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A report of the 2002–2008 paternity testing workshops of the English speaking working group of the International Society for Forensic Genetics

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ABSTRACT

The English Speaking Working Group (ESWG) of the International Society for Forensic Genetics (ISFG) offers an annual Paternity Testing Workshop open to all members of the group. Blood samples, a questionnaire and a paper challenge are sent to the participants. Here, we present the results of the 2002–2008 Paternity Testing Workshops with the objective to evaluate the uniformity of DNA-profiling and conclusions of the participating laboratories as well as to clarify tendencies in typing strategies and biostatistical evaluations of the laboratories. The numbers of participating laboratories increased from 46 in 2002 to 68 in 2008. The results showed an increasing degree of concordance concerning methods and DNA systems used and a high degree of uniformity in typing results with discrepancies in 0.1 and 0.3 % of all submitted PCR-based results. The paper challenges showed uniformity in the calculation of the weight of evidence for simple cases with straight-forward genetic constellations. However, a high degree of variation existed in complex scenarios with rare genetic constellations such as genetic inconsistencies/possible silent alleles, rare alleles and haplotypes.

1. Introduction

Since 1991, The English Speaking Working Group (ESWG) of the International Society for Forensic Genetics (ISFG) has organized an annual, collaborative workshop concerning genetic analysis in paternity testing [1–4]. The workshops are performed with the aim to enable inter-laboratory comparison, which is essential for modern, accredited laboratories. The workshop is divided into three parts. One part is the paternity testing exercise, in which blood samples from fictive paternity cases are distributed to the participating laboratories that are asked to perform genetic investigations according to their usual protocols. With the aim to compare laboratory strategies and biostatistical evaluations among the participants, the second part of the workshop is a questionnaire. The third part is a paper challenge concerning biostatistical calculations. As laboratories use different systems for typing as well as different frequency-databases for their calculations, comparisons of calculated likelihood ratios (LR) of the performed paternity tests are unattainable. Thus, from 2000, a paper challenge has been included in the workshop. This allows for comparison of the biostatistical calculations of both routine combinations and rare events such as genetic inconsistencies/possible silent alleles and haplotypes.

Here, we present the results of the 2002–2008 Paternity Testing Workshops of the ESWG. The report describes tendencies in methods and kits used for DNA-typing, information concerning strategies for biostatistic calculations of the weight of evidence and requirements for issuing a report with an excluded/non-excluded man. Also, concordances/discordances in phenotyping results are presented. Finally, the divergence in biostatistical calculations of the weight of evidence among the laboratories, highlighted by the paper challenges, is presented.

2. Material and methods

Blood samples for the paternity testing exercise were distributed to the participants along with paper challenges and questionnaires. The laboratories were asked to perform testing according to their usual strategies and methods. Until year 2004, the participants reported the results of the paternity tests in their report. From 2005, the participants have reported the results, the answers to the questionnaire and the paper challenge online. The participating laboratories are listed in Appendix A. The results were analysed and presented at the annual ESWG meetings (Appendix B).

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Table 1
Methods available for genetic investigations in paternity testing.

Methods	2002 (%) N^a = 46	2003 (%) N^a = 51	2004 (%) N^a = 55	2005 (%) N^a = 62	2006 (%) N^a = 64	2007 (%) N^a = 69	2008 (%) N^a = 68
Autosomal STR kits	91	100	100	98	100	100	99
Y-chromosomal STRs	20	39	64	71	78	86	81
kits only	–	18	45	61	67	78	77
X-chromosomal STRs	–	8	15	10	20	26	35
kits only	–	–	–	4	13	19	32
VNTR-systems (RFLP)	43	25	29	18	16	13	12
mtDNA sequencing	7	16	36	31	34	30	29
Autosomal SNPs	–	–	9	9	5	7	7
Y-chromosomal SNPs	–	–	11	11	8	6	6
X-chromosomal SNPs	–	–	2	3	–	1	–
mtDNA SNPs	–	–	2	–	2	4	4

[a] Number of participating laboratories.

In 2002, blood samples were drawn from a child, the biological mother and two alleged fathers. In 2003 and 2004, blood samples were drawn from a child, the biological mother and an alleged father. In 2005, blood samples were drawn from two children, the biological mother and an alleged father, who was the brother of the biological father. In 2006, blood samples from two twins, their biological mother and an alleged father were provided. In 2007 and 2008, blood samples were drawn from a child, the biological mother and an alleged father.

In the paper challenges, all laboratories investigated the same hypothesis using the same phenotyping data and information (numbers of alleles in a database), and it was left to the laboratories to use this information according to their usual procedures.

3. Results

3.1. Accreditation

The number of participating laboratories increased from 46 in 2002 to 68 in 2008. In this time span, the percentage of laboratories with accreditation increased from 46% to 59%. The main accreditation standard was ISO17025 according to which 95% of the laboratories were accredited in 2008 compared to 71% in 2004. In 2006 and 2007, 12% and 11%, respectively, were accredited according to the ISO15189 standard. In 2008, only 5% were accredited according to this standard.

