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The 1998–1999 collaborative exercises and proficiency testing program on DNA typing of the Spanish and Portuguese Working Group of the International Society for Forensic Genetics (GEP-ISFG)

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Abstract

A total of 28 laboratories (labs) submitted results for the 1998 collaborative exercise and the proficiency testing program of the Spanish and Portuguese Working Group of the International Society for Forensic Genetics (GEP-ISFG) group. This number increased to 46 labs in 1999. Six bloodstains were submitted, each one with 200 μl soaked in cotton except the sample no. 6 submitted for DNA quantification which had 2 μl. One of the samples was a mixed stain. A paternity testing case and a criminal case in the 1998 trial (GEP'98) and two paternity testing cases in 1999 (GEP'99) were included and the statistical evaluation of the evidence was requested in both cases. In the GEP'99 trial, a theoretical paternity testing case was included. A total of 52 DNA genetic markers were used by the participants in the GEP'98 trial, which increased to 101 in GEP'99. Despite this increasing number of participating labs, results remained quite satisfactory. All the labs used PCR-based DNA polymorphisms with an increasing number of markers, obtaining good results. SLPs were used by a decreasing number of labs but the results indicated a good level of expertise despite the different protocols used.

Good results were also obtained for mtDNA despite the difficulties presented by the samples due to the presence of length heteroplasmy in some samples in both trials. The detection of heteroplasmy should, however, be improved.

Similar conclusions were reached for both, the paternity and the criminal case by all the labs. Common methodologies for the statistical evaluation of the paternity case were used and the paternity index and the probability of paternity (with an a priori value of 0.5) reported by most of the labs. Also, a great uniformity was found in the evaluation of the criminal case despite the lack of a specific hypothesis in the design of the exercise. Some errors in statistical programs or in calculations were detected in a theoretical paternity case included in the GEP'99 trial for statistical analysis.

Keywords: DNA polymorphisms; Standardization; Collaborative exercise; Proficiency testing; GEP-ISFG

1. Introduction

The Spanish and Portuguese Working Group (GEP) of the International Society for Forensic Genetics (ISFG) comprises forensic genetic laboratories (labs) from Spain, Portugal, France and most of the Portuguese and Spanish speaking countries in America. A total of 60 labs from 15 Iberoamerican countries are members of the group.

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The same as other working groups of the ISFG [1], since 1992, the GEP-ISFG 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 [2,3]. A total of seven exercises (GEP'93–GEP'99) have been carried out until now. One of the consequence of these exercises was the creation of a proficiency testing programme in Spain and Portugal in 1995 which was carried out simultaneously with the GEP collaborative exercises.

The number of participating labs increased from 10 in the first exercise (GEP'93) to 28 in GEP'98 and 46 in GEP'99. In this paper, the results of the exercises are summarized and the characteristics of the proficiency testing programme described.

2. Material and methods

2.1. Samples and forensic cases

A total of six bloodstains were distributed to participants in both exercises. Each bloodstain was prepared by applying 200 μl of whole blood onto cotton cloth and was air dried before distribution. Sample no. 6 was included for DNA quantification purposes and consisted of only 5 μl.

There are 31 labs agreed to participate and 28 sent results within the deadline previously fixed for the exercise in GEP'98 and 54 labs agreed to participate in GEP'99 with results received from 46 labs (Fig. 1).

Participants and people involved in the development of these exercises are listed in Appendix A.

A criminal case and a paternity case were included in the GEP'98 trial and two paternity cases (one an inclusion and the other an exclusion) in the GEP'99 exercise.

The labs were free to use DNA polymorphisms currently in use in their protocols, including SLPs, STRs of autosomal and Y chromosome and mtDNA (HV I and HV II regions).

All labs were given an anonymous number and they were requested to fill in a questionnaire with all the technical details as well as statistical details of the analysis.

The whole process was previously discussed and agreed to by the GEP-ISFG assembly and the organization as well as the proficiency testing validation was carried out by the Centro de Garantía de Calidad (National Institute of Toxicology, Ministry of Justice, Madrid, Spain).

