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# The effect of various stain carriers on the quality and quantity of DNA extracted from dried bloodstains*, **

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**Summary.** Bloodstains were made with 200 µl blood on each of 11 different common substrates to examine the effect of the stain carrier on the amount and quality of DNA recoverable. High-molecular-weight DNA was extracted from all samples after 2 days. The yield of DNA from each sample varied considerably, not only between the different stain carriers but also within a given category. With a DNA yield of up to 10 µg, paper, glass, nylon, wood, smooth leather and wool gave the best results, followed by blue denim and wallpaper (up to 6 µg), cotton fabric and carpeting (up to 4 µg) and suede (up to 2 µg). For several stain carriers the DNA-containing solution was contaminated by chemical substances, which in the case of the blue denim, suede, and carpet samples inhibited the digestion of the DNA with restriction enzymes and prevented DNA typing. The different textures of the stain carriers tested and (as for varying yields on the same carrier) the differing degree of loss of DNA during extraction and the physiological variation in the number of leukocytes in human blood are discussed as possible reasons for the wide range of variation in the amounts of DNA it was possible to extract.

**Key word:** DNA extraction, Bloodstains

**Zusammenfassung.** Auf elf verschiedenen, häufig vorkommenden Materialien wurden aus 200 µl Frischblut Spuren angelegt, um den Einfluß des Spurenträgers auf Menge und Qualität extrahierbarer DNA festzustellen. Nach der Lagerzeit von zwei Tagen konnte aus allen Proben hochmolekulare DNA gewonnen werden. Die jeweils isolierten Mengen schwankten sowohl innerhalb einer Kategorie, als auch zwischen den verschiedenen Spurenträ-

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gern erheblich. Mit bis zu 10 µg extrahierbarer DNA zeigten Papier, Glas, Nylon, Holz, glattes Leder und Wolle die besten Resultate, gefolgt von Bluejeansgewebe und Tapete (bis zu 6 µg), Baumwollstoff und Teppichboden (bis zu 4 µg) und Wildleder (bis zu 2 µg). Die erhaltene DNA-Lösung war in einigen Fällen durch aus dem Träger stammende chemische Substanzen verunreinigt, wodurch für die Spurenträger Bluejeansgewebe, Wildleder und Teppichboden das Schneiden der DNA mit Restriktionsenzymen und somit die Typisierung verhindert wurden. Als Ursache für die unterschiedlichen DNA-Ausbeuten werden die Trägereigenschaften und – wie auch für unterschiedliche Ergebnisse für den gleichen Träger –, der differierende Verlust bei den verschiedenen Extraktionsschritten und die physiologische Variation der Leukozytenanzahl der Spenderblute diskutiert.

**Schlüsselwort:** DNA-Extraktion, Blutspur

## Introduction

As in the serological examination of bloodstains, successful DNA profiling is dependent on correct treatment of the samples and the choice of an appropriate extraction method. The requirements that must be met for DNA analysis are purification of a sufficient amount of high-molecular-weight DNA and the absence of restriction enzyme-inhibiting substances in the DNA-containing solution.

Several studies have been published reporting that, except under humid conditions, the integrity of the DNA is sufficient even after several years [1–3], though the yield of DNA recovered changes with time. It has also been reported that the amount of DNA that can be extracted from bloodstains is dependent on the stain carrier [4]. Though in forensic practice it would be helpful to be able to judge the probability of achieving a successful DNA profile from different amounts of blood on different substrates, as far as we know, no study has been published that deals with the connection between the quality and quantity of the isolated DNA and the stain carriers. To obtain the necessary information, we extracted DNA from bloodstains of a fixed size and age on 11 different everyday materials and analyzed the concentration and integrity of the DNA recovered and the completeness and specifity of the restriction enzyme digestion.

## Materials and methods

The following 11 commercially available materials were tested: writing paper, glass, nylon, unpainted wood, leather, lambswool, white cotton fabric, blue denim, wallpaper, suede and carpeting (wool/synthetic mix). Different types of wood, leather, wool, suede and carpeting were tested.