3.2. Available methods

Table 1 shows the methods available for genetic investigations in the participating laboratories. Since 2003, all laboratories have analysed STR-systems. From 2002 to 2008, the use of RFLP-based VNTR analysis decreased notably from 43% to 12%. The use of HLA typing decreased from 17% in 2002 to 2% in 2004 and is no longer used for paternity testing by the participants. The availability of mtDNA sequencing as an additional test has increased from 7% of the laboratories in 2002 to 36% in 2004 and has remained constant since then. In 2003, the first laboratories started to report results of X-STR systems increasing to 35% in 2008. Likewise, Y-STR analysis was only available in 20% of the laboratories in 2002 compared to 86% and 81% in 2007 and 2008, respectively. As seen in Table 1, the use of SNP analysis as an additional test was first reported in 2004, but its use has not increased since then.

There is an obvious tendency towards the use of commercial kits both for autosomal STR-systems and for Y- and X-chromosomal STR systems. In 2002, 91% of the participants used commercial kits. Since 2003, all participants have used commercial autosomal-STR kits, except for a single laboratory in 2005 and 2008. Table 2 shows the most frequently used kits. The two autosomal kits, PowerPlex 16 System (Promega) and Amp*Fl*–STR Identifiler (Applied Biosystems-AB), are the most frequently used kits. The use of SGM Plus and Profiler Plus (AB) has decreased from 67% and 36%, respectively, in 2002 to 35% and 10% in 2008. The use

Table 2
The most frequently used commercial STR-kits for paternity testing.

STR-kits	2002(%)	2003(%)	2004(%)	2005(%)	2006(%)	2007(%)	2008(%)
Autosomal kits	N^a = 42	N^a = 51	N^a = 55	N^a = 61	N^a = 64	N^a = 69	N^a = 67
PowerPlex 16 (Promega)	48	55	64	68	70	71	76
Identifiler (AB[b])	10	27	49	52	45	57	57
SGM Plus (AB)	67	47	45	47	44	43	35
FFFL (Promega)	14	22	24	24	28	28	25
SEfiler (AB)	–	–	13	13	16	20	21
Profiler (AB)	14	–	18	15	11	12	13
Profiler Plus (AB)	36	16	20	18	9	10	10
Power ES System (Promega)	2	–	9	10	13	13	10
Humantype Chimera (Biotype)	–	–	–	2	6	10	9
MiniFiler (AB)	–	–	–	–	–	–	10
Y-chromosomal kits		N^a = 9	N^a = 29	N^a = 39	N^a = 48	N^a = 54	N^a = 55
Powerplex Y (Promega)	–	–	96	99	75	59	62
Y-filer (AB)	–	–	–	13	38	53	56
DYSplexI /II(Serac)	–	44	14	13	4	–	–
X-chromosomal kits				N^a = 6	N^a = 13	N^a = 16	N^a = 22
Mentype ArgusX-UL (Biotype)	–	–	–	100	100	100	92
Mentype ArgusX-8 (Biotype)	–	–	–	–	–	–	8

[a] Number of laboratories.
[b] Applied Biosystems.

Table 3
Biological material used for paternity testing.

Biological material used for PCR	2004 (%)	2005 (%)	2006 (%)	2007 (%)	2008 (%)
	N^a = 55	N^a = 62	N^a = 64	N^a = 69	N^a = 68
Buccal swabs	76	84	86	87	85
Whole blood	65	66	64	55	53
Blood on FTA cards	25	27	27	25	29
Buccal cells on FTA cards	20	19	20	22	22
Blood on filter paper	20	21	20	16	15

[a] Number of participating laboratories.

Table 4
Statistics reported in paternity testing.

Statistics reported	2002	2003	2004	2005	2006	2007	2008
	N^a = 46	N^a = 51	N^a = 55	N^a = 62	N^a = 64	N^a = 69	N^a = 68
	%	%	%	%	%	%	%
PI (Paternity Index)	80	71	69	69	70	67	71
W (Probability of Paternity)	70	61	78	79	86	86	85
EM Value	13	6	16	11	13	13	12
Probability of Exclusion	11	8	9	5	11	7	4
No statistic	2	10	5	2	–	–	–

[a] Number of participating laboratories.

of FFFL (Promega) has increased moderately from 2002 to 2008. Some of the laboratories, 25 % in 2008, used single STR systems not included in kits, mostly as additional tests or in special cases. The two single systems most frequently used were SE33 and F13A. The use of F13A has however decreased from 20% in 2002 to 6% in 2008.

Y- and X-chromosomal STR systems were mainly typed by use of commercial kits. The most frequently used Y-chromosomal STR kits were PowerPlex Y (Promega) and Y-filer (AB), used by 62% and 56% respectively, of the participants in the 2008. DYSplex I and II, used by 44 % in 2003, are no longer available. Mentype ArgusX-UL (Biotype) was used by all of the participants reporting results on X-chromosomal STR-systems from 2005 until 2007. In 2008, 8% used Mentype ArgusX-8 (Biotype).

Since 2004, the biological material used for PCR-based analyses has mainly been buccal cells on swabs and whole blood. Blood or buccal cells on FTA cards were used by more than 20 %. As seen in Table 3, the use of biological material has remained fairly unaltered since 2004, although a tendency towards using buccal swabs instead of whole blood was observed.

