A REVIEW OF THE 1991-1994 PATERNITY TESTING WORKSHOPS OF THE ENGLISH SPEAKING WORKING GROUP

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INTRODUCTION

The ability of any laboratory offering paternity tests to correctly assign relationship is an essential attribute that needs to be tested by a quality control programme. Such a programme could involve regular internal laboratory testing of blinded samples and involvement in an external testing programme. Since 1991 members of the English Speaking Working Group (ESWG) of the International Society for Forensic Haemogenetics (ISFH) have organised and participated in an annual Paternity Testing Workshop that fulfils the latter requirement in part. The exercise was not intended, however, to be concerned only with proficiency, but also to provide a forum for the discussion of paternity related topics, whether it be current and developing methodology, anomalous results or measurement variation, at the annual ESWG meeting. On behalf of the ESWG we present below a report of the last four workshops.

METHODS

Blood samples from a 'family' were sent to participating laboratories in the Spring of each year, from 1991 to 1994, with the request that they perform a paternity investigation and make a report in their usual manner. In addition laboratories were asked to complete a questionnaire to provide more detailed information on their current routine methodology and what additional testing might be carried out if a laboratory found it difficult to resolve the case. Nine laboratories were approached for the initial workshop in 1991; since then ten other laboratories who came to the first paternity workshop forum, or who had heard about the workshop, have asked to participate. Although samples were not presented blind, only the two named authors were aware of the true family relationships and did not participate in the testing process.

Workshop Families

1991: Two possible fathers, mother and child. One of the possible fathers was a sibling of the other but information about this relationship was not provided to the testers.

1992: Two possible fathers and child. One of the possible fathers was a sibling of the mother, who had not been presented for testing, but again this information was withheld from the testers.

1993: One possible father, mother and child. The possible father was a sibling of the true father (information not provided to testers).

1994: One possible father, mother and child.

RESULTS

The number of participating laboratories has increased from 9 (1991) to 13 (1992), 16 (1993) and 19(1994). All laboratories performed some form of DNA testing, with around 75% using DNA single locus probes (SLP). About 40% of laboratories reporting SLPs have continued to report other conventional test systems (red cell and white cell antigens, red cell enzymes and serum protein polymorphisms). Since 1993 the small number of laboratories using multi-locus probe (MLP) testing as their main testing process have also used other test systems as 'back-up', principally SLPs. YNH24, MS43a, MS31 and G3 have remained the most popular probes in use throughout, followed by MS1, which has decreased in popularity, and MS205 which has increased in popularity. Eight other probes have been used routinely, with pMLJ14, MS8 and TBQ7 being the most popular. Chemiluminescent labelled probes have increased in popularity. In 1991 only radioactively labelled probes were used but in 1992 over 50% of laboratories were using chemiluminescent systems and that increased significantly to around 80% in 1993 and 85% in 1994. Hinf I was the restriction enzyme favoured by around 70% in 1991, increasing to 80% in 1994. Others use Alu I or Hae III. Since 1992 there has been an increase in the number of laboratories developing PCR based systems (under 10% in 1992 to over 40% in 1994), with short tandem repeat (STR) systems gaining in popularity over other PCR based systems. No laboratories were reporting PCR systems as part of their routine paternity investigation up to 1994.

Four 'families' have been tested and reported, all without conflicting conclusions. The 1991 case, in which the two possible fathers presented were brothers, proved difficult to resolve with one laboratory only obtaining a single exclusion after seven SLP probes and no laboratory found exclusions on more than two SLP test systems. About 80% of the laboratories also suggested that the two men were related. Since 1991 all laboratories who provided a report came to the same conclusion about the paternity of the men tested. Over 80% of laboratories, in writing their report, provide the raw results on which their conclusion is based; the remainder provide either their conclusion alone, or indicate fragment size rank only.