2.2. DNA extraction and quantification

The majority of laboratories (26/28 in GEP'98, 42/46 in GEP'99) used phenol–chloroform (60%) and Chelex 100 (40%). In some cases, phenol–chloroform extraction was followed by Centricon-100 purification. Some of the labs used both phenol–chloroform and Chelex.

The majority of laboratories (25/28 in GEP'98, 40/46 in GEP'99) carried out quantification of extracted DNA, slot–blot with Quantiblot (Perkin-Elmer) being the method most commonly used (12/28 in GEP'98, 18/40 in GEP'99), spectrometry–fluorometry was used by eight labs in GEP'98 and four in GEP'99 and other hybridization methods of quantification (Dynaquant, Genequant) used by the rest of participating labs. Important differences, both in GEP'98 and GEP'99, were observed in the total amount of DNA recovered from sample no. 6.

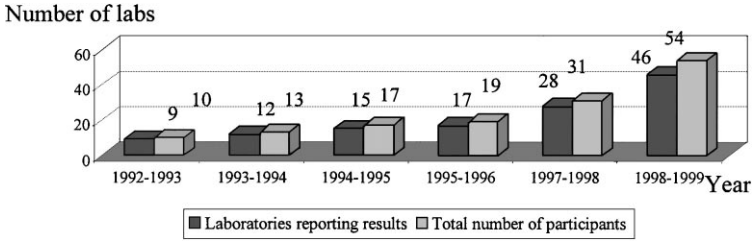

Fig. 1. Laboratories reporting results and total number of participants in the annual exercises of the Spanish and Portuguese Working Groups of the International Society for Forensic Genetics (GEP-ISFG).

2.3. DNA polymorphisms

All the participating labs used STRs. SLPs were used by 5/28 labs in GEP'98 and 8/46 labs in GEP'99. The mtDNA was also reported by five labs in GEP'98 and by 16 labs in GEP'99. Table 1 summarizes the systems utilized by the different labs.

2.4. RFLP methodology

The number of labs using RFLP methodology continuously decreased during the last four exercises (45% in GEP'94, 30% in GEP'96, 18% in this exercise).

The five participating labs followed very different protocols. Different types of agarose and different agarose concentrations (from 0.7–1%) were used as well as different gel thickness (from 0.3 to 14 mm), different ladders, different electrophoretic as well as temperature conditions and different running distances (115–280 mm) and times (from 15 to 48 h). Ethidium bromide was only used after running the gels.

Sizing of fragments was carried out using a variety of methods (manual and automated systems).

Table 1
DNA markers used by the participating laboratories

Systems[a]	GEP'98	GEP'99	Systems[a]	GEP'98	GEP'99	Systems[a]	GEP'98	GEP'99
YNH24	5	5	D21S11	8	18	IFNAR-ALU	–	1
MS43	4	4	D1S1656	5	7	DXS8076	–	1
MS31	4	3	D5S818	7	21	DXS1050	–	1
MS1	4	5	ACTBPII(SE33)	4	4	DXS8114	–	1
MS205	1	3	Col2A1	1	–	DXS995	–	1
MS8	2	2	ApoB	1	1	DXS1002	–	1
G3	3	–	YNZ22	1	–	D18S1270	–	1
V1	1	–	DYZ1/DXS424	1	10	D6S366	–	1
LH1	2	–	HUMPRTB	3	14	DYS388	–	1
TBQ7	1	–	D16S539	2	24	D12S1090	–	3
D17S79	1	–	D19S253	3	3	D3S1744	–	3
PH30	1	–	D18S535	2	3	D18S849	–	3
CEB42	2	–	ph30	1	–	D1S533	–	3
HLADQA1	21	26	HUMFABP	1	2	D9S304	–	3
D1S80	16	22	DYS 19	2	12	DPB1	–	1
HUMFES/FPS	24	39	DYS 385	2	3	DQB1	–	1
HUMTH01	26	42	DYS 389-I	2	9	DRB1	–	1
HUMF13A01	25	38	DYS 389-II	2	9	DRB3	–	1
HUMVWA	28	43	DYS 390	3	11	DRB4	–	1
D12S391	7	11	DYS 391	1	9	D17S250	–	1
HUMTPOX	19	39	DYS 392	1	7	D4S174	–	1
CSF1PO	17	37	DYS 393	3	10	GLUT2	–	1
D3S1358	10	19	D6366	1	–	VWF	–	1
FIBRA/FGA	14	23	D1S1612	–	1	D2S428	–	1
HUMF13B	8	16	D2S1353	–	1	D1S549	–	1
HUMLPL	5	15	D3S2387	–	1	D5S1457	–	1
Amelogenine	15	34	D3S2406	–	1	D6S502	–	1
LDLR	17	24	D4S2431	–	1	D7S796	–	1
GYPA	18	24	D4S1644	–	1	D2S1327	–	1
HBGG	18	24	D5S2501	–	1	D2S441	–	1
GC	18	24	D6S1031	–	1	D8S1106	–	1
D7S8	18	24	D8S1119	–	1	CD4	–	2
D7S820	9	36	D10S2325	–	1			
D13S317	10	35	D10S1237	–	1	mtDNA	5	15
D18S51	5	18	D14S742	–	1			
D8S1179	5	17	D15S657	–	1			