3.3. Inter-laboratory comparison of biostatistic evaluation

Since 2006, all of the participating laboratories have reported biostatistics either as the paternity index (PI) or as the probability of paternity (W) (Table 4). The requirements for issuing a report with positive weight for paternity have not changed much over the years. Table 5 shows the requirements when the results speak for paternity in an ordinary case with a mother, child and an alleged father. There was a high degree of uniformity in the requirements for writing a report with weight against paternity. From 2002 to 2008, the percentage of laboratories requiring three or more genetic inconsistencies increased from 61% to 76%, and the percentage of laboratories requiring only two inconsistencies decreased.

Table 6 shows that the number of laboratories considering the possibility of silent alleles in systems with opposite homozygosity between man and child increased from 24% in 2004 to 68% in 2008. Likewise, the percentage of laboratories considering the possibility of silent alleles between woman and child increased from 16% to 60%. In 2008, only 15% of the laboratories considered the possibility of a silent allele in all cases when homozygosity was present in accordance with the recommendations of ISFG [5]. As seen in Table 7, there was high variability in the formulas used for calculating the probability of a rare allele; the most frequently used formula being $5/2N$ followed by $(x+1)/(N+1)$ that is recommended by the ISFG [5].

Most laboratories use computer software for biostatistical calculations. As seen in Table 8, the use of computer software for biostatistical calculations has remained fairly constant since 2005 although the use of Familias [6] has increased.

3.4. Inter-laboratory comparison of results of PCR typing

In 2008, a total of 68 laboratories reported results for 33 autosomal STR systems. Of these, 13 systems were reported by more than 90% of the laboratories, whereas 15 systems were reported by less than 9%. Likewise, a total of 24 laboratories reported results for 17 Y-STR systems. A total of 12 systems were

Table 5
Requirements for issuing a report with positive weight for paternity.

PI-values	W-values[a]	2002 (%)	2003 (%)	2004 (%)	2005 (%)	2006 (%)	2007 (%)	2008 (%)
		N^b = 46	N^b = 51	N^b = 55	N^b = 62	N^b = 64	N^b = 69	N^b = 68
100–1000	99%–99.9%	20	10	5	6	0	6	10
1000–10,000	99.9%–99.99%	15	20	33	23	28	22	21
10,000–100,000	99.999%–99.999%	33	45	33	40	41	42	46
>100,000	>99.999%	9	16	27	21	23	24	21

[a] The posteori probability of paternity, W_{post}, is calculated based on the priori probability of paternity, W_{prior}, and the PI, which is a true likelihood ratio. By tradition, the W-value is calculated under the assumption that $W_{prior} = 0.5$.
[b] Number of participating laboratories.

Table 6
Silent alleles criteria used in paternity testing.

Silent alleles are considered in systems with:	2004 (%)	2005 (%)	2006 (%)	2007 (%)	2008 (%)
	N^a = 55	N^a = 62	N^a = 64	N^a = 69	N^a = 68
Homozygosity	15	15	11	9	15
Opposite homozygosity between man and child	24	58	66	64	68
Opposite homozygosity between woman and child	16	53	67	64	60
Not considered	25	21	13	16	18

[a] Number of participating laboratories.

Table 7
Calculation of rare allele probability in paternity testing.

Rare allele propbability	2002 (%) N^a = 46	2003 (%) N^a = 51	2004 (%) N^a = 55	2005 (%) N^a = 62	2006 (%) N^a = 64	2007 (%) N^a = 69	2008 (%) N^a = 68
x^b/n^c	11	4	2	–	–	–	–
$5/2n^c$	9	12	18	11	17	19	19
$1/n^c$	4	8	16	8	5	7	7
$(x^b + 1)/(n^c + 1)$	4	4	9	15	9	9	6
$1/(n^c + 1)$	–	2	7	5	9	12	6
$5/n^c$	4	4	2	3	3	1	3
$1/2n^c$	–	2	5	5	5	4	1
$3/n^c$	2	2	4	3	3	1	1
Fixed min. probability	40	32	34	27	28	35	32
Other	26	30	3	23	21	12	25

[a] Number of participating laboratories.
[b] Number of observed alleles in the database.
[c] Total number of alleles in the database.

Table 8
Computer software programs used for calculation of biostatistical parameters in paternity testing.

Computer software	2005 (%) N^a = 62	2006 (%) N^a = 64	2007 (%) N^a = 69	2008 (%) N^a = 68
In house	24	27	33	22
DNA-VIEW[b]	23	22	20	21
Familias (PATER)[c]	10	17	22	19
Program by Max Baur[d]	6	9	9	7
EasyPat[e]	5	5	4	3
Genotype 5.0 or 5.1[f]	3	3	3	3
Other programs	18	25	20	25
Not applied	23	17	20	19

[a] Number of participating laboratories.
[b] Software by Charles Brenner: http://dna-view.com/.
[c] Software by Egeland, Dalen and Mostad: T. Egeland et al. [6] http://www.math.chalmers.se/~mostad/familias/.
[d] Software by Max Baur: N. von Wurmb-Schwark et al. 2005 [7].
[e] Software by Michael Krawczak: http://www.uni-iel.de/medinfo/mitarbeiter/krawczak/download/.
[f] Software by s.r.o. Kvant: www.lims.sk/typo3/lims.sk/fileadmin/pdf/genotype-final.pdf.

used by more than 95% of the laboratories, four systems were used by 60%, and the remaining system was used by 8%. Only eight laboratories reported results on X-STR systems of which four systems were reported by all laboratories. No laboratories submitted result from mtDNA-typing or from SNP-typing.

