Int J Leg Med (1991) 104:273–275

International Journal of
Legal Medicine


# DNA profiling of bloodstains on linen pretreated with remedies used for cleaning and maintaining clothes

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Received April 4, 1991

**Summary.** Linen was pretreated with 20 remedies for cleaning and maintaining clothes and 20 µl blood was applied on each sample and dried. Restriction enzyme digest of bloodstain DNA was irregularly inhibited by highly concentrated residues of 2 detergents and a stain-remover and colour-brightener. An additional dialysis step to purify DNA (Gill 1987) reliably prevented disturbance. High molecular DNA was obtained in every case and bandshifts were not observed.

**Key words:** DNA profiling – Bloodstains – Stain carrier

**Zusammenfassung.** Leinenstoff wurde mit 20 Mitteln, die zur Wäschereinigung und -pflege verwendet werden, in normaler und sehr hoher Konzentration behandelt, absichtlich unzureichend oder nicht gewaschen, getrocknet und mit 20 µL Blut versetz. Hohe Rückstandskonzentrationen von zwei Waschmitteln und eines Aufhellers führten unregelmäßig zu partieller und totaler Inhibition der Restriktionsspaltung. Durch einen Dialyseschritt bei der Reinigung der DNA (Gill 1987) konnten die Störungen zuverlässig verhindert werden. Hochmolekulare DNA konnte in jedem Fall gewonnen werden, Bandshifts traten nicht auf.

**Schlüsselwörter:** DNA Profiling – Blutspuren – Spurenträger

## Introduction

The influence of chemicals on investigations of biological evidence is well known, e.g. for the proof of human protein, ABO blood grouping (Coombs and Dodd 1961; Nickolls and Pereira 1962; Outteridge 1963), test for seminal stains, semen and spermatozoa (Fletcher et al. 1989; Feenstra et al. 1990). Unfavourable effects on DNA profiling have also been reported (Holtz and Olek 1988; Prinz and Berghaus 1990; Scheithauer and Weisser 1991a, b). The most frequent stain carriers in casework are clothes, and it is possible that residues of remedies used for cleaning and maintaining clothes are present. To imitate casework conditions, bloodstains were applied on linen stain carriers, that had been pretreated with 20 of these remedies.

## Materials and methods

*Preparation of stain carriers and bloodstains.* Linen cloth was treated with 20 different impregnators, fabric softeners, detergents, finishes, and stain-removers. Concentrations were applied

**Table 1.** Remedies for cleaning and maintaining cloths used in overdosed concentration to prepare the linen stain carriers

| | Solution | Rinsed | |
|---|---|---|---|
| | | Yes | No |
| *Impregnation remidies* | | | |
| M + S spray | * | | X |
| Colonil spray | * | | X |
| Imprägnol fluid | 5% | | X |
| *Fabric softeners* | | | |
| Quanto fluid | 10% | | X |
| Lenor fluid | 10% | | X |
| *Detergents* | | | |
| Tandil | 1% | X | |
| Dalli | 2% | X | |
| Henko | 2% | | X |
| Rei | 0.5% | | X |
| Saptil | 1.5% | | X |
| Luhns soap flakes | 2.5% | | X |
| neutral soft soap | 20% | | X |
| semi-solid soft soap | 3% | | X |
| fluid soft soap | 6% | | X |
| *Finishes* | | | |
| Hoffmans starch | 5% | | X |
| Apretta spray | * | | X |
| Perla fluid | 1.5% | | X |
| *Stain-removers* | | | |
| K2R | conc. | | X |
| Dr. Beckmann | conc. | | X |
| Baby-weiß | 2% | | X |

* Sprayed from both sides until thoroughly soaked

**Table 2.** Number of investigations performed with bloodstains on pretreated carriers

| Stain carriers treated with | DNA purification by dialysis | Number of investigations Concentration of the remedies | |
|---|---|---|---|
| | | Low | Overdosed |
| All 20 remidies | yes | 0 | 3 |
| | no | 2 | 3 |
| In addition: | | | |
| Tandil[a] | yes | 0 | 12 |
| | no | 10 | 10 |
| Dalli[a] | yes | 0 | 12 |
| | no | 10 | 10 |
| Baby-weiß[b] | yes | 0 | 12 |
| | no | 10 | 10 |

[a] Detergent (washing-powder)
[b] Stain-remover and colour-brightener

according to the manufacturers manual for the first series of experiments and cloth samples were dried after short, intentionally incomplete washing. For a second series, linen was treated with at least a 10-fold higher concentration of the remedies (Table 1). Samples of boiled linen were used as controls and 20 µl of fresh, native blood was applied on the linen immediately after removal. DNA profiling was performed after storage at room temperature from 2 days up to 2 months.

