

PCR DNA Typing of Washed Stains

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Introduction

Agressors who get victim's blood on their cloth may try to wash the blood stains, either by superficial cold water rinsing or using a regular washing machine. On the other hand, rape victims sometimes wash their semen-stained underwear to get rid of any contamination related to the offence. In all these situations, it might be hastily concluded that nothing can be recovered from the stains at least if they are no more superficially detectable. In fact, stains submitted to normal washing procedures still contain measurable amounts of DNA. The results mean that the main problem may finally be the simple detection of the presence of the stains.

Material/Methods

Stains : A series of 0.1, 1 and 10 μ l blood, semen and saliva stains were made onto different substrate materials and allowed to dry. The substrates were:

- cotton - white, 100% cotton
- rayon - pale yellow, 100% rayon
- denim - pale blue, 100% cotton
- silk - orange
- "wool" - green, 50% lambswool, 30% polyacrylic, 20% angora

Washing : Three washing temperatures were used: 40°C, 60°C and 95°C, under two main conditions: washing with water or using a standard biological detergent (Biancomat (Coop Konsumentendienst, Basel, Switzerland) composed of 13% active ionic and non-ionic detergent of which 1.5% is soap; 21% water softener (zeoliths and polycarboxylates); 13% basic components including carbonates, silicates; 16% bleaching agents; 34% neutral salts; 3% specialist substances including enzymes, optical brighteners). All the experiments were made in triplicates and the results present the mean of the three measurements.

DNA extraction : all the stains were extracted using a Chelex extraction protocol [2], amputated of its preliminary water wash step. For semen stains, the extraction was made in the presence of 90mM DTT and 180 μ g/ml proteinase K.

DNA quantification : The DNA yield was estimated by slot-blot hybridization using the "Human DNA Quantitation System" (GIBCO-BRL, Gaithersburg, MD, USA), which makes use of the D17Z1 probe [3].

PCR amplification of the *D1S80* locus : The D1S80 locus was amplified according to the method of Kasai [1] with minor modifications. The PCR products were analysed on 2% agarose gel followed by staining with ethidium bromide.

Results

Surprisingly large amounts of DNA can be recovered from semen, blood and even saliva stains after washing. For semen stains, the yield is in the nanogram range with stains corresponding to less than 1 μ l. The yield is about one order of magnitude lower for blood stains and two orders of magnitude lower for saliva stains (Fig.1). The influence of a few parameters on the yield was examined ; their effects correspond to what could be expected:

- The yield was inversely proportional to the washing temperature. Several 95°C washed stains did not yield any DNA (Fig.2).
- The use of washing powder substantially reduced the yield, with a few unexplained exceptions (Fig.3).
- The support of the stains is of great importance. The larger yields were obtained with stains on cotton and especially on blue denim (Fig.4).

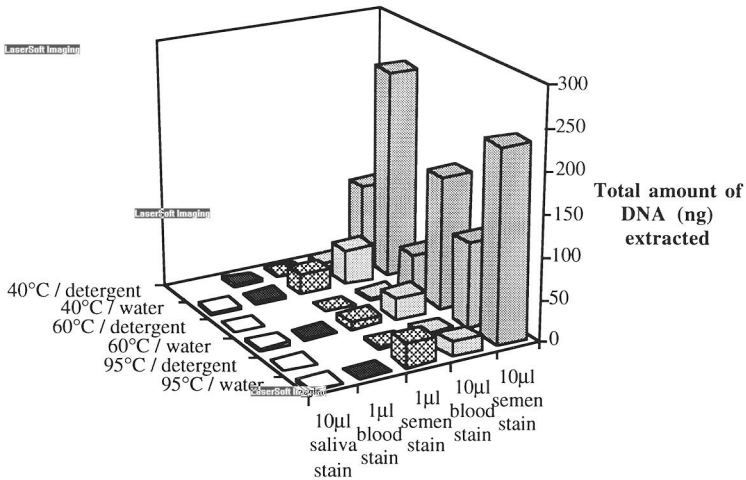


Fig.1 Overview of the DNA yield from washed stains of various types and sizes on cotton