Bloodstains were prepared by applying 200 µl fresh blood to the substrate and letting it dry at room temperature. DNA was isolated 2 days after application of the blood. To achieve realistic conditions blood was taken from different donors and the macroscopically clean carriers were not washed or sterilized.

*Isolation of DNA*

The DNA was isolated using proteinase K digestion [5] and following the procedure described elsewhere [1, 3] for bloodstains with several modifications (see Discussion).

Stains were shredded or scraped off and presoaked in 0.001 *M* EDTA at 4°C for 4 h. For the proteinase K reaction, DNA lysis buffer and proteinase K were added to give a final concentration of 0.1 *M* Tris-HCl pH 8.0, 0.1 *M* EDTA, 0.1 *M* NaCl, 1% SDS, and 200 µg/ml proteinase K. Reaction took place at 37°C, with constant shaking, overnight. The total volume of the incubation mixture varied according to the stain carrier and ranged from 2.4 ml for stains on glass to 13 ml for stains on carpeting.

After incubation the stain substrate was separated from the DNA solution by two centrifugation steps. After pelleting the substrate, the supernatant was transferred to a new tube. The pellet was resuspended in a small volume of distilled water and after piercing of the bottom of the tube the remaining liquid was centrifuged down to a second tube and pooled with the first extract. Samples were extracted once with phenol and twice with phenol/chloroform and dialyzed against TE buffer (0.01 *M* Tris-HCl, 0.001 *M* EDTA) at 4°C overnight. Phenol, chloroform, and dialysis tubing were pretreated as described by Maniatis et al. [6]. Before ethanol precipitation the volume was reduced by several extractions with *n*-butanol. DNA was precipitated by adding 0.1 volume of 3 *M* sodiumacetate and 2.5 volumes of 99% ethanol, cooling at −20°C overnight and centrifuging at 15000 *g* for 30 min. The pellets were resuspended in 70% ethanol and centrifuged as above. The dry pellets were dissolved in 500 µl TE buffer.

*Quantification and determination of molecular weight*

The amount of recovered DNA was determined by photometry at 260 nm and by comparison of the ultraviolet fluorescence of an aliquot of each sample with the fluorescence of known quantities of lambda DNA in a gel stained with ethidium bromide. Samples and 20, 50, 100, 300, and 500 ng lambda DNA were loaded onto a 0.8% agarose gel and electrophoresis was carried out for 2 h at 100 V. The result of the comparison was calculated for the total volume. The molecular weight was determined by the migration of the samples in relation to that of the 40-kb-long lambda DNA.

*DNA restriction and control reaction*

A 3-µg aliquot of DNA from each sample was digested in a 300-µl volume using 5 units/µg *Hae*III (Boehringer Mannheim) in accordance with the manufacturer's instructions. The restriction fragments were precipitated by adding 3 *M* sodium acetate and 99% ethanol, cooling for 2 h, at −20°C and centrifuging for 15 min at 15000 *g*. The dry pellet was redissolved in 40 µl TE buffer and loaded onto an 0.8% agarose gel. Electrophoresis was carried out with TBE buffer (0.089 *M* Tris-HCl, 0.089 *M* boric acid, 0.002 *M* EDTA [6]) at 30 V for 18 h. After staining of the gel in ethidium bromide the result of the restriction enzyme reaction can be judged under UV light. The DNA was blotted on a nitrocellulose membrane (pore size 0.45 µm, Schleicher & Schuell) according to Southern [7]. Sex determination was carried out according to Cooke et al. [8] using the probe Y-chromosome-specific repeat (Amprobe No. RPN.1305, Amersham) and the Nonradioactive DNA Labeling and Detection Kit (Boehringer Mannheim). Prehybridization, hybridization and washing of the blot were performed following the Boehringer manual.

