

# EXTRACTION OF DNA FROM COAGULATED BLOOD SAMPLES

Richard Scheithauer, Hans-Joachim Weisser

Institute for Forensic Medicine  
University of Freiburg, Albertstr. 9, 7800 Freiburg, FRG

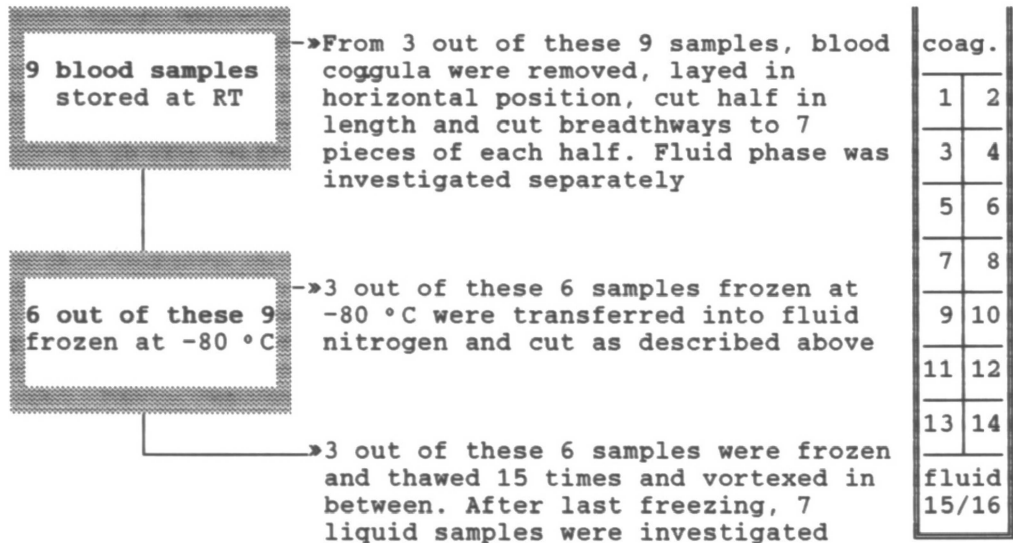
## INTRODUCTION

In forensic medicine, blood samples available for case work are coagulated predominantly. Following routine manuals, yield of DNA is irregular and sometimes low.

## MATERIALS

■ 9 blood samples of ca. 10 ml were taken from one donor at one time. No anticoagulating agent was present. Tubes were stored vertically for one night at room temperature. Following procedures are shown in the figure.

*Subdivision of blood samples*



■ Other samples were dried as a whole and grinded.

## METHODS

- All samples were incubated overnight in an extraction buffer (Bär et al. 1988) containing 400 µg/ml Proteinase K at 56 °C.
- Just one series was pretreated prior to DNA extraction with Protease Type VIII from *bacillus licheniformis* for 5 h at 37 °C with a concentration of 400 µg/ml (7 pieces of the coagula each of the first three blood samples stored at RT and cut half in

length; pieces No. 2,4,6,8,10,12, and 14 and the fluid phase 16; see figure).

- Deproteinization by one phenol/chloroform/isoamyl alcohol and one chloroform/isoamyl alcohol extraction. The isolated DNA was resuspended in 40  $\mu$ l TE pH 7,4, applied on a membrane filter (Type VM, pore size 0,05  $\mu$ m; Millipore) and dialyzed for 2 h against TEN buffer pH 7.4 (10 mM Tris-HCl, 1 mM EDTA, 10 mM NaCl) at RT (Gill 1987).
- DNA concentration was determined by UV spectrophotometry (260 nm)

## RESULTS

		Series 1a Stored at RT Not pretreated	Series 1b Stored at RT Pretreated	Series 2 Stored at -80 °C Fluid nitrogen
coag.	1 2	2,37	2,98	3,11
	3 4	2,87	3,25	3,64
	5 6	2,59	3,43	3,64
	7 8	3,00	3,28	2,83
	9 10	2,93	3,19	3,75
	11 12	3,26	3,82	4,60
	13 14	2,57	3,61	3,51
	fluid 15/16	1,07	1,03	
		Series 3 15 freeze/ thaw cycles	Series 4 Dried blood coagula	Series 5 Dried blood fluid phase
All samples		6,44	2,17	1,16

Average amount of DNA obtained from pieces of coagula and remaining fluid in  $\mu$ g/100 mg liquid blood

## CONCLUSIONS

- No characteristic distribution of DNA concentration was found in blood coagula, although blood samples were stored in vertically standing tubes immediately after removal
- Yield of DNA recovered from coagula is irregular
- Pretreatment of coagula with a protease prior to extraction showed slight effect
- Liquid phase contains only little DNA
- Drying and grinding blood is not advantageous
- Reproducible high yield was obtained after numerous freeze/thaw cycles and vortexing in between

## REFERENCES

- Andersson M, Böhme J, Andersson G, Möller E, Thorsby E, Rask L, Peterson PA (1984) Genomic hybridization with class II transplantation antigen cDNA probes as a complementary technique in tissue typing. *Human Immunol* 11:57-67
- Baechtel FS (1988) Recovery of DNA from human biological specimens. *Crime Lab Digest* 15:95-96
- Bär W, Kratzer A, Mächler M, Schmid W (1988) Postmortem stability of DNA. *Forensic Sci Int* 39:59-70
- Gill P (1987) A new method for sex determination of the donor of forensic samples using a recombinant DNA probe. *Electrophoresis* 8:35-38
- Jeanpierre M (1987) A rapid method for the purification of DNA from blood. *Nucl Acid Res* 15:9611
- Kanter E, Baird M, Shaler R, Balazs I (1986) Analysis of restriction fragment length polymorphisms in deoxyribonucleic acid (DNA) recovered from dried bloodstains. *J Forensic Sci* 31:403-408
- Kunkel LM, Smith KD, Boyer SH, Borgaonkar DS, Wachtel SS, Miller OJ, Breg WR, Jones HW, Ray JM (1977) Analysis of human Y-chromosome-specific reiterated DNA in chromosome variants. *Proc Natl Acad USA* 74:1245-1248
- Prinz M, Berghaus G (1990) The effect of various stain carriers on the quality and quantity of DNA extracted from dried blood stains. *Z Rechtsmed* 103:191-197