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SHORT COMMUNICATION

Transfer of biological stains from different surfaces

Peter Wiegand · Christian Heimbold · Rachel Klein · Uta Immel · Dankwart Stiller · Michael Klintschar

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Abstract Highly sensitive short tandem repeat-based stain analysis of weak biological stains has been improved during the last years. The risk of transfer of cellular material from handled items contaminated with biological stains such as blood and saliva is of forensic relevance. Although the policemen working with crime scene items are very careful, there exists a potential risk of transfer contamination. To obtain estimates for the risk of stain transfer by handling, we have carried out an experimental study on 288 dried blood and saliva stains in two laboratories. DNA quantification showed only small amounts of DNA that could be transferred, especially from stains on paper and cotton. The saliva and especially the blood stains from plastic surface resulted in higher amounts of transferred DNA, depending on the relation of blood volume to included area: Of 192 direct transfers, 17% gave extracts above 10 pg DNA/μl, and only 3% of 96 secondary transfers resulted in amounts above 10 pg DNA/μl.

Keywords DNA typing · Secondary stain transfer · Blood · Saliva

P. Wiegand · R. Klein
Institute of Legal Medicine,
Prittwitzstr 6,
89075 Ulm, Germany

U. Immel · D. Stiller
Institute of Legal Medicine,
Franzosenweg 1,
06112 Halle, Germany

C. Heimbold
Institute of Legal Medicine,
Robert-Koch-Str. 40,
37075 Göttingen, Germany

M. Klintschar
Institute of Legal Medicine,
Carl-Neuberg-Str.1,
30625 Hannover, Germany

P. Wiegand (✉)
Institute of Legal Medicine, University Hospital,
Albert-Einstein-Allee 11,
89081 Ulm, Germany
e-mail: peter.wiegand@uniklinik-ulm.de

Introduction

The increasing sensitivity of short tandem repeat (STR)-based stain analysis during the last years has enabled typing of very weak biological stains [1–7]. However, this improvement may jeopardise the reliability of DNA typing as epithelial cells or, more likely, blood or saliva could have been secondarily transferred from stains to skin areas of persons and from there to other items without notice of the recipient [3]. This is relevant for the police work at a scene of crime, where it is crucial to avoid handling procedures carrying the risk of secondary transfer contamination and also for persons who may have been involved in crime scene situations [5]. To obtain estimates for the risk of stain transfer, we have carried out an experimental study on dried blood and saliva stains in two laboratories. Based on the results of a pilot test (unpublished results) and common sense, we came to the conclusion that contact with wet blood and saliva stains enables an intensive secondary stain transfer leading to full STR profiles. Based on this

conclusion, the main study was focussed on dried blood and saliva stains.

Materials and methods

Aliquots of 50 µl of saliva or venous ethylenediaminetetraacetic acid (EDTA) blood from the male donors were transferred onto paper, cotton cloth and plastic surfaces by equally moistening an area of 2×2 cm. After air drying overnight, the stains were touched (a) for 2 s by pressing the thumb on it and (b) 10 s of rubbing with the thumb under low pressure. This procedure was performed with and without gloves (Sempercare, powder-free latex gloves; Semperit, Vienna, Austria). The stains were recovered from the thumbs using moistened cotton wool swabs (direct transfer). In a second experimental series, stains were transferred after 10 s of rubbing by the test person with the thumb to the surface of a paper—then the contact area was swabbed (secondary transfer). Four test persons for each laboratory (two female and two male persons) were included in the stain contact series. Stains were removed from the thumb/gloves/paper using cotton wool swabs moistened with sterile water. After each stain contact, the skin of the thumb was intensively cleaned with water on paper towel. To test whether it is possible to extract the probands' own DNA, the thumb of each proband was swabbed after cleaning; these previous investigations gave no detectable amounts of DNA. All steps of the experimental series have been carried out subsequently.