## Results

### *Quantity of DNA recovered*

For most samples the photometric determination of DNA concentration was disturbed by ultraviolet-absorbing substances deriving from the stain carriers

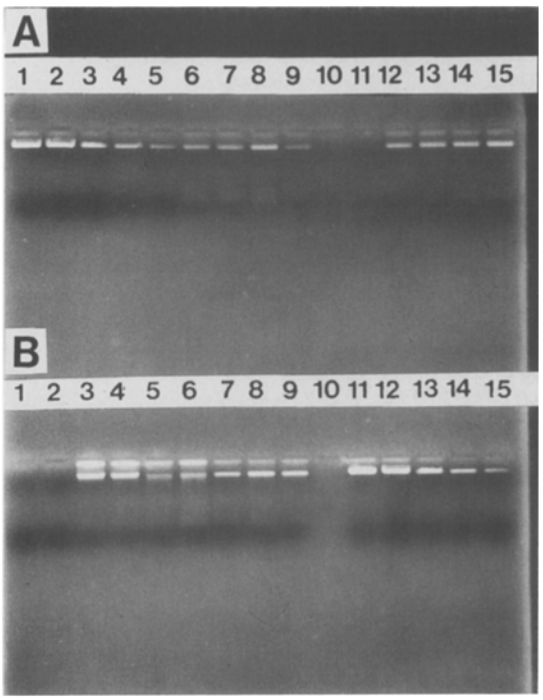


**Fig. 1.** Ethidium bromide-stained agarose gel to determine DNA concentration and molecular weight. *Lanes A1–5* and *B11–15*, Lambda DNA dilution steps (500, 300, 100, 50, 20 ng); *A6–9*, suede samples; *A10–11*, suede blind samples; *A12–15*, wallpaper samples; *B1, 2* paper blind samples; *B3, 4*, paper samples; *B5–9*, cotton fabric samples (20 µl aliquots of each)

and persisting in the DNA solution in spite of the multistage purification. This was especially true for wood, suede, blue denim and carpeting, in none of which a DNA peak was distinguishable at 260 nm. For other substances (e.g., wool) there was a peak at 260 nm, but since the basic absorbance line was increased calculation of the DNA concentration produced false results. Consequently, we will present and discuss only the more reliable semiquantitative results obtained by evaluating the intensity of ethidium bromide staining.

For all substrates examined it was possible to recover DNA from 200 µl bloodstains. No DNA was detected after digestion with proteinase K of a piece of each substrate with no blood applied (blind sample). Figure 1 shows an example of a DNA test gel to which aliquots of several samples were applied. The lambda dilution steps are in lanes A1–5 and B11–15. Blind samples have been applied in lanes A10–11 (suede without bloodstains) and B1–2, (paper without bloodstains). Comparison of the amount of DNA makes the differences between, for example, lanes A6–9 (blood on suede) and B3–4 (blood on writing paper) obvious. The yield of the DNA extraction varied considerably, not only between the different substrates but also between the separate samples from one substrate. In Table 1 the maximum and minimum recovery of DNA and the resulting average yields are listed. For some substrates the observed range is very wide (e.g. 2–10 µg per 200 µl blood). For other substrates, e.g., suede and carpeting, the recovery rate is generally low. The different types of wood, wool, carpeting, and suede we examined showed no systematic differences in the yield of DNA.

**Table 1.** Yield and quality of DNA extracted from dried bloodstains (2 days old, stored at room temperature) by stain carrier (6–8 samples per carrier)

| DNA | Stain carrier | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Paper | Glass | Nylon | Wood | Leather | Wool | Cotton fabric | Blue denim | Wallpaper | Suede | Carpeting |
| Amount of DNA (μg) | 2–10 | 2–10 | 2–10 | 2–10 | 4–10 | 2–10 | 2–4 | 1–6 | 2–6 | 0.4–2 | 1–4 |
| Average (μg) | 7 | 7 | 5 | 8 | 8 | 6 | 3 | 3 | 3 | 1 | 2 |
| High molecular weight[a] | + + | + + | + + | + + | + + | + + | + + | + + | + + | + + | + + |
| Degree of impurity[b] | − − | − − | − − | + | + | + + | − | + + | + | + + | + + |
| Restriction enzyme reaction | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Not complete | Yes | Not always | No |

[a] + +, intact
[b] − −, pure; −, very low; +, some; + +, high

*Quality of DNA recovered*

The results concerning the quality of DNA are listed in Table 1. For all substrates, the DNA recovered was of high molecular weight.

