

EXPERIENCES WITH SIX SINGLE LOCUS PROBES IN PATERNITY TESTING

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INTRODUCTION

Recent developments in molecular biology allow the rapid detection of specific DNA-polymorphisms and DNA-testing is also used in paternity testing. Two different approaches are in use: Multilocus probes (MLP's) and single locus probes (SLP's). We report our experiences with 6 SLP's in cases of disputed paternity. Apart from DNA-testing most cases were also investigated with the conventional systems (red cell and serum groups, enzyme polymorphisms) and some of them with the HLA-system.

MATERIAL AND METHODS

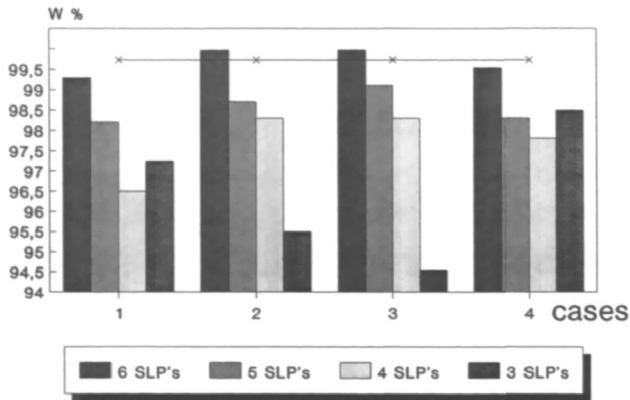
For DNA-analysis the following biotin labelled SLP's were used: pS 194 (5.0-11.0 Kb), pL 336 (2.0-6.0 Kb), pL 159-1 (4.0-5.5 Kb), pL 355-8 (6.0-8.0 Kb), pL 427-4 (1.4-3.4 Kb) and pR 365-1 (1.3-3.5 Kb).

DNA digest was performed by adding 25 units of Pst I to 10 µg of genomic DNA, followed by gelelectrophoresis (42-66 hours), hybridization for 16 hours, 3x washing, 10 min. incubation with streptavidin, again 3x washings, thereafter alkaline phosphatase followed by 4x washings, finally NBT and BCIP was added. In all cases the bands could be easily identified.

RESULTS AND DISCUSSION

A total of 23 cases of disputed paternity were investigated. In 13 cases in which 6 SLP's were applied no exclusion of the alleged men was observed. In these cases biostatistical calculation was performed using ESSEN MÖLLER probability (W). In 10 of 13 cases (76.92%) the biostatistics revealed $W \geq 99.73\%$. However, when only 3 SLP's were used $W \geq 99.73\%$ was observed in 6 of 13 cases. In 4 cases a remarkable difference exists between 6 and 3 SLP's. For comparison these four cases were investigated with 6,5,4 and 3 SLP's. The data are shown in fig. 1. In two cases (No. 2 and 3) a significant difference exists between the W-values calculated after applying 3 and 6 SLP's, whereas the differences in cases 1 and 4 are rather small. It should also be noted that in cases in which DNA-testing did not result in W-values of $\geq 99.73\%$ the combination with the HLA-system or the conventional systems (red cells, serum groups or enzyme allotypes) gave W-values of $\geq 99.73\%$.

Comparison between 6, 5, 4 and 3 SLP's in cases of disputed paternity



In table 1 10 of 23 cases of disputed paternity are listed with exclusion. The second column shows the number of probes applied in each case. Except two cases (No. 6 and 38a) exclusion was obtained with two or more SLP's.

Table 1

Exclusion in 10 cases of disputed paternity

Case No.	Probes investigated n	Exclusions	
		n	(%)
4	6	3	(50%)
6	4	1	(25%)
8	5	5	(100%)
10	6	5	(83.3%)
15	5	2	(40%)
20	3	3	(100%)
22	3	3	(100%)
29	5	4	(80%)
33	5	5	(100%)
38a	4	1	(25%)

Table 2 summarizes the exclusion rates obtained with the six SLP's used in this study. As expected the exclusion rates varied between the various probes. Probe 427-4 resulted in all cases applied in exclusion of the alleged men, whereas the lowest exclusion rate (25%) was observed with probe 365-1. This probe had also the lowest rate (51.70%) as indicated by the manufacturer.

Table 2

Exclusion rates in cases of disputed paternity

Probes	cases investigated	exclusion rate n	exclusion rate %	exclusion rate % (manufacturer)
pL 427-4	10	10	100	83.27
pR 365-1	8	2	25	51.70
pL 355-8	6	3	50	67.82
pL 159-1	11	8	72.7	43.54
pL 336	6	5	83.3	88.88
pS 194	10	7	70	79.08

A case of disputed paternity with consanguinity (uncle-niece) could be clarified by DNA-analysis, excluding the alleged man (uncle) with 4 probes.

SUMMARY

Six SLP's were applied in 23 cases of disputed paternity:

1. Exclusion of the alleged men was observed in 10 cases, including a case with consanguinity between mother and alleged man (uncle/niece).
2. Exclusion rates varied between 25% and 100% of the SLP's applied.
3. In 10 of 13 cases (76.92%) with no exclusion the biostatistical calculation resulted in W-values of $\geq 99.73\%$.
4. When DNA-analysis was combined with the results of HLA-testing or with the data obtained with conventional systems W-values of $\geq 99.73\%$ resulted in all cases.
5. DNA-analysis with SLP's is a reliable technique which can be applied in cases of disputed paternity.