

Surprising stability of DNA in stains at extreme humidity and temperature

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Abstract. The stability of DNA in air-dried blood and buccal stains at various conditions of relative humidity and temperature was tested by the ability to amplify 1600 and 273 bp DNA fragments by PCR and a 147 bp DNA fragment by real time PCR. Surprisingly it was observed that even the long DNA fragment was amplifiable after incubation for more than 1 month at 100% humidity and temperatures up to 65 °C. The 273 bp fragment was amplifiable after 3 months at 65 °C, 100% humidity. Fungal/microbial growth was observed on some stains after incubation at room temperature or 35 °C at 100% humidity, but never at lower humidity. However, in wet stains DNA degraded rapidly. Thus, even at extreme humid and hot conditions simple air-drying is adequate for the preservation for month of stains and reference samples to be analysed for STRs and SNPs. © 2003 Elsevier B.V. All rights reserved.

Keywords: DNA; Stability; Stains; Humidity; Temperature

1. Introduction

Most forensic laboratories have experienced the inability to detect or amplify DNA from moist biological stains or reference samples on cotton buds, which had been stored and/or shipped at soaking wet conditions. It has been shown that the inactivation rate of a protein (acid phosphatase) in semen stains is exponentially related to the relative humidity, the rate being 1,000,000-fold higher at 100% humidity than at 40% [1]. The degradation of DNA often parallels the degradation of proteins, and many of the processes that degrade DNA depend on the presence of water [2,3]. Therefore, we expected that DNA in biological stains and reference samples would show a rapid decay at high relative humidity. To see if this was true, stains of whole blood or buccal cells were incubated at various conditions of relative humidity and temperature. The quality and quantity of the remaining DNA was assessed by PCR.

2. Methods and materials

Whole blood (5 µl) or buccal cells were spotted onto pieces (4 × 4 mm) of Whatman filter paper No. 3 and air-dried overnight. The stains were incubated in closed boxes at 0%,

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50%, 80% and 100% relative humidity at room temperature, 35, 45, 55 and 65 °C. Constant humidity was maintained by the inclusion of H₂O, saturated solutions of (NH₄)₂SO₄ (80% humidity), NaHSO₄, H₂O (50% humidity) or silica gel. One series of bloodstains on microscopic slides was transferred to soaking wet (Milli-Q grade water) cotton buds and incubated at 100% humidity at room temperature. DNA was extracted from the stains using QIAamp® DNA Mini Kit (Qiagen, Germany). PCR of a 1600 bp segment was performed using primers targeting the human ACPI locus from intron 4 to exon 5 and a 273 bp segment was amplified using primers targeting exon 4 at the human HFE-locus. Real time PCR was performed using ABI-Prism-7000 SDS (Applied Biosystems) and primers and probe targeting a 147 bp segment in exon 4 of the human HFE-gene.

3. Results

3.1. Dry bloodstains

Five sets of bloodstains on filter paper were prepared during the spring, summer and autumn month and incubated at various nonsterile conditions of temperature and relative humidity. Amplification of the 1600 bp fragment was possible from all dry stains after incubations for up to at least 1 month (Fig. 1A) even at the most extreme conditions (65 °C and 100% humidity). The 273 bp fragment was amplifiable after 3 months, even at 65 °C, 100% humidity. Fungal and/or microbial growth was observed on some of the bloodstains at 100% humidity after 1 month at room temperature or 35 °C. Real time PCR showed a decrease in the number of DNA templates in 5 µl extract from about 3000 copies to about 30 copies after incubation for 6 months at 35 °C, 100% humidity, and

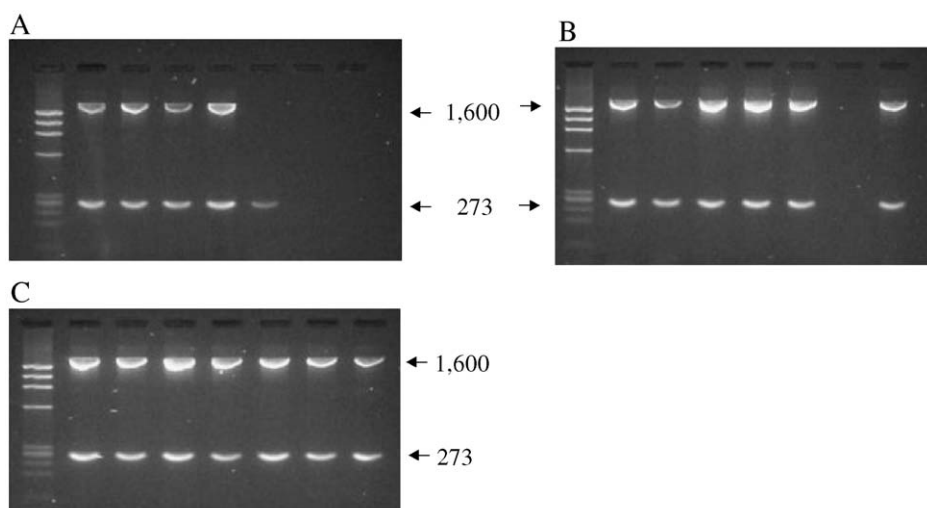


Fig. 1. Bloodstains incubated at 35 °C and controlled relative humidity. PCR of 1600 and 273 bp fragments (37 cycles). Lane assignments (left to right) 1: Φ X174 RF DNA HaeIII-digested; 2: 0 month; 3: 1 month; 4: 2 months; 5: 3 months; 6: 4 months. (A) 100% humidity. Lanes 7–8: 6 months; 7 months. (B) 80% humidity. Lane 7: void; Lane 8: 1 ng DNA template. (C) 50% humidity. Lanes 7–8: 6 months; 7 months.

approximately 10 copies after 7 months. As expected, the stability of the DNA increased at lower humidity and fungal and/or microbial growth was not observed at these conditions (Fig. 1B and C).

3.2. *Dry buccal stains*

Buccal stains were incubated at 35 °C and the long DNA fragment was amplifiable up to 3 months at 100% humidity; the short fragment was amplifiable after 6 months, 100% humidity.

3.3. *Wet cotton buds with blood*

Rapid degradation of DNA was observed in soaking wet cotton buds with blood. After 1 week at room temperature it was no longer possible to amplify the long fragment, and the short fragment was only amplifiable up to 4 weeks of incubation.

4. Discussion

The results showed a surprising stability of DNA in stains even at extreme environmental conditions (35–65 °C, 100% relative humidity), provided the stains had been dried prior to incubation. We had expected a rapid decay of DNA at high relative humidity but observed, on the contrary, that even long DNA fragments were amplifiable after month of incubation. The appearance of fungal/microbial growth on some of the stains at 100% humidity may explain the decrease in amplifiable DNA observed for these stains. However, the average environmental humidity is well below 100% even during wet seasons, e.g. the average value is 90% for the winter month in Denmark [4] and it does not exceed 82% at any time of the year in tropical Darwin, Australia [5]. Therefore, even in humid climates simple air-drying seems to be adequate for the preservation for month of DNA in stains and reference samples to be analysed for STRs and SNPs. This may be especially attractive to developing countries that want to implement modern DNA-profiling techniques but find the purchasing of expensive sampling kits prohibitive.

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