DNA from blood and from buccal swabs was extracted using the Chelex method described elsewhere [8]. DNA from saliva and blood stains was extracted with the First-DNA all-tissue DNA kit (GEN-IAL® GmbH, Troisdorf) in laboratory 1 according to manufacturer's protocol, while laboratory 2 used the DNA IQ® extraction protocol (Promega, Mannheim, Germany).

In both laboratories, DNA extracts were diluted in 40-µl extraction volume; 5 µl was used for real-time polymerase chain reaction (RT-PCR) quantification (PCR cycler ABI 7500; Plexor DNA Quantification Kit; Promega—reliable detection limit, 6 pg DNA/µl). RT-PCR quantification was carried out for two times in each laboratory. Altogether, 288 stains were extracted and subsequently quantitated. Selected DNA extracts within the range between 2 and 15 pg/µl were amplified with the SEfilerPlus kit (Applied Biosystems, Weiterstadt, Germany) according to the manufacturer's recommendations (30 PCR cycles) using 7 µl of DNA extract in 12.5 µl PCR volume.

The PCR reactions were analysed with capillary electrophoresis (ABI 3130, Weiterstadt, Germany). Analysis was carried out only for alleles with signal intensities above 50 rfu.

Results

DNA quantification showed comparable results in both laboratories; the quantification data were combined in one figure for each surface/stain combination (Fig. 1a–f).

Saliva Direct transfer of saliva from paper and cotton after 2 s of low pressure and 10 s of rubbing with the thumb showed nearly for all test persons detectable but low DNA concentrations (<10 pg/µl). Stains on gloves vs. direct transfer onto the skin of the thumb resulted in comparable amounts of DNA (Fig. 1a, b). Secondary transfer of these stains (10 s with rubbing and transfer to paper) gave only approximately 50% of the stains detectable and very low amounts of DNA in the range of 0–1 pg/µl.

In contrast, transferred saliva stains from plastic showed DNA concentrations up to 100 pg/µl and also lead to secondary transferred DNA in the range of 0–10 pg/µl (Fig. 1c).

Blood Direct and secondary transferred blood stains from cotton resulted in DNA concentrations mostly close to 0.1 pg/µl (Fig. 1e). Only direct transfer on gloves gave low amounts of detectable DNA up to 10 pg/µl for single probands. Clearly, higher concentrations of DNA could be quantified for blood from paper and especially from plastic (Fig. 1d, f). More than 100 pg/µl was detectable for a higher number of probands for blood stains on gloves. Also, secondary transfer reached relevant values, in one instance, of more than 100 pg/µl (Fig. 1f).

For this reason, we extended the experimental scheme for the plastic surface in the way that 50 µl of EDTA blood was transferred on larger areas: (a) 4×4 and (b) 8×8 cm. In this extended investigation, only two probands were included. Only for the 4×4-cm area detectable amounts of DNA were found in the range between 3 and 8 pg/µl (2 and 10 s without gloves). The other constellation gave no quantification results.

To further investigate whether the amounts of DNA transferred were sufficient for successful DNA typing, a selection of samples with a concentration of 2–15 pg/µl DNA was analysed using STR typing. From both laboratories, 15 samples were analysed by STR typing for this critical range to obtain the information whether such limited amounts of DNA may lead to interpretable STR profiles. For stains containing 10 pg/µl of DNA, it was possible to get full profiles using SEfilerPlus (Fig. 2). Lower DNA concentrations (5–10 pg/µl) led to less balanced and often partial STR profiles.

Of 192 direct transfers, 33 resulted in DNA extracts above 10 pg/µl and, thus, concentrations that are in our hands gave full DNA profiles on a regular basis.