[a] Total systems=94.

The local reciprocal method [4] was used for calculations.

2.5. PCR methodology

2.5.1. DQA1 and Polymarker

Although a decreasing number of labs used DQA1 and Polymarker it should be said that they are still commonly used (GEP'98: 21 labs out of 28 participants, GEP'99: 18 labs out of 46 for DQA1, and 18/28 in GEP'98 and 24/46 using Polymarker). 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).

2.5.2. Minisatellites

PCR-based minisatellite analysis was performed by a more reduced number of labs. Col2A1, ApoB and YNZ22 were reported by only one lab in GEP'98 and also one lab reported results for ApoB and YNH22 in GEP'99. D1S80 was reported by 16 labs (GEP'98) and 22 labs (GEP'99).

All participating labs carried out the D1S80 amplification by using the AmpliFPD1S80 Amplification Kit (Perkin-Elmer Corp., Norwalk, CT). All the labs reporting results used the commercial D1S80 ladder of 27 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 fluorochrome-based detection systems.

2.5.3. STRs

STRs were used by all the participating labs in both trials. In the GEP'98 trial, the STR most widely used was VWA (28 labs), followed by TH01 (26 labs), F13A01 (25 labs) and FES/FPS (24 labs). There were 21 other STRs used by more than four labs and six other STRs by three or less labs. In GEP'99, the STR most widely used was also VWA (43/44 labs), followed by TH01 (42 labs), TPOX and FES/FPS (39 labs), F1301 (38 labs) and CSF1PO (37 labs). There were 24 other STRs used by more than three labs and 28 STRs by three or less labs.

There were eight Y STRs (not reported in the previous exercises of our group) used by three labs in GEP'98 and by nine labs in GEP'99.

Results from the amelogenin system were reported by 15 labs in GEP'98 and 34 labs in GEP'99. Most of the labs have used Perkin-Elmer or Promega kits to analyze this marker.

In general, amplification of STR markers was performed using common primers but different electrophoretic systems and allelic ladders. Commercial kits were more widely used than in previous exercises and the majority of labs (except for some specific STRs) used Promega or Perkin-Elmer kits.

There are 50% labs used automatic sequencers (ABI 373/377, ABI 310, ALF and ALF express) and the other 50% denaturing polyacrylamide gels followed by silver staining. Isotopic methods of detection were used by one single lab in both exercises. In general, the labs using manual methods used Promega kits and the labs using ABI sequencers, Perkin-Elmer kits.

All the labs reported having used sequenced allelic ladders provided by commercial companies (80%) and in some cases (20%) obtained in their own lab or from colleagues.

The tendency to use sequenced allelic ladders continues and now 100% of the labs follows the ISFG recommendations concerning their use in comparison with only 70% in GEP'94.

