Int J Legal Med (1997) 110 : 273–277

TECHNICAL NOTE

J. Gómez · M. S. Rodriguez-Calvo · C. Albarrán · A. Amorim · J. Andradas · C. Cabrero · R. Calvet D. Corach · M. Crespillo · C. Doutremépuich · O. García · H. Geada · M. Gené · S. Jimenez · J. A. Lorente S. M. Marques-Santos · B. Martínez-Jarreta · M. Martínez de Pancorbo · F. Montes · J. M. Ruíz de la Cuesta P. Sanz · M. F. Terra-Pinheiro · M. C. Vide · A. Carracedo

A review of the collaborative exercises on DNA typing of the Spanish and Portuguese ISFH working group

Received: 7 February 1997 / Received in revised form: 17 April 1997

J. Gómez · C. Albarrán
Instituto Nacional de Toxicología, Madrid, Spain

M. S. Rodriguez-Calvo · A. Carracedo (✉)
Instituto de Medicina Legal, F. Medicina, E-15705 Santiago, Spain
FAX: +34 (81) 580 336

A. Amorim
IPATIMUP, Porto, Portugal

J. Andradas
Comisaría General Policía Científica, Laboratorio de ADN, Madrid, Spain

C. Cabrero
Dpto. Biología Molecular, Pharma Gen, Madrid, Spain

R. Calvet
U. Medicina Legal, Facultad de Medicina, Santander, Spain

D. Corach
Servicio de Huellas Digitales Genéticas, Facultad de Farmacia, Buenos Aires, Argentina

M. Crespillo
Instituto Nacional de Toxicologia, Barcelona, Spain

C. Doutremépuich
Laboratoire d'Hematologie, Faculté de Pharmacie, Bordeaux, France

O. García
Area de Laboratorio Ertzaintza, Bilbao, Spain

H. Geada
Instituto de Medicina Legal, Lisboa, Portugal

M. Gené
Cátedra de Medicina Legal, F. Medicina, Barcelona, Spain

S. Jimenez
Cátedra de Medicina Legal, Alicante, Spain

J. A. Lorente
Cátedra de Medicina Legal, F. Medicina, Granada, Spain

S. M. Marques-Santos
Laboratorio de Policia Cientifica, Policia Judiciaria, Lisboa, Portugal

B. Martínez-Jarreta
Cátedra de Medicina Legal, F. Medicina, Zaragoza. Spain

M. Martínez de Pancorbo
Dpto. Biología Celular y Ciencias Morfológicas, F. Medicina, Leioa (Pais Vasco), Spain

F. Montes
Dpto. de Análisis, Dirección General de la Guardia Civil, Madrid, Spain

J. M. Ruiz de la Cuesta
Dpto. de Medicina Legal y Toxicología, F. Medicina, U. Complutense, Madrid, Spain

P. Sanz
Instituto Nacional de Toxicologia, Sevilla, Spain

M. F. Terra-Pinheiro
Instituto de Medicina Legal, Porto, Portugal

M. C. Vide
Instituto de Medicina Legal, Coimbra, Portugal

Abstract Since 1992 the Spanish and Portuguese Working Group (GEP) of the International Society for Forensic Haemogenetics (ISFH) has been organizing collaborative exercises on DNA profiling with the aim of making progress on standardization and discussing technical and statistical problems in DNA analysis. A total of four exercises (GEP-92 to GEP-95) have been carried out until now. A consequence of these exercises was the creation of a quality control programme in Spain and Portugal in 1995 which was carried out simultaneously with the GEP-95 exercise. The number of participating laboratories increased from 10 in the first exercise (GEP-92) to 19 in the last exercise (GEP-95). Despite this increasing number of participating laboratories, results remained satisfactory. In the last exercises, all the laboratories used PCR-based DNA polymorphisms with an increasing number of markers obtaining good results. SLPs were used by only 30% of laboratories in the last two exercises but the results indicated a good level of expertise in most of these laboratories. The reasons for these successful results are the common use of the EDNAP protocol for SLP analysis and commercially available kits or common sequenced allelic ladders for PCR-based DNA polymorphisms.

Key words DNA polymorphisms · Standardization · Collaborative exercise · Forensics

Introduction

The Spanish and Portuguese Working Group (GEP) of the International Society for Forensic Haemogenetics (ISFH) comprises forensic genetic laboratories from Spain, Portugal, France, and some South and Central American countries. The majority of all the casework in forensic genetics (criminal casework and paternity testing) in Spain and Portugal is carried out in these GEP laboratories.

