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Population and family data of RFLP's using selected single- and multi-locus systems

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Summary. The multi-locus systems (MLS) 33.15 and 33.6 (Jeffreys et al 1985a) and the single-locus systems (SLS) MS 1, MS 8, MS 31 and MS 43 (Wong et al. 1987) were investigated. The number of bands and the rate of band sharing were determined for both multi-locus systems and compared to the results of an English survey. Additionally 73 families were investigated using the multi-locus probes and the results compared to those obtained for the traditional blood grouping systems. The results were in full agreement so that no evidence of new mutations could be found. The fragment distribution was calculated for each of the 4 single-locus systems and compared to the values reported in an English survey (Smith et al. 1990b). Distinct discrepancies were seen in the systems MS 1 and MS 8. Family analyses were carried out for the 4 single-locus systems and compared to English data (Jeffreys et al. 1988) to look for any indications of possible new mutations. Only one isolated exclusion could be demonstrated with MS 1.

Key words: RFLP's – Multi-locus systems – Single-locus systems – Forensic efficiency

Zusammenfassung. Untersucht wurden die von Jeffreys et al. (1985a) entdeckten Multi-Locus-Systeme (MLS) 33.15 and 33.6 sowie die Single-Locus-Systeme (SLS) MS 1, MS 8, MS 31 and MS 43 (Wong et al. 1987). Für die beiden Multi-Locus-Systeme wurden die Bandenzahlen und die Band-Sharing-Raten ermittelt und mit einer englischen Stichprobe verglichen. Zusätzlich erfolgte für 73 Familien ein Vergleich der MLS-Untersuchungsergebnisse mit konventionellen Gutachten. Die Ergebnisse stimmten in allen Fällen überein; es fand sich kein Hinweis auf Neumutationen. Für die 4 Single-Locus-Systeme wurde die jeweilige Fragmentverteilung dargestellt und mit den Werten einer englischen Stichprobe (Smith et al. 1990b) verglichen. Auffällige Abweichungen zeigten sich bei den Systemen MS 1 and MS 8. Desweiteren erfolgte eine Gegenüberstellung der errechneten Heterozygoten-Frequenzen beider Stichproben (Münster-England). Um Hinweise auf mögliche Neumutationen zu erhalten, wurden für die 4 SLS Familienanalysen durchgeführt und mit englischen Daten (Jeffreys et al. 1988) verglichen. Lediglich in MS 1 ließ sich ein isolierter Ausschluß zum Eventualvater feststellen.

Schlüsselwörter: RFLP's – Multi-Locus-Systeme – Single-Locus-Systeme – Forensische Effizienz

Introduction

The application of DNA minisatellite probes (Jeffreys et al. 1985a) has greatly improved the efficiency of forensic haemogenetics. The inclusion of DNA analysis has also brought a controversial discussion over their evidential value (Brinkmann et al. 1989; Kömpf and Driesel 1989; Lander 1989a). The extremely high variability and efficiency is counteracted by the argument that an unequivocally defined genetic model is lacking. For this reason their use in paternity and stain diagnostics must be appraised in different ways. The aim of this investigation was to evaluate the frequency and family data for 6 highly polymorphic systems and to compare them with previously published data.

Materials and methods

Blood samples were collected in EDTA from routine paternity and criminal cases. DNA was isolated by haemolysis of erythrocytes in 50 m*M* KCl and digestion in Tris buffer containing Proteinase K (2 mg/ml, Boehringer Mannheim, FRG) and SDS (final concentration 0.5%) overnight at 37°C. Extraction was carried out in phenol/chloroform 1:1 (twice) and chloroform/isoamyl alcohol 24:1 (once) and subsequent precipitation by addition of 1/10 vol. 3 *M* sodium acetate (pH 5.2) and 2.5 vol. absolute alcohol. After air-drying, the precipitate was redissolved in TE buffer (10 m*M* Tris, 1 m*M* EDTA pH 7.4). The concentration of DNA was measured spectrophotometrically by absorption at 260 nm (Davies et al.

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1986). Estimation of high molecular weight DNA was carried out by loading 1 µg DNA on a 0.8% agarose gel and visualised by addition of ethidium bromide (0.5 µg/ml). The concentration was visualised and compared to a 1 µg Lambda DNA control (Pharmacia) after Maniatis et al. (1982). Digestion of high molecular weight DNA was carried out with Hinf I (Gibco, BRL; 3.5µg DNA/10 U Hinf I) by incubation overnight at 37°C.

