FORENSIC DNA-TYPING IN THE SPANISH POLICE USING VNTRs SINGLE PROBES

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Introduction.

In the last years, our Laboratory has included DNA profiling in forensic case work as a substitutive technique of traditional analysis of proteins. This technique has been employed primarily in case of sexual assault; more than 230 stains and other biological specimens deriving from 125 Spanish crime cases were subjected to this technique, during 1994.
Variable number of tandem repeats (VNTRs) base secuences are one of the most informative genetic DNA Polymorphism for indentification purposes. We have implemented the analysis of VNTRs loci at the Spanish Police using the single locus probe hybridization technique; this analysis includes four independent DNA VNTRs systems: D1S7 (Probe MS1); D2S44 (Probe YNH24); D12S11 (Probe MS43a) and D7S21 (Probe MS31).
This paper describes the case types in which DNA profiling was carried out and shows the rate of obtained profiles from evidence samples in reference to suspect's profile.

DNA extraction.

The extraction of DNA in samples containing semen is done in a differential way. Vaginal cells found in washings and swabs are lysed in presence of Proteinase K and SDS. A second lysis of the spermatozoa is done later in presence of DTT and Proteinase K. Purification is carried out by standard Phenol-Chlorophorm methods, or by ultrafiltering by Centricon 100 (Amicon, USA). The presence of high molecular weight DNA was determined electrophoretically.

Electrophoretic system and DNA Probes.

DNA aliquots were partially digested with Hinf I and quantificated using fluorometric methods. Afterwards, DNA was completly digested and fragments were separated by gel electrophoresis, according to the EDNAP protocol. Gels contained: Lambda DNA/Hind III, to see the movility of the fragments; DNA control Ladders (BRL), as marker and DNA from cell line K562/Hinf I, as allelic control. After electrophoresis, the DNA was transfered to a nylon membrane, by Southern blotting. Hybridization was done using oligonucleotide probes, which were already labelled with alkaline phosphatase. The membrane was sprayed with Lumi-Phos 530 (Lumigen, Inc.) and the light emission was detected with X-ray film by exposing overnight. (Fig. 1).

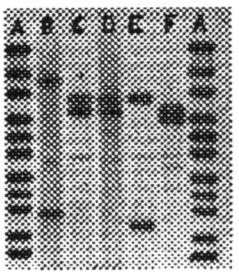

Fig. 1. Hinf 1 / MS43a profile. A)Ladder. B) Allelic control. C) Suspect blood. D) Semen Evidence. E) Victim blood. F) Anonymous Evidence.

Analysis of band position.

The length of DNA-fragments was calculated (Elder 1983) by reference to the DNA control ladder (BRL) using a computerized scanner system (EQUIDNA Pharmagen/Filosoft). This system allows many profile comparison possibilities. Generally, the suspect's profile (if it is available) is compared with the profile obtained in the evidence. This comparison is made band to band using a previously defined coincidence margin (criterion of

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defined coincidence margin (criterion of matching). If the band sizes are coincident, the identification is established and using the sliding window method, the possibility of finding the identified profile among the Spanish population is calculated. If the suspect's sample is not available, then the profile obtained from the evidence is stored as anonymous in a special data-base which allows the relationship between different offences. (Fig. 2).

Results and discussion.

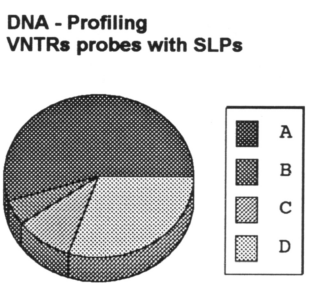
The types of semen stained exhibits examined at the DNA Laboratory of the Spanish Police are recorded in table N. 1.

Table 1. Exhibit types examined

EXHIBIT TYPE	NUMBER	SUCCESS RATE (%)
Vaginal swabs or washings	89	73
Knickers	30	96
Fabric clothing	30	96
Bed linen	12	83
Other semen remains	1	100
Condoms	2	100
Papers	12	75

Vaginal swabs or washings are the most common items submitted for DNA profiling in rape cases. The DNA is generally of good quality because swabs are taken in controlled conditions so that there is little opportunity for the DNA to degrade, but however the samples are sometimes contaminated, for instance with blood, which does not suppose any problem or with microbic that can increase the amount of extracted DNA.

The preferential extraction procedure for separating spermatozoa from epithelial cells is always used and in most cases no vaginal DNA was observed on the autoradiograph. However, in cases where a high number of vaginal cells is observed, a mixed profile can be obtained, (Rand 1991). It is in these cases that a control sample is requested from the donor of the swabs. If the DNA is seen to be of good quality on a test gel prepared during the profiling procedure, very low amounts will give results. Our Laboratory has obtained profiles from swabs which yield less than 350 ng. of DNA.



A) Anonymous profiles 55 %
B) Exclusion-suspects 5 %
C) Linked cases 10 %
D) Inclusion-suspects 30 %

Fig. 2. Cases when DNA-profile was obtained (Sexual assaults).

Knickers with semen stains in the cruch and fabric clothing are commonly submitted for DNA profiling and also give a very high success rate. This is an option when there is no semen on washings or vaginal swabs (Greenhalgh 1992). Analysis of these stains is easier now, by the use of Laser Technology, that let us see possible semen stains non visible with the naked eyes. Although stains on fabric are likely to be composed of semen without contaminating vaginal material, the preferential extraction procedure is still performed in some cases. The major problem with this sort of stains is that many fabrics, especially dark colours, are not colour fast and the dyes coextract with the DNA. The most successful method found to overcome this problem is to wash the spermatozoa from the fabric by vortexing in cold water; the fabric is then removed and the cells sedimented by preferential extraction procedure.

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Bed linen does not present any particular problem, yielding good results in general (83% success rate). Pollutants that inhibit the restriction process can appear in the papers used to recover semen remains in floors.

Conclusions.

A wide range of semen stained exhibit types is suitable for profiling. The present amount of DNA has some influence on the chances of obtaining a result but it is not the only factor to consider. Small quantities of DNA can give results using SLP analysis if the DNA is undegraded and there are no contaminants present that inhibit the restriction process. If only highly degraded DNA can be isolated it is unlikely that conclusive results will be obtained from SLP analysis. In these cases one possibility is the use of PCR better than SLPs.

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