

## USE OF PCR IN FORENSIC CASEWORK IN SOUTHERN SPAIN.

Sanz P, Prieto V.

Instituto Nacional de Toxicología, Sevilla, Spain.

### INTRODUCCION

For the last two years our laboratory has included PCR amplification techniques in forensic casework as a supplement to traditional diagnostic procedures. Up to this moment the following genetic markers have been used: DQA1, D1S80 and when required, DYZ3 for sex determination. In this paper we present some statistical data from our casework which illustrates the development in PCR implementation and strategy in our Institute. A selection of the most representative cases is also reported.

### MATERIAL AND METHODS

Our Institute is located in Southern Spain and covers about one third of the Country (128,859 square Km) and the Canary Islands. (Fig 1). Forensic samples are sent to the laboratory from any town or village in the area by different transport means. Facilities of some local Police or Forensic Services are often precarious. Paternity samples: blood samples are usually taken by venipuncture at our laboratory. Occasionally (i.e. from Canary Islands) they are correctly sent according to a preestablished protocol.

DNA extraction: In general extraction protocols have been taken from AmpliType user guide, Version 2 (Cetus Corporation)

Amplification and typing: Perkin Elmer Cetus DNA Thermal Cycler 480

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  $\phi$  x 174 (Promega)

### STATISTICS

### IMPLEMENTATION RATE

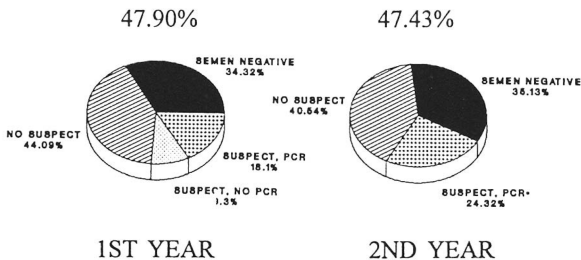
#### FORENSIC CASES

##### NUMBER OF CASES WHERE PCR WAS INCLUDED

Global from May 1991 to May 1993	24.4%
1st year	22.54%
2nd year	32.69%

#### A.- SEXUAL ASSAULTS

##### N° CASES



\* in many cases there was no suspect, but some evidence was left (cigarette ends, hairs...)

## B.- CRIMINAL ASSAULTS

	1st year	2nd year
N° Cases	52.10%	52.57%
N° Cases with PCR	21.92%	62.50%

## PATERNITY CASES

PCR has been applied routinely since September 1991  
25.45% were exclusions

<u>N° PAT. EXCLUSION</u>	<u>CONVENTIONAL</u>	<u>DQA1</u>	<u>D1S80</u>
6*	X		
2	X	X	X
3	X	X	
3		X	X

In two cases there was consanguinity between parents  
PCR always confirmed inclusions  
SL was only irreplaceable in very conflictive cases

PCR TYPING SUCCESS RATES

(per cent of successfully typed samples in cases where PCR technique was applied)

## A.- BLOOD SAMPLES

	PATERNITY CASES	FORENSIC CASES
	% SUCCESS	% SUCCESS
AcP	100.00	100.00
Gc	100.00	94.20
PGM	100.00	97.10
DQA1	100.00	92.95
D1S80	100.00	85.92

COMMENTS: Typing failures in forensic blood samples were frequently due to poor conditions of blood taken from corpses. Recently, whenever possible, PCR amplification is achieved in fixed specimens sent for histopathological study. (DNA extraction is carried out according to Gill et al., 1992). Good results were obtained with samples from exhumations made after several weeks; muscle, skin and hair yielded the best results.

## B.- BLOOD STAINS

		% FAILURE CAUSE VERSUS TOTAL FAILURE	
	% SUCCESS	INSUFFICIENT AMOUNT OF SAMPLE	UNDETECTED
AcP	42.80	68.30	31.70
Gc	50.00	52.00	48.00
PGM	29.70	58.70	41.20
DQA1	83.83		
D1S80	58.10		

COMMENTS: The success of PCR was mainly related to the correct collection and preservation of samples. Wet conditions were the first cause of failure.

Difficulties were also found with different kinds of supporting materials such as soils and stones, probably due to adsorption mechanisms. Because of the adherence properties of plastic materials, good results were only obtained when blood crust was discarded and the plastic support was used for the analysis.

Failure was more frequent with D1S80 than with DQA1. We never obtained positive results with D1S80 if DQA1 was unsuccessful so, routinely DQA1 is always done first.

### C. SEMEN

		% SUCCESS
DQA1	SEMEN	67.0
	VAGINAL CELLS	84.6
D1S80	SEMEN	44.4
	VAGINAL CELLS	75.0

COMMENTS: As with blood stains wet conditions were the main cause of failure, especially in blood stained samples.

Variability of stained material was even greater than with blood stains, due to incorrect sampling collection and storage conditions previous to reception in the laboratory.

No significant differences in success rate were found between two different extraction techniques: phenol extraction followed by Centricon-100 ultrafiltration or chelating resin extraction.

### CASE REPORTS

CASE 1.- A woman was found dead on a bank. She was completely naked. A preservative found near the corpse and blood from the victim were sent to the laboratory.

DNA typing was successfully carried out in semen and in epithelial cells recovered from the outside of the preservative which coincided with blood typing of the victim.

CASE 2.- A man disappeared and his car was found burnt. Minute blood stains were collected from different objects on the outside and in the surroundings of the car: lateral strip, a plastic bottle and a beer can. Blood from the mother and the brother were also sent for identification purposes.

Analysis: ABO, AcP, Gc, PGM1, DQA1, D1S80, DYZ3.

Results:

	ABO	AcP	Gc	PGM1	DQA1	D1S80	DYZ3
Mother	B	BB	1S1F	1+1+	1.1, 4	T29 T31	
Brother	B	BB	1S1F	1+1+	1.2, 4	T29 T29	
Blood stains	B	B	1S1F	-	2, 4	T29 T29	XY

X/Y= 24685

CASE 3.- A sexual assault was reported.

Samples: Blood from the victim, vaginal swabs, pubic hairs. No suspect.

Spermatozoa were found in the the vaginal swab and DNA typed.

Two pubic hairs were identified different from the victim. DNA typing of one of them coincided with semen, the other one was different. It could be concluded that two different individuals were implicated in the sexual assault.

### REFERENCES

- Cetus Corporation (1990) AmpliType User Guide, Emeriville.
- Gill P., Kimpton C.P., Sullivan R. (1992) Electrophoresis, 13, 172-175.
- Witt M., Erickson R.P. (1989) Human Genetics, 82, 271-274.