The error rate over the years is presented in Fig. 1.The error rate was divided into discrepancies caused by differences in nomenclature, difference in phenotyping results and clerical errors. The classifications of the errors were based on the information given by the laboratories when the cause of errors was investigated. Differences in nomenclature have not been observed since 2005. Very few errors were caused by actual phenotyping errors during the last years. However, the error rate remained constant due to increasing numbers of clerical errors. The shift from answering the paternity testing exercise as an ordinary report into typing in the results in an online questionnaire may explain the increased rate of clerical errors. Yet, in a paternity case, clerical errors are as crucial as phenotyping errors.

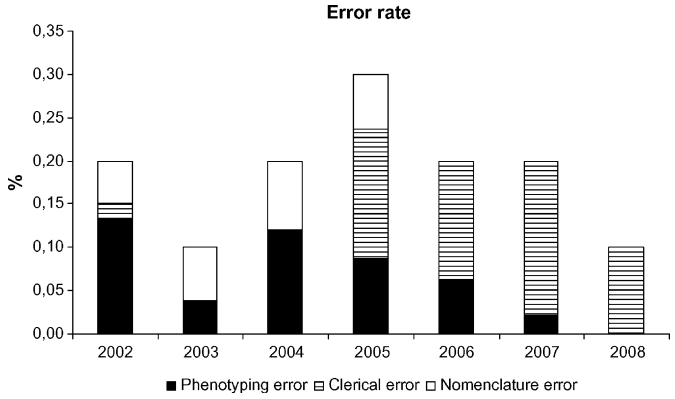

Fig. 1. The bars represent the error rates for each year divided into errors in phenotyping, clerical errors and errors in nomenclature. The error rates were calculated as the numbers of inconsistent allele results divided by the numbers of all submitted allele results.

3.5. Conclusions of the paternity testing exercise

In 2002, all of the participating laboratories agreed that the results were in favour of paternity of the same alleged father and of exclusion of the other alleged farther. Likewise, in 2003 and 2004, all laboratories that offered a verbal conclusion agreed that the results were in favour of paternity of the alleged father. In 2005, the alleged father was excluded from being the biological father of Child 1 and 2 by 74% and 76%, respectively. However, 3% (two laboratories) concluded incorrectly that the results were in favour of paternity of Child 1. Phenotyping results from the two laboratories showed two and three inconsistencies between Man and Child 1, respectively. One of these laboratories concluded incorrectly that the results were in favour of the mans's parentage of Child 2. The phenotyping results showed three inconsistencies between the man and Child 2. A total of 15 STR systems were analysed by both laboratories. The alleged father was a close relative of the biological father, which was correctly hypothesised by 76% of the participants. In 2006, 2007 and 2008, all laboratories that submitted a verbal conclusion agreed correctly that the results were in favour of paternity.

4. Paper challenge

4.1. Comparison of results

A summary of the results for each year is shown in Table 9. The table is divided into results from autosomal STR systems only and results including Y-STR or X-STR data. In general, the paper challenges showed a significant variation in PIs and LRs. Some of the variation in PIs and LRs was caused by single laboratories with unique biostatistical formulas. The variation was mainly caused by differences in calculations when rare genetic events occurred such as rare alleles, silent alleles and inconsistencies, but also biostatistical calculations based on haplotypes led to increased variation of the submitted formulas. When excluding these systems, the general inter-laboratorial consistency in the choice

Table 9
Reported biostatistical values and conclusions of the paper challenges in the paternity testing workshops.

	2002	2003	2004	2005	2006		2007	2008
	Trio[a]	Motherless[b]	Fatherless[c]	Trio[d]	Fatherless[e]		Fatherless[f]	Fatherless[g]
Autosomal STRs[h]	(*N* = 23)	(*N* = 32)	(*N* = 21)	(*N* = 27)	(*N* = 33)	(*N* = 37)	(*N* = 28)	(*N* = 28)
No. of combined LR-values	21	22	10	11	31	21	24	23
Highest consistency[i]	9%	23%	48%	26%	6%	16%	11%	11%
LR range	$2–1^{E+5}$	$1^{E-3}–3^{E+3}$	$1^{E+3}–2E^{+5}$	$2^{E-6}–6^{E+4}$	$1^{E-2}–2^{E+6}$	$2^{E+3}–6^{E+5}$	$2^{E-20}–6^{E+11}$	$2^{E-7}–4^{E+7}$
In favour of parenthood	9%	–	92%	–	–	82%	100%	41%
Against parenthood	–	5%	–	50%	–	–	–	6%
Inconclusive	88%	95%	8%	50%	100%	18%	–	35%
X- or Y STRs included[i]		(*N* = 10)	(*N* = 9)	(*N* = 19)	(*N* = 23)	(*N* = 26)	(*N* = 19)	(*N* = 11)
No. of LR-values	–	10	7	10	11	20	18	11
Highest consistency[i]	–	10%	22%	32%	9%	12%	11%	–
LR range	–	$3^{E-4}–5^{E+4}$	$4^{E+4}–5^{E+5}$	$2^{E-4}–6^{E+6}$	$7^{E+4}–8^{E+8}$	$9–4^{E+6}$	$2^{E+3}–2^{E+8}$	$4^{E-3}–4^{e+11}$
In favour of parenthood	–	10%	67%	5%	39%	62%	63%	64%
Against parenthood	–	–	–	74%	–	–	–	–
Inconclusive	–	90%	33%	21%	61%	38%	26%	36%

