TECHNICAL NOTE

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PCR-Based Human Leukocyte Antigen (HLA) DQα Typing of Blood Stained Light and Dark Blue Denim Fabric

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ABSTRACT: Obtaining typable PCR products from DNA purified from blood stained blue denim has been difficult since inhibitors of PCR in blue denim apparently co-purify with the DNA. Organic and chelex extraction methods were tested for their ability to purify typable DNA from either light or dark blue denim fabric both stained with blood. DNA purified from the light blue denim using either method was successfully used in obtaining correct HLA-DQα typing results. The chelex, but not the organic, procedure was able to yield typable DNA when the dark blue denim was the substrate. Therefore, the chelex method may be more effective than the organic method in preventing compounds that inhibit PCR from co-purifying with the DNA.

KEYWORDS: forensic science, DNA, HLA-DQA1, DNA typing, polymerase chain reaction, denim

Blood-stained fabrics are common evidentiary material for DNA analysis. DNA typing by restriction fragment length polymorphism (RFLP) is routinely used to match a genotype of a suspect or victim with that of the blood on the fabric. However, RFLP analysis is time consuming, expensive, and requires a minimum of 50 ng of undergraded DNA (1). The polymerase chain reaction (PCR) is often used in place of RFLP analysis since it is faster, more sensitive, and more amenable to degraded DNA (2). However, PCR amplification can be adversely affected by a large number of factors such as, detergents, porphyrin compounds derived from heme, and proteinase K (2). Therefore, inhibitors such as these must be removed. Current protocols for PCR-based analysis of DNA from blood stained substrates require DNA to be extracted from the material using either an anion-exchange resin, such as chelex, or organic, such as phenol (3,4). In most cases, extracted DNA produces robust PCR products for typing.

It has been reported that DNA extracted from blood stained denim, especially the blue-colored variety, using either organic or chelex could not be amplified and typed (5–7). In fact, Jung et al. established that the chelex method alone was insufficient for producing DNA to be amplified and typed for the human leukocyte antigen (HLA) DQα locus (7).

In this report, studies were performed to evaluate a modified organic purification (8) and chelex extraction for purifying DNA associated with blood stained 100% cotton dark and light blue-colored denim. The purified DNA was then amplified and typed to evaluate the effectiveness of the extraction methods (for example, removal or prevention of dyestuff, PCR inhibitors, from co-purifying with the DNA). The difference in color intensity of blue denim is determined by the concentration of indigo dye that is present on the fabric (9–11). Since there is less indigo dye, which may be responsible for inhibiting PCR, associated with the light blue denim, it seemed plausible that blood-stained light-blue denim may not be as problematic for DNA amplification as the dark-colored denim. To address this theory, DNA from both light and dark blue denim punches, stained with blood from different individuals, was purified using either the chelex or the modified organic method. The resultant DNA was amplified and typed for the HLA-Dα locus.

Methods and Materials

DNA Samples

Blood samples were collected from laboratory staff, spotted on 3 cm × 3 cm pieces of dark blue 100% cotton denim and light (pre-washed) 100% cotton denim, and allowed to dry. The blood stained fabric was placed in a foiled envelope and stored at room temperature for 2 months.

DNA Purification

Modified Organic Method—Sample punches (4 mm) were taken from dark or light blue-colored blood stained denim, and placed in 1.7 mL microcentrifuge tubes. Five hundred microliters of buffered phenol (GIBCO-BRL) was added to each tube and incubated at 55°C for 1 h. The punches were washed three times with 500 mL solution B (75% isopropanol, 25% 0.1 M potassium acetate, pH 7.8), incubated at room temperature for 20 min in solution C (75% isopropanol, 25% 0.01 M magnesium acetate), washed once with isopropanol, dried at 80°C for 10 min. The fabric is directly added to a microamp tube (Perkin Elmer) for PCR.

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Chelex Extraction Method—Sample punches (4 mm) were taken from blood stained dark and light blue denim. Each individual punch was placed in 1.7 mL microcentrifuge tube. The DNA was purified by chelex extraction as described by Walsh et al. (4) except that a longer incubation time of 2 h was used. Ten microliters of extract, corresponding to approximately 50 ng of DNA, was transferred to a microamp tube for amplification.

DNA Quantitation

DNA obtained from the punches by chelex extraction was quantitated using the GeneQuant DNA/RNA calculator (Pharmacia Biotech).

