USE OF PCR FOR FORENSIC ANALYSIS OF DNA IN CIGARETTE ENDS.

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INTRODUCCION

DNA polymorphic sequences can be amplified up to one million fold by the polymerase chain reaction (Mullis and Faloona, 1987). It is possible to characterise minute samples (a single hair, a diploid cell, or single sperm (Higuchi et al., 1988; Li et al., 1988) as long as the target fragment of DNA remains intact. Good results can also be obtained from very old or deteriorated samples, in which DNA is highly degraded (Beroldingen et al., 1989). Due to the remarkable sensitivity of PCR and in spite of its stringent methodology, use of this technique is rapidly becoming widespread in forensic laboratories, among other reasons because it allows cases to be solved in which sample shortage, age or deterioration impede solution by conventional techniques, often inapplicable. With relative frequency cigarette ends are important biological evidence in forensic casework, sometimes the only clue left by a criminal at the scene of the crime. This work presents the results of identification of the individual from traces of saliva left on a cigarette end, in experimental samples and in two forensic cases, using PCR techniques (DQA1, D1S80, DYZ3 for sex determination).

MATERIAL AND METHODS

- Experimental samples: blood and cigarette ends from 4 individuals (2 cigarettes per person)
- Forensic samples: cigarette ends taken from the scene of crime in criminal or sexual assaults; blood samples from suspect and victim, bloodstains.
- DNA Extraction: Blood and bloodstains: 3 μL blood or 3 mm^2 stain were extracted with chelating resin (Imidoacetic Acid, Sigma) according to Cetus Corporation Technique (Amplitype User Guide Version 2)
- Cigarrete ends: From the proximal end of the cigarette paper, 1/2 cm was cut off, and one half of this was introduced in to a 1.5 mL eppendorf. Four extraction techniques were assayed in experimental samples according to Cetus Corporation protocols: phenol extraction followed by Centricon-100 ultrafiltration and chelating resin extraction were carried out with and without previous proteinkinase digestion.
- Spectrofluorometric DNA quantification: in a DNA fluorometer, TKO, Heofer Scientific Instruments. Fluorescence reagent: Bis-benzimide (Hoescht 33258, Sigma)
Amplification: A Perkin Elmer Cetus DNA Thermal Cycler 480 was used.
- DQA1: Amplitype HLA DQA1 Forensic DNA Amplification and Typing kit (Perkin Elmer). PCR product was detected by hybridisation with allele specific oligonucleotide probes
- D1S80: The D1S80 DNA Amplification Reagent Set (Perkin Elmer) was used, PCR product was detected by flat bed SDS-PAGE followed by silver staining (gel: 8-18% SDS PAA; gel buffer: 0.12 M Tris-Acetate pH 6.4; electrode buffers: anode: 0.3 M Tris-Acetate,4 g/L SDS, pH 6.4, cathode: 0.08 M Tris, 0.8 M Tricine, 4 g/L SDS, pH 7.1; Electrophoretic conditions: 400V, 100 mA, 30 W, 50-55 min).
- DYZ3: According to Witt and Erickson (1989). PCR product was detected by agarose gel (3% Nusieve GTG + 1% low EEO), stained with Ethidium bromide, using as size marker Hinf I digested φ x 174 (Promega)

RESULTS

The EXTRACTION TECHNIQUE of cigarette ends which yielded the best results in quantification (Table I) and amplification of experimental samples was chelating resin extraction of proteinkinase digests.

TABLE I **QUANTIFICATION**

SAMPLE	EXTRACTION METHOD	ADN (ng/μL)
1	PHENOL: NO PK (A.1)	2.0
	PK (B.1)	0.8
	CHELATING RESIN: NO PK (A.2)	1.6
	PK (B.2)	3.8
2	PHENOL: NO PK (A.1)	1.0
	PK (B.1)	0.4
	CHELATING RESIN: NO PK (A.2)	6.4
	PK (B.2)	5.6
3	PHENOL: NO PK (A.1)	0.4
	PK (B.1)	0.4
	CHELATING RESIN: NO PK (A.2)	3.0
	PK (B.2)	2.0
4	PHENOL: NO PK (A.1)	1.2
	PK (B.1)	1.6
	CHELATING RESIN: NO PK (A.2)	3.8
	PK (B.2)	1.8

* The following PROTOCOL was established and used for forensic samples: Pipette 1 mL of sterile destiled water into a 1.5 mL microcentrifuge tube. Add one half of 0.5 cm cigarette end paper and mix gently; Incubate overnight at 4°C; Spin in a microcentrifuge for 2-3 min; Remove supernatant (all but 50μl) and discard; Add sterile distilled water up to 200μL and 2μL of 10 mg/mL proteinase K. Mix gently; Incubate at 37°C for about 2 hours; Add 50 μL of 20% Chelating Resin; Incubate at 37°C for about 1 hour; Vortex at high speed for 5 to 10 seconds; Spin in a microcentrifuge for 10 to 20 seconds; Incubate 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. Samples ready for amplification. Store and reuse as usual.

* Table II shows the results of individual characterization of experimental samples.

TABLE II **TYPING RESULTS**

	INDIVIDUAL	DQA1	D1S80	DYZ3
1	BLOOD	1.1, 1.2	T24 T36	
	CIGARETTE END	1.1, 1.2	T24 T36	XX
2	BLOOD	1.3, 2	T18 T21	
	CIGARETTE END	1.3, 2	T18 T21	XY
3	BLOOD	1.1, 1.3	T23 T31	
	CIGARETTE END	1.1, 1.3	T23 T31	XX
4	BLOOD	4, 4	T24 T34	
	CIGARETTE END	4, 4	T24 T34	XX

CASE N°1: A woman was found dead in her apartment. The body showed many wounds and bone fractures. No weapon was found. There was no suspect. Besides toxicological and histopathological analysis, the police wanted to know how many people had been in the apartment.
SAMPLES: 24 smoked cigarette ends (tobacco and marihuana) found in different ash-trays.

RESULTS: PCR showed that several cigarette ends had been smoked by more than one person. One or two persons, besides the victim, were identified. One of them was a man.

CASE N°2: Several sexual assaults were committed in the same area. Circumstances suggested they could be related.
SAMPLES: vaginal swabs, hair, cigarette ends found at the scenes of the crimes, blood from one suspect, cigarette ends known to have been smoked by each of three other suspects.
Genetic typing of the victims was achieved from epithelial cells and/or pubic hair (we did not received blood samples from any of the victims).
RESULTS: Results from semen identification were not clear (scarce remains, only a very small number of spermatozoa heads, poorly preserved, were found in the samples from one of the victims).
According to DQA1 typing of cigarette ends and hairs, one of the suspects was excluded from the scenes of crimes. The other three suspects and two other persons were probably present (TABLE III).

TABLE III

SAMPLES		DQA1
PERSONS INVOLVED:	VICTIMS 1	4, 4
	2	1.1, 1.1
	SUSPECTS 1	1.1, 1.2
	2	4, 4
	3	1.1, 4
	4	1.1, 3
EVIDENCES:	CIGARETTE ENDS	1.1, 1.2
		1.2, 1.1, 4
		1.1, 1.2
		1.2, 2
		1.2(?), 1.1, 4, 2
	HAIRS FOUND IN A CAR	1.2, 2
		4, 4
	PUBIC HAIR: HAIR COMBING VICTIM 1	1.1, 4
	ON VAGINAL SWAB VICTIM 1	1.3, 2

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