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A report of the 1995 and 1996 Paternity Testing Workshops of the English Speaking Working Group of the International Society for Forensic Haemogenetics

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Abstract

We report the results of the 1995 and 1996 Paternity Testing Workshops of the English Speaking Working Group of the International Society for Forensic Haemogenetics. In 1995, 18 laboratories participated and in 1996, 21 laboratories participated. Each year, blood samples from three persons (child, mother and alleged father) were sent to participating laboratories which performed paternity testing according to their usual protocols. The results and answers to questionnaires concerning methods were compiled and are presented in this report. From the questionnaires, a general tendency to a more frequent use of polymerase chain reaction (PCR) based methods was seen. In 1996, 62% of the laboratories used PCR based methods. Ten per cent of the laboratories used only PCR based methods. The remaining 90% of the laboratories performed restriction fragment length polymorphism (RFLP) investigations of variable numbers of tandem repeat (VNTR) loci with single locus probes (SLPs) either alone or in combination with PCR based typing, multi locus probing, classical systems (ABO etc.), or serological HLA typing. In 1996, typing with classical systems was used in 29% of the laboratories. The majority of the laboratories performed RFLP typing of VNTR loci using very similar methods. The results and the

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inter-laboratory variations of the measured lengths of the DNA-fragments of the VNTR regions D2S44, D7S21, D7S22, and D12S11 of the trios were analysed. The overall coefficient of variation was 2.15% in 1995 and 1.43% in 1996. During the period 1991–1996, the inter-laboratory variation has decreased, most probably due to the fact that the methods have now been optimised and the majority of the participating laboratories have adopted the standardised method for RFLP typing with SLPs which was agreed upon for investigations in crime cases by the European DNA Profiling Group. In 1996, eight laboratories reported the results of PCR based typing of the short tandem repeat (STR) locus HumTH01, six laboratories reported results of HumVWA31A typing, and five laboratories reported the results of typing of the STR locus HumF13A1 and the VNTR locus D1S80. The results were concordant although the nomenclature was slightly inconsistent concerning the classification of an irregular repeat of the HumTH01 system.

Keywords: Paternity testing; Single locus probes; DNA profiling; Inter-laboratory comparisons; DNA; RFLP; VNTR; STR; PCR

1. Introduction

Since 1991, the English Speaking Working Group (ESWG) of the International Society for Forensic Haemogenetics (ISFH) has, once a year, offered its members an exercise concerning genetic analysis in paternity testing [1]. The exercises are performed in order to enable the laboratories undertaking paternity testing to compare their strategies and results to those of other paternity testing laboratories. Such inter-laboratory comparisons are essential for modern quality controlled laboratories. The exercises are made simple by sending blood samples from a paternity trio (mother, child and alleged father) to the laboratories, which are then asked to perform genetic investigations according to their usual protocols. Although a number of different genetic tests are used, the final conclusion concerning paternity may be compared between the laboratories.

Analysis of variable number of tandem repeat (VNTR) DNA regions with the restriction fragment length polymorphism (RFLP) technique and single locus probes (SLPs) is performed in almost all paternity testing laboratories. The great majority of the participating laboratories perform these investigations according to the standardised methods of the European DNA Profiling (EDNAP) Group, which is a working group under the ISFH collaborating on the exploration of new methods for DNA investigations in crime cases [2,3]. Direct comparisons between the laboratories of the results of the measurements of the sizes of the DNA-fragments have been undertaken.

Previous analyses of the variation of the measurements of the lengths of DNA-fragments in RFLP investigations of VNTR regions showed that, in the period from 1991 to 1994, the variation between the results obtained in the participating laboratories was reduced and, in 1994 the coefficient of variation of the results was below 2% [1].

On behalf of the ESWG, we present a summary of the measurement data as well as the information concerning strategies and methods of the participating laboratories (see Appendix A) which was collected from the exercises in 1995 and 1996.