Amplification and Typing

DNA amplification and typing were performed using the Amplitype™ HLA-DQα Amplification and Typing Kit (Perkin-Elmer) according to the manufacturer's instructions. Amplifications were achieved in a total volume of 50 μL that contained either 10 μL of chelex extract or 10 μL of distilled water and a punch purified by the organic method. All amplifications were performed using the Perkin-Elmer 9600 thermal cycler.

Results and Discussion

Two types of blue denim, light (pre-washed) and dark denim were assessed. DNA purified from the light blue denim by either extraction method was amplified and typed for the human leukocyte antigen (HLA) DQα locus. The reverse dot blot strips indicated that DNA was successfully typed with both purification methods when the light blue denim was the substrate (Table 1; Fig. 1*a* and 1*c*). The modified organic method resulted in no detectable signal on the strips, when dark blue denim was the substrate (Table 1; Fig. 1*d*). With respect to previous reports (5–7), it was surprising how efficient the DNA, purified from the dark blue denim with chelex, amplified and typed (Table 1; Fig. 1*b*). All the HLA-DQα genotypes, as indicated by the robust signals on the reverse-dot blot strips, were identical to the known genotypes of the individuals (Table 1).

TABLE 1—*HLA-DQα DNA amplification and typing from DNA purified from blood stained-light and dark blue denim.*

Sample	Purification Method	Type of Blue Denim	Genotype Known	Known Genotype*
1	Chelex	Light	1.2, 2	1.2, 2
		Dark	1.2, 2	
	Organic	Light	1.2, 2	
		Dark	Negative†	
2	Chelex	Light	1.1, 4	1.1, 4
		Dark	1.1, 4	
	Organic	Light	1.1, 4	
		Dark	Negative†	
3	Chelex	Light	4, 4	4, 4
		Dark	4, 4	
	Organic	Light	4, 4	
		Dark	Negative†	
4	Chelex	Light	2, 3	2, 3
		Dark	2, 3	
	Organic	Light	2, 3	
		Dark	Negative†	

*The known genotypes had been determined using a validated protocol in which DNA was purified from whole blood using chelex.

†There was no signal on the reverse-dot blot strips.