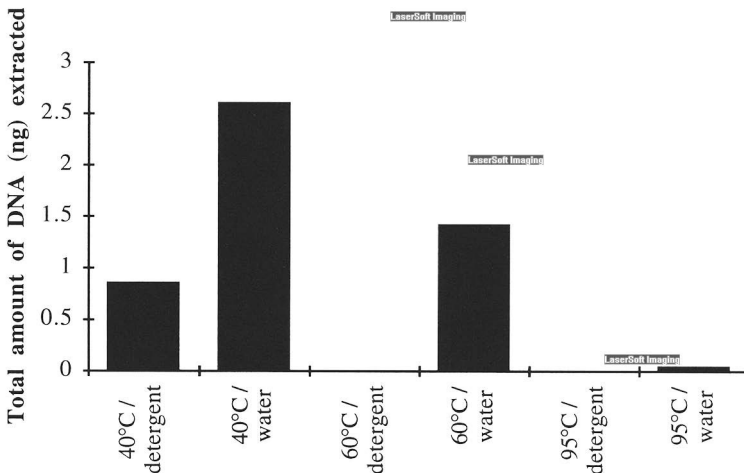


Fig.2 Influence of the washing temperature on the DNA yield from washed 1µl blood stains on cotton

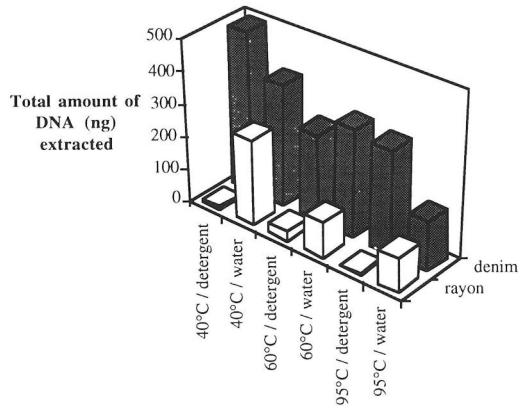


Fig.3 Influence of the use of washing powder on the DNA yield from washed 10µl semen stains on rayon and denim. On denim, the washing with water seems to be curiously more effective than the washing in the presence of detergent.

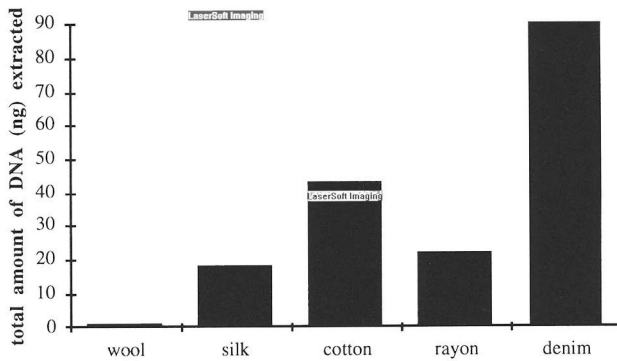


Fig.4 Influence of the support of the stain on the DNA yield from 10µl blood stains washed at 40°C with water.

Washed stains are definitely a useful source of DNA for forensic identification. Even tiny washed stains allow successful PCR DNA typing (data not shown). The main remaining problem consists in the mere detection of the presence of the stains.

References

- [1] Kasai K, Nakamura Y, White R (1990) Amplification of a variable number of tandem repeats (VNTR) locus (pMCT118) by the polymerase chain reaction (PCR) and its application to forensic science. *J Forens Sci* 35(5):1196-1200
- [2] Walsh P.S., Metzger D.A., Higuchi R. (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechnique* 10(4) 506-513
- [3] Waye J.S., Michaud D., Bowen J.H., Fournay R.M. (1991) Sensitive and specific quantification of human genomic deoxyribonucleic acid (DNA) in forensic science specimens: casework examples. *J. Forens. Sci.* 36(4):1198-1203