2. Material and methods

Blood samples (mother, child and alleged father) and questionnaires were sent to the participating laboratories from either The Department of Haematology, St. Bartholomew's and the Royal London School of Medicine and Dentistry, London, (1995) or from The Department of Forensic Genetics, Copenhagen, (1996). In both exercises the alleged father was considered to be the biological father of the child. The participating laboratories are listed in the appendix. In 1995, 18 laboratories participated in the exercise and, in 1996, 21 laboratories participated. The participating laboratories were asked to perform the paternity testing according to their usual strategies and methods, and to make a report in their usual manner. The laboratories listed the test systems which they had available for paternity investigation. Not all laboratories will use every system in each case, but they have them available for use where necessary. In the present exercises, however, many of the laboratories reported results of all their available test systems in order to facilitate a more extensive comparison even though some of the test systems are not routinely used by the laboratories. Results were collected and analysed in Copenhagen.

The measurements and the variation of the lengths of the DNA-fragments [kilobase (kb)-values] of the commonly used VNTR loci D2S44, D7S21, D7S22, and D12S11 were analysed. For each VNTR system, a laboratory wise comparison was performed. The data of each VNTR system was analysed for each laboratory as well as for all the laboratories and, for each laboratory, the relative deviation from the mean of the results of all laboratories were calculated in per cent. For each of the VNTR systems, a combined analysis of the variation of the results of all laboratories submitting data was performed and the variation was expressed as the coefficient of variation in per cent, i.e., the standard deviation in per cent.

No data was excluded except that the measurements for D2S44 from one laboratory in 1995 were excluded from the analysis because the fragment sizes reported were highly discrepant from those reported by the remaining laboratories and this was shown to be most probably due to clerical errors.

The data of the workshops were presented and discussed by the members of the ESWG of the ISFH in 1995 (Santiago de Compostela, Spain) and in 1996 (Orlando, USA).

3. Results

3.1. Methods used for genetic investigations

Table 1 shows the methods available for use in genetic investigations in paternity testing. From 1995 to 1996, the use of classical systems decreased from 44% to 29%. In 1995, all laboratories performed SLP-testing, either routinely or additionally. In 1996, 19 of the 21 laboratories performed SLP-testing. Multi locus probes (MLPs) were used by

Table 1
Methods available for use in genetic investigations in paternity testing

Methods	1995 ($N=18$)		1996 ($N=21$)	
	Number[a]	%	Number	%
Conventional systems	8	44	6	29
HLA systems (serology)	4	22	6	29
SLP systems	18	100	19	90
MLP systems	2	11	4	19
PCR based analysis	9	50	13	62
VNTR/STR systems (PCR)	9	50	12	57
PolyMarker (PCR)	2	11	4	19
HLA systems (PCR)	3	17	9	43

[a] Number of laboratories.

four laboratories in 1996. The use of polymerase chain reaction (PCR) based investigations increased from 50% to 62%.

3.2. Strategies of investigations

Table 2 shows an overview of the strategies of the participating laboratories. The laboratories which used MLPs all supplemented the investigations with SLP-testing. In both the 1995 and 1996 exercises, the majority of laboratories undertook testing using all available methods, i.e., classical/serological HLA, SLP/MLP, and PCR based investigations. In 1996, two laboratories used PCR based methods only.

3.3. RFLP typing with SLPs

A total of 14 different VNTR systems were used by the participating laboratories. Table 3 shows the VNTR systems most commonly used for SLP typing. In the 1995 and 1996 exercises, all but one laboratory used the restriction enzyme *Hin*fI. Other restriction enzymes used were *Alu*I and *Hae*III.

Table 4 shows a summary of the methods used for SLP investigations. Just over half of the laboratories used organic solvents for the extraction of DNA. In 1996, one laboratory alone used only radioactively labelled probes. All laboratories supplying

Table 2
Combinations of methods available for use in genetic investigations in paternity testing

Methods	1995 ($N=18$)		1996 ($N=21$)	
	Number	%	Number	%
PCR systems only	0	0	2	10
SLP systems only	3	17	4	19
SLP+MLP systems only	2	11	2	10
SLP and/or MLP+PCR systems	4	22	5	24
Classical and/or serol. HLA+SLP and/or MLP	4	22	2	10
Classical and/or serol. HLA+SLP and/or MLP+PCR	5	28	6	29