Since 1992 the group has been organizing collaborative exercises on DNA profiling with the aim of progressing on standardization as well as for the discussion of technical and statistical issues in DNA analysis (Gómez and Carracedo 1996) and four exercises (GEP-92 to GEP-95) have been carried out. As a consequence of these exercises, a quality control programme has been set up since 1995 in Spain and Portugal, which was carried out simultaneously with the GEP-95 exercise.

In this paper the results of the exercises are summarized and the characteristics of the quality control programme described.

Material and methods

Samples

A total of six bloodstains were distributed to participants. Each bloodstain was prepared by applying 400 µl of whole blood onto cotton cloth and air dried before distribution.

DNA extraction and quantification

Phenol-chloroform was used by the majority of laboratories (10/19) in the last exercise usually followed by centricon-100 purification but chelex extraction was also used by many laboratories (9/19) especially by those reporting results for only PCR-based systems. Quantification of extracted DNA was carried out by the majority of laboratories, slot-blot being the method most commonly used.

RFLP methodology

SLPs were reported by 7 out of 19 participating labs in GEP-95 (7 out of 15 in GEP-94). Most of the laboratories (except one in GEP-95) followed the EDNAP electrophoresis protocol (Gill et al. 1992). The agarose concentration varied from 0.7 to 1%. The BRL ladder (Life Technologies S.A., Barcelona, Spain) was used by the majority of laboratories. Ethidium bromide was only used after running the gels. Sizing of fragments was carried out by a variety of methods (manual and automated systems) using the local reciprocal method (Elder and Southern 1987) for calculations.

PCR methodology

DQA1 and Polymarker

All participating laboratories used the DQA1 system. The markers included in the Polymarker system were reported by a total of 13 laboratories. All participants performed the amplification and typing of these systems by using reverse dot-blot with the AmpliType HLADQA1 and PM Forensic DNA Amplification and Typing Kits (Perkin Elmer Corp., Norwalk, CT).

D1S80

All participating laboratories carried out the D1S80 amplification by using the AmpliFPD1S80 Amplification Kit (Perkin Elmer Corp., Norwalk, CT). The commercial D1S80 ladder of 27 alleles was used by 13 labs, 1 laboratory used the old commercial Perkin-Elmer ladder of 13 alleles and another used a ladder composed of 8 sequenced alleles.

A variety of electrophoretic and detection methods were used for typing this system, including native PAGE (both vertical and horizontal) and silver staining, SDS-PAGE and silver staining, metaphor agarose and EtBr, and automated sequencers with fluorocrome-based detection systems.

STRs

STRs were used by the majority of participating laboratories (15/19). The systems of choice were HUMTH01 (15 labs), HUMFES and HUMVWA31A (14 labs) and HUMF13A1 (13 labs). Generally, amplification of STR markers was performed using common primers but different electrophoretic systems and allelic ladders. Basically, laboratories can be classified into those that used the GenePrint STR System (Promega Corporation, Madison, WI, USA) followed by silver staining detection, and those that used primers and allelic ladders included in the EDNAP exercises (Gill et al. 1994; Kimpton et al. 1995) in conjunction with automated sequencers. However, all laboratories used sequenced allelic ladders in GEP-95 (11 vs 3 used sequenced allelic ladders in GEP-94). Also all the laboratories used denaturing polyacrylamide gels in GEP-95 (12 vs 2 used denaturing conditions in GEP-94).

Amelogenin

Results from the amelogenin system were reported by only five laboratories. Denaturing PAGE and silver staining was the method most commonly used for analysing this marker.

Results

Since 1992 the Quality Control Centre of the National Institute of Toxicology has been distributing six samples to participants, usually by December. Data from laboratories were reported by May of the following year, and the results were discussed in June during the Annual Meeting of the GEP-ISFH group. In the last exercise three of the six samples corresponded to a paternity case.

Each laboratory was given a code in order to preserve anonymity. Participants in the exercise were supplied with a data sheet with methodological questions for the DNA loci included in each exercise (i.e. primers, ladders, buffer, gel composition, detection system).

The number of participating laboratories (Table 1) increased from 10 in the first exercise (GEP-92) to 19 in the last exercise (GEP-95). In the last two exercises, results from 15 laboratories out of 17 participants (GEP-94) and 17 laboratories out of 19 participants (GEP-95) were reported.