The degree of impurity refers to chemical contaminations detected by running a photometric spectrum of the sample solution. Two minus signs mean a pure preparation. Two plus signs indicate a high degree of impurity, as in the case of the dyed substrates wool, blue denim and suede. The different types of carpeting also could not be purified further. There was a difference in the extent and the composition of chemical contamination between the different sorts of suede, carpeting, and wood.

Most of the samples could be cut with restriction enzyme *Hae*III and showed no unexpected signals after hybridization with the sex determination DNA probe. In some cases (e.g., wool) the high degree of impurity did not inhibit the restriction enzyme. Because the DNA could not be digested, sex determination was not possible in stains on blue denim, the carpeting, and one type of suede.

## Discussion

Compared with previously published methods for extracting DNA from dried bloodstains [1, 3], the method used here has four major modifications: the presoaking of the stains, the two-step centrifugation, the volume reduction, and the extended centrifugation time after ethanol precipitation. Various preparat-

ory experiments in our laboratory, in which each modification was tested separately, showed that the recovery rate could be improved in this way. The presoaking buffer contains EDTA to inhibit possible DNase activity [6]. After removal of the stain substrate there is always a residual amount of DNA-containing extract in the pellet. Resuspension of the stain substrate reduces this loss of DNA. Depending on the absorbency of the material, the volume of the proteinase K reaction mix has to be adapted to guarantee complete immersion of all bloodstained pieces. The resulting concentration of DNA expected is often very low ($<1\,\mu g/ml$), which means that the efficiency of ethanol precipitation can be as low as 70% [9]. To avoid this loss of DNA the volume reduction with *n*-butanol was introduced and the centrifugation time of 15 min was extended to 30 min.

The variation in the amounts of isolated DNA from the different stain substrates is probably caused by three facts.

Firstly, there are carrier-specific difficulties in resolving the leukocytes from each carrier. From carriers with a smooth surface where the blood cannot soak in, e.g., writing paper, glass, wood, and leather, high yields of DNA can be obtained. On the other hand, the blood soaks into cotton fabrics and roughly textured wallpaper, which reduces the recovery rate of DNA. We consider this to be the main reason for the different average yields.

Secondly, the DNA extraction involves several steps (e.g., extraction with phenol) in the course of which a loss of DNA is almost unavoidable. The consequence of losing different amounts of DNA during the extraction steps is the impossibility of reproducing a specified yield. This is also one explanation for the wide range of different quantities purified from different samples of the same stain carrier.

The third reason for the variation observed lies in the variability of the number of DNA-containing cells (leukocytes) in human blood. We noted, for instance, that the amount of DNA recovered from dried bloodstains from certain donors was consistently higher than that from other donors on the same carriers. For healthy adult people the number differs from 5000 to 9000 leukocytes per µl blood [10] and is influenced even by minor infections. Based on a content of 6.2 pg DNA per cell [11] the theoretical amount of DNA in 200 µl of blood ranges from 6.2 to 11.2 µg DNA. In relation to this theoretical yield the extractions with 10 µg DNA gave a very good result. To obtain reproducible results this variation would have to be taken into account. This of course does not lead to a more reliable estimation in practical case work, where the whole range possible has to be considered.

To distinguish between usable and problematic stain carriers the quality has to be taken into account. With regard to a high yield of DNA and no impurities, paper, glass and nylon are non-problematic stain carriers. Since considerable contamination does not inhibit restriction enzymes, wood and leather are also usable. Carriers with a texture allowing the blood to soak in and that have been chemically treated generally do not allow recovery of good-quality DNA. This is especially true for suede leather, which is produced using aggressive tanning agents, e.g., formaldehyde derivatives and chromic acid salts, or for industrially fabricated carpeting that is not only glued to a foam underlayer, but also im-

pregnated with fungicides and bactericides. The presence of organic dyes can, but does not always, disturb DNA analysis, as observed with the blue denim and wool samples. For each category of stain carrier tested, a wide range of varieties was observed so that the results presented here can be generalized only to a certain degree. Furthermore, our DNA yields were obtained under favorable conditions (clean material, only 2 days old, stored dry and at room temperature), which will hardly ever be obtained in a real-life situation.

Nevertheless they provide a helpful orientation. Further studies dealing with storage conditions and age of the forensic samples should follow.

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