

The degradation of a VNTR polymorphic site in various biological specimens when subjected to different temperature conditions

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SUMMARY

Liquid blood and semen samples were left at room temperature, 37°C and outdoors temperature for four weeks.

Stains of blood and semen were left for up to four weeks at room temperature.

After digestion with AluI, extracted DNA samples were analysed by mini gel electrophoresis for degradation and then the polymorphic VNTR locus MS43 was investigated by Southern blot electrophoresis.

In addition to the finding of evidence of degradation of the DNA, the appearance of bands additional to and stronger than the expected bands after probing with MS43, was an unexpected observation.

INTRODUCTION

Both discrimination power and stability are major criteria relating to the usefulness of polymorphic genetic markers in forensic problems.

There is some evidence for extreme stability of DNA from workers who have extracted small fragments of intact DNA from mummified material (Paabo, 1989).

High molecular weight DNA is required for the single locus polymorphisms currently widely used and whilst some workers have reported successful storage of extracted DNA for up to 20 years at -70°C (Madisen et.al. 1987) and others produced profiles from blood and semen stains up to four years old (Gill et.al. 1985), case work material can be subjected to various unfavourable conditions.

The experiments described here investigate the effect of one condition, temperature, over a period of four weeks.

MATERIALS AND METHODS

Paired EDTA blood and semen samples from 3 individuals were aliquoted into tubes and stored at room temperature, 37°C and outdoors for up to four weeks (in September/October).

Stains of the blood and semen were made on cotton cloth, air dried and stored at room temperature for up to four weeks.

DNA was extracted from 200ul of liquid blood and semen samples by a conventional phenol/chloroform method except that with the semen samples the addition of 20ul of DTT (10mg/ml stock) was included in the proteinase K digestion step.

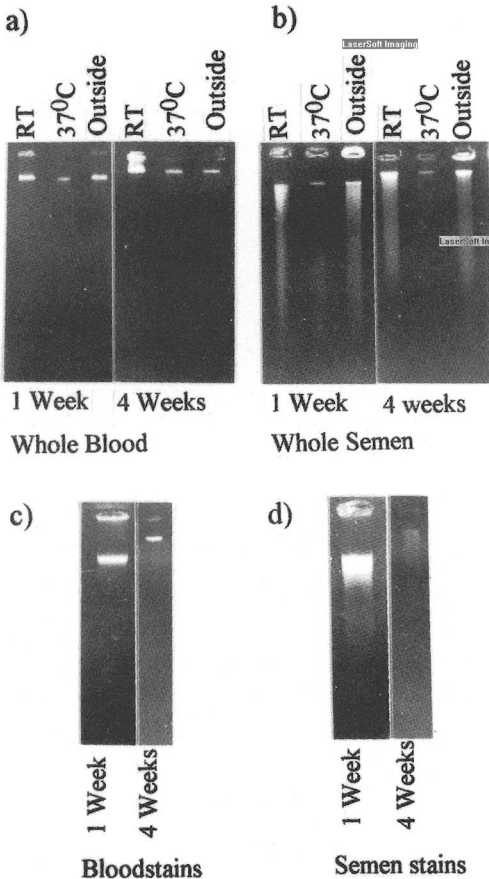
Stain material measuring 1 cm by 1 cm was extracted overnight at 37C in 400ul extraction buffer* plus 40ul each of proteinase K (10mg/ml) and DTT. Subsequent extraction was as for liquid blood and semen.

All samples were subsequently digested with Alu 1, analysed by mini-gel electrophoresis and then subjected to Southern blot analysis using the MS43 probe.

*100mM Tris, 10mM Na₂ EDTA, 100mM NaCl, 2% SDS

RESULTS

Figure 1 Quality of DNA recovered



Analysis of the extracted DNA by mini gel agarose electrophoresis with ethidium bromide provided information on the quality of the DNA extracted.

Figure 1a and 1b show example results from liquid blood and semen samples from one individual stored for up to 4 weeks under three different temperatures.

DNA from the semen shows degradation after one week.

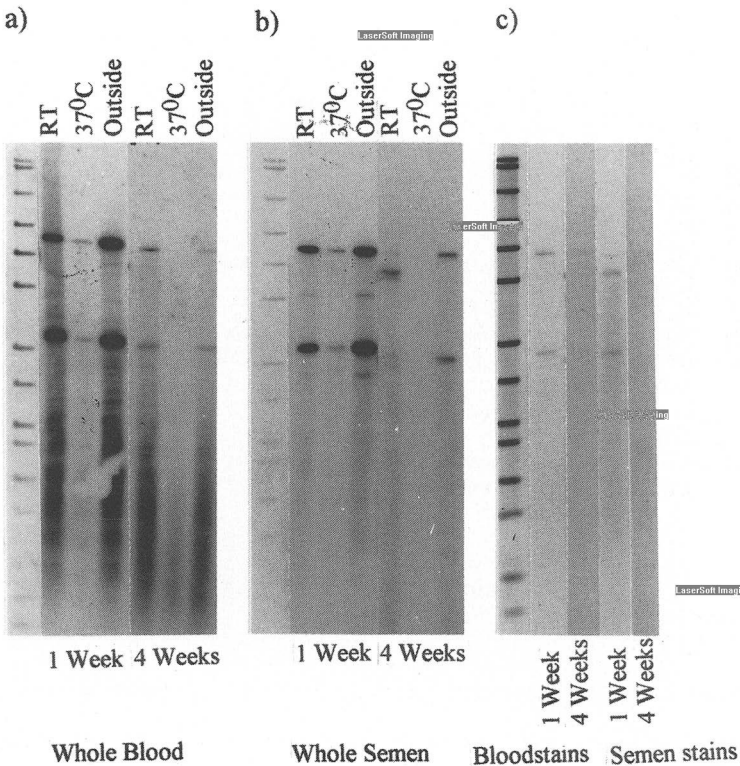
DNA from blood shows a discrete band of high mol. weight DNA after four weeks, even when stored at 37°C.

Figures 1c and 1d show example results from stain material.

DNA from the bloodstain gives clean, high mol. weight DNA after four weeks.

DNA from the semen stains show some degradation even after 1 week.

Figure 2 shows examples of MS43 probe test results



- Panel a shows example results from liquid blood.
 1 week - Satisfactory detection of bands.
 4 weeks - Expected bands detected when stored at room temperature and outdoors but no detectable bands from material stored at 37°C.
- Panel b Example results from liquid semen
 1 week - Expected bands are clearly detectable.
 4 weeks - No bands detected in material stored at 37°C. Expected bands detected from material stored at room temperature but additional bands also seen.
- Panel c Example results from stains stored at room temperature.
 Semen stains show additional band after 1 week and no bands after 4 weeks.

CONCLUSIONS

These results indicate DNA may be more labile than previously observed.

DNA from liquid semen shows some degradation even within 1 week.

Material extracted from liquid blood and semen stored at 37°C shows weak band patterns after one week and no bands at all were detected after 4 weeks.

DNA in bloodstains is considerably more stable and typing possible after 4 weeks.

The observation in some instances in material from liquid semen and semen stains, both stored at room temperature, of bands additional to those expected gives cause for concern and warrants further investigation. In this limited study although such bands were seen in association with the bands which were expected to be present in the sample they could appear stronger than the expected bands.

References:

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