The variation between laboratories has been examined with time, comparing fragment sizes provided by laboratories using the same restriction enzyme and the four most popular probes (YNH24, MS43a, MS31 and G3). Figure 1 presents the range of coefficient of variation results for each fragment reported. There is a highly significant inverse correlation between the year of testing and coefficient of variation (Spearman's Rank Order correlation = -0.65, $p<0.000001$), with the decrease in coefficient of variation being highly significant between 1992 and 1993 (Scheffé's Post-Hoc Test, $p<0.001$). The maximum coefficient of variation fell from around 7% in 1991 to under 2% in 1994 when we observed a percentage difference in fragment size measurements, between laboratories, of 1.6% to 5.0%, dependent on fragment size. The measurements from one laboratory providing results in 1994 were excluded from this analysis since the fragment sizes given were highly discrepant (about 1-2 kb) away from the consensus for the majority of the test systems used.

DISCUSSION

This review of the activities of the ESWG in paternity testing illustrates the continuing popularity of DNA SLP testing, with conventional systems frequently being used in addition. MLP testing seems no longer to be performed in isolation and frequently SLP tests are used to support the MLP interpretation. Four probes (YNH24, MS43a, MS31 and G3) have maintained their popularity. MS1 usage has decreased, probably because of the high rate of mutations found, but use of MS205, a newer probe, is becoming more common. More laboratories are experimenting with PCR based systems and it is

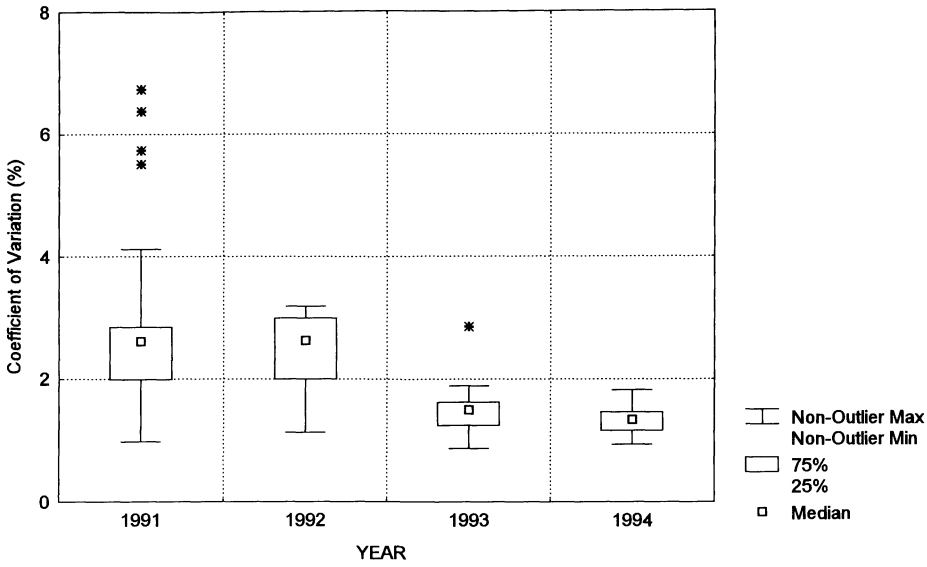

Figure 1: Box and whisker plot showing median, interquartile range, non-outlier range and outliers (*) of measures of coefficient of variation, between laboratories, for each fragment measured in any particular test year.

possible that we will see such systems being used routinely in the future. The general decreased variability in fragment size measurement seen between laboratories over the four years is likely to be due principally to the increased use of chemiluminescent techniques, providing much clearer band patterns and enabling more precise measurements to be made, but increased experience may also be important. Although one family proved particularly difficult to resolve, consensus concerning the conclusion was achieved in all cases that were reported and the meetings have proved a useful forum for discussion of paternity related matters, particularly in relation to the methodologies being applied. Comparison of different laboratory's results, whether it be DNA SLP fragment sizes or other genetic markers, can provide independent quality criteria for laboratories. Laboratory identity is kept anonymous to encourage participation in the exercises. Participation in external quality tests is also an important feature of many accreditation schemes and The English Speaking Working Group Paternity Workshop has proven itself both useful in this aspect and inexpensive to run.

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