FORENSIC USE OF PCR DNA ANALYSIS IN HAIRS, ENVELOPES AND CIGARETTE ENDS

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INTRODUCTION

The advances in the polymerase chain reaction (PCR) development enables a great discrimination in analysis of forensic traces. Samples such as bloodstains and semen stains can be identified, but it also allows to have a great information about forensic samples that contain a limited amount of DNA, such as single hair, salival cells in cigarette ends, stamps or envelopes and skin residues found in nails although they were old samples with degraded DNA.

This work is a revision of forensic traces analysed in our laboratory, the majority resulting from forensic cases, in the last three years.

MATERIALS AND METHODS

Samples preparation

Hairs: They are washed with distilled water and are examined by microscopy to ensure that root is present. They are cut 0.5cm from the root and put in an eppendorff tube.

Salival cells in envelopes, stamps and cigarettes ends: the sample is cut and put into an eppendorff tube with distilled water.

Extraction

Chelex 100 extraction (Walsh 1991)

Phenol-Chloroform extraction procedure is based on the related by Higuchi (1988) with minor modifications according to the samples.

Amplification

Amplification of specific regions of the following genetic loci HLA-DQA1, LDLR, GYPA, HBGG, D7S8 and GC is performed using the Amplytipe® HLA-DQα Forensic DNA Amplification and Typing Kit and Amplitype® PM PCR Amplification and Typing Kit supplied by Perkin-Elmer. Typing of these six loci is performed according to the kits protocols.

D1S80 Forensic DNA Amplification Reagent Set (Perkin-Elmer) is used to amplify the alleles at the D1S80 locus according to the protocol. The amplified products are analysed by vertical electrophoresis using 0.75 mm thick native polyacrylamide gel (GeneAmp Detection Gel, Perkin-Elmer). The electrophoresis runs for one hour at a constant voltage or 500 Volts. To size de PCR

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products, a 27-allelic ladder, supplied by the manufacturer, is used. After electrophoresis the DNA fragments are silver stained (Bassam 1991).

HUMTHO1 locus is amplified in 25 ml of PCR mix (Gene Print STR systems Promega) according to the manufacturers instructions. The amplified products are analysed by vertical electrophoresis on 0.75 mm thick 4% denaturing polyacrylamide gel (19:1 acrylamide: bisacrylamide, 7 M urea, 24 cm length) and silver stained. The electrophoresis runs for one hour at constant voltage of 1000 Volts with a fixed temperature of 51°C. Alleles were assigned by a ladder supplied by Promega.

All the DNA amplifications are carried on a Linus DualCycler Thermocycler.

RESULTS AND DISCUSSION

DNA extraction method differs according to the samples. At first, chelating resin was used for minute samples because is a simple and fast procedure. At present, this is the used method in cigarette butts, stamps, envelopes and salival cells in fabrics, often combined with Microcon 100 in order to concentrate and purify. In analysis of single hairs, the DNA is obtained by phenol-chloroform extraction because it provides better results in VNTR analysis.

The found hairs in crime scenes are not, in most of cases, freshly plucked hairs samples but shed hair and contain too little DNA amounts to analyse all the markers and often only HLA-DQA1 and PM can be analysed.

SAMPLES	N. SAMPL.	DNA EXTRACTION		POSITIV. EXTRAC.	DQA1	PM	D1S80	TH01
		CHELEX	PHE/ CHLOR					
HAIRS	88	36	52	64	44	6	1	1
CIGARETTE BUTTS	28	28	0	25	16	6	1	1
SALIVA	8	8	0	8	8	8	1	1
ENVELOPES	4	4	0	4	4	4	1	0
STAMPS	4	4	0	4	4	4	1	1
TISSUES REMAINS	3	1	2	3	3	3	0	0
TOTAL	135	81	54	108	79	31	5	4

Table 1 shows the different kind of samples, extraction methods and loci studied in this sort of evidences, in the last years.

The progressive use of VNTR loci at the same time as DQA1 and PM loci can be seen on Fig. 1. In 1994 we began to analyse D1S80 and HUMTH01 in hairs, salival cells and cigarette butts.

Figure 2 shows the rate of successful DNA extraction. In 1994, DNA extraction seems to give worst results because we began to receive some older and older samples so as re-examined cases.

In Fig. 3, the different sort of samples are related.

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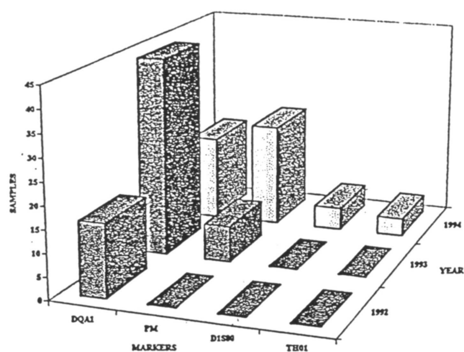

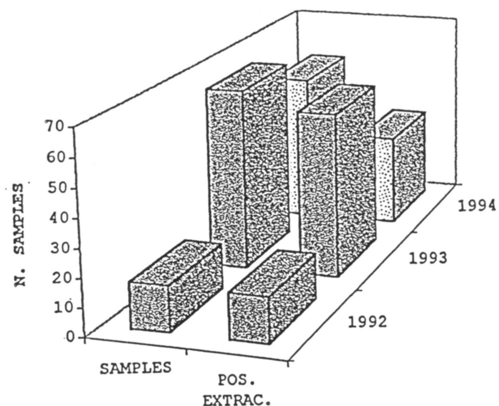

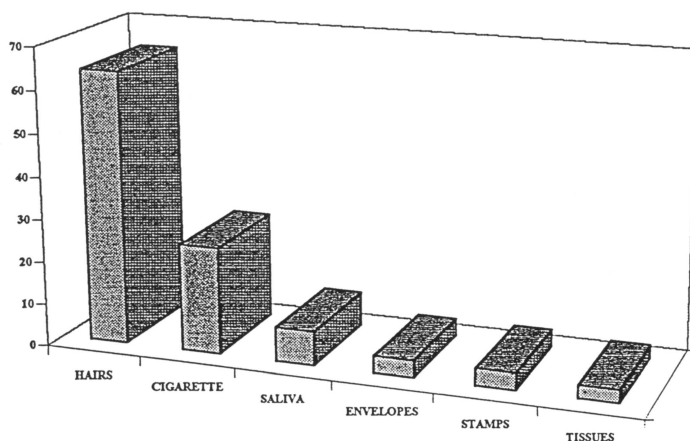

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