Table 3
The most frequently used VNTR systems for SLP typing

VNTR systems	Probe	1995 ($N=18$)		1996 ($N=21$[a])	
		Number	%	Number	%
D2S44	YNH24	18	100	18	86
D12S11	MS43a	17	94	17	81
D7S21	MS31	17	94	17	81
D7S22	g3	13	72	14	67
D16S309	MS205	13	72	12	57
D5S110	MS621	9	50	10	48
D1S7	MS1	9	50	7	33
D5S43	MS8	6	33	6	29
D4S139	pH30	5	28	4	19

[a] RFLP typing with SLPs was not performed in two laboratories.

information used the DNA Analysis Marker System (DAMS) from BRL as reference (ladder) for the estimation of the molecular weight. Approximately $\frac{2}{3}$ of the laboratories used DNA from the commercially available cell line K562 as reference (''positive control''). Others used genomic DNA, usually from a staff member.

Table 4
Methods used for purification of DNA, labelling of probes, and ladder for RFLP analysis

	1995 ($N=18$)		1996 ($N=21$[a])	
	N	%	*N*	%
DNA purification				
Organic	–[b]	–	11	53
Non-organic	–[b]	–	7	33
Unknown	–[b]	–	1	5
No RFLP typing	–[b]	–	2[a]	10
Labelling of probes				
Alkaline phosphatase	7	39	11	52
Chemiluminescence	1	6	1	5
Nice	3	17	2	10
Nice and P^{32}	2	11	4	19
Radioactive	4	22	1	5
No RFLP typing	0	0	2[a]	10
No information	1	6	0	0
Ladder				
DNA Analysis Marker System—DAMS (BRL)	12	67	12	57
DAMS + MW100 probe	5	28	6	29
No RFLP typing	0	0	2[a]	10
No information	1	6	1	4
Positive control				
K562	13	72	14	67
Genomic DNA	5	28	4	19
No RFLP typing	0	0	2[a]	10
No information	0	0	1	4

[a] RFPL typing with SLPs was not performed in two laboratories.
[b] No information.

Table 5
Methods for measurements of RFLP fragments, match criteria, and frequency windows

	1995 ($N=18$)		1996 ($N=21$[a])	
	N	%	*N*	%
Method of sizing				
Video-camera	9	50	12	57
Digitizer	2	11	3	14
Manual	7	39	3	14
No RFLP typing	0	0	2[a]	10
No information	0	0	1	5
Match criteria				
Fixed match criteria	11	61	11	52
Visual	6	33	8	38
No RFLP typing	0	0	2[a]	10
No information	1	6	0	0
Frequency window				
Match criterion = window size	3	17	5	24
Match criterion < window size	15	83	14	67
No RFLP typing	0	0	2[a]	10
Database				
Own	15	83	12	57
Other	2	11	2	10
No RFLP typing	0	0	2[a]	10
No information	1	6	5	23

[a] RFLP typing was not performed in two laboratories.

Table 5 shows the methods for determination of the positions of DNA-fragments on the lumigraphs/autoradiographs. The majority of the laboratories used a video camera based system and, in 1996, only three laboratories used a ruler.

Approximately half of the laboratories used a fixed criterion for the evaluation of match or non-match (Table 5). There was no uniformity concerning the definition of match criteria.

In 1996, the window size for assessing the frequency of matching alleles in the reference database when no exclusion was detected was the same as the match criterion in 24% of the laboratories and was greater in the remainder.

The great majority of the laboratories which supplied information had established own data bases with allele frequencies.

3.4. RFLP typing with MLPs

In 1996, four laboratories used MLP analysis. The restriction enzymes used were *Hin*fI and *Hae*III and the probes were ‘‘33.6’’, ‘‘33.15’’, and ‘‘MZ1.3’’.