*DNA profiling.* The technique used was similar to that described by Bär et al. 1988: Stains (Table 2) were cut into small pieces and incubated in 500 µl buffer [*Tris*–HCl 10 m*M* pH 7.6; EDTA 10 m*M*; NaCl 100 m*M*; SDS 2%; DTT 40 m*M*, Proteinase K 400 µg/ml (Boehringer)] for 16 h at 37°C. Deproteinization was performed by one penol/chloroform/isoamyl alcohol and one chloroform/isoamyl alcohol extraction for 10 min in a rotating mixer, followed by centrifugation at 8.000 g for 5 min. If dialysis was performed, the isolated DNA was resuspended in 40 µl TE (pH 7,4), applied on a membrane filter (Type VM, pore size 0,05 µm; Millipore) and dialyzed for 2 h against TEN buffer pH 7.4 (10 m*M Tris*-HCl, 1 m*M* EDTA, 10 m*M* NaCl) at room temperature (Gill 1987). DNA was digested with *Hae*III (Boehringer) according to the manufacturer's recommendations. Test gels were run to check the quantity of high molecular weight DNA and the quality of restriction enzyme digest. After analytical gel electrophoresis in 0.8% agarose, DNA was transferred to a positively charged membrane (Immobilon N, Millipore) by vacuum blotting. Hybridization was performed with [$^{32}$P] labeled (Amersham) probe YNH24 (gift of Dr. Nakamura).

## Results

Test gels for high molecular weight DNA and for restriction enzyme digest showed no significant differences in the extracted amount and quality of the DNA for all stain carriers pretreated with "low" concentrations of the remedies. DNA profiles showed no difference when compared with the untreated linen controls. Dialysis was not performed in this group.

No influence on the DNA extracted from stain carriers with concentrated remedies was seen in test gels and autoradiographs from 17 out of 20 remedies. Restriction enzyme digest was disturbed by 2 washing-powders and one stain-remover and colour-brightener, if extracts were not purified by dialysis (Table 3).

**Table 3.** Results of bloodstains on carriers pretreated with the 3 out of 20 remidies that caused disturbance if applied in excess

| Stain carrier treated with | Restriction enzyme digest | | |
|---|---|---|---|
| | Totally inhibited | Partially inhibited | Not affected |
| Tandil[a]; dialyzed | 0 | 0 | 15 |
| Dalli[a]; dialyzed | 0 | 0 | 15 |
| Baby-weiß[b]; dialyzed | 0 | 0 | 15 |
| Tandil[a]; not dialyzed | 1 | 1 | 11 |
| Dalli[a]; not dialyzed | 11 | 1 | 1 |
| Baby-weiß[b]; not dialyzed | 4 | 0 | 9 |

[a] Detergent (Washing-powder)
[b] Stain-remover and colour-brightener

Bandshift did not occur in any case. All bands obtained in 14 blots were within a limit of 25 bp from the mean values (2905 bp and 2095 bp). Standard deviation was 18,4 bp = 0,63% and 14,5 bp = 0,69% respectively.

## Discussion

The influence of remedies used for cleaning and maintaining clothes upon conventional serology is well known. Scheithauer and Schilling (1990) used 20 of those remedies to prepare linen as carriers for bloodstains, and reported severe problems with ABH grouping, especially from textiles with highly concentrated residues. A similar set of stain carriers were prepared to test the possible influence of these residues on DNA profiling. Inhibition of the restriction endonuclease with bloodstains on filter paper was reported by Holtz and Olek 1988; disturbance by blue-jeans, suede and carpet as stain carrier by Prinz and Berghaus 1990, who also obtained an irregular yield of high molecular DNA extracted under same conditions from bloodstains applied on the same carrier.