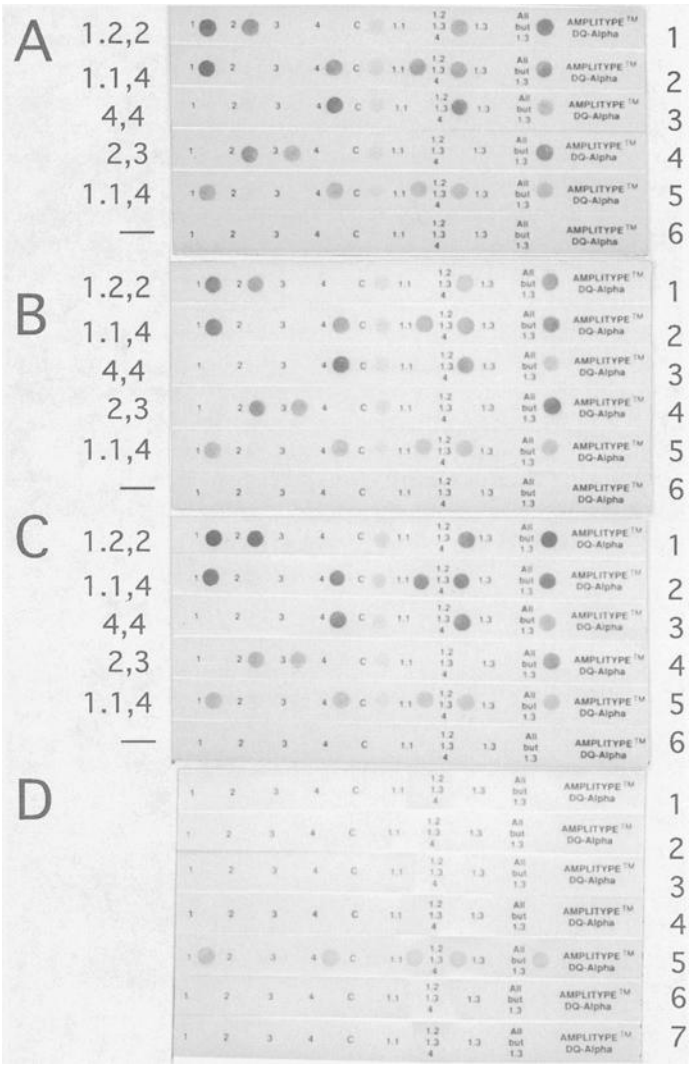

FIG. 1—*Typing of amplified HLA-DQα products obtained from purified DNA. Shown are typical reverse dot blot strips from one set of experiments. Blood stained light* [A,C] *or dark* [B,D] *(except sample 7)] blue denim punch DNA from 4 different individuals (strips 1–4) was purified by the chelex extraction method* [A,B] *or the modified phenol method.* [C,D *(except sample 7)]. Each sample was prepared and typed at least 5 times. Shown are typical reverse dot blot strips from one set of experiments. Strips 5 and 6* [A,B,C,D] *were the positive control (DNA 1 supplied with the kit) and negative control (no DNA) respectively. Strip 7* [D] *was the positive control DNA that was PCR amplified with a piece of unstained blue denim in the same reaction tube. HLA-DQα typing results* (indicated on the left) *were compared with the known genotypes of the individuals, and are summarized in Table 1. The known genotypes had been determined using a validated method in which DNA was purified from whole blood using chelex. Note that there was no detectable signals for strips 1–4, 6 and 7 in panel* D.

The modified phenol extraction, which is routinely used to purify blood stain punches from blood storage cards is unique in that it removes PCR inhibitors leaving the DNA tightly associated with the fabric (8). The blood stain punches, which are stained red with blood, are white as a result of this purification process. Applying the same method to the dark blue denim, resulted in the removal of the heme and metal ions, however, the blue dye was not removed. Since amplifiable DNA was obtained only when the light blue

denim was the substrate, the levels of indigo dye in the material may be directly related to PCR inhibition. This is consistent with a previous finding where positive typing results were obtained from white, but not blue, denim when various organic methods were tested (5). To determine if there was a trans-acting PCR inhibitor associated with the dark denim, a PCR was performed in which control DNA (DNA 1), supplied with the Amplitype™ HLA-DQα typing kit, and a 4 mm piece of dark blue denim was placed in the same reaction tube. As expected, no amplified DNA was detected by the typing assay (Fig. 1*d*, sample 7). This suggests that a compound associated with the dark blue denim, such as indigo dye or its derivatives, inhibits PCR when present at concentrated levels.

Overall the chelex extraction was a more reliable method since it yielded typable DNA regardless of the type of blue denim. Therefore, the chelex method may be more effective than the organic method in preventing compounds that inhibit PCR from co-purifying with the DNA.

References

(1) Budowle B, Baechtel FS. Modifications to improve the effectiveness of restriction fragment length polymorphism typing. Appl Theoret Electrophoresis 1990;1:181–87.
(2) Rolfs A, Schuller I, Finckh U, Weber-Rolfs I. PCR: clinical diagnostics and research, Springer-Verlag, New York, 1992.
(3) Schwartz EI, Khalchitsky SE, Eisensmith RC, Woo SL. Polymerase chain reaction amplification from dried blood spots on guthrie cards. Lancet 1990;336:639–40.
(4) Walsh PS, Metzger DA, Higuchi R. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 1991;10:506–13.
(5) Comey CT, Budowle B. Validation studies on the analysis of the HLA DQα locus using the polymerase chain reaction. J Forensic Sci 1991;36:1633–48.
(6) Koblinsky L. Recovery and stability of DNA samples of forensic science significance. Forensic Sci Rev 1992;4:67–87.
(7) Jung JM, Comey CT, Baer DB, Budowle B. Extraction strategy for obtaining DNA from blood stains for PCR amplification and typing of the HLA-DQa gene. Int J Legal Med 1991;104:145–48.
(8) Belgrader P, Del Rio SA, Turner KA, Marino MA, Weaver KR, Williams PE. Automated DNA purification and amplification from blood stained cards using a robotic workstation. BioTechniques 1995;19(3):426–32.
(9) Johnson AJ. Indigo dyes. The chemistry of synthetic dyes and pigments. Reinhold, New York, 1955.
(10) Society of Dyers and Colorists. Color index 3rd Edition, Humphries, North Carolina, 1971.
(11) Zollinger H. Indigo and its derivatives. Color chemistry: synthesis, properties and applications of organic dyes and pigments, VCH, New York, 1987.

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