QUALITY CONTROL:
INTERLABORATORY COMPARISON OF RFLP RESULTS.

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INTRODUCTION

Interlaboratory quality control programmes for RFLP analysis have been established by some European forensic laboratories. Two such programmes (EDNAP 1-2) and 3 German programmes were published [1-3]. The evaluation of proficiency testing represents an important part of such programmes, and it is the aim of this report to draw attention to weaknesses in the methods of comparison applied and to suggest a better approach.

METHODS AND DATA

Data from the second EDNAP exercise were used as published in appendices to the report [2]. The data comprise analyses from 12 laboratories of 4 different samples of DNA using 2 probes, YNH24 and MS43a. Each sample was supplied as a bulk restricted (*Hin*fI) sample and as an unrestricted sample. There was no significant difference between these two series of determinations which consequently were combined in this study.

It should be well known that the measurement error in terms of basepairs (bp) and also in terms of bp% increases with increasing fragment length. To circumvent this obvious inconvenience we have transformed all fragment lengths into normalized migration distance. After transformation the measurement errors were normally distributed and independent of the fragment length [4].

In the second EDNAP exercise the results of the different laboratories were compared by a special test for match. For each sample and for each probe all possible combinations of bandpairs were tested for match using windows ranging from 2 to 8 bp%. A few determinations of high molecular weight fragments from one of the laboratories were not accepted by an 8 bp% window, and all the results from this laboratory were omitted from the statistical treatment in the report. In the present study all results are included.

RESULTS AND DISCUSSION

The fragment lengths varied from 1,935 to 14,018 bp. The coefficient of variance in terms of basepairs increased with increasing fragment length (range: 0.47-2.85) and it does not make sense to calculate an average. After transformation the standard deviation (SD) in terms of mm was independent of the fragment length. The SD ranged from 0.33 to 0.68 mm and the average was 0.49 mm. In our laboratory the SD for determinations on different plates is 0.5 mm.

The match plot used in the EDNAP report is identical with the cumulative frequency distribution of the absolute differences between all possible combinations of fragment lengths. With a normal distribution of the differences this means that 99.7 % of the differences will be less than 3 SD, or that 99.7 % match will be expected with a window of 3 SD.

When the differences were measured in terms of bp% - as in the EDNAP report - the SD and the window width increase with increasing fragment length. For the 14 kb fragment the 99.7 % window will be approximately 12 bp%, for the 9 - 10 kb fragments it will be 6 - 8 bp% and for smaller fragments it will be approximately 2.5 bp%. This was confirmed by testing 4 fragments of different size (Fig. 1A). The width of the window increased with increasing fragment length which strongly indicates that the test is inadequate. In the German study [3] the interlaboratory comparison was performed on the basis of SD values which increased with increasing fragment length.

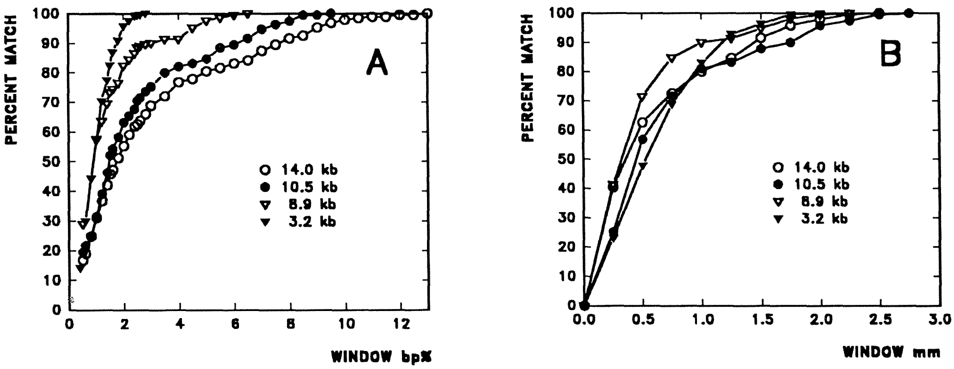

FIG. 1. MATCH TEST WITH 4 FRAGMENTS OF DIFFERENT LENGTH
Fragment lengths: 3.2 - 14 kb.
A. Window width in bp%. B. Window width in mm

When the differences were measured in terms of normalized migration distance they were normally distributed with an SD of 0.7 mm (0.5 x√2) and a 99.7% window of 2.1 mm. This was confirmed by a test performed with all determinations (number of comparisons: 3184). When the analysis was performed on single fragments, *e.g.* those analysed in Fig. 1A, minor deviations were seen (Fig. 1B). The maximum deviation was 2.6 mm.

In the EDNAP report the test involved both bands of a bandpair. This, however, is of no significance because the smaller fragment always will be accepted by the window size determined by the larger fragment. It would be more relevant to investigate whether the deviations were correlated. To this purpose the data from all 4-band profiles obtained with the two probes were analysed as described previously [5]. For 53 % of the comparisons the deviations were insignificant, *i.e.* their coordinates fell within the central part of the accept area which accepts measurement errors of ± 1 mm (±1.4 SD). For the remaining profiles the coordinates for both probes fell either within the upper accept area (31 %) or within the lower (16 %) accept area (Fig. 2). This means that all deviations were correlated which is in agreement with the finding of Evett *et al.* [6].

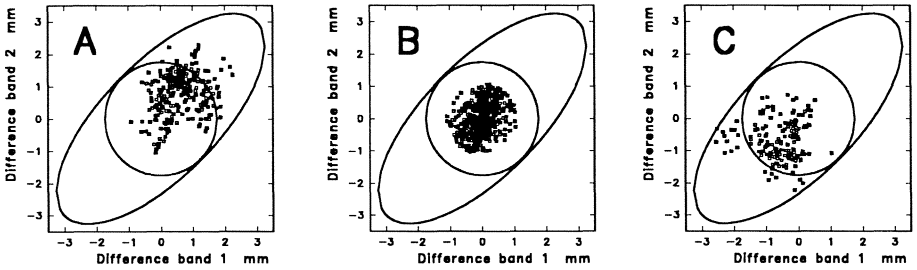

FIG. 2. CORRELATION OF TRANSFORMED DEVIATIONS.
For each set of determinations of 3 samples with MS43a and YNH24 all size differences were calculated. The difference between the HMW bands was plotted against the difference between the LMW bands. A: positive differences. B: insignificant differences. C: negative differences. SD = 0.7 and ρ = 0.7.

CONCLUSION

Interlaboratory comparisons cannot be performed on the basis of the coefficient of variation in terms of basepairs (bp%). Deviations will be underestimated for small fragments and overestimated for larger fragments. However, an 'accept-corridor' [3] based on fragment length dependent SD-values can be used. We suggest the use of transformed [4] fragment lengths, or of normalized migration distances corrected for transversal skewness. The data should be tested for correlation.

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