The number of DNA polymorphisms included in the exercises increased from four in GEP-92 to five in GEP-93, and nine in GEP-94. In GEP-95, laboratories were free to submit results for the DNA systems currently used in their casework, and a total of 25 systems were included. The systems used in each exercise are listed in Table 1.

Table 1 The systems used in each exercise (from GEP-92 to GEP-95). The figures at the bottom indicate the total number of participating labs for each GEP-exercise

GEP-92	GEP-93	GEP-94	GEP-95		
MS43a	MS43a	MS43a	MS43a	DQA1	SE33
YNH24	YNH24	YNH24	YNH24	D1S80	HUMLIPOL
DQA1	DQA1	DQA1	MS31	HUMTH01	HUMFIBRA
D1S80	D1S80	D1S80	MS1	HUMFXIIIA1	D21S11
	HUMTH01	HUMTH01	MS205	HUMVWA	D12S391
		HUMFXIIIA1	MS8	HUMFES/FPS	HUMLPL
		HUMVWA	G3	HUMTPOX	Amelogenin
		HUMFES/FPS	MS621	HUMCSF1PO	
		Polymarker	Polymarker	HUMF13B	
10	15	17	19		

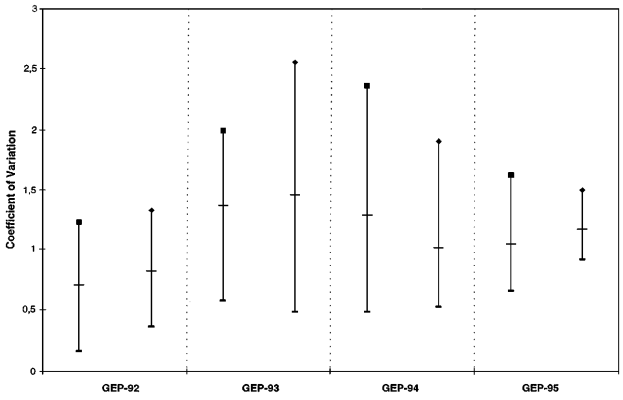

Fig. 1 Mean and total range of coefficient of variation among laboratories for two SLPs (MS43a and YNH24). ■ MS43a; ◆ YNH24

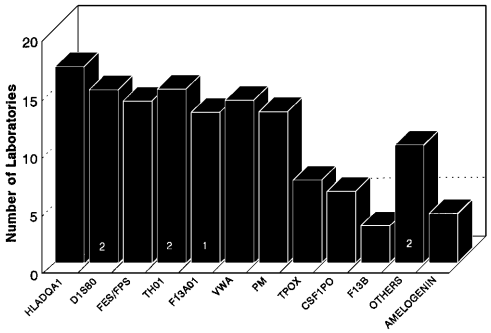

Fig. 2 Results of the GEP-95 exercise for the PCR-based systems. Bars indicate the number of labs performing each system. Errors found are indicated by numbers within bars

SLPs

Figure 1 shows the mean and the total range of coefficient of variation among laboratories for two SLPs (MS43a and YNH24). Detailed data for each band, locus and laboratory as well as a complete statistical analysis are available upon request.

The mean laboratory deviation (Deviation δ from the mean: $\delta\ (\%) = (\overline{M} - F)/\overline{M} \times 100$) (Gill et al. 1992) for MS43a and YNH24 ranged between 0.14–0.92 (GEP-92), 0.07–2 (GEP-93), 0.22–1.58 (GEP-94) and 0.22–1.71 (GEP-95). These results are similar to the GEDNAP (Puers et al. 1992; Bär et al. 1992; Brinkmann et al. 1993; Wiegand et al. 1995), ESWG (Syndercombe-Court and Lincoln 1996), and the second EDNAP exercise (Gill et al. 1992), and considerably better than the EDNAP I trial (Schneider et al. 1991). The interlab variation was 4.11% in one isolated case. Except in this particular case the interlaboratory variation was low and 100% of matches achieved using a guideline of 2%. It is necessary to keep in mind that fragments were sized in each participating laboratory.

Other probes were included in the GEP-95 exercise (MS31, MS1, MS205, MS8, G3 and MS621) with similar results.

PCR-based systems

Results of the GEP-95 exercise are summarized in Fig. 2. No typing errors were found in dot-blot based systems (DQA1 and Polymarker) for a total of 612 samples (17 laboratories).

Only 2 laboratories reported isolated errors from a total of 15 participants. The lack of detection of an allele larger than 41 by one laboratory in sample 2 and a mistyping in sample 3 in other laboratory (23–25 instead of the correct 23–27) were the reasons for the errors found in the trial.