The completeness of digestion was controlled by running all samples in a 0.8% agarose gel. DNA was further purified by extracting twice in phenol/chloroform and once in chloroform/isoamyl alcohol and precipitated as previously described. After centrifugation, (12,000 rpm, 4°C) the DNA was air-dried and resuspended in 15 µl TE buffer. Restriction fragments were separated in a 0.7% gel (20 × 23 cm) in TBE at constant current (65 mA) for approx. 24 h or until the 2.3 Kb fragment of the Lambda marker had migrated 20 cm. After electrophoresis, gels were depurinated by immersion in 0.25 *M* HCl for 15 min, denatured for 30 min in a solution of 0.5 *M* NaOH and 1.5 *M* NaCl and neutralised for 30 min in a solution of 3 *M* NaCl and 0.5 *M* Tris (pH 7.5).

Transfer was performed onto nylon membranes (Hybond-N, Amersham-Buchler) using Southern blotting in 20 × SSC (Maniatis et al. 1982). The membranes were washed (5 min in 2 × SSC), air-dried and baked (4 h at 80°C) to fix the DNA.

Hybridisation was carried out using the primer extension method (Feinberg and Vogelstein 1983) to a specific activity of 2×10^8 cpm/µg for MLP's and 4×10^8 cpm/µg for SLP's (DNA polymerase I, Boehringer, α^{32}P d GTP for MLP's, α^{32}P d CTP for SLP's, Amersham-Buchler 3,000 Ci/mmol).

Nylon membranes were prehybridised in the appropriate hybridisation solution (MLS: 0.2% BSA, 0.2% Ficoll 400, 0.2% PVP, 6% PEG 6,000, 0.1% SDS, 50 µg/ml degraded herring sperm DNA; SLS: 5 × Tris, 10% dextran sulphate, 1 *M* NaCl, 30% formamide, 10 µg/ml sonicated salmon sperm DNA) at 62°C for MLP's and 50°C for SLP's, followed by hybridisation under the same conditions.

For MLP's the nylon membranes were washed 2–3 times at 62°C for 30 min in wash solution (20 × SSC, 50 µg/ml degraded herring sperm DNA, 10% SDS) and once in 3 × SSC for 1 min.

For SLP's the membranes were washed once at 20°C (10 min in 2 × SSC) and twice at 65°C (30 min in 0.1 × SSC, 0.2% SDS).

Autoradiography was carried out at −80°C for 1–10 days with intensifying screens.

Results

Multi-locus systems

In this investigation only the bands above 4 kb were evaluated (Fig. 1). The area below 4 kb was not taken into consideration because here the bands were obviously more diffuse and under the conditions used in this study a much higher band sharing rate could be recognized.

The number of bands for each individual and the band sharing rate between individuals were calculated for both multi-locus probes and were found to be smaller than those from an English survey (Jeffreys et al. 1985b; Table 1).

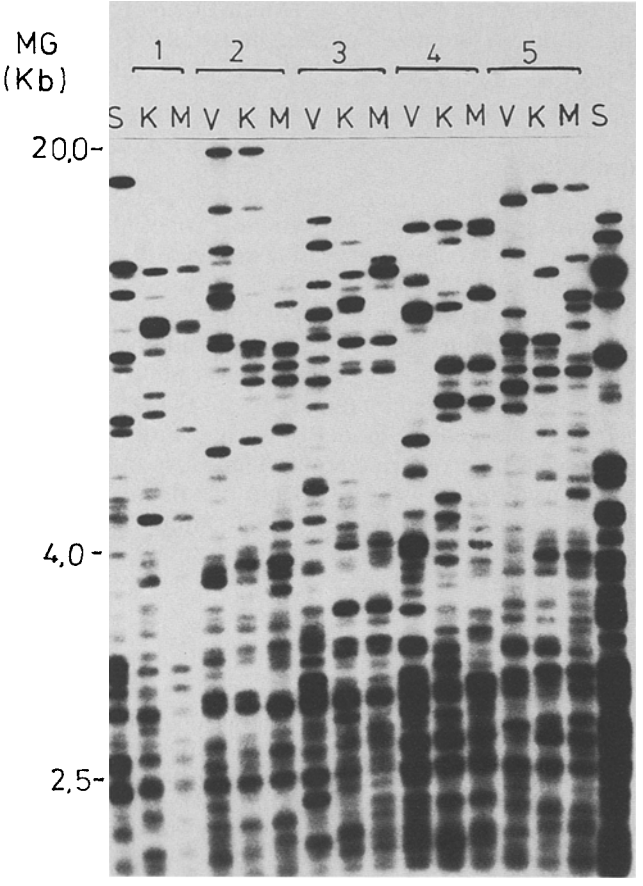

Fig. 1. Autoradiogram of DNA labelled with the probe 33.15: restriction enzyme Hinf I. Families are grouped together (M = mother, K = child, V = putative father, S = control DNA). The standard kb-control can be seen on the left hand side