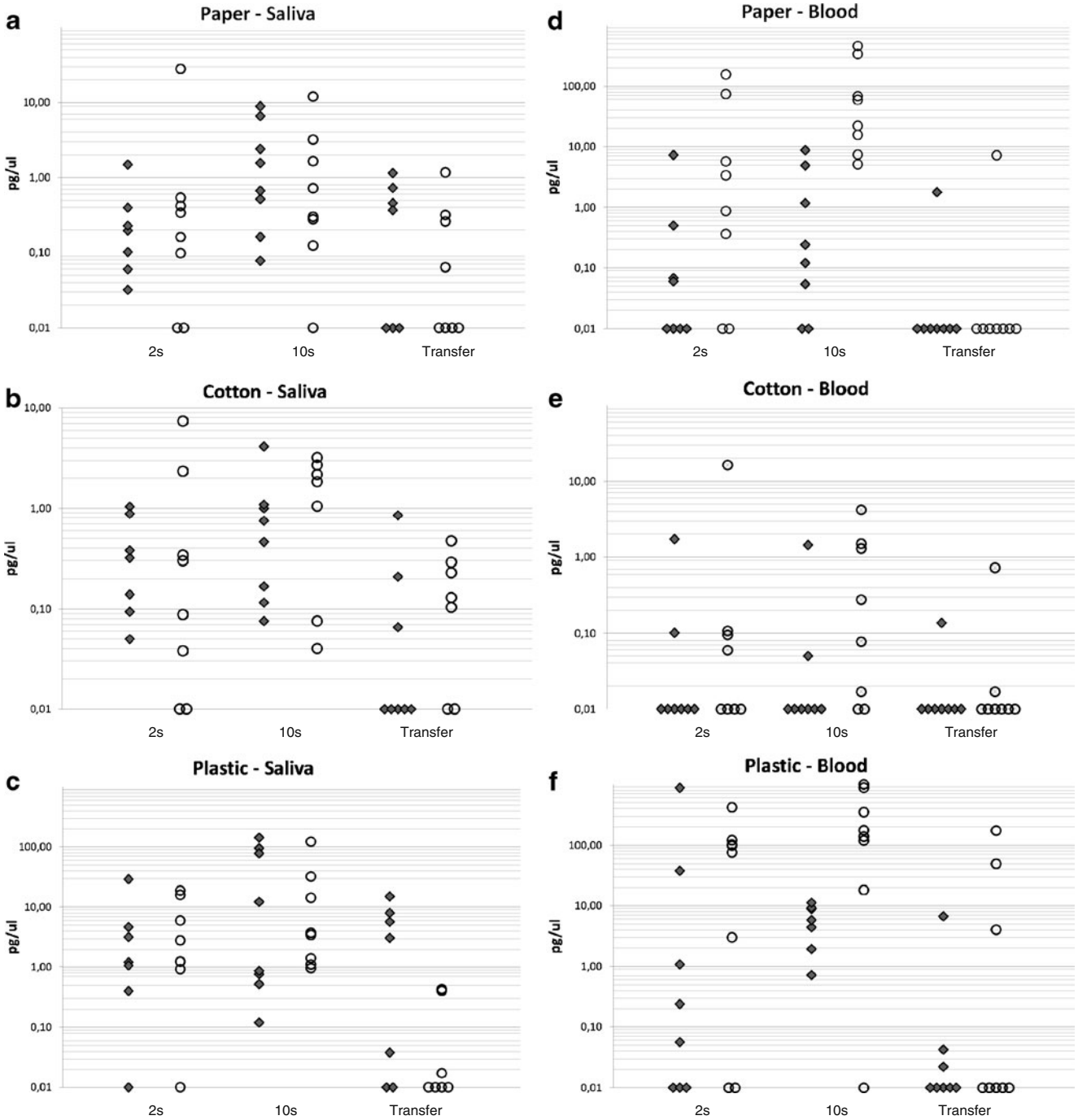

Fig. 1 **a**–**f** Direct and secondary transferred saliva (**a**–**c**) and blood (**d**–**f**) stains on different surfaces (paper, cotton and plastic). *Diamonds*: transfer by skin (thumb) without gloves; *circles*: transfer by thumb using gloves. 2 s=2 s by pressing the thumb; 10 s=10 s rubbing with the thumb under low pressure; transfer=secondary transfer after 10 s of rubbing with the thumb under low pressure and subsequent stain transfer on paper. *y*-axis: DNA concentration in pg/µl. The value 0.01 pg/µl represents data which are below the detection limit=<0.01 pg/µl

Eleven more direct transfers led to DNA amounts of 5–10 pg/µl, concentrations for which we often had weak or partial profiles.

