Routine Use of a Single Locus DNA Probe

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INTRODUCTION

This report evaluates the results of using a single locus DNA probe on routine parentage testing cases in addition to a battery of tests expected to exclude at least 95% of falsely accused men.

METHODS

Samples from 1189 trios were tested for markers in the following systems: ABO, Rh, MNSs, Kell, Duffy, ACP, PGM1, GC, BF, F13B, PLG, and TF. DNA was extracted from each sample following the procedure described by Miller (1988). A standardized quantity of DNA was restricted with Pst I. Samples from each member of the trio and a mixture of DNA from the alleged father and child were run in adjacent lanes on the same gel. After electrophoresis and transblotting samples were probed with pS194 (locus: D7S104) obtained from Collaborative Research (Bedford, MA). The detection method (Dykes 1988) utilized a nonisotopic label. The position of each band was determined from a digitised video picture using an image analysis routine developed inhouse. Calculation of the paternity index (PI) was based on population frequency values that include bands located within +/- 1% of the measured location of the bands detected.

RESULTS

Table one shows that 30.9% of the tested trios were excluded by combining the single locus DNA probe with the other test systems. Of trios excluded, approximately 30% had an exclusion in one or more of the other systems but were not excluded by pS194. In 19 of these 107 cases a single exclusion was observed [13 direct, 2 indirect (JK, PLG), and 4 (3 PLG , 1 F13B) with a very high residual index and a probable null allele]. In 13 trios (5.0% of cases with a DNA exclusion) pS194 provided the only evidence of non-paternity. In 61 trios (23.5% of cases with a DNA exclusion) one other system in addition to pS194 provided evidence of non paternity. In 14 of these trios (23%) the other exclusion was indirect (4 MNSs, 3 GC, 2 PGM1, 1 Kell, 1 PLG, 1 F13B) and would have required additional tests or new samples to confirm non-paternity.

Combining the results of pS194 with the other test systems increased the PI to greater than 100 in 324 (77.5%) of 418 cases where it fell below 100 when only the results of red cell antigen, red cell enzyme and serum protein systems were used for the calculation (Table Two). In 60 additional cases the PI was increased to a value between 50 & 100. Thus of the 822 trios without an exclusion, a W $\geq$ 98% (prior probability = 0.5) was obtained in 95.9% of the cases.

DISCUSSION

The 1189 parentage cases reported here illustrate the usefulness of a single locus DNA probe as part of the battery of tests used in resolving disputed paternity. The addition of an RFLP marker system (pS194) with an expected exclusion probability of greater than 0.8, made it possible for us to reduce the number of systems tested, while increasing the cumulative probability of exclusion (CPE) of our routine test battery. The RFLP system did not

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Edited by H. F. Polesky and W. R. Mayr

give evidence of exclusion in as many of the non fathers (cases with an exclusion in another system) as expected; however, pS194 did provide the only evidence of non paternity in 3.5% of the exclusion cases and confirmed a single exclusion with another system in 16.6% of these cases. The finding of an exclusion with the DNA system significantly reduced the number of cases in which we would have requested additional samples for further testing.

The addition of a highly polymorphic DNA RFLP system to a group of less polymorphic systems, as expected, increased the PI. In 88.6% of the cases without an exclusion the value reported was over 100. Though this increase in observed index values is probably not significant in determining paternity, it is helpful in obtaining parentage estimates that are mandated by statutory requirements of several US states. The higher PI values found by using pS194 also decreased the number of cases in which we would have tested additional systems or requested additional samples.

Several problems have been encountered in routinely using a DNA marker system. In some trios it has been difficult to recover adequate amounts of DNA from one or more of the individuals to be tested. Usually this has been related to the age or amount of sample available. On several occasions reagent related problems have occurred, most frequently this has been failure of the commercially obtained restriction enzyme to perform as expected. Careful attention to technical details including handling of gels, temperature of the stringency wash, and preparation of reagents is essential.

SUMMARY

Routine use of a single locus DNA probe enhances the results found with other marker systems. As additional probes are used or combined to resolve cases of disputed parentage, it is important that the outcome of the testing be validated by comparison with results obtained by well established marker systems.

Table One

Results in 1189 Cases Tested with pS194 and other Systems

	Number of Trios	% of Total	% of Excl Trios
Total with an exclusion	**367**	**30.9**	**---**
Excl by multiple systems	**335**	**28.2**	**91.3**
Excl by pS194	**260**	**21.8**	**70.7**
Excl by pS194 & one other system	**61**	**5.1**	**16.6**
Excl only by pS194	**13**	**1.1**	**3.5**
No exclusion observed	**822**	**69.1**	**---**

Table Two

Effect of DNA Testing on the Paternity Index (PI)

	Observed Value			
	Excluding DNA		Including DNA	
Index	No. of Cases	%	No. of Cases	%
< 100	418	51	94*	11.4
> 100	404	49	728	88.6

* In 34 cases the PI < 50.

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