Table 1. Comparison of the average number of bands, band sharing and discrimination index (DI) from unrelated individuals in Münster and England[a], calculated according to Jeffreys et al. (1985b)

	33.15	33.6
n	247	219
No of bands	13 (16)[b]	17 (18)[b]
Band sharing	15% (21%)	18% (23%)
DI	1.9×10^{-11} (1.4×10^{-11})	2.2×10^{-13} (3.2×10^{-12})

[a] Smith et al. 1990a

[b] The number of bands observed in the English survey were in the range 2.5–20 kb (in brackets), in the survey from Münster in the range above 4 kb

Table 2. Comparison of the average number of bands, band sharing and discrimination index (DI) from unrelated individuals in Münster and England[a], calculated according to Jeffreys et al. (1985b)

	Family studies		
	33.15/ 33.6[a]	33.15[b]	33.6[b]
1) Exclusion with conventional systems and DNA (*n* = 39 families)	18	15	6
2) No exclusion with conventional systems and DNA (*n* = 34 families)	18	11	5

[a] Families investigated with both probes in combination

[b] Families investigated with only 1 probe

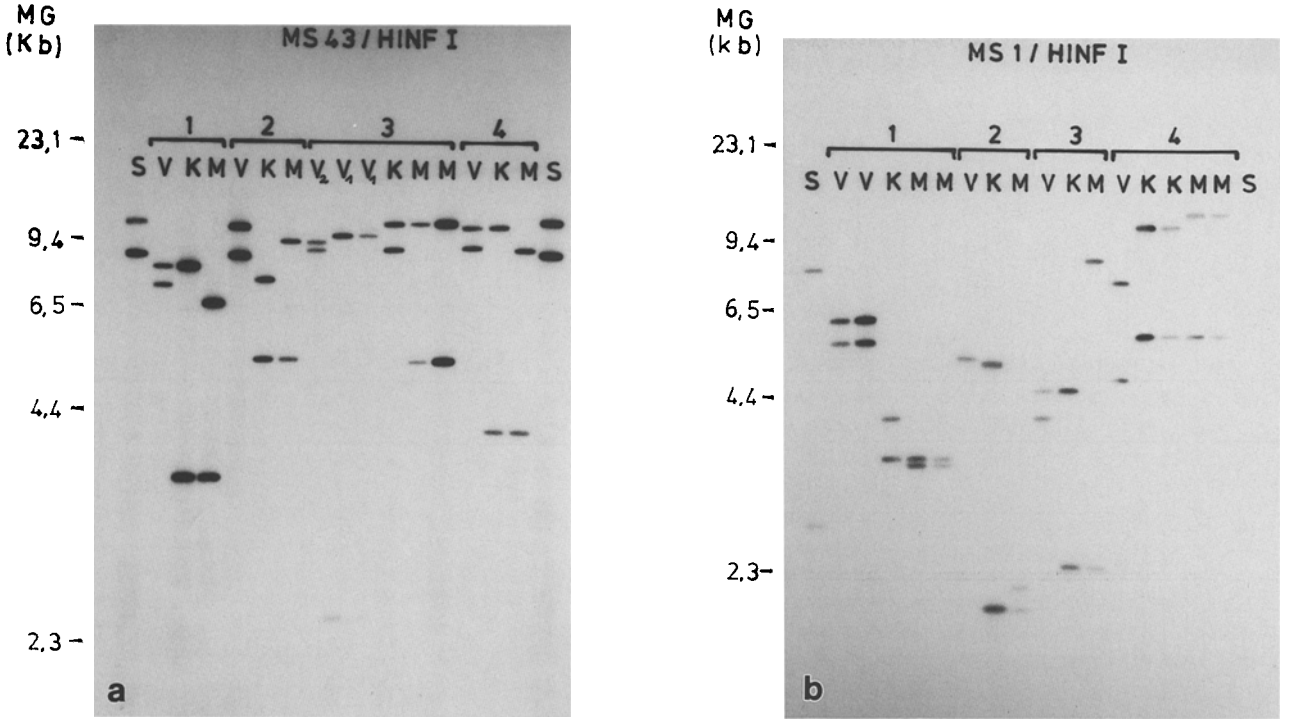

Fig. 2a, b. Autoradiogram of the single locus systems MS 43 (2a) and MS 1 (2b)