Of 96 secondary transfers, only three DNA extracts gave values above 10 pg/µl. Four direct transfers resulted in concentrations of 5–10 pg/µl (Fig. 1a–f).

Discussion

Direct transfer of blood and saliva resulted most often in DNA concentrations below the threshold of successful STR typing or in concentrations that enabled only partial STR profiles; however, 17% of direct transfers (33 of

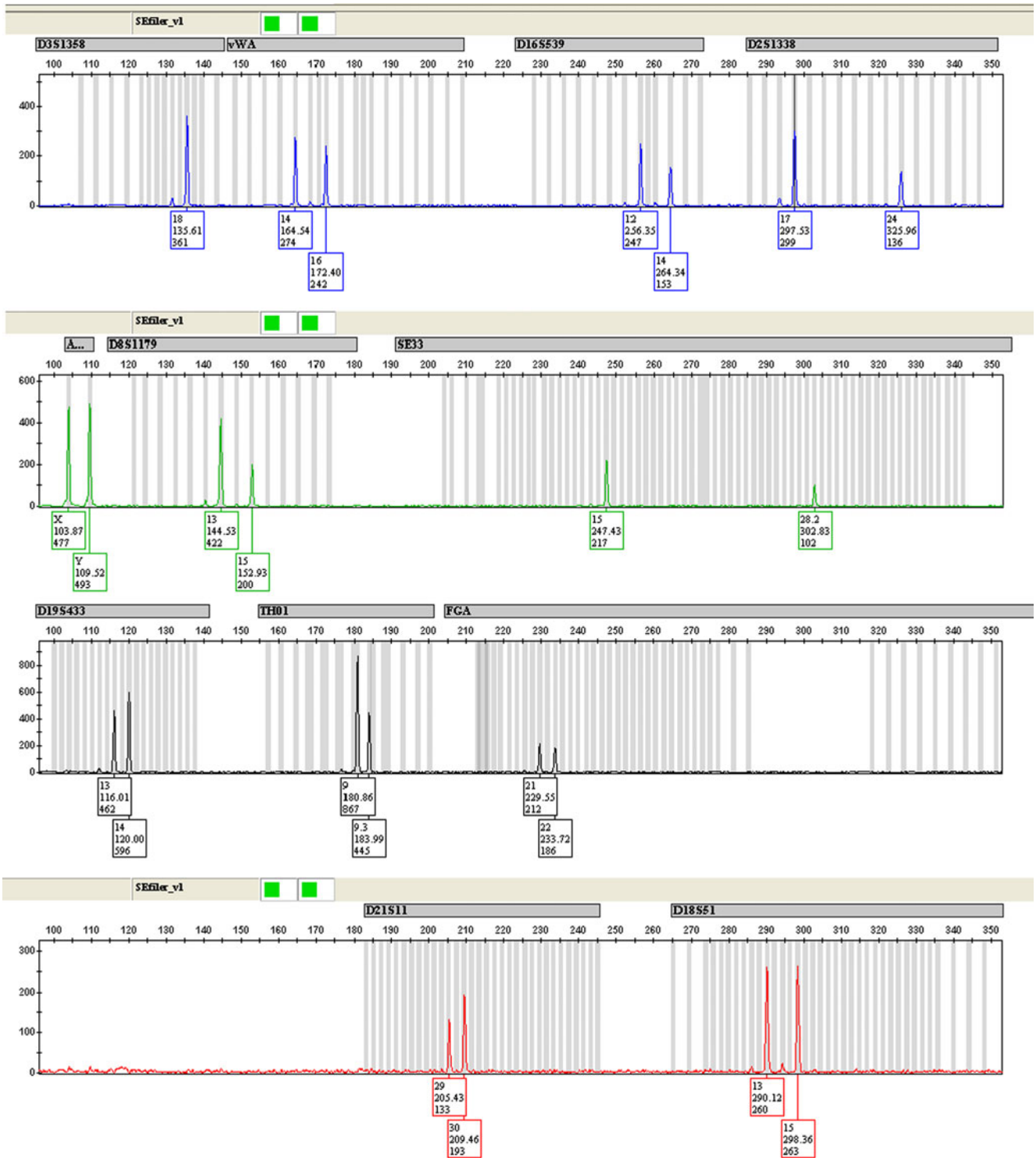

Fig. 2 The SEfilerPlus electropherogram shows a DNA profile of a secondarily transferred saliva stain with the concentration of approximately 10 pg/μl. Polymerase chain reaction was carried out with 7 μl DNA extract using 30 cycles

192) resulted in amounts of DNA that enabled complete profiles.

Secondary transfer, on the other hand, gave only in rare instances DNA concentrations sufficient for complete DNA profiles (three of 96). All these were transfers from stains on plastic, one saliva stain and two blood stains.

Obviously, the absorptive capacity of the material from which the transfer is done is of crucial importance. The

contact of the thumb (with and without gloves) to the blood stains on the nonporous plastic surface resulted, in most of the cases, in an impression of the surface of the blood stains followed by a more or less concentrated transfer of dried blood particles onto the thumb/glove. In two cases, these dried blood particles could be secondarily transferred in higher amounts to the paper surface. If the distribution area of the 50-µl blood stains was enlarged (4×4 or 8×8 cm), no impression of the surface of the blood stain was visible which resulted in clearly lower amounts of direct transfer and no detectable DNA upon secondary transfer. Interestingly, the amount of transferable blood was higher wearing gloves than wearing no gloves for blood, but not for saliva. This phenomenon appears to be the result of tiny dried blood particles adhering to rubber gloves more than to naked skin.

