

EFFICIENCY OF 6 STR SYSTEMS, HLADQ α AND THE POLYMARKER SYSTEMS (PM) IN PATERNITY TESTING

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Introduction

In order to test the efficiency of PCR based DNA systems in paternity testing the following 12 systems were selected: the 6 STR systems SE33, D21S11, HUMTHO1, HUMVWA, HUMF13A1 and HUMFES, HLADQ α and the 5 Polymarker systems (PM) LDLR, GYPA, HBG, D7S8 and GC. This study presents the results of 47 cases of disputed paternity (26 nonexclusion and 21 exclusion cases) which were examined with the 12 PCR systems. The results were compared with the efficiency of the 4 single locus VNTR systems MS43A, MS31, G3 and yNH24 routinely used in paternity cases since 1991.

Methods

a) STR-Systems:

Primers: SE 33 (Polymeropoulos et al. 1992), D21S11 (Sharma and Litt 1991), HUMTHO1 (Edwards et al. 1991), HUMVWA (Kimpton et al. 1992), HUMF13A1 (Polymeropoulos et al. 1991), HUMFES (Polymeropoulos et al. 1991).

Amplification conditions (Thermocycler: Biometra Triothermoblock):

SE33 and HUMTHO1: 94°C - 45 sec., 60°C - 30 sec., 72°C - 30 sec; 30 cycles.

D21S11, HUMVWA, HUMF13A1, HUMFES: 94°C - 45 sec., 55°C - 30 sec., 72°C - 30 sec.; 30 cycles.

Analysis and fluorescent detection: The PCR-products were analyzed and detected on an ABI 373A automated DNA sequencer (ABI GeneScan 672). The electrophoresis was carried out on 6 % denaturing polyacrylamide gels according to the ABI protocol (running conditions: 1600 V, 24mA, 7-8hs). Allele designations were determined by comparison with an allelic ladder.

b) HLA DQ α and PM Systems:

Amplification and typing reactions were performed according to the Cetus Protocol (HLA DQ α Forensic DNA Amplification & Typing Kit, AmpliType PM PCR Amplification and Typing Kit).

c) RFLP:

Paternity investigations using the four single locus probes MS43A, MS31, G3 and yNH24 has been published previously (Bär and Kratzer 1992).

Results

1. Nonexclusions

26 cases were analysed. The biostatistical evaluation of a nonexclusion was performed according to Essen-Möller (1938). Table 1 shows the calculated W-values using different sets of combinations of PCR based systems in comparison to 4 SLPs. The results demonstrate that all 12 investigated PCR systems are usually necessary to reach a conclusive probability of above 99.8 % in all cases, whereas DNA analysis with 4 single locus systems led to W-values of above 99.8 % in all cases.

The average contribution of each system is expressed in the average EM-value which is shown in table 2 and 3. The results show that the STR system SE33 is comparable with any of the single locus system.

Table 1:
W-Values resulting after applying different test batteries of VNTRs in 26 cases

W-Value	4 STRs* PM, DQ α	6 STRs*	6 STRs* PM, DQ α	4 SLP
< 99.8 %	15	3	0	0
\geq 99.8 %	11	23	26	26

*4 STRs: THO1, VWA, F13A1, FES

*6 STRs: SE33, D21S11, THO1, VWA, F13A1, FES

Table 2: Average EM-Values of 12 PCR systems (N = no of cases)

System	SE33	D21S11	THO1	VWA	F13A1	FES	DQ α	PM
EM	8.055	9.3085	9.5775	9.5468	9.4116	9.6734	9.5520	9.3694
N	27	27	27	28	28	28	37	27

Table 3: Average EM-Values of the single locus systems (N = no of cases)

System	MS43A	MS31	G3	yNH24
EM	9.0101	8.9869	8.9175	9.1044
N	105	105	104	104

2. Exclusions

21 exclusion cases were analysed with the above described 12 PCR systems. Table 4 and 5 show the results of the exclusion efficiency in comparison with the single locus systems. The power of exclusion differs considerably and does not reach the high efficiency of any single locus systems. The highest value was observed for the STR system SE33. Multiple exclusions (3 and more exclusions per case) were only obtained using all 12 PCR systems whereas DNA analysis with 4 single locus probes always led to multiple exclusions (≥ 3).

Table 4: Average power of exclusion (PE)

PCR	SE33	D21S11	THO1	VWA	F13A1	FES	DQ α	PM
PE	79%	62.5%	71%	57%	52%	43%	53%	61%
SLP	MS43A	MS31	G3	yNH24				
PE	90%	95%	89%	96%				

Table 5: Number of exclusions (Excl.) per case (N = 21)

Systems	no Excl.	1 Excl.	2 Excl.	3 Excl.	≥ 4 Excl.
PM	9	10	2	0	0
PM+DQ α	2	12	6	1	0
6 STR's	0	1	3	5	12
6 STR's, PM, DQ α	0	0	0	4	17
4 SLP	0	0	0	1	20

Discussion

With the exception of the STR system SE33 all PCR systems studied have a considerable smaller efficiency than each of the 4 single locus VNTR systems. SE33 is comparable with a single locus VNTR system. For paternity testing at least 12 PCR systems must be applied to obtain a comparable efficiency as with a battery of the 4 single locus VNTR systems.

The PCR systems are useful and reliable marker systems for paternity testing with many advantages: e.g. discrete alleles, high sensitivity and the technique is rapid and less expensive. However only a considerable number of PCR systems are capable to match the high efficiency of the polymorphic single locus systems. In an intermediate phase combinations of SLP and PCR systems are useful.

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