[a] Trio case with mother, child and two alleged fathers.
[b] Motherless case with a boy and an alleged father.
[c] Deficiency case with a mother her daughter, and brother and sister of missing, alleged father.
[d] Trio with mother, child and an alleged father (East African origin).
[e] Deficiency case with mother, son and daughter, and parents of the alleged father, the two LRs are for the son and daughter, respectively.
[f] Fatherless case with mother, child, and two other children of the alleged father (assumed to have the same mother).
[g] Fatherless case with two children and an alleged mother (the children are assumed to have different fathers).
[h] Number of laboratories that calculated the paper challenge.
[i] Highest consistency: Percentage of laboratories submitting the same combined LR.

Table 10
Comparison of biostatistical calculations for systems with genetic inconsistencies, silent alleles, rare alleles, and Y-chromosome haplotypes.

	2002 (N^a = 23)	2003 (N^a = 32)	2004 (N^a = 21)	2005 (N^a = 27)	2006 (N^a = 33)	2007 (N^a = 28)	2008 (N^a = 28)
Inconsistencies							
No. of reported formulas	14	11	–	8	9	–	15
Frequency of most commonly used formula	13%	18%	–	37%	24%	–	18%
Mutation not considered	–	14%	–	4%	–	–	14%
Not calculated	13%	13%	–	37%	15%	–	4%
LR[b] range within one system	143	17	–	1667	349		3193
Shared Silent alleles/Inconsistency							
No. of reported formulas	4	8	–	–	10	15	–
Frequency of most commonly used formula	65%	33%	–	–	21%	21%	–
Calculated as a silent-allele	4%	33%	–	–	6%	7%	–
Calculated as a mutation	17%	40%	–	–	76%	68%	–
Other	65%	27%	–	–	16%	14%	–
Not calculated	14%	–	–	–	2%	11%	–
LR[b] range within one system	2	10	–	–	15	400,000	–
Rare allele							
No. of reported formulas	5	–	–	–	16	–	–
Frequency of commonly used formula	48%	–	–	–	21%	–	–
Not calculated	32%	–	–	–	–	–	–
LR[b] range within one system	12	–	–	–	52	–	–
Y-chromosome haplotype							
No. of reported formulas	–	5	6	6	9	7	–
Most commonly used formula	–	$1/(1/(n^d + 1))$	$\mu^e/(x^c/n^d)$	$1/(x^c/n^d)$	$\mu^e/(x^c/n^d)$	$1/(x^c/n^d)$	
Frequency of most commonly used formula	–	26%	19%	52%	35%	36%	–
Not calculated	–	26%	–	26%	21%	32%	–
LR[b] range within one system	–	5	5	2000	48,120	5	–
Systems without rare events							
Percentage of laboratories submitting the same formula[f]	70–96%	90–94%	62–92%	84–88%	97–100%	54–57%	68–78%[g]

[a] Number of laboratories submitting formulas.
[b] The range is calculated as: the highest LR-value divided by the lowest LR-value.
[c] Number of observed haplotypes in the database.
[d] Number of haplotypes in the database.
[e] μ: Mutation rate.
[f] Percentage of laboratories submitting the same formula in systems without rare events.
[g] Percentage of laboratories reaching one of the two most commonly used formulas based on the two alternative hypotheses used.

of formulas was between 62–100% as seen in Table 10. The 2007 paper challenge was an exception. This paper challenge included a more complicated deficiency case where children of the missing, alleged father were included. The variation in PI was as high as 31 orders of magnitude for the autosomal systems, highlighting the very different approaches for this type of deficiency case. Another exception was the 2008 paper challenge, where the inter-laboratory consistency was below 40%. However, examination of the submitted formulas suggested that two different alternative hypotheses were used. Taking this into consideration, the variation in LR within these two main groups was below two orders of magnitude and the percentage of laboratories reaching one of the two common formulas for the autosomal STR systems was 66%–78% when omitting systems with genetic inconsistencies.

4.2. Rare alleles

Table 10 lists the main sources of variation in calculation of the weight of evidence, i.e. rare alleles, inconsistencies, shared silent alleles and haplotypes. As seen, rare alleles were included in the 2002 and 2006 paper challenges. In 2002, the variation of the PI-values was mainly caused by different ways of calculating the probability of a rare allele: x/n (70%), $(x+1)/(n+1)$ (13%). In the 2006 paper challenge, all laboratories agreed on a single formula for a system with a rare allele, but 16 different probabilities of the rare allele were used, resulting in PI-values ranging from 19.2 to 1000 for the given system. The most frequently used formula for the probability of a rare allele was $5/n$ (21%), followed by $1/n$ (18%), whereas a fixed minimum probability was applied by 31%.

