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Simultaneous labeling of multiple components in a single fingermark ☆

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ABSTRACT

A fingermark contains important forensic information of the donor, not only in its ridge pattern, but also in the chemical composition of its secretion. Detection and identification of these secretions can be done by immunolabeling. In this study, we describe for the first time a reproducible immunolabeling method that allows the simultaneous detection of multiple components of interest. This method not only reduces the manipulation of fingermarks, but also different types of information can be obtained about the donor in one labeling session. To prove the concept of this technique, we selected two general components as antigens of interest, dermcidin and the human serum albumin. Conjugation of both antibodies to two different synthetic fluorophores, followed by simultaneous incubation of both conjugated antibodies, resulted in successful multiple immunolabeling of fingermarks left on a porous nitrocellulose membrane and on a non-porous glass slide surface. In order to minimize false positives to prevent non-specific binding of antibodies to fingermarks and surface carriers, careful blocking and washing steps were found crucial. With this reproducible protocol, high quality images could be obtained from the multiple labeled fingermarks. In conclusion, simultaneous multiple immunolabeling of antibodies in fingermarks can identify specific components in the secretion of the fingermark, including components related to hygiene, diet, time of day, contacts gender and drug use. Multiple immunolabeling therefore has the potential to make a major impact in the forensic field.

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1. Introduction

Fingermarks found at crime scenes and on crime-related objects, contain skin ridge patterns that are used for database searches and identification purposes. Their DNA content allows personal identification as well [1,2]. Secreted components of fingermarks that originate from skin glands have a chemical composition that includes lipids, amino acids, proteins, and also exogenous components like debris and cosmetics, which are affected by personal hygiene, diet, time of the day and type of contact [3–5]. Thus, analysis of the composition could provide intelligence information about the donor, like gender, diet and/or presence of drug metabolites [3,6–8]. A method to retrieve this information is immunolabeling.

Not all fingermarks found at a crime scene will lead to the identification of the donor. Profiling information will then be of great forensic value, for example to include or exclude potential donors. For forensic purposes antibodies (ABs) can be used to detect specific components of interest and/or to enhance the visibility or develop fingermarks [9–16]. Recently, we have described how immunolabeling is compatible with two common fingermark visualization methods, ninhydrin spraying and powder dusting [9]. This finding is a major advantage, since the fingermark cannot only be used for identification purposes using the ridge pattern, but, when using immunolabeling, also for the detection of antigens of interest. An important issue then is to minimize the manipulation of the fingermark. Therefore, we have sought to develop a method that is reproducible and able to detect two or more antigens in a fingermark simultaneously without physically affecting the fingermarks.

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To prove the principle of multiple and simultaneous immunolabeling, single fingermarks were directly incubated with a mixture of two antibodies to obtain multiple and simultaneous immunolabeling. Two generally present peptides of endogenous origin were selected as antigens of interest, first dermcidin, an antimicrobial peptide and, second, the human serum albumin (HSA), an abundant carrier protein [12,17–20]. Both antigens are identified to be present in eccrine sweat. Dermcidin and HSA have been successfully detected in fingermarks by Drapel et al. and Reinholz [12,15,17,18]. We emphasize that there are no fundamental reasons to be limited to only two antigens. In this study, fingermarks were left on porous nitrocellulose membranes and on common non-porous glass slides. Positive and negative controls were performed to investigate the specificity of the antibodies used during the experiments.

2. Materials and methods

All experiments were performed under room temperature unless mentioned otherwise.

Buffer solutions were prepared according to the description in Table 1.

2.1. Fingermark collection

Fingermarks were collected during working hours. Donors were asked to deposit a fingermark on nitrocellulose membranes (0.45 μm, Millipore, Merck KGaA, Germany) or on glass slides (Superfrost plus, Gerhard Menzel GmbH, Germany). Fingermark depositions were completely natural; no special instructions were given to the donor. Before treatment, samples were left to dry for 24 h. For every experiment at least eight fingermarks of different donors were included, as shown in Table 2.

2.2. Incubation of primary and secondary antibodies on fingermarks left on nitrocellulose membrane

After one day, fingermarks deposited on nitrocellulose membrane were incubated for one hour in blocking buffer 1 (Table 1). Next, the membrane was incubated with 100 μl primary AB for one hour. Mouse monoclonal IgM anti-dermcidin (G-81) (SC-33656, Santa Cruz Biotechnology, Inc., USA) was diluted in blocking buffer 1 at a concentration of 1:20 (v/v). Mouse monoclonal IgG2a anti-HSA (A6884, Sigma–Aldrich, Germany) was diluted in blocking buffer 1 at 1:100. After incubation, the membrane was washed three times for two minutes with washing buffer. Next, the membrane was incubated with 100 μl secondary AB, goat anti-mouse IgM FITC (115-095-075, Jackson Lab, Brunschwig, Switzerland) or goat anti-mouse IgG FITC (ab6785, Abcam, UK) both diluted in blocking buffer 1 at 1:100 (v/v) for thirty minutes. After incubation the membrane was washed three times for two minutes with washing buffer. Negative controls included isotype controls: mouse IgM FITC (553474, BD Biosciences, USA) and mouse IgG2a (MCA929F, Bio-connect, The Netherlands) and the exclusion of the primary AB and the exclusion of both primary and secondary ABs.