Isolated errors were found in TH01 (2 laboratories from a total of 15) and F13A01 (1 laboratory out of 13 participants). Errors in TH01 were due to an incorrect denomination of the alleles in the allelic ladder in one case and a mistyping of sample 6 by another laboratory (6–9.3 instead of the correct 6–9). In general, mistyping errors were combined with allelic ladders of poor quality or not correctly designated.

Differences in nomenclature were reported by two participating laboratories for the complex STRs ACTBP2 and D21S11.

No errors were reported in the following STRs: FES/FPS, VWA, TPOX, CSF1PO, F13B, LIPOL, FIBRA, D12S391 and LPL. Also, no errors were found in the amelogenin system.

In GEP-92 three errors in the DQA1 system and four in the the D1S80 system (mistyping of allele 28 for 29) were

reported. In GEP-93 only one error in the D1S80 system was found and in GEP-94 discrepancies in the D1S80 system (1), Polymarker (2) and HumVWA (2) were reported.

Statistical evaluation of the paternity case

Statistical results for the paternity case included in the GEP-95 exercise were reported by 12 laboratories. No exclusions were found by any of the participants. Laboratories reported IP values and W values (a priori of 0.5) taking into account their own frequencies (usually the frequencies of the area estimated by the laboratory). All the participating laboratories reported W values over 99.9%. W values greater than 99.999% were reported by the majority of participating laboratories (7/12), two laboratories reported W values higher than 99.99% and three laboratories reported W values higher than 99.96%.

The quality control scheme

In conjuction with the GEP-95 exercise, a quality control programme was set up as shown in Fig. 3. The Quality Control Centre of the National Institute of Toxicology (the official laboratory for Forensic Sciences of the Spanish Ministry of Justice) distributes samples to participants including a paternity testing case. Laboratories can submit their results for validation or just participate in a collaborative exercise. Discussion in the working group about the results of the collaborative exercise, the validation, and the QC scheme itself will continuously improve the system.

Out of the 17 laboratories submitting results 12 asked for validation under the quality control programme, 4 other laboratories applied for validation of at least one marker. Another laboratory submitted results only for the collaborative exercise.

Accreditation reports include only the markers successfully reported. There was a general agreement to only use the markers validated under the QC scheme for casework.

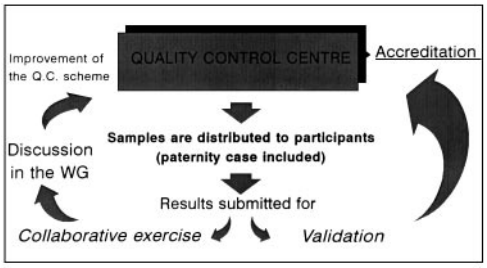

Fig. 3 Spanish and Portuguese ISFH WG Quality Control Scheme

Discussion

The experience of the GEP exercises has shown that in spite of the increasing number of participants, the quality of the results has remained within the acceptable standards.

The number of laboratories submitting results for SLPs analysis remained constant. However, the number of laboratories submitting results for PCR-based analysis increased continuously, specially in the last exercise (GEP-95). Also, there was a significant increase in the number of systems, specially in GEP-95 where a total of 25 DNA polymorphisms were tested. The continuous increase in the number of DNA polymorphisms carried out is another factor to consider in the urgent need for standards in forensic genetics in addition to other reasons already highlighted in other reports (Brinkmann 1991; Carracedo et al. 1996). Quality control programmes can only be implemented if participants would agree to use a limited number of systems.

Results for SLPs remained within reasonable standards in all exercises. The use of a common electrophoresis protocol together with the expertise of the participating laboratories are the main factors to explain these results. In fact, laboratories using electrophoresis protocols different from that of EDNAP have shown greater deviations from the mean in previous exercises. In the last two exercises a single laboratory used a protocol different from that of EDNAP and had the highest difference in results. Data from PCR-based polymorphisms were promising with only a few errors detected despite the high number of systems studied in the last trial. No errors were reported in systems analysed with reverse dot-blot and ASO probes. The few errors detected in D1S80, TH01, and F13A1 were due to preferential amplification of a heterozygote with a heavy allele in the D1S80 system, mistyping errors, and incorrect designation of the allelic ladder. In these last two cases, the human factor in reading results or making the ladders can be regarded as the major error-prone causative agent. The progress in automation will probably avoid these errors in the future. The quality of the results attained can be attributed to the use of commercial dot-blot systems with appropriate controls and the use of sequenced allelic ladders.

