Int J Legal Med (1999) 112 : 388–390

TECHNICAL NOTE

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An improved method for STR analysis of bloodstained denim

Received: 8 December 1997 / Received in revised form: 13 August 1998

Abstract Indigo dye is used to dye denim and other fabrics. It is now accepted that if this is co-extracted with the DNA, it may inhibit PCR amplification. A simple, improved method is described for the extraction of DNA from bloodstained denim for PCR amplification and short tandem repeat (STR) analysis. The DNA was extracted by constructing a blotting system using capillary action to draw a saline solution through the denim. The transferred material was collected onto nylon membranes and these were processed by chelex extraction. A variety of coloured denim substrates and other heavily dyed fabrics, including case work samples were used. In all cases the DNA was extracted, amplified and typed correctly.

Key words Denim · Bloodstains · PCR

Introduction

In forensic analyses the development of the polymerase chain reaction (PCR) has allowed the analysis of small quantities of DNA that may also be degraded [1]. Forensic material can contain contaminating and/ or inhibitory substances. For PCR to succeed on DNA extracted from such material it is desirable that these substances be removed or significantly reduced prior to the PCR reaction [2,3].

Bloodstained denim is a frequently encountered piece of evidentiary material. Denim is a tight twill cloth, where the difference in shade of blue denim is determined by the levels of indigo dye present [4]. It is accepted that the presence of indigo dye or its derivatives in the DNA extraction solution are likely to inhibit PCR and often a profile is not obtained [2,4] when sufficient DNA appears to be present. Other dyes and dyed material often show the same effect. Indigo dye is one of the oldest dyes known to man and was originally extracted as a glucoside from various plant species. Indigo is a dark blue powder that is practically insoluble, but when dissolved in highly polar solvents produces a blue solution [5] that can be used to dye denim cloth. A variety of extraction protocols have been tested to try and extract the DNA content of bloodstains from stained denim, without co-extracting the indigo dye [2–4]. Most have had some limited success, but still have difficulty in obtaining DNA profiles from bloodstained dark blue and black denim.

We describe a method for the removal of blood from a variety of denim material without co-extraction of the indigo dye and subsequent DNA profiling following a standard 5% chelex extraction protocol. This method has also been used on a black cotton T-shirt where it appeared that the co-extraction of the dye was inhibiting the PCR reaction.

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Materials and methods

Sample preparation

Bloodstains were made on white, dark blue, black, purple, light blue denim and blue corduroy. A reference bloodstain was also prepared on clean white cotton cloth. All bloodstains were air dried for 24 h and stored at –20° C until testing. These fabrics were new and had not been washed prior to laboratory use. Bloodstains from case work that had failed to amplify were also examined. These included a bloodstained black cotton T- shirt and bloodstains on black denim jeans.

Blotting procedure

Duplicate stains were processed in all cases except those where insufficient material remained. A blotting chamber was set up using saline solution as the transfer solute in a similar method to a Southern Blot [6]. All containers were rinsed with 70% ethanol prior to use and all solutions were freshly prepared.

An approximately 5×5 mm square of the bloodstained material was placed bloodstain up on a wick of blotting paper. This was pressed gently until the denim was damp. Hybond-N (hybridisation transfer membrane, Amersham), wet with saline solution, was

placed over the bloodstain and any air bubbles removed quickly. A second square of Hybond-N membrane was placed over the first, followed by 3–4 squares of dry Whatman filter paper (Grade 1, Qualitative) to allow a capillary action to be established. In comparative studies, the Hybond-N nylon membrane was replaced with Hybond-N+ or filter paper. Once the top layers of filter paper were wet, the transfer was stopped. Each membrane and the filter paper squares along with the denim square could be processed separately. The nylon membranes may accumulate some fibres during the transfer which can be carefully removed with forceps to prevent any unnecessary presence of the potential inhibitor.

Extraction

The nylon membranes and filter paper squares were extracted with 5% chelex as described previously [7].

Quantification

The amount of DNA recovered was estimated using the ACES 2.0^{+} system (Advanced Chemiluminescent Enhancement System Human DNA Quantification System, Gibco BRL Life Technologies) as described in the manufacturers protocol.

Amplification and analysis

Amplification was conducted for the loci CSF1PO, TPOX, THO1 (known as the CTT triplex) and an Amelogenin sex test, using a Promega Kit following the manufacturers recommendations. Between 0.2 μg and 1 μg of DNA was amplified on a Perkin Elmer 480 thermal cycler. The products of the PCR reaction were run on a 4% acrylamide gels followed by silver staining for detection [8].

Results and discussion

A variety of denim materials were examined to provide a practical range of the different concentrations of indigo dye that may need to be overcome to produce a STR profile. In total, five varieties of denim, a blue corduroy and a black cotton fabric were used. Blood on each fabric was extracted using three methods (unblotted control, Hybond-N membrane and filter paper only). Each experiment as described in the methods was repeated 5 times.