2.3. Positive control experiment

Hundred microgram of dermcidin, DCD-1L (SP2420a, Abgent, USA) was diluted in 100 μl MilliQ (Millipore, Merck KGaA, Germany) to obtain a concentration of one mg/ml. One mg of albumin from human serum (A9511-100 mg, Sigma–Aldrich, Germany) was diluted in one ml MilliQ to obtain a concentration of one mg/ml. To obtain two spots for each protein solution, 2 μl were applied to the nitrocellulose membranes. Immunolabeling was performed according to the protocol described in Section 2.2. Instead of using a secondary antibody, conjugated with a fluorescent dye, a 1:2 (v/v) diluted goat anti-rabbit secondary antibody conjugated to horse radish peroxidase (HRP) (prediluted) was used. After incubation, the membrane was washed three times for two minutes with the washing buffer. HRP was activated with 3,3′-diaminobenzidine (DAB) (DAB plus, powerDAB, Immunologic, Netherlands). DAB was prepared according the manufacturer's protocol. After activation, the membrane was washed three times with washing buffer.

Table 1
Specification of used buffer solutions.

Buffer solutions	Contents
Washing buffer	Phosphate buffer saline (PBS)[a] + 0.1% Tween-20[b]
Blocking buffer 1	PBS[a] + 0.1% Tween-20[b] + 5% skim milk powder (SMP)[c]
Blocking buffer 2	PBS[a] + 5% SMP[c]

[a] Biowhittaker, Lonza Cologne GmbH (Germany).
[b] Merck KGaA (Germany).
[c] Sigma–Aldrich (Germany).

2.4. Conjugation of antibodies to fluorophores

Multiple labeling was obtained by conjugation of the ABs to different fluorophores. Anti-dermcidin was conjugated to Dylight®350, anti-HSA to Cy3, using Lightning-Link® Rapid DyLight®350 and Lightning-Link®Rapid Cy3 (Innova Biosciences, UK), respectively. The conjugations were prepared according to the manufacturer's instructions.

2.5. Multiple labeling performed on fingermarks left on nitrocellulose membrane

One day after deposition, fingermarks on a nitrocellulose membrane were incubated with blocking buffer 1 for one hour. Next, the membrane was incubated with a mixture of both conjugated primary ABs anti-dermcidin 1:20 (v/v), anti-HSA 1:100 (v/v) (dilutions of total multiplex solution) for one hour. After incubation, the membrane was washed three times for two minutes with the washing buffer. Negative controls included isotype controls, the exclusion of the primary ABs and the exclusion of both primary and secondary ABs.

2.6. Multiple labeling performed on fingermarks left on glass slides

One day after deposition, fingermarks on glass slides were fixated in methanol (Sigma–Aldrich, Germany) for twenty minutes at −20 °C. Next, the slides were rinsed three times for two minutes in MilliQ. Samples were air-dried for 10–15 min. A layer of fixogum (Marabu, Germany) was applied around the fingermark and dried for thirty minutes. The fixogum serves as a water-repellent barrier that keeps staining reagents localized on the fingermark sections. MilliQ was added to cover the section for five minutes. After that, PBS was applied to the samples and left for five minutes. Samples and surface were blocked with blocking buffer 2 for one hour. The mixture of both conjugated primary ABs (anti-dermcidin 1:20 (v/v), anti-HSA 1:100 (v/v)) (dilutions of total multiplex solution) were applied to the fingermark and left overnight in a wet chamber, using moistened tissues. After overnight incubation the samples were washed three times for two minutes in PBS. Fixogum was removed and a drop of DAKO fluorescent mounting media (Dako, Corp., Denmark) was applied to every glass slide, and cover slips were put on. Samples were dried and analysis was done by fluorescent and bright field microscopy. Negative controls included isotype controls, the exclusion of the conjugated primary ABs and the exclusion of both primary and secondary ABs.

2.7. Imaging

Samples were dried. The presence of ABs was confirmed by obtaining fluorescence images using a Nikon Eclipse E600 microscope (Dylight®350 (blue channel): excitation filter 340–380 nm, dichroic mirror 400 nm, barrier filter 435–485 nm; FITC (green channel): excitation filter 465–495 nm, dichroic mirror 505 nm, barrier filter 515–555 nm; Cy3 (red channel): excitation filter 510–560 nm, dichroic mirror 575 nm, barrier filter 590 nm and a Nikon Coolpix 990 digital camera. Fluorescent overall images of fingermarks were obtained using the blue/green (excitation: 460–510 nm) Crime-lite®2 torch (Foster and Freeman, UK). Digital images were taken using the yellow filter (GG495, Foster and Freeman) and a Nikon D40X digital camera (Nikon, Japan). To distinguish fluorescence by the labeling from autofluorescence, the FITC channel was used to monitor the autofluorescence when performing multiple labeling.

3. Results

Single labeling of specific components in fingermarks, left on a nitrocellulose membrane, was possible using immunostaining with anti-dermcidin and anti-HSA, as shown in Fig. 1. Nitrocellulose membrane binds proteins and amino acids with a high affinity and is normally used to check the specificity of ABs [21]. As depicted in Fig. 1A, dermcidin is only spread around the pore-sites of the fingermark and provides therefore information that can help in obtaining third level details from fingermarks. Fig. 1B shows that HSA is found at the pore-sites of the fingermark deposition and along the ridges.

Differences in fluorescence intensity could be observed in and between anti-dermcidin and anti-HSA labeled fingermarks. Lower fluorescence intensity was obtained in all fingermarks when detecting HSA, compared to dermcidin detection. Possible explanations for this observation can be found in (i) the different affinity of the antibodies to their epitope, (ii) the amount of the antigen present in the fingermark deposition, (iii) the isotype of the antibodies (IgM vs IgG) and (iv) antigen presentation. These results

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