Additionally, 73 families were investigated with both multi-locus probes and the results could be divided into two groups:

(1) 39 families with an exclusion using conventional systems. These families were investigated either using 1 probe only or using both probes in combination. With each multi-locus probe additional multiple exclusions could be found (i.e. exclusions with more than 2 bands in 1 system).

(2) 34 families where no exclusion was found using conventional systems. The probability of paternity using traditional systems was >99% in all cases. No exclusions were found by the additional investigations with the multi-locus probes and also no "single exclusions" could be found as indication of new mutations (Table 2).

Single-locus systems

The band patterns revealed by rehybridisation were also clearly visible after the last rehybridisation and could easily be evaluated (Fig. 2). The measurement error was found to have a standard deviation of 2.3%. In the population studies performed different distributions could be demonstrated for each polymorphism (Fig. 3): Indications of a uni-modular type of distribution (MS 31), the presence of 3 cluster regions (MS 8), exceptionally frequent fragments lying close together in the lower kb region (MS 1) and a more irregular distribution (MS 43). The smoothed frequency curves taken from the histograms showed good agreement in some areas when compared to the English survey but deviations in others (Fig. 4): General agreement was observed with MS 43, MS 31 and MS 1. Partial differences were found with MS 43 and MS 1 but only in 1 region of the histogram (Fig. 4).

With MS 8 a definite similarity in the overall profile was observed so that no indications of displacement between both populations (Fig. 4) were found. In the English survey however, 2 definite peaks were found with maximum frequencies of 15 and 24%, whereas in the Münster survey 3 or 4 peaks could be recognized with maximum frequencies between 8 and 10% As the position of 2 of the peaks was very similar, the differences would not seem to be due an inbuilt measurement error but more to a variation between the populations. The frequency of the heterozygosity also showed a corresponding deviation between the 2 surveys (Table 3).

In family analyses (Table 4) only 1 isolated exclusion could be found with MS 1 in spite of otherwise "matching" results in all other DNA systems and in conventional systems (Essen-Möller >99.9%). This exclusion was attributed to a new mutation. The new mutation rate in this system should be approximately 5% (Jeffreys et al. 1988).

Discussion

The band sharing rate and the number of bands calculated from our investigations lead to discrimination indices by which proof of individuality is basically possible (Jeffreys et al. 1985b) but the accuracy of this calculation using the formula given by Jeffreys is debatable because of unknown linkage ratios. In our opinion the order of magnitude would show no substantial change even when it is taken into consideration that some linkage would lead to