3.5. PCR based typing

A total of 32 different PCR based systems were used. Of these, 17 were used in only one of the participating laboratories. Table 6 shows the most frequently used genetic

Table 6
The most frequently used DNA systems for PCR based typing

Systems	1995 ($N=18$)		1996 ($N=21$)	
	Number	%	Number	%
HumTH01	6	33	11	52
HumVWA31A	5	28	9	43
D1S80	5	28	6	29
HumFES/FPS	2	11	6	29
ACTBP2	3	17	5	24
HumF13A1	1	6	5	24

In 1996, 32 PCR based DNA systems were used by the participating laboratories. Only systems used by five or more laboratories are listed.

systems including the VNTR system D1S80 and five short tandem repeat (STR) systems. In 1996, dot blot based PCR methods were used by 43% of the laboratories (Table 1). The technique was used for a number of test systems including investigations of HLA-DRB1, -DQA1, -DQB1, -DPB1, and the PolyMarker system, each of which were used by only a few laboratories. All laboratories using PCR-HLA systems and three of four laboratories using the PolyMarker kit used also PCR-VNTR/STR systems.

3.6. The reports of the investigations, statistics and verbal interpretation of the results

In both 1995 and 1996, all participating laboratories concluded correctly that the man could be the father.

In the 1996 exercise, genetic inconsistency was observed between the child and the man in the ACTBP2 system. In both laboratories which investigated this system, the phenomenon was interpreted as a genetic recombination.

In 1996, 90% of the laboratories reported statistical assessment to evaluate the weight of the evidence. Table 7 shows the paternity indices (PI)- and probability of paternity (W)-values reported.

Although the verbal expressions of the conclusions of the paternity investigations differed between the laboratories, they could be classified into two major groups: approximately half of the laboratories stated that ‘‘the man is not excluded from paternity’’; the statements of the other half of the laboratories could be categorised as ‘‘very strong evidence for paternity’’ (Table 8).

3.7. Inter-laboratory comparisons of results of RFLP typing with SLPs

The estimated kb-values of DNA-fragments submitted for the four most frequently used VNTRs (D2S44, D7S21, D7S22, and D12S11) were compared. Figs. 1–4 show the

Table 7
Values of PI- and W-values

PI-values	W-values	1995 ($N=18$)		1996 ($N=21$)	
		N	%	*N*	%
100–1000	99%–99.9%	0	0	3	14
1000–10 000	99.9%–99.99%	2	11	5	24
10 000–100 000	99.99%–99.999%	8	44	6	29
100 000–1 000 000	99.999%–99.9999%	1	6	3	14
>1 000 000	>99.9999	2	11	2	10
No statistics		5	28	2	10

Table 8
Verbal expression of the conclusion of the investigations

	1995 ($N=18$)		1996 ($N=21$)	
	N	%	*N*	%
''Not excluded''	8	44	9	43
''Very strong evidence''	8	44	9	43
No information available	2	11	3	14

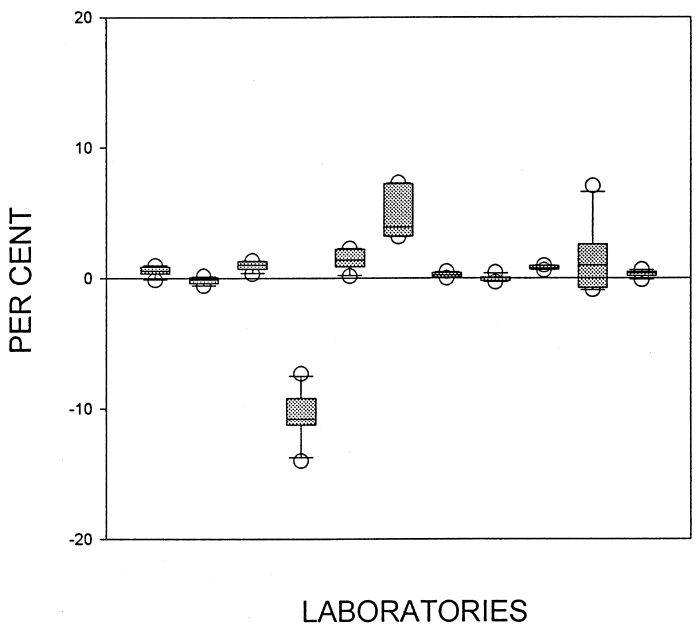

Fig. 1. Inter-laboratory variations of the measurements in 1995 of the lengths of DNA-fragments of the VNTR locus D2S44 (DNA probe: YNH24). Each laboratory submitted data on six D2S44 fragments from the mother, child, and man. The lower and upper boundaries of the box indicate the 25th and 75th percentiles, respectively. Error bars below and above the box indicate the 10th and the 90th percentiles. The middle bar indicates the mean. Open circles indicate values outside the 10th and the 90th percentiles.

