Application of conventional polymorphisms and single locus DNA probes in cases of disputed paternity

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A series of 151 cases of disputed paternity have been investigated using up to 5 single-locus DNA probes and also a battery of 15-20 systems of red cell antigen, red cell enzyme, serum protein polymorphisms and also, but rarely, HLA. The DNA probes used were Muc 7, MR24/1, 3'α-HVR (Amersham Int), TBQ7 and YNH24 (Promega Corp).

We have assessed the combined use of both conventional and DNA testing in this context. Also the cases have been used to determine the power of exclusion of wrongly named men by the construction of false trios.

METHODS

3μg samples of Alu1-digested DNA from whole blood samples were electrophoresed, blotted and hybridised using the probes named above. Fragment sizes were estimated by manual measurement of migration distances using the local form of the reciprocal relationship $c=(m-m_o)(L-L_o)$ as described by Elder and Southern (1987). The DNA size markers used were a 14-rung ^{35}S-labelled ladder. (Amersham Int). A named man is considered a possible father if he has a band within +/-2.5% of the paternal band in the child, when the samples have been run on the same gel. The frequency of occurrence of the paternal band in the child was estimated from databases compiled in this laboratory using a window of +/-5% of the paternally contributed band size, summing the number of bands in this range. Databases are of 150-250 European caucasians.

RESULTS

114 of the 151 cases gave no exclusions on conventional or DNA testing, using up to 5 probes. Clear exclusions were found using conventional testing in 25 cases, and all of these were detected with two or more DNA probes.

In 12 cases the conventional testing did not allow a clear conclusion to be made but DNA testing was useful (see Table 1).

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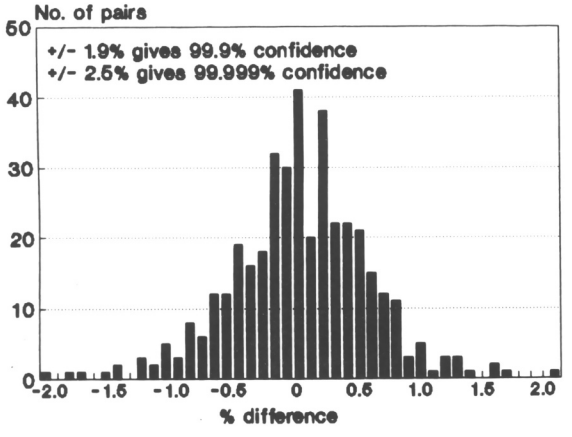

Fig. 1. Frequency distribution of % size difference between 394 mother-child pairs showing the within-gel variation experienced

To assess the variation seen when samples are run on different gels, two control samples were electrophoresed on 30 gels carrying case samples. Figure 2 shows the variation for each of the five probes used. The larger variation seen with 3'α-HVR and TBQ7 is due mainly to the very small fragments seen here. We currently use a window of +/-5% when using databases or matching samples from different gels, but it is likely that this window will be altered to reflect the variation seen over a particular fragment size range.

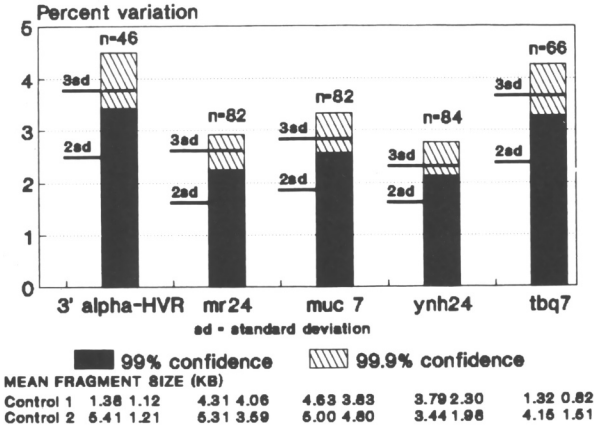

Fig. 2. Bar chart showing the amount of between-gel variation for each probe

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It is important on some occasions to be able to compare samples that have been processed and measured in different laboratories using different techniques. The data was examined initially for outliers by plotting mean fragment size against difference in fragment size. Five outliers were identified, all relating to measurement made by image analysis. The discrepancies were due to wrongly identified bands, poorly resolved bands and problems associated with sizing when there are not sufficient ladder rungs. Obviously assiduous checking of results, whatever method of measurement is used, is essential to avoid errors. Also, a large between-laboratory difference, in the sizes of the two very large bands detected, was related to the different resolution in the large fragment region of the gels run in the separate laboratories. Therefore it would also seem that rigorous standardisation is important if there is going to be a meaningful exchange of results or sharing of databases.

Figure 3 shows that, when results from the two laboratories were compared, there was minimal variation (around +/-1.5%) in measurements produced by manual (ruler) and automatic (image analysis) methods of measurement and both performed similarly. Figure 4 shows that, using this set of probes, up to a +/-5% variation can occur in fragment sizes obtained from tests conducted in two different laboratories.

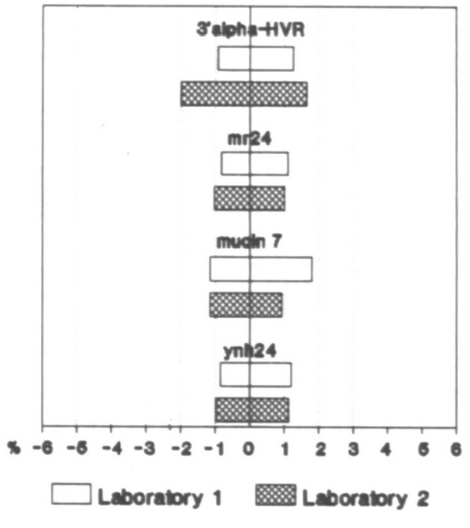

Fig. 3. 99% confidence intervals for the between measurement method % differences

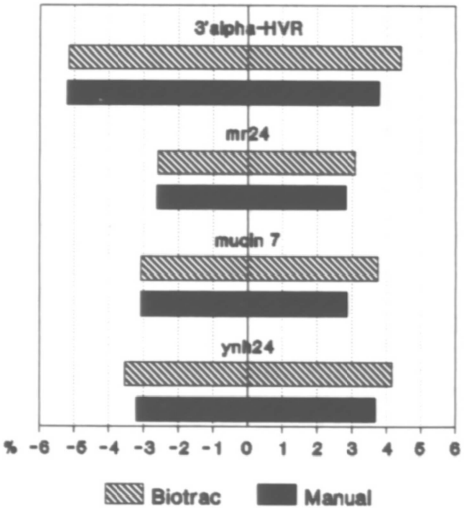

Fig. 4. 99% confidence intervals for the between laboratory autoradiograph % differences

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