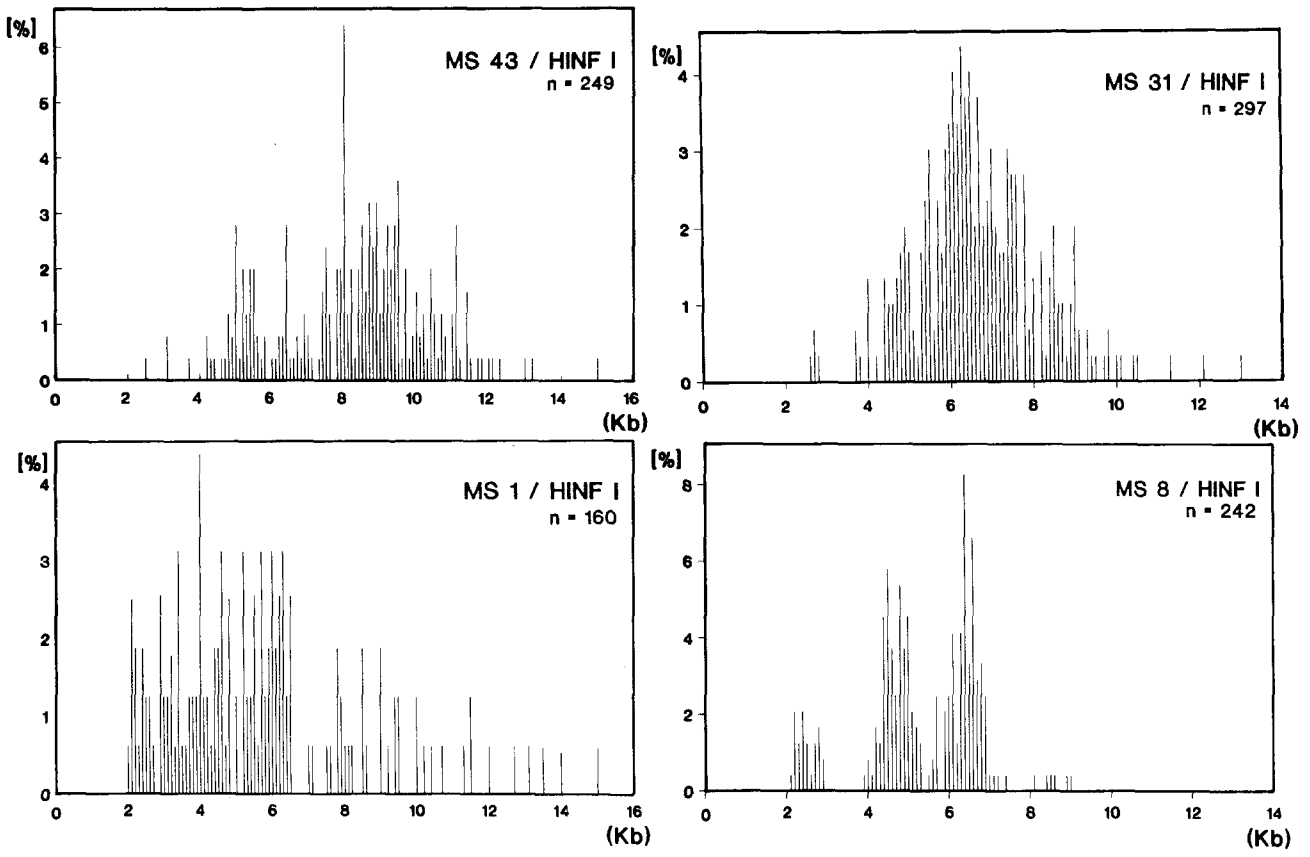

Fig. 3. Frequency of the measured fragment sizes (>2 kb) on the polymorphism MS 1, MS 8, MS 31, MS 43; divisions are to the nearest 0.1 kb