4.3. Genetic inconsistencies

Some laboratories did not calculate the LR for systems with inconsistencies. Among the laboratories that did calculate the LR, the majority considered the possibility of mutational events. However, a large number of different formulas for encountering this possibility were used as seen in Table 10. The variation in the calculated LR was generally very high, thereby having a considerable impact on the conclusion drawn from the genetic data. The small fraction of laboratories that did not consider the possibility of mutational events, seemed to calculate the LR without taking the genetic inconsistency in consideration.

4.4. Silent ('null') alleles

Only very few of the laboratories considered the presence of silent alleles in cases of equivalent homozygosity between parent and child. The LRs in these cases were in very close proximity to calculations neglecting the possibility of silent alleles (not shown). However, in cases of opposite homozygosity between a parent and the child, the differences in likelihood ratios were considerable. As shown in Table 10, the 2002, 2003, 2006 and especially the 2007 paper challenge demonstrated a high variation in the LR (up to 5 orders of magnitude) caused by very different statistical approaches, predominantly considering the event as genetic inconsistency rather than a shared silent allele. This result was surprising since more than 64% answered that they considered silent alleles in cases of opposite homozygosity between woman/man and child in 2006 and 2007.

4.5. Haplotypes

Y-STR haplotypes were included in the paper challenges of 2003, 2005, 2006 and 2007. In 2006, a genetic inconsistency was present. In 2003, the most frequently used formula was $1/(x/(n+1))$, where x is the number of the observed haplotype in the database, and n is the total number of haplotypes in the database. In 2005 and 2007, the most frequently used formula was $1/(x/n)$.

In the 2008 paper challenge, information on 8 X-chromosomal STR systems along with information on their pair wise close linkage was provided. Out of the 11 laboratories submitting biostatistical formulas for these systems, six considered the information on linkage groups whereas 5 considered the systems as being unlinked.

5. Discussion

Participation in proficiency testing has gained more interest as more laboratories have become accredited. The number of participating laboratories in the Paternity Testing Workshop of the ESWG has increased from only nine in the beginning in 1991 [1] to 69 in 2007 and 68 in 2008. The PTC of the ISFG recommends that paternity testing be performed in accordance with the ISO 17025 standard that specifies general requirements for the laboratories for competent performance of test. Recommendations concerning selected areas of special importance to paternity testing can be found in Morling et al. [7]. In 2008, 59% of the laboratories were accredited, all except one according to the ISO17025 standard.

The questionnaire showed that the classical blood grouping methods are no longer in use in the participating laboratories and that the use of RFLP-based methods has decreased notably. PCR-based analysis of STRs by fragment length separation in capillary-electrophoresis is available in all laboratories. The use of X-STR analysis as additional test is increasing. Likewise, Y-STR analysis is available in more than 80% of the laboratories. There is an obvious tendency towards the use of commercial kits for both autosomal and sex-chromosomal STR systems. Currently, mtDNA sequencing is available as an additional test in approximately one third of the laboratories, whereas SNP analysis is still not commonly used.

All of the participating laboratories report biostatistics either as a PI or as a W-value. There is a high variation in the requirement for issuing a report with positive weight for paternity. However, there is a tendency towards requiring an index of more than 10,000 (a probability of more than 99.99%, assuming a priori=0.5). On the other hand, there was high uniformity in the criteria for paternity exclusion, with three or more inconsistencies as the preferred requirement based on at least 15 investigated STR systems. The recommendation of the PTC of ISFG is establishment of exclusion criteria in terms of a LR threshold (e.g., PI $< 1/1000$) [5].

The results of the Paternity Testing Workshops 2002–2008 showed an increasing degree of concordance concerning methods and applied systems. The observed errors counted for only 0.1%–0.3% of all PCR-based STR allele results. Today, most discrepancies are due to clerical errors rather than differences in phenotyping or nomenclature. Naturally, the tendency of using the same commercial kits accounts for some of the uniformity in the obtained results. Also, recommendations for nomenclature policy have contributed to improved consensus [8,9].

In general, there was high uniformity in the conclusions of the paternity testing exercises. The only discrepancy was seen in the 2005 exercise, where the alleged father was closely related to the biological father. Still, the majority of the laboratories correctly excluded paternity and hypothesised a close genetic relationship between the biological and the alleged father.

Most laboratories use computer programs for biostatistical calculations. Even so, the paper challenges showed a considerable variation in the formulas used among the participants, especially when rare alleles, inconsistencies or silent alleles were present. Most laboratories did consider the possibility of mutational events, but a large number of different formulas were used for calculating the LR leading to considerable variation in the total LRs. Silent

alleles arise when the allele size is outside the range of measurement or, when mutations in primer-binding sites have occurred. The recommendation from the PTC of the ISFG is to consider the possibility of silent alleles when only one allele is observed [5]. Only few participants currently follow this recommendation, but the majority consider silent alleles in cases of opposite homozygosity between parent and child according to the questionnaires. Nonetheless, very different approaches were used in the paper challenge in cases of opposite homozygosity and most laboratories treat the event as a possible mutation. Finally, there is a high variability in how the participants calculate the probability of a rare allele resulting in very different LRs for the given system.