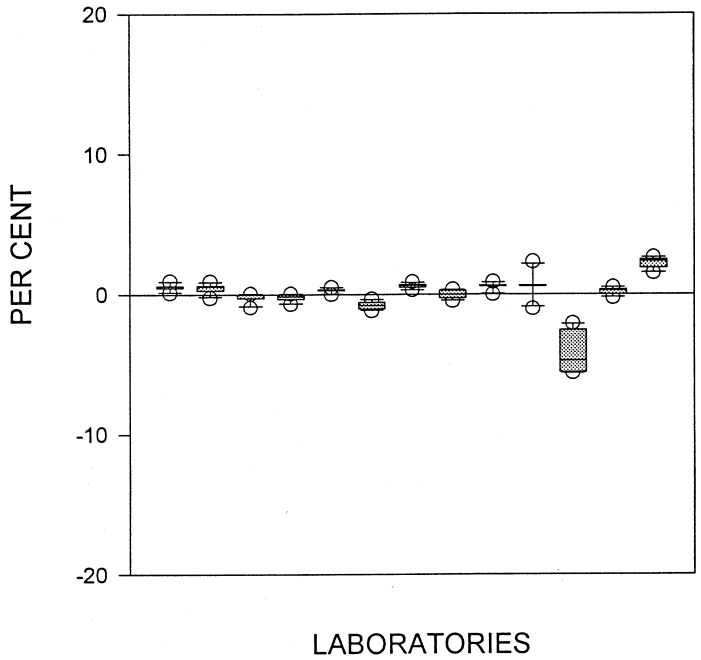

Fig. 2. Inter-laboratory variations of the measurements in 1996 of the lengths of DNA-fragments of the VNTR locus D2S44 (DNA probe: YNH24). For symbols, see legend to Fig. 1.

comparisons of the variations of the results of D2S44 and D12S11 typing in 1995 and 1996. Comparisons of the two other VNTR systems showed similar results although the deviations from the mean kb-values were less than those of the 1995 results of D2S44 typing.

Fig. 5 shows a summary of the variations observed for each of the four most commonly used VNTR systems and the overall variations in 1995 and 1996. A slight decrease in the overall coefficient of variation and in the range of variations between the laboratories was observed from 1995 to 1996.

3.8. Inter-laboratory comparisons of results of PCR based VNTR and STR typing

In 1996, results of the STR system HumTH01 were reported by eight laboratories. The results were concordant although the nomenclature was slightly inconsistent. The HumTH01 system includes a frequent, irregular repeat, TH01 9.3, which was reported as ‘‘9.3’’, ‘‘10’’, or ‘‘9.3/10’’. Six laboratories reported HumVWA31A types, and results of D1S80 and HumF13A1 typing were reported by five laboratories. All these results were concordant. When only one allele was detected in a test system, the types were reported by some laboratories as a phenotype and by others as a genotype, e.g., either D1S80 24 or D1S80 24/24.