In our investigations, stain carriers which contained residues of "normal" concentrated remedies, applied as recommended by the manufacturer, had no influence upon the results. High concentrations of the washing-powders *Tandil* and *Dalli* and the stain-remover and colour-brightener *Baby-weiß* disturbed restriction enzyme digest. The amount of the residues present due to intentional lacking or insufficient washing of the stain carriers were extremely high and would hardly be reached in practice. The irregular results with the problematic stain carriers were obviously not caused by irregular laboratory work, as they were obtained repeatedly in several series and independently of the technician. One can speculate that the amount of residues from the stain carrier present in the reaction vessel is decisive for the inhibition effect. In comparison with the above cited ABH investigation (Scheithauer and Schilling 1990), where only 3 out of the 20 remedies did *not* influence the results, DNA profiling proved to be an extremely robust method, as only 3 of the 20 remedies caused disturbances. Bandshift did not occur in any case.

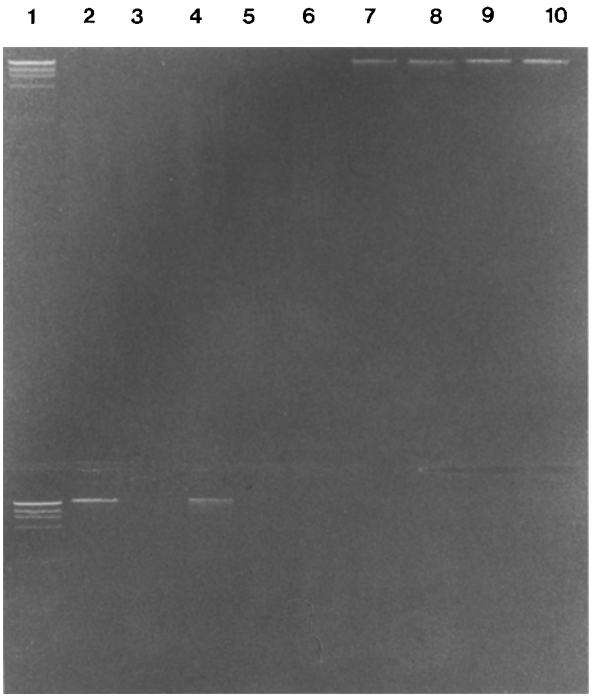


**Fig. 1.** Test gel after restriction enzyme digest. Extraction without dialysis. *Top:* Slot 1: lambda *Hin*dIII; Slots 2–6: Washing-powder *Tandil*; Slots 7–10: Washing-powder *Dalli*. *Bottom:* Slot 1: lambda *Hin*dIII; Slot 2: Washing-powder *Dalli*; Slots 3–7: Colour-brightener *Baby-weiß;* Slot 8: Control (untreated)

Test gels for high molecular DNA were inconspicuous in all cases. Disturbances of the restriction enzyme digest, as shown in Table 3, could easily be recognized in the test gels. The supplement of additional restriction enzyme and further incubation for 6 h, as performed with a few samples that were conspicuous in the test gels after restriction enzyme digest, did not show any influence.

Dialysis of the DNA as proposed by Gill 1987, prevented the inhibitory influence of the remedies and correct results were obtained in every case. This effect is not self-evident, as e.g. blue denim and other anilin coloured clothes can cause bandshifts that cannot be reliably prevented by dialysis (Scheithauer and Weisser 1991a, b). The positive effect of dialysis has to be weighed against the disadvantage of an additional step and the, at least theoretical, loss of DNA. Obviously, the remaining amount of DNA on the dialysis membrane is very low, as we did not succeed in visualizing the residues by ethidium bromide staining. The tactile and visually recognizable properties of the cloth samples are not reliable criteria for the decision to perform dialysis. A check for the necessity of dialysis by a test of a control blood sample applied on the stain carrier prior to the case work investigation would be time-consuming, and, according to our irregular results, unreliable. Autoradiographs of samples which obviously showed incomplete digestion in the test gels (Fig. 1) and were dialized after restriction enzyme digest, followed by another restriction enzyme digest, showed no difference to the controls.

*Acknowledgements.* Authors thank Mrs. Andrea Laube for her skilful technical assistance and Dr. Nakamura for a sample of his probe YNH24.

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