Fingermarks, more than just a ridge pattern

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This section contains the results of the negative, isotype and positive controls. Also, a table including the camera settings is applied within this section. Importantly, to use immunolabeling for donor profiling in fingermarks, the labeling must be specific. Non-specific binding leads to false positive results. Negative controls and isotype controls are performed parallel with each immunolabeling experiment. In total four different fingermarks were obtained from each donor at the same time per experiment.

1. Direct labeling
2. Negative control (exclusion of primary antibody)
3. Negative control (exclusion of both primary and secondary antibody)
4. Isotype control

Positive controls were performed in triplicate. Within each series eight spots were applied to the nitrocellulose membrane, four spots containing diluted dermcidin (1 mg/ml) and the other four spots containing diluted HSA (1 mg/ml).
SUPPORTING FIGURES

In images S1 to S8: A and D: (Blue channel) Excitation filter 340-380 nm, dichroic mirror 400, barrier filter 435-485 nm; B and E: (Green channel) Excitation filter 465-495, dichroic mirror 505 nm, barrier filter 515-555. C and F: (Red channel) Excitation filter 510-560 nm, dichroic mirror 575 nm, barrier filter 590 nm.

Figure S1. Fluorescence images of a negative control for detection of dermcidin and HSA in fingermarks left on nitrocellulose membranes. In this negative control, the primary antibody was excluded from the protocol (anti-dermcidin and anti-HSA). This negative control is necessary to find out whether the secondary antibody has the ability to bind non-specific to the fingermark and/or whether background staining can be observed. No non-specific binding of the secondary antibody can be observed. Autofluorescence of the fingermark can be noticed and is observable in all three different channels.

Figure S2. Fluorescence images of the negative control for detection of dermcidin and HSA in fingermarks left on nitrocellulose membranes. In this negative control, both primary and secondary antibodies are excluded. Therefore the fluorescence observed is only originating from the fingermark or background itself. Autofluorescence can be observed in all the three different channels.
Figure S3. Fluorescence images of the isotype control for detection of dermcidin left on nitrocellulose membranes. This control included the incubation of an isotype control mouse IgM FITC conjugated, similar to the isotype of anti-dermcidin. The isotype control is used to observe whether the primary antibody has the ability to bind non-specific to the fingerprint or surface. No non-specific binding of the isotype mouse IgM can be observed. Autofluorescence of the fingerprint can be observed in the three different channels.

Figure S4. Fluorescence images of the isotype control for detection of HSA left on nitrocellulose membranes. In this control an isotype similar to the primary antibody anti-HSA was incubated with the fingerprint, an isotype control mouse IgG2a conjugated with FITC. No non-specific binding to the fingerprint or background can be observed. Autofluorescence can be observed in the three different channels.
Figure S5. Fluorescence images of the negative control for detection of dermcidin and HSA in fingermarks left on glass slides. This negative control included the exclusion of the primary antibodies (anti-dermcidin and anti-HSA). Fingermarks were incubated with the secondary antibody. No non-specific binding of the secondary antibody can be observed. Autofluorescence of the fingermark can be noticed and is observable in all three different channels.

Figure S6. Fluorescence images of the negative control for detection of dermcidin and HSA in fingermarks left on glass slides. This negative control included the exclusion of both primary and secondary antibodies. Autofluorescence of the fingermark can be observed and is visible in the three different channels.
Figure S7. Fluorescence images of the isotype control for detection of dermcidin in fingermarks left on glass slides. This control included the incubation of an isotype control mouse IgM FITC conjugated, similar to the isotype of anti-dermcidin. The isotype control is used to observe whether the primary antibody has the ability to bind non-specific to the fingermark or surface. No non-specific binding of the isotype mouse IgM can be observed. Autofluorescence of the fingermark can be observed in all three different channels.

Figure S8. Fluorescence images of the isotype control for detection of HSA left on glass slides. In this control an isotype similar to the primary antibody anti-HSA was incubated with the fingermark, an isotype control mouse IgG2a conjugated with FITC. No non-specific binding to the fingermark or background can be observed. Autofluorescence can be observed in all three different channels.
Figure S9. Brightfield images of the positive control experiments. Dermcidin was diluted in MilliQ (1 mg/ml) and 2 µl per spot was applied to the nitrocellulose membrane (fig. S9-A and B). HSA was diluted in MilliQ and spots of 2 µl were applied to the nitrocellulose membrane (fig. S9-C and D). Anti-dermcidin was applied to spots placed in area A and C, a positive result with dermcidin was obtained (fig. S9-A) and a negative result was obtained with HSA (fig. S9-C) after applying the secondary AB and the chemical reagent DAB. Anti-HSA was applied to spots B and D, a negative result was obtained with dermcidin (fig. S9-B) and a positive result was obtained when incubated with HSA (Fig. S9-D) after applying the secondary AB and the chemical reagent DAB.

Table S1. Camera settings

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<th>Camera settings</th>
<th>Exposure time</th>
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<th>Aperture</th>
<th>Magnification Microscope</th>
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<td>F/2.7</td>
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