The highly reproducible results achieved in the paternity case, despite the different frequencies used, is a remarkable finding and a good signal towards the progress in statistical standardization.

These collaborative exercises together with the Quality Control Programme have proved to be extremely valuable and clearly improve the quality of the medico-legal expertise in forensic genetics in Spain and Portugal.

References

Bär W, Brinkmann B, German U, Henke L, Kratzer A, Puers C, Scheithauer R, Wenzel R, Wiegand P (1992) GEDNAP (German DNA Profiling Group) II. Rechtsmedizin 3:6–9

Brinkmann B (1991) Quality control in DNA investigations: results of blind trials in Europe. Proceedings from the Second International Symposium on Human Identification. Promega, Madison WI, pp 211–225

Brinkmann B, Bär W, Feuerbach M, Henke L, Hochmeister M, Kratzer A, Rand S, Rothämel T, Weisser HJ, Wiegand P (1993) GEDNAP III. Rechtsmedizin 3:33–39

Carracedo A, Rodriguez-Calvo MS, Pestoni C, Lareu MV, Bellas S, Salas A, Barros F (1996) Standardization of forensic DNA analysis in Europe. Proceedings from the first European symposium on human identification. Toulouse. (In press)

Elder JK, Southern EM (1987) Computer-aided analysis of one-dimensional restriction fragment gels. In: Bishop MJ, Rawlings CJ (eds) Nucleic acid and protein sequence analysis. IRL Press, Oxford, pp 165–172

Gill P, Woodroffe S, Bär W, Brinkmann B, Carracedo A, Erikssen B, Jones S, Kloosterman A, Ludes B, Mevag B, Pascali V, Rudler M, Schmitter H, Schneider P, Thompson J (1992) A report of an international collaborative experiment to demonstrate the uniformity obtainable using DNA profiling techniques. Forensic Sci Int 53:29–43

Gill P, Kimpton C, d'Aloja E, Andersen J, Bär W, Brinkmann B, Holgerson S, Johnsson V, Kloosterman AD, Lareu MV, Nellemann L, Pfitzinger H, Phillips CP, Schmitter H, Schneider PM, Stenersen M (1994) Report of the European DNA profiling group (EDNAP)-towards standardisation of short tandem repeat (STR) loci. Forensic Sci Int 65:51–59

Gómez J, Carracedo A (1996) A review of the collaborative exercises of the Spanish and Portuguese ISFH working group. In: Carracedo A, Brinkmann B, Bär W (eds) Advances in forensic haemogenetics 6. Springer, Berlin Heidelberg, pp 695–699

Kimpton C, Gill P, d'Aloja E, Andersen J, Bär W, Holgerson S, Jacobsen S, Johnsson V, Kloosterman AD, Lareu MV, Nellemann L, Pfitzinger H, Phillips CP, Rand S, Schmitter H, Schneider PM, Stenersen M, Vide MC (1995) Report on the second EDNAP collaborative STR exercise. Forensic Sci Int 71:137–152

Puers C, Wiegand P, Bär W, Brinkmann B, Gujer HR, Kloosterman AD, Rand S, Rothämel T, Schmitter H, Weisser HJ (1992) GEDNAP (German DNA Profiling Group)-Studie I. Rechtsmedizin 2:99–103

Schneider PM, Fimmers R, Woodroffe S, Werrett DJ, Bär W, Brinkmann B, Eriksen B, Jones S, Kloosterman A, Mevag B, Pascali V, Rittner C, Schmitter H, Thompson J, Gill P (1991) Report of a European collaborative exercise comparing DNA typing results using a single locus VNTR probe. Forensic Sci Int 49:1–15

Syndercombe-Court D, Lincoln P (1996) A review of the 1991–1994 paternity testing workshop of the English speaking working group. In: Carracedo A, Brinkmann B, Bär W (eds) Advances in forensic haemogenetics 6. Springer, Berlin Heidelberg, pp 683–686

Wiegand P, Ambach E, Augustin C, Bratzke H, Cremer U, Edelmann J, Eriksen B, Germann U, Haas H, Henke L, Holtz J, Keil W, Kreike J, Nagy M, Prinz M, Rand S, Rothämel T, Scheithauer R, Schneider HR, Schürenkamp M, Teifel-Greding J, Bär W (1995) GEDNAP IV and V. The 4th and 5th stain blind trials using DNA technology. Int J Legal Med 108:79–84