When omitting these more complicated genetic events, there was a general high uniformity in how the statistics were calculated.

6. Conclusion

The results of the 2002–2008 Paternity Testing Workshops revealed high uniformity in the methods and genetic systems used by the laboratories as well as in phenotyping results and conclusions. There was a general agreement in how the weight of evidence was calculated in routine genetic constellations, but there was a large variation in the biostatistical calculations in case of rare events such as rare alleles, silent alleles and genetic inconsistencies.

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Appendix A

A.1. Participating laboratories

- Amtlich benannter Sachverständiger für Abstammungsgutachten, Zentrallaboratorium, Berlin, Germany (2002–2008)
- Anglia DNA Services Limited, Norwich Research Park, Norwich, UK (2005)
- Antwerp Blood Transfusion Center, Edegem, Belgium (2002–2003)
- Arzt für Laboratoriumsmedizin, Heidelberg, Germany (2002–2008)
- Aurigen, Lausanne, Switzerland (2008)
- BJ Diagnostik GmbH, Giessen, Germany (2002–2008)
- Complement Genomics Ltd., 128 Bioscience Centre, Business & Innovation Centre, Sunderland, UK (2008)
- Crucial Genetics Ltd., F1 House, Winsford Industial Estate,Winsford, Cheshire, UK (2008)
- Department of Biochemistry, Olomouc, Czech Republic (2005)
- Department of Forensic Medicine and Criminology, School of Medicine, University of Zagreb, Zagreb, Croatia (2002–2008)
- Department of Forensic Medicine, Medical Academy of Bialystok, Bialystok, Poland (2003–2007)
- Department of Forensic Medicine, Medical Academy of Lodz, Lodz, Poland (2002–2008)
- Department of Forensic Medicine, Warsaw Medical School, Warsaw, Poland (2002–2008)
- Department of Forensic Medicine, Medical University, Lublin, Poland (2004–2007)
- Department of Forensic Medicine,Medical University of Gdansk, Gdansk, Poland (2003–2008)
- Department of Forensic Medicine,University of Helsinki, Helsinki, Finland (2004–2008)
- Department of Forensic Medicine & Science, The University of Glasgow, Glasgow, UK (2002)
- Department of Genetics, Institute of Legal Medicine, Bucharest, Romania (2002–2008)
- Department of Haematology, St. Bartholomew's and The Royal School of Medicine and Dentistry, London, UK (2002–2008)
- Department of Immunogenetics, CLB, Amsterdam, Netherland (2004–2006)
- Department of Legal Medicine, University of Rome "La Sapienza", Rome, Italy (2008)
- Department of Molecular Biology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia (2002–2004)
- Department of Molecular Genetics, Institute of Haematology and Blood Transfusion, Praha, Czech Republic (2002–2007)
- Department of Medical Genetics, Ghent University Hospital, Ghent, Belgium (2004–2008)
- Department of Paternity Testing, Diagnostic Services Sanquin, Amsterdam, Netherland (2005–2008)
- DNA Analysis Laboratory, Athens Department of Legal Medicine, Athens, Greece (2002–2008)
- DNA Database, SPSA Forensic Service, Dundee, UK (2003–2008)
- DNA Diagnostics Ltd., Biosciences Building, Liverpool, UK (2005–2008)
- DNA Diagnostics Ltd., Panmure, Auckland, New Zealand (2003–2008)
- DNA Section, Forensic Science Laboratory, Garda Headquaters, Dublin, Eire (2003–2008)
- DNAtest s.r.o., Bratislava, Slovakia (2005–2008)
- Dynamic Code AB, Linköping, Sweden (2006–2008)
- Forensic DNA Laboratory, Universitair Ziekenhuis Antwerpen, Edegem, Belgium (2004–2008)
- Forensic Division, Department of Chemistry, Jalan Sultan, Petaling Jaya, Selangor, Malaysia (2007)
- Forensic Laboratory for DNA-Research, Leiden University Medical Center, Netherland (2002–2008)
- Forensic Science Service, QA Group, Priory House, Birmingham, UK (2002–2008)
- Gemeinschaftspraxis für Laboratoriumsmedizin, Mikrobiologie und Humangenetik, Mönchen, Germany (2008)
- Genetech Ltd., Budapest, Hungary (2008)
- Genetic Technologies (DNA-ID Labs), Fitzroy, Australia (2002–2007)
- GENNET, s.r.o., Prague 7, Czech Republic (2008)
- Genedia AG, München, Germany (2002–2007)
- Genodia Molecular Diagnostics, Budapest, Hungary (2005)
- Hospinvest Diagnostics, Paternity and Kinship Testing Unit, Budapest, Hungary (2007)
- Human Genetics Lab., Human Sciences Department, Loughborough University, Loughborough, UK (2007)
- Human Identity, Orchid Cellmark, Abingdon Business Park, Abingdon, UK (2005–2008)
- IGNA, Nantes, France (2004–2008)
- Institut de Médecine Légale, Genève 4, Switzerland (2002–2008)
- Institut für Blutgruppenforschung LGC GmbH, Köln, Germany (2002–2008)
- Institut für Rechtsmedizin, Johannes Gutenberg Universität Mainz, Mainz, Germany (2002–2008)
- Institut für Rechtsmedizin, Kantonsspital St. Gallen, St. Gallen, Switzerland (2002–2008)
- Institut für Rechtsmedizin, Klinikum der Universität zu Köln, Köln, Germany (2005–2008)
- Institut für Rechtsmedizin, Ludwigs-Maximilians-Universität München, München, Germany (2005–2008)
- Institut für Rechtsmedizin, Universität Bern, Bern, Switzerland (2007–2008)