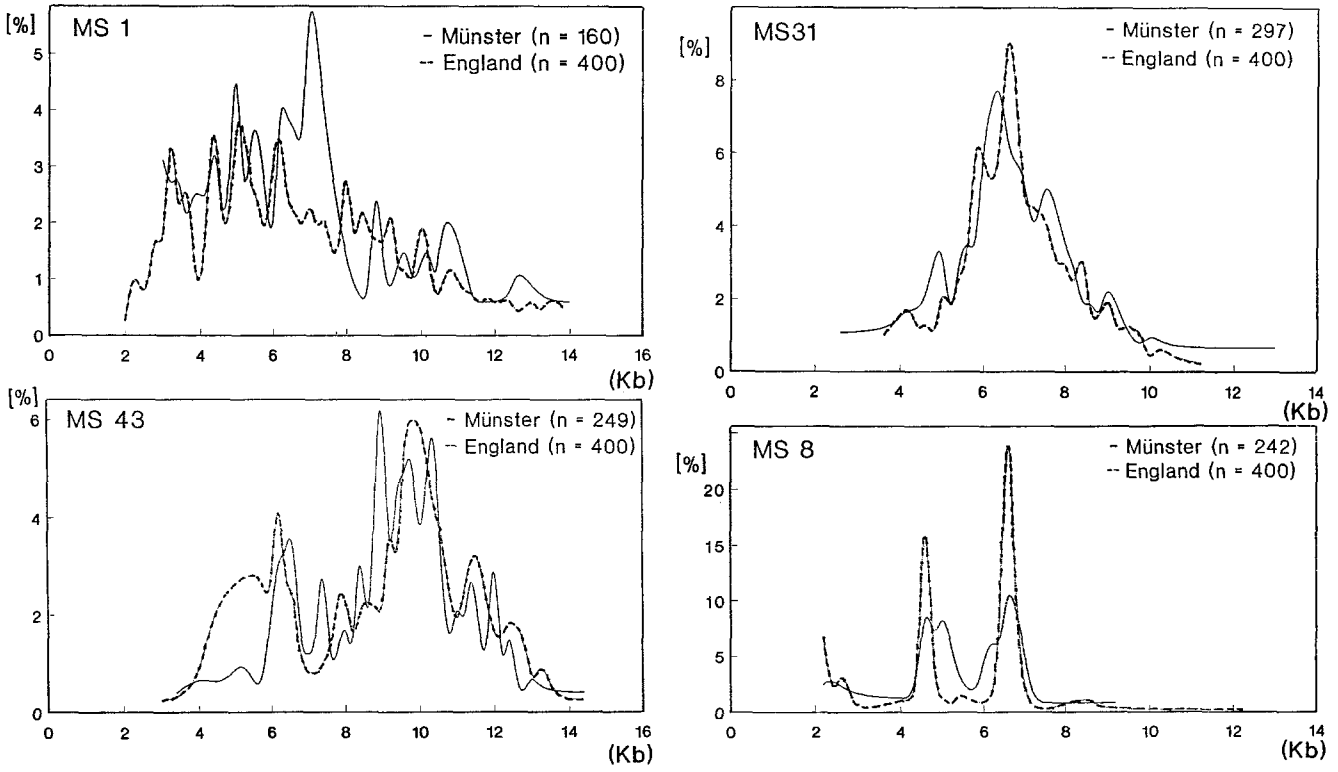

Fig. 4. Comparison of the frequency curves of surveys from Münster and from England (Smith et al. 1990b)

Table 3. Comparison of the heterozygosity rates in this study and an English survey (Smith et al. 1990b)

	Heterozygosity	
	London	Münster
System		
MS 1	99%	99%
MS 8	86%	92%
MS 31	98%	95%
MS 43	97%	96%

Table 4. Comparison of the mutation rates between an English survery and one from the Münster area (n = number of offspring)

	Mutation rate			
	London[a]	(n)	Münster	(n)
System				
MS 1	0.052	(344)	0.026	(38)
MS 8	0	(344)	0	(58)
MS 31	0.007	(344)	0	(60)
MS 43	0	(344)	0	(58)

[a] Jeffreys et al. 1988

Table 5. Discrimination indices calculated for 4 single locus systems[a]

	Disc. index
System	
MS 1	0.0003
MS 8	0.0123
MS 31	0.0041
MS 43	0.0036
	4×10^{-11}

[a] Method of Wong et al. (1987)

other values. In our opinion the domain of the multi-locus probes lies more in stain analysis, at least when a side-to-side comparison is possible.

Special attention must of course be paid to the problem of shifting of band patterns and of comparison of bands in stains. This is especially the case when only a few bands are found in a stain and these bands correspond of those a possible suspect.

The use of highly polymorphic single-locus systems leads to comparably high discrimination indices (Table 5). Because there is at present no known linkage between the systems a combined frequency can be estimated (Wong et al. 1987). When a side-to-side comparison with stains is not possible the fragment size can be estimated and when the size of the measurement error is taken into consideration a comparison with other results is still possible. If the frequency of the single fragments is known then the frequency of the corresponding haplotype combinations can be calculated. However due to the measurement error of approximately 2.3%, the measured values of neighbouring fragments will overlap each other. At present no method of certain discrimination exists, even though a variety of methods have already been published (Baird et al. 1986; Gjertson et al. 1988; Morris et al. 1990). Only by the use of an extremely conservative approach, such as assuming a frequency of 10% for each fragment, which must be greater than the highest observed frequencies, can a total frequency be calculated which would not lead to discrimination against the accused. This obviously would not be valid for the English figures for MS 8 where frequencies up to 24% occur.

The differences between this survey and the English one make it evidently necessary that each laboratory must carry out frequency analyses in the relevant catchment area and using a defined method.

In our opinion the 4 single-locus systems investigated are highly efficient tools in forensic haemogenetics for application to paternity testing and to stain cases. The validation of the genetic model, with verification of the Hardy-Weinberg equilibrium has not yet been completely fulfilled and is at present not possible because of the aforementioned reasons. Therefore the evidental value in paternity testing is still under discussion. In general, the following points must be taken into consideration: When frequencies are estimated it is necessary to know from which population the figures must be taken to avoid problems of differences between populations and genetic drift (Lander 1989b). Population studies must also be made on adequately large sample sizes (>500) where the individuals are randomly selected and in Hardy-Weinberg equilibrium.

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