DNA TYPING - STRATEGIES IN PATERNITY AND FORENSIC CASEWORK

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Since 1972 we have tested 7272 cases of doubtful paternity. Most cases were adequately resolved by testing the conventional red cell antigen, plasma protein, red cell enzyme and HLA polymorphisms. During the last year we have added DNA typing using single locus VNTRs in all cases not resolved by conventional methods or where the probability of paternity was too low.

DNA testing in forensic casework was recently introduced in our laboratory. This was particularly as a result of an increasing demand due to newspaper publicity of developments in DNA technology. The only other laboratory doing DNA tests in forensic casework in South Africa is the State forensic laboratory.

MATERIALS AND METHODS

Paternity cases:

The salting-out method (Miller *et al*, 1988) was used to obtain genomic DNA from peripheral blood. The DNA was digested using restriction enzymes *Pvu*II and *Hae*III, Southern blotted and hybridised to six VNTR probes YNH24 (D10528), CMM101 (D14513), TBQ7 (D10528),(Promega Corporation), Mucin, HaRas and 3'alpha globin. Fragment sizes were determined using a digitised video-image system developed in our laboratory. A fixed-bin approach was used in which the increments were 2%.

The results in 300 paternity disputes were used to evaluate probe and enzyme combinations in the local populations.

Forensic cases:

We have done a variety of forensic case studies over the last 12 months. These include rape, murder and body identification. DNA samples have been isolated successfully from vaginal swabs, underwear/clothing, organs/muscle/brain, drug abuser's syringes, amniotic fluid, bone marrow and wax-embedded tissue.

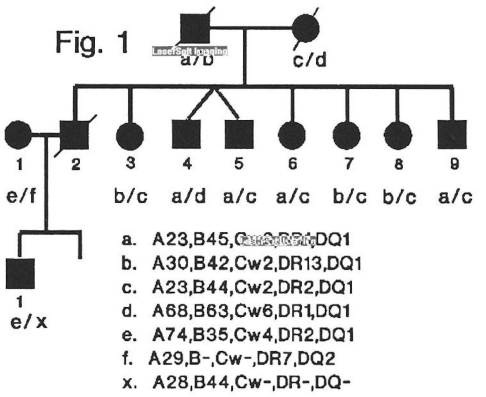
The problems were resolved using a combination of techniques, which include the reverse dot-blot technique (Cetus HLA DQA Forensic DNA Typing Kit), PCR-SSP, PCR-RFLP, PCR-SSOP as well as single and multilocus VNTR analysis.

EXAMPLES

Case 1

A woman claimed that a millionaire, who had been murdered and buried a year earlier, had fathered her son. This was disputed by the other heirs and the insurance company. Exhumation seemed unnecessary as the deceased had seven siblings. The red cell and plasma protein results were uninformative. The red cell enzyme PGD*AC phenotype, present in the child was absent in the siblings of the deceased as well as the mother (all phenotype PGD*A). This was not conclusive and hinted at a possible exclusion. The HLA haplotype "x" which the child must have inherited from his biological father was not present in the siblings of the deceased putative father (Fig 1). The HLA "d" haplotype of the twin brothers of the deceased was an added proof that they all had the same parents. The results (not shown) of at least 2 VNTR probes also enabled us to exclude the child from the family.

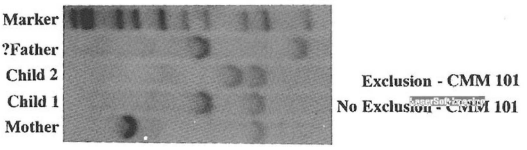
Fig. 1



Case 2

In a paternity dispute involving twins the putative father was excluded as the father of the one twin, II-2, (Fig 2), in the HLA system as well as with the restriction enzyme *Hae*III and the VNTR probes YNH24, CMM101 (Fig 3) and TBQ7.

Fig. 3



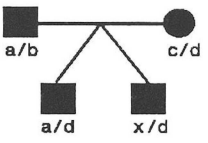


Fig. 2

a. A23,B57
b. A30,B-
c. A23,B49
d. A24,B18
x. A1,B52

Case 3

Prostatic chips from two patients were accidentally mixed in a surgical pathology laboratory. Histological examination revealed malignancy in some of the chips. Our laboratory was asked whether it was possible to match the DNA types of the benign and malignant tissues to the bloods of the two patients. The PCR-SSOP based HLA-DQA DNA typings (Fig 4) of the wax-embedded tissues and bloods of the two patients resolved the problem(Table 1).

CONCLUSION

Our experience illustrates that a variety of techniques and strategies are essential to resolve clinical as well as forensic problems.

Table 1 HISTO-PATHOLOGY CASE
HLA-DQA DNA TYPING

Patient	Blood	Tissue	
		Benign	Malignant
P1	1.2, 2	1.2, 2	-
P2	4, 4	-	4, 4

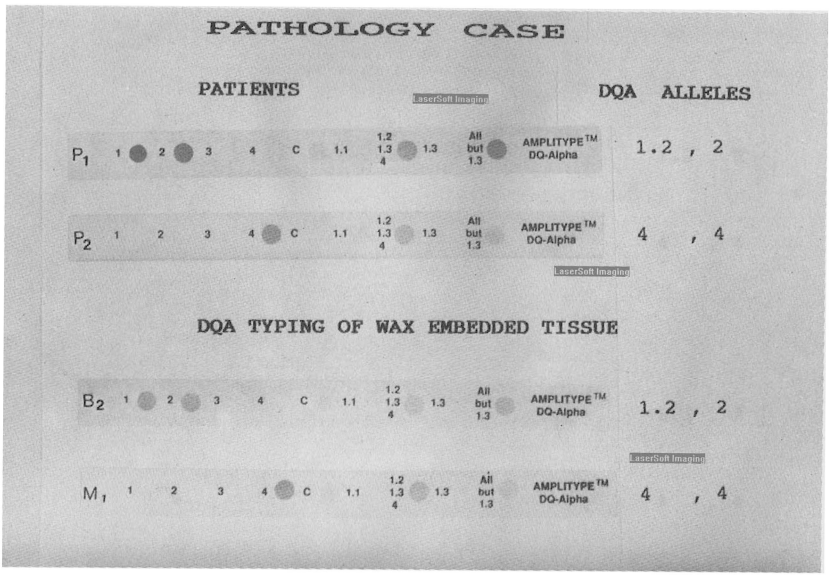


Fig. 4

REFERENCES

Miller SA, Dykes DD, Polesky HF (1988) A simple salting-out procedure for extracting DNA from nucleated cells. Nucleic Acids Research 16: 1215.