- Institut für Rechtsmedizin, Universität Zürich-Irchel, Zürich, Switzerland (2002–2008)
- Institut National de Transfusion Sanguin, Paris Cedex, France (2002–2008)
- Institute for Human Genetics, Hannover Medical School, Hannover, Germany (2003–2008)
- Institute of Forensic Medicine, Budapest, Hungary (2002–2008)
- Institute for Medical Diagnostics (IMD), Berlin, Germany (2004–2007)
- Instituto de Medicina Legal de Lisboa, Servico de Biologia Forense, Lisboa, Portugal (2002–2008)
- Labor Dr. Wagner, Stibbe und Partner, Labor Dr. Glaubitz, Hannover, Germany (2003–2008)
- Laboratoire Codgene, Strasbourg, France (2003–2008)
- Laboratoire de Genetique Moleculaire, Institut de Biologie, Nantes, France (2002–2003)
- Laboratoire de génétique forensique, Institut universitaire de Médecine Légale, Université de Lausanne, Switzerland (2002–2008)
- Laboratoire d'Identification Génétique, INCC/NICC, National Institute of Forensic Science, Bruxelles, Belgium (2002–2008)
- Laboratoire IDNA, Batiment C Campus Erasme, Brussels, Belgium (2002–2008)
- Laboratoire Police Scientifique de Lyon, France (2007–2008)
- Laboratorio di Diagnostica Molecolare, Lugano, Switzerland (2008)
- Laboratory of Molecular Genetics, National Institute of Chemical Physics and Biophysics, Tallinn, Estonia (2002–2007)
- Laboratory of Paternity Testing, Medical Academy of Wroclaw, Wroclaw, Poland (2002–2007)
- Laboratory for Forensic Genetics and Molecular Archaelogy, Center for Human Genetics, K.U. Leuven, Belgium (2002–2008)
- Laboratory for Tissue Immunology, Falmouth Building, Falmouth Road, Cape Town, South Africa (2002–2003)
- Laborigo Molecular Diagnostics, Budapest, Hungary (2006)
- Leiterin Forensische Genetik, Institut für Rechtsmedizin der Univerität Basel, Basel, Switzerland (2005–2008)
- Medical University, Lublin, Poland (2002–2003)
- Micropathology Ltd. University of Warwick Science Park, Barclays Venture Centre, Coventry, UK (2002–2008)
- Ministry of the Interior Police, POLICE, Forensic Science Centre, Ljubljana, Slovenia (2006–2007)
- Netherlands Forensic Institute, Netherlands Ministry of Justice, Rijswijk, Netherland (2002–2005)
- Neodiagnostica, SL, Laboratoria de Análisis de ADN, Lleida, Spain (2007–2008)
- Palacky University, Olomouc, Czech Republic (2006–2008)
- Paternity Laboratory, Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland (2002–2008)
- Paternity Testing Laboratory, Labim NV, Meerdonk, Belgium (2002–2008)
- Rättsmedicinalverket, Avdelingen för rättsgenetik och rättskemi, Linköping, Sweden (2002–2008)
- Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health Sciences, University of Copenhagen, Denmark (2002–2008)
- Seksjon for Familiegenetikk, Rettsmedisinsk Institutt, UiO, Rikshospitalet, Oslo, Norway (2002–2008)
- Synergene Technologies Ltd., Attard, Malta (2003–2006)
- South African National Blood Service, Pinetown, South Africa (2005–2008)
- TDL Genetics, London, UK (2008)
- Unidad de Garantía de Calidad, Departamento de Madrid, Spain (2005–2008)
- Unit Manager Genotyping, Eurofins Medigenomix GmbH, Martinsried, Germany (2007–2008)
- University Diagnostics Ltd., LGC Building, Middlesex, UK (2002–2008)
- Verilabs, Leiden, Netherland (2002–2008)
- Zentrum der Rechtsmedizin, J W Goethe Universitaet, Frankfurt, Germany (2004–2008)

Appendix B

Results of annual workshops were presented at:

- 20th ISFG Congress, 9–13 September, 2003, Arcachon, Bordeaux, France.
- ESWG Meeting, 17–19 June, 2004, Zandvoort, Holland.
- 21st ISFG Congress, 13–17 September, 2005, Ponta Delgada, Azores, Potugal.
- ESWG Meeting, 8–11 June, 2006, Helsinki, Finland.
- 22nd ISFG Congress, 20–22 August, 2007, Copenhagen, Denmark.
- ESWG Meeting, 17–21 June 2008, Sinaia, Romania.

Appendix C. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fsigen.2009.01.016.

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