITC, which is supported by literature [23-24]; nevertheless, both observations were not valid for labeling on aluminum foil.
gave a good contrast when FITC was used as visual tag, this in contrast with the plastic surfaces. When choosing a fluorescent tag characteristics such as emission wavelength and quantum yield are the first selection criteria. However, these characteristics are influenced by environmental conditions. Thus in general, we propose to always test a number of fluorescent tags when developing a new method as the results might be counterintuitive.

In this study we used fresh fingermarks. Fingermarks left on non-porous surfaces were treated one hour after deposition, whereas fingermarks left on porous surfaces were treated one day after deposition to give the material time to adsorb the fingermark. Our main goal was to evaluate whether immunolabeling could be applied to fingermarks left at different surfaces than glass slides and nitrocellulose membrane. For this study the age of the fingermark was not relevant. However, in preliminary work (not shown) dermcidin could easily be detected in fingermarks of an age up to one month on glass slides and nitrocellulose. The stability of dermcidin is influenced by the type substrate and environmental factors. Therefore, we expect that dermcidin can minimally be detected up to one week and even more. Since, we did not investigate this for the different types of substrates, we can only speculate.

To evaluate immunolabeling of fingermarks left on different surfaces dermcidin is a good target. For the visualization of fingermarks left on thermal paper, dermcidin and albumin can be targeted. However, dermcidin and albumin do not give donor profiling information. To use immunolabeling as a new visualization method more experiments should be included and also other targets should be investigated. Sears et al. describes a good protocol to develop and investigate the efficacy of new visualization methods [25]. In future research the method described by Sears et al. can be used to investigate the possibilities of the immunolabeling technique as visual developer [25]. In our current and future studies, instead of anti-dermcidin and anti-albumin, other antibodies will be used, which attach to antigens that can provide donor information, like gender, drug use, smoking habits and food habits [3-6, 11, 13-14]. Currently, immunolabeling guided profiling is not yet possible, but could become interesting in the nearby future. Future research should focus on the detection of antigens that provide such information about the donor. An important advantage of immunolabeling is that it is not limited to fingermarks, but can also be applied to other traces, like minimal contact traces, blood, sperm and saliva [26]. Immunolabeling is a technique that can easily be applied to fingermarks and other traces. With the described protocol, immunolabeling takes up to a day. The actual hands on laboratory work is less than one hour, the rest is accounted by incubation times. The method can be speeded-up by increasing the operating temperature to 37°C and/or coupling of a visual enhancers directly to the primary antibody (15). The wide applicability of the immunolabeling technique makes this technique an excellent method to use for donor profiling and/or (re)developing fingermarks in the forensic field.
5. CONCLUSION
We have shown successful immunolabeling of fingermarks left on aluminum foil, stainless steel keys, plastic sheets, different colored garbage bags, sandwich bags, Ziploc bags, white tiles, and thermal paper. These findings demonstrate that immunolabeling is compatible with a wide variety of backgrounds, including non-porous, semi-porous and porous backgrounds. Our results broaden the applicability of this technique in the forensic field for instance: to retrieve information about the donor and to (re)develop fingermarks. On the problematic substrate, thermal paper, our immunolabeling method appears to be an excellent manner to develop latent fingermarks. The fast and easy performance, the applicability on multiple surfaces and the compatibility with fingerprint visualization techniques, makes immunolabeling a promising technique in the forensic field.

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