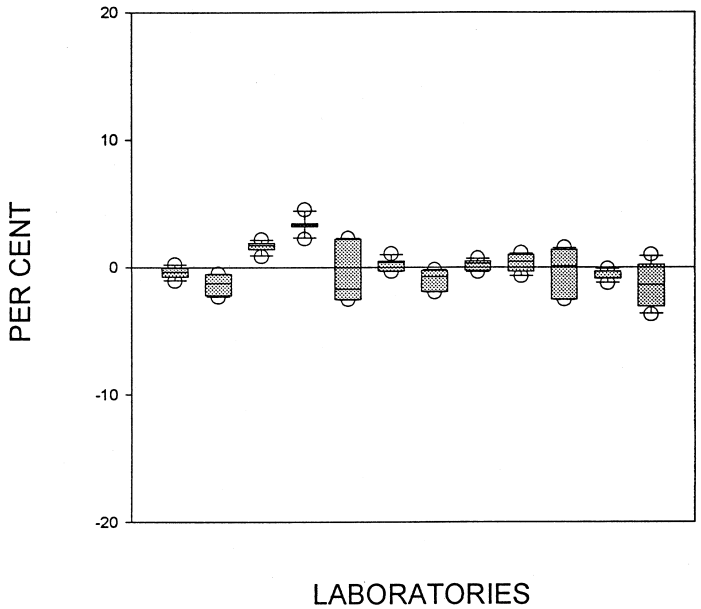

Fig. 3. Inter-laboratory variations of the measurements in 1995 of the lengths of DNA-fragments of the VNTR locus D12S11 (DNA probe: MS43a). For symbols, see legend to Fig. 1.

4. Discussion

Although paternity testing laboratories rarely have the need to exchange results with other laboratories, an interest in comparability exists in such laboratories. In many countries, there is an increasing focus on the quality of the genetic investigations and the demonstration of technical competence and traceability of the results. This is becoming increasingly important for the laboratories which wish to gain accreditation. It is also of great interest for laboratories to be aware of the trends in methods for paternity testing and to have the opportunity to compare results through participation in proficiency testing exercises such as the one offered by the ESWG or by the College of the American Pathologists [4,5].

The answers to the questionnaires showed that the use of classical methods (blood grouping, etc.) has decreased. RFLP typing with SLPs is still used by the great majority of the laboratories. MLP-testing is used by a few laboratories, but they all use other methods (SLPs and PCR) as a supplement. The PCR based methods are becoming popular and are now used by 62% of the laboratories. Methods detecting sequence variation with hybridisation techniques like HLA-DQA1 typing and methods detecting fragment length variations of VNTRs and STRs are commonly used. It is remarkable that, in 1996, 29% of the laboratories still had all the techniques (conventional and/or HLA typing, SLP and/or MLP, and PCR based typing) available for use.

The methods used for RFLP typing have reached considerable uniformity. The VNTR systems D2S44 (probe YNH24), D12S11 (MS43a), D7S21 (MS31), and D7S22 (g3) are

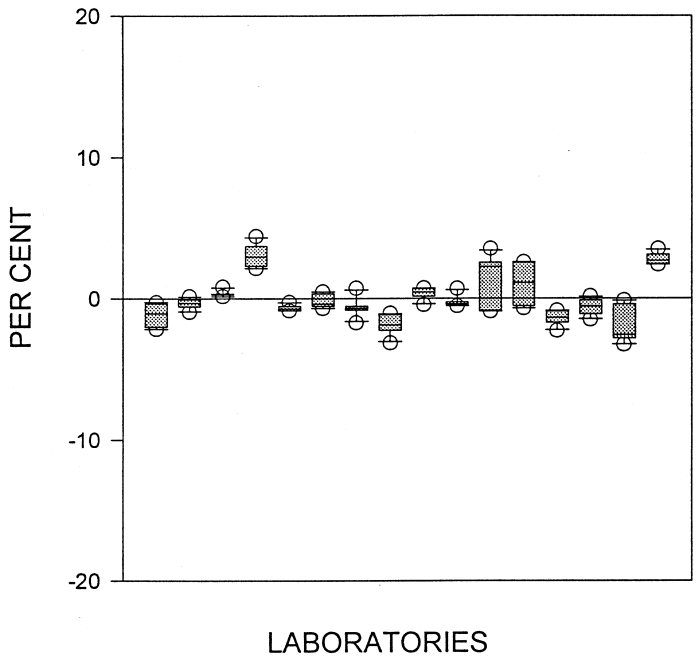

Fig. 4. Inter-laboratory variations of the measurements in 1996 of the lengths of DNA-fragments of the VNTR locus D12S11 (DNA probe: MS43a). For symbols, see legend to Fig. 1.

still very popular and the restriction enzyme *Hin*fI is used routinely by almost all laboratories. Non-organic methods like 6 M saline for extraction of DNA are used by a number of laboratories and may be of interest to others wishing to avoid the biohazards of organic extraction solvents. Labelling of probes with non-radioactive methods is now more common. The non-radioactive methods are without the biohazards of radioactivity and allow a faster development of thinner bands which make a more precise determination of the DNA-fragment lengths possible.

