

CASEWORK EXPERIENCE WITH SINGLE LOCUS DNA PROBES AND CHEMILUMINESCENT DETECTION IN SPAIN

C. Albarrán, A. Alonso, P. Martín and M. Sancho.

Sección de Biología. Instituto de Toxicología. Ministerio de Justicia. Luis Cabrera, 9. 28002 Madrid. Spain.

INTRODUCTION

DNA profiling using oligonucleotide coupled with alkaline phosphatase single locus probes (MS1, MS31, MS43a, MS8 and YNH24) has been in use in casework at the forensic biology laboratory of the National Institute of Toxicology since 1991.

Here, we present our DNA profiling experience in cases of sexual assaults, body identifications and paternity testing from fetal remains.

MATERIAL AND METHODS

Types of cases examined

DNA profiling has been employed in over 200 cases. Of these, the majority have been sexual assaults, body identifications and paternity testing on fetal tissues (Table 1).

CASE TYPE	NUMBER
Sexual assaults	110
Body identifications	28
Paternity testing on fetal tissues	15

Table 1. Case types examined at the INT of Madrid.

DNA profiling procedure

In summary, DNA was extracted by the standard phenol/chloroform protocol [1] with some modifications depending on the sample (see below). DNA was restricted with Hinf I. Separation of the DNA fragments was performed by agarose gel electrophoresis in TBE buffer according to the EDNAP group protocol. Gels contained a control DNA size marker (Hind III/ λ), three DNA control ladders (BRL) and two genomic controls (K562). Gels were depurinated, denatured and Southern blotted onto nylon membranes. Membranes were hybridised sequentially with the probes MS1, MS31, MS43a, MS8 (Cellmark Diagnostic) and YNH24 (Promega Corporation) according to the Non-Isotopic Chemiluminiscent Enhancement protocol (Cellmark Diagnostic). Autoradiography was carried out for 2-5 days. All autoradiographs were analysed using the Biimage equipment (Millipore) using the method Elder and Southern [2] for band size calculation with reference to the control DNA ladder at three differing positions on the autoradiographs.

RESULTS

SEMEN STAINS

The analysis of seminal stains is the most common type of case analyzed in our laboratory. In these cases, the preferential extraction procedure for separating spermiatic DNA from the DNA of ephitelial cells is normally used [3]. The phenol/Chloroform extract was washed and concentrated by ultrafiltration (Centricon-100 concentrator) instead of ethanol precipitation. This procedure has two main advantages over ethanol precipitation:

- The DNA is always maintained in solution and the redissolution problems are abolished.
- The ultrafiltration method allows microsoluble removal which can be of importance to prepare the DNA extract for PCR amplification.

Table 2 shows the range of exhibits examined in our laboratoty. As can be seen, the vaginal samples and the knickers are the most common items submitted for DNA profiling.

EXHIBIT	NUMBER	SUCSEFUL RATE (%)
Vaginal swabs or washing	44	66
Knickers	30	77
Other clothing	39	54
Other semen remains	5	100
Anal swabs	4	0
Condoms	2	50

Table 2. Exhibit types examined at the INT of Madrid.

Sperm concentration on the exhibits was assesed microscopically. The relationship between sperm concentration and successful rate is shows in Fig 1.

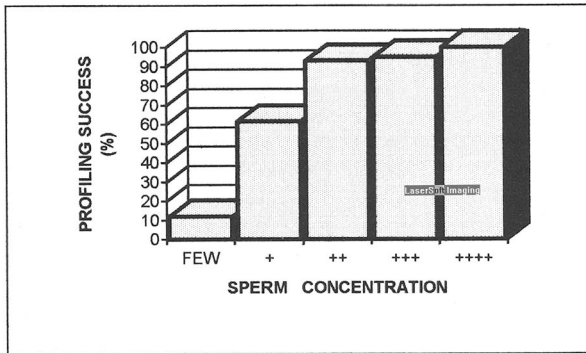


Fig. 1. Relationship between sperm concentration and profiling success with single locus probes. Scale ranges from "few" when there are very low numbers of visible espermatozoa on the whole slide to "++++" when there are many visible in every field of view. (This clasification has been established according to the criteria used by the Metropolitan Police Laboratory [4]).

In cases where DNA profile were obtained (73.4%), one or more suspects were excluded in 25% and 75% gave a profile matching the suspect or suspects. However, in 26.6% cases where no results were obtained for the RFLP technique (due to insufficient or totally degraded DNA), the PCR technology allowed to obtain a 47 % of success rate. Therefore, the successful rate with both techniques was 86.2%.

BODY IDENTIFICATIONS

Other important application of the single locus DNA profiling technique carried out in our laboratory has been the identification of cadaveric remains (carbonized bodies, dismembered corpses...) by comparison of the DNA profile obtained from the evidence to the DNA profile of family members.

The following cases and samples has been analyzed:

CASE TYPE	NUMBER	SAMPLE ANALYZED	SUCCESSFUL RATE
Carbonized bodies	12	Blood and muscle	75 %
Dismembered corpses	4	Blood, muscle, skin, hair, liver...	100 %
Other unidentified cadavers	12	Blood and muscle	100 %

We have obtained a DNA profile in 23 cases and in 7 cases, the persons have been identified by paternity testing.

FETAL REMAINS

The analysis of fetal tissues in rape cases and deserted newborn in cases of ilegal abortions and infanticides has been another application of the RFLP technology carried out in our laboratory.

The following table shows the type of sample and ssucceful rate:

SAMPLE ANALYZED	NUMBER	SUCCESSFUL RATE
Fetal remains	12	83 %
Newborns	3	100 %

ACKNOWLEDGEMENTS.

We are grateful to Cristina Agüero for her coloboration in the microscopical analysis of semenstains.

REFERENCES

- [1] Smith JC, Newton CR, Alves A, Anwar R, Jenner D and Markham AF (1990). J. Forensic. Sci. Soc., 30: 3.18.
- [2] Elder JK and Southern EM (1983). Anal. Biochem., 128: 227-231.
- [3] Gill P, Lygo J, Fowler S and Werrett D (1987). Electrophoresis, 8: 38-44.
- [4] Greenhalgh M, Burridge F and Willott G (1992). Forensic. Sci. Int., 57: 29-37.