Hybond-N, Hybond-N+ and filter paper alone were compared for their relative effectiveness in this process. The yield recovered from each varied but on average the nylon membranes provided the greater yields of DNA. Between 0.2 ng and 24 ng of DNA was recovered from the filter paper samples compared to 20 ng–134 ng for the samples recovered from the nylon membranes. In comparison, the yields of DNA recovered from fabric that was extracted without blotting were from 60 ng–280 ng.

STR profiles were produced more often when Hybond-N was used (results not shown). Profiles could be obtained from the filter paper used above the nylon membrane. When using Hybond-N+ there was no detectable transfer of DNA to the overlying filter paper. The dye and haem components of the extracted bloodstained denim did not transfer to the blotting membrane.

The STR profiles obtained from the bloodstains on all of the substrates tested were consistent with the reference blood profile (Table 1).

Table 1 Allelic profiles obtained from bloodstained denim and other heavily dyed fabrics used in experimental and case work material following blotting procedure and methods described

Substrate	THO1	TPOX	CSF1PO	AMEL
Reference blood	6, 7	8, 8	10, 10	XX
Blood on denim(1)	6, 7	8, 8	10, 10	XX
Blotted denim(2)	6, 7	8, 8	10, 10	XX
Victim's blood	7, 8	8, 10	12, 12	XY
Unblotted cotton T-shirt	– –	8, 8	– –	XY
Blotted cotton T-shirt	7, 8	8, 8	10, 11	XY
Victim's blood	6, 8	8, 9	11, 11	XX
Bloodstained black denim	– –	– –	– –	– –
Blotted black denim	6, 8	8, 9	11, 11	XX

(1) In some cases blood from unblotted denim may amplify and be typed correctly but these results are not consistently obtained
(2) Blotted white, dark blue, black, purple, light blue denim and blue corduroy

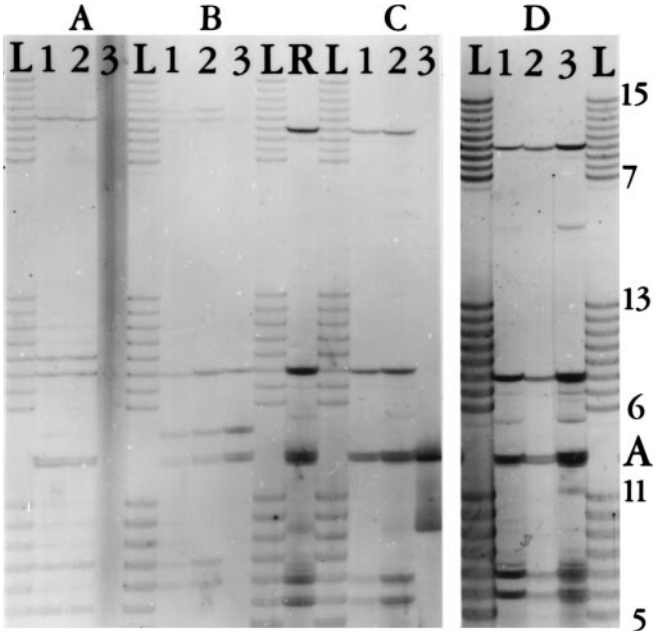

Fig. 1 The effect of different extraction procedures on the analysis of blood on denim material and a black cotton T- shirt. The CTT triplex was used in combination with amelogenin as described in materials and methods. Lanes labelled L contain the allelic ladders of the loci being analysed. Set A is blood on the back of black denim jeans. Set B is blood on a black cotton T- shirt. Set C is blood on light blue denim. Set D is blood on white denim. Lanes labelled 1 are processed filter paper used to create the capillary action as described in materials and methods. Lanes labelled 2 are the processed Hybond-N nylon membranes. Lanes labelled 3 are unblotted material. Numbers to the left indicate the minimum and maximum number of repeats in each loci ladder. "A" denotes the amelogenin locus

In some cases, unblotted bloodstained denim could be amplified and typed correctly, but this was not a consistent result. Unblotted stains did not always amplify at all the STR loci, although an amelogenin typing result was often obtained. This is evident in unprocessed light blue denim (Fig. 1, set C, lane 3). This appears to be a frequent

occurrence with denim and is likely to be an artefact of the presence of indigo dye. In some cases a high degree of background staining and stuttering was also noted in unprocessed denim samples.

This method is an improved technique for the profiling of bloodstains recovered from denim material. The technique has worked on older case samples and in situations where there is a limited supply of biological material. It is relatively quick and easy to set up and perform when compared to methods such as phenol-chloroform extraction and centricon concentration treatments and appears to overcome the associated problems of the co-extraction of the indigo dye.

Acknowledgements This work was conducted as part of a Master's of Science (Forensic Science) Degree for the University of Auckland, New Zealand and is run in conjunction with the Institute of Environmental Science and Research Ltd (ESR). We thank Dr. Douglas Elliot for his comments on the manuscript.

Names of commercial manufacturers and suppliers are provided for identification only and their inclusion does not imply endorsement by the Institute of Environmental Science and Research Ltd or the authors.

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