All laboratories supplying information about ladder used the DAMS (BRL), a major factor contributing to the uniformity of the results obtained. The great majority of the laboratories also used a common ''positive control'', i.e., DNA from the cell line K562 which is commercially available. The measurements of the positions of the bands on the lumigraphs/autoradiographs are performed with automated (video-camera) or semi-automatic techniques (digitiser) in the majority of the laboratories. This not only contributes to the ease of the work but also aids precision of the measurements and avoidance of clerical measurement errors.

Complete uniformity does not exist concerning the interpretation of a match and the calculation of the probability of a match in the random population. However, the majority of the laboratories use a fixed match criterion while some laboratories require visual inspection only to determine a match. The frequency of a match among unrelated individuals is calculated based on allele frequencies, determined from a database using a

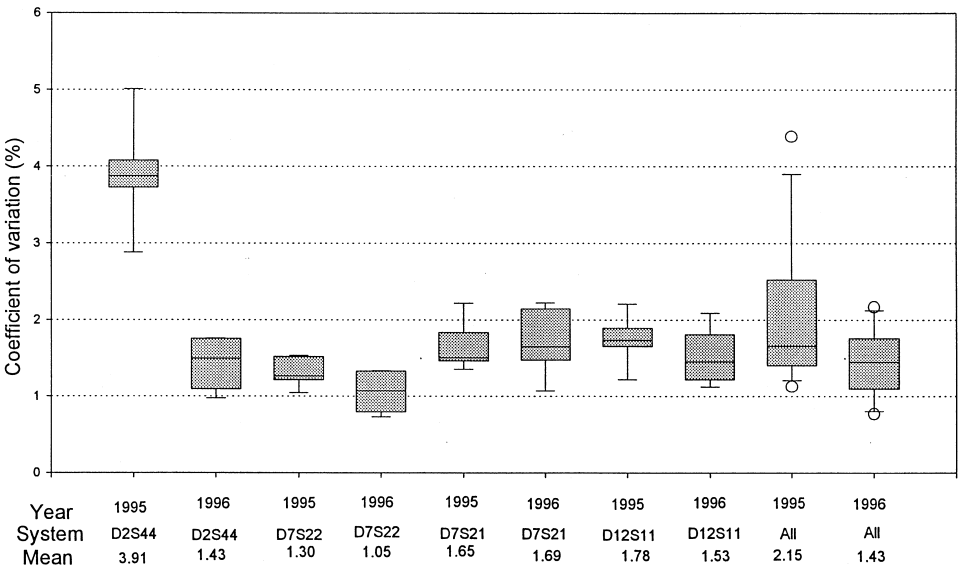

Fig. 5. Inter-laboratory variation of the measurements of the length of DNA-fragments of four VNTR systems and the overall variation in 1995 and 1996. Each laboratory submitted data on six DNA- fragments for each of the VNTR systems D2S44, D7S21, D7S22, and D12S11 from the mother, child, and man. For symbols, see legend to Fig. 1.

window which is equal to the match window in a minority of the laboratories. In the remaining laboratories, a window which is larger than the match window is used in accordance with a general conservative interpretation of the weight of the evidence of the results. It is generally recommended that local databases of allele frequencies are established in the paternity testing laboratories; such databases have been established in the majority of the participating laboratories.

The PCR based methods primarily include methods detecting sequence variations with hybridisation techniques, many of which are commercially available as kits, and methods detecting fragment length variations of VNTRs and STRs. Reagents for some of the VNTR and STR systems are commercially available. The methods for VNTR and STR typing are based on electrophoresis which, in 1996, was performed in polyacrylamide (PAGE) in all participating laboratories. The investigation may be performed using manual or automated techniques. In 1996, seven laboratories used DNA sequencers and all of these used an ABI 310, 373, or 377 DNA sequencer, which allow multi-colour detection of DNA-fragments, and two laboratories used in addition a Pharmacia ALF DNA sequencer. The most commonly used VNTR and STR systems investigated included the VNTR system D1S80 as well as the STR systems HumTH01, HumVWA31A, HumFES/FPS, and HumF13A1, which are commercially available and may be investigated as a quadruplex [6], and the highly polymorphic ACTBP2 system.