In comparison to the blood stains, the surface condition of the saliva stains appears to be different. Dried saliva stains proved to be hard to remove by contact from porous surfaces such as paper and cotton, which allowed only minute amounts of DNA to be transferred (below 2 pg/µl). Relevant amounts of saliva cell transfer were possible, however, from plastic, although in smaller concentrations than for blood. Secondary saliva transfer from plastic gave even smaller concentrations, although in three cases, concentrations of more than 5 pg/µl could be quantified (see Fig. 1c).

Other forensic groups have investigated the risk of secondary transfer of biological stains containing epithelial cells [1, 3, 5, 7]. Secondary stain transfer could be demonstrated, but only very weak patterns were detected after 34 PCR cycles [6]. Additionally, it has to be kept in mind that secondary transfer can be obtained if forensically relevant crime scene items were sampled without changing gloves [5]. Furthermore, such kind of stain cell transfer may be possible by using crime lights, which may have had a short accidental contact with a crime scene item (e.g. clothes), enabling subsequent (secondary) transfer of such stain material to items of another case [5]. The same risk may occur if microscopical examinations are carried out with gloves and epithelial cells are transferred during microscope handling. Secondary transfer is also possible from a camera while taking photographs from crime scene item if the camera has had previous contact with unprotected hands. Additionally, secondary cell transfer could also be a defence strategy to explain stains on a crime scene item under the assumption that the suspect has not been at the scene. For such scenarios, one relevant aspect is the amount of DNA. If a complete STR stain profile could be typed, secondary transfer is much more unlikely for dried stains compared with direct transfer.

For the relevant kind of stains in this study (blood and saliva), it should be kept in mind that normally considerably more cells were concentrated on the surfaces after drying compared to epithelial cell stains. Such stains could be removed and perhaps transferred after short contact with fingers.

In conclusion, we could demonstrate a potential risk of transfer contamination depending on surface conditions and the concentration of these stain categories on the specific areas.

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