The Usefulness of Chelating Resins for DNA Extraction from Forensic Material Prior to PCR Amplification

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INTRODUCTION

DNA extraction procedures prior PCR have been simplify since Singer-Sam et al. (1989) reported the use of chelating resins which prevents the degradation of DNA allowing the DNA extraction at high temperatures. In this paper we report a comparison between different methods of extraction, including chelatin resins, boiling with water and TE and phenol-chloroform extraction prior to the amplification of HLA DQA1 and pMCT118 systems.

MATERIAL AND METHODS

Samples.- Bloodstains [Recent (up to 6 months old); old (10 years old)] and hair

DNA extraction methods

Bloodstains:

A.- Chelating resin

Pippete 1 ml of water into a sterile 1.5 ml microfuge tube. Add bloodstain. Incubate at room temperature for 15 to 30 minutes. Mix occasionally by inversion or gentle vortexing. Spin in a microcentrifuge for 2 to 3 minutes at 10.000 to 15.000 xg. Remove supernatant (all but 20 to 30 µl) and discard. Add 5% chelating resins to a final volume of 200µl. Incubate at 56°C for 15 to 30 minutes. Vortex at high speed for 5 to 10. Boil in a boiling water bath for 8 minutes. Vortex at high speed for 5 to 10 seconds. Spin in a microcentrifuge for 2 to 3 minutes at 10.000 to 15.000 xg. Add 20 µL of supernatant to the PCR mix.

B.- Conventional method with phenol-chloroform

C.- Water boiling.- Chelating resin is substituted in method A by distilled water.

D.- TE boiling.- Chelating resin is substituted in method A by TE.

Hair:

E.- Chelating resin

Use a clean scalpel to cut a 5 to 10 mm portion from the root end of the hair. Add the root portion of the hair to 200 µL of 5% chelating resin in a 1.5 mL microfuge tube. Incubate at 56 °C overnight (at least 6-8 hours). Vortex at high speed for 5 to 10 seconds. Boil in a boiling water bath for 8 minutes. Vortex at high speed for 5 to 10 seconds. Spin in a microcentrifuge for 2 to 3 minutes at 10.000 to 15.000 xg.Add 40 µL of the supernatant to the PCR mix.

F.- Conventional method with phenol-chloroform

PCR.- HLA DQA1: Primers and amplification conditions according to Cetus AmpliType protocol. Dot-blot with Aso-probes (Amplitype). pMCT-118: Primers

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and amplification conditions according to Budowle et al. (1990). Detection by SDS-PAGE, T= 10-15, 100 Vh, followed by silver-staining according to Barros et al. (1991).

RESULTS

Table 1.- RECENT-OLD BLOODSTAIN COMPARISON

METHOD / BLOODSTAIN (50µl)	CHELATING RESINS		PHENOL-CHLOR.	
	DQA1	pMCT-118	DQA1	pMCT-118
RECENT	+++	+++	+++	+++
OLD (10 years old)	++	++	-	-

Table 2.- MINUTE BLOODSTAINS

METHOD / BLOODSTAIN (2 years old)	CHELATING RESINS		PHENOL-CHLOR.	
	DQA1	pMCT-118	DQA1	pMCT-118
0.5 µl	++	++	-	-
1 µl	++	++	-	-
5 µl	+++	+++	-	-

Table 3.- CHELATING RESINS/WATER/TE COMPARISON

METHOD / BLOODSTAIN	CHELATING RES.		WATER		TE	
	DQA1	pMCT	DQA1	pMCT	DQA1	pMCT
0.25 cm^2	++	++	+	+	-	-
0.50 cm^2	++	++	+	+	-	-
1 cm^2	+++	+++	+	+	-	-

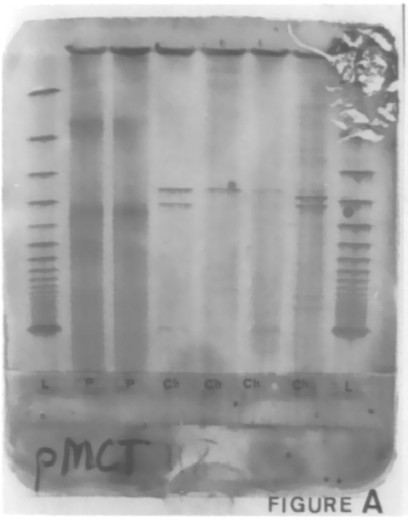
Table 4.- ROOT HAIRS

METHOD / SAMPLE	CHELATING RESINS		PHENOL-CHLOROFORM	
	DQA1	pMCT-118	DQA1	pMCT-118
ROOT HAIR	++	++	-	-

CONCLUSIONS

1.- Extraction with chelating resins provided positive results in 10 years old bloodstains whereas phenol-chloroform extraction gave negative results.

2.- Extraction with chelating resins provided positive results in minute bloodstains 2 years old whereas phenol-chloroform extraction gave negative results.
3.- Chelating resins increases the PCR signal when compared with a simple water extraction (boiling). TE gives negative results.
4.- Extraction with chelating resins provided positive results in root hairs whereas phenol-chloroform extraction gave negative results.



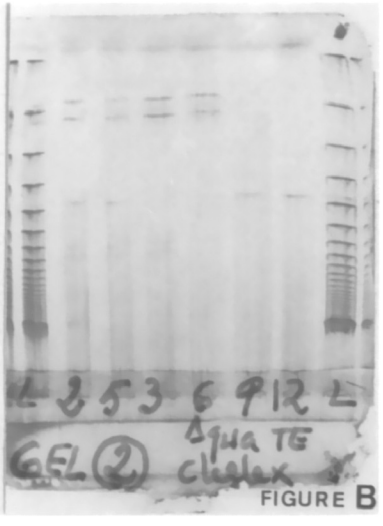


Figure A.- Comparison between chelating resin and phenol-chloroform methods in old bloodstains and hair. P= phenol-chloroform extraction, Ch= chelating resin extraction, L= 123bp DNAladder (BRL). (Hair: lanes 2,4; bloodstains: lanes 3,5,6,7).

Figure B.- Comparison between differents DNA extraction methods in old bloodstains. (Water: lanes 2,5; chelating resin: lanes 3,6; TE: lanes 9,12)

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