The presentation of the conclusion of the investigations varied between the laboratories. However, the great majority presented an estimate of the weight of the evidence, either as the paternity index, PI, or as the probability of paternity, W, under the assumption of an a priori probability of 0.5. PI-values depend on the extent of investigations. The PI-values varied from 135 to above 1 000 000, probably reflecting that difference. The extent of investigation ranged from a limited number (four or five) of VNTR or STR systems to more than 30 genetic markers using all presently available techniques. PI-values in the range 10 000–100 000 were those most frequently reported. The laboratories verbalised their conclusions in different ways; some simply stating that the alleged father was not excluded from paternity, and others stating that the results could be considered very strong evidence for paternity.

Previous assessments in the period 1991 to 1994 of the inter-laboratory variation in estimated DNA-fragment sizes of VNTR systems between paternity testing laboratories showed that the mean coefficient of variation was reduced from approximately 2.6% to approximately 1.3%. In 1995 and 1996, we observed coefficients of variations of 2.15% and 1.43%. Figs. 1–4 show that, for D2S44 and D12S11, the majority of the variation was due to results from a few laboratories deviating substantially from the consensus values. The same phenomenon was observed for the results of the two other VNTR systems investigated (data not shown).

In a collaborative exercise organised by the EDNAP Group, where only the restriction enzyme, VNTR systems, probes, and ladder were standardised, the results obtained from the participating laboratories were within a match window of 10% [2]. When a second, similar exercise was performed under standardised conditions, including the use of common ladder, electrophoresis buffer, composition of the agarose gel etc., the variability was greatly reduced, and the mean deviation from the consensus mean was below 1% for 11 of 12 laboratories resulting in a mean coefficient of variation of 0.67% for all the participating laboratories [3].

The results of PCR based typing submitted were limited and only results from VNTR and STR systems dominated by simple, regular repeats such as D1S80, HumTH01, HumVWA31A, and HumF13A1 were sufficiently abundant for conclusions to be drawn. The results submitted were concordant. Previous collaborative exercises in the EDNAP Group have demonstrated a general agreement from typing of HumTH01 and other simple repeat systems [7–9]. In our exercise, however, it was clear that some inconsistencies concerning the nomenclature of irregular repeats of simple, regular STR systems exist in spite of the fact that this problem has been addressed by the DNA Commission of the ISFH [10]. In general, such minor inconsistencies are without practical consequences if all samples in a paternity test are investigated in the same laboratory.

In conclusion, all laboratories participating in the 1995 and the 1996 exercises drew correct conclusions from the investigations of the paternity trios. The reported lengths of DNA-fragments obtained by RFLP investigations with SLPs were very similar. The results of PCR based investigations of VNTR and STR systems with simple repeats were concordant although some minor inconsistencies of the nomenclature of irregular repeats existed.

Acknowledgements

Our thanks are due to the staff from the participating laboratories.

Appendix A

Participating laboratories

Antwerp Blood Transfusion Centre, Edegem;
Arzt für Laboratoriumsmedizin, Heidelberg;
Cellmark Diagnostics-Zeneca, Abingdon;
Department of Forensic Genetics, University of Copenhagen;
Department of Haematology, St. Bartholomew's and The Royal London School of Medicine and Dentistry, London;
Department of Human Genetics, University of Newcastle (only 1995);
Department of Immunogenetics, CLB, Amsterdam;
Etablissement de Transfusion Sanguine, Lille;
Forensic Science Laboratory, Dundee;
Forensic Science Service, Wetherby;
Institut de Médecine légale, Lausanne;
Institute of Forensic Medicine, Oslo;
Institut für Blutgruppenforschung, Köln and Düsseldorf;
Institut für Rechtsmedizin, Mainz;
Institut für Rechtsmedizin, St. Gallen;
Institut für Rechtsmedizin, Zürich;
Institut National de la Transfusion Sanguine, Paris;
Institut Universitaire de Médecine légale, Genève;
National Institute of Forensic Science, Bruxelles;
National Public Health Institute, Helsinki;
State Institute of Serology, Linköping;
University Diagnostics Limited, London.

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