2.6. mtDNA

Five labs submitted results for mtDNA in GEP'98 and 16 labs in Gep'99. Most of the labs used automated sequencers (mainly ABI systems) a single lab reported having used manual sequencing with isotopic methodology. Most of the labs used the primers and conditions described in Wilson et al. (1995) for sequencing the HV I and HV II regions. Cycle sequencing was used by all the labs with Rhodamine terminators, BigDye terminators or Thermosequenase.

2.7. Validation

Results were considered to be correct when more than two labs submitted results and the majority of the labs agree with the result. In the case of doubt or inconclusive results, typing was submitted to independent labs for reference.

3. Results

3.1. SLPs

Detailed data for each band, locus and lab as well as a complete statistical report are available upon request and can also be obtained in http://www.usc.es/gep-isfh/.

Despite all the differences in methodologies, a great uniformity of results was obtained. Interlaboratory variation was low, 100% of matches achieved using a guideline of 2.5% in the GEP'98 trial. The same was true for the GEP'99 trial with the exception of two labs, one particularly out of range. It is necessary to keep in mind that fragments were sized in each participating lab.

Although it was previously thought that the good results obtained with SLPs was in part due to the use of common protocols (Gómez and co-workers [2,3]), it is now clear that even with very different protocols, similar results can be obtained and that the probable reason is simply the greater experience of the participating labs.

A lesser number of probes (six probes) was used in GEP'98 compared to previous exercises but the number increased again to 13 probes in GEP'99. However, in the latter trial, only six probes (YNH24, MS43a, MS31, MS1, MS205 and g3) were used by more than three labs.

3.2. PCR-based systems

The results of GEP'98 were as follows. No typing errors were found in dot–blot-based systems (DQA1 and Polymarker) in a total of 550 analyses. Nor were any typing errors found in D1S80, TH01, VWA, D12S391, TPOX, D3S1358, FIBRA/FGA, F13B, D13S317, D21S11, D18S51, D1S1656, D7S820, D8S1179, D5S818, D18S535, HPRTB, D16S539, DYS19, DYS389I, DYS389II, DYS385, DYS390, DYS393. No errors were found in the amelogenin system either.

Only a few isolated errors were reported which were mainly due to the use of poor quality or incorrectly named allelic ladders, to the lack of detection of intermediate alleles and also transcription errors. Errors were detected in the following systems: FES/FPS (one lab in 25 participating labs), F13A01 (one lab in 25 participating labs), D19S253 (one lab in four participating labs), CSF1PO (one lab in 17 participants), F13A01 (one lab in eight participants) and ACTBP2 (one lab in four participants).

Since the 28 labs analyzed a total of 401 STRs and 2005 samples, the total number of mistakes was of 32/2005 (1.60%) including labs using incorrectly named allelic ladders (which means errors were present in the five samples analyzed) and including laboratory 1 with a general management problem. If laboratory 1 is excluded, the total number of mistakes is 17/1965 (0.86%).

Results for DYS391, DYS392, Col2A1, ApoB, YNZ22, D6S366 and HUMFAPB were considered to be inconclusive since they were reported by only one lab.

For the GEP'99 trial, the results were as follows. An isolated error was reported in dot–blot-based systems (a mistake in the GC system in one sample). No errors were reported in HLADQA1 (18 labs), LDLR, GYPA, HBGG and D7S8 (24 labs).

Concerning the STRs, a total of 468 STRs and 2430 samples were analyzed (excluding the STRs being reported by less than three labs), the total number of mistakes was 41 (1.68%). It was a tendency towards the concentration of errors in the same labs. If two labs having reported a high number of mistakes are excluded, the rate of errors is 0.87% similar to GEP'98.

The causes for errors were the same as in the previous exercise: poor quality ladders or techniques, transcription errors and lack of detection of intermediate alleles. However, most of the labs reported correct results.

3.3. mtDNA

The results almost coincide in all participating labs despite the difficulties presented by some samples. In GEP'98 samples, two and three presented, in addition to two changes in relation with the reference Anderson sequence, length heteroplasmy in an homopolymeric tract of Cs (16,184–16,194). However, this heteroplasmy was detected by most of the labs (4/5). Sample no. 5 was also complicated with up to nine changes in comparison to the Anderson sequence for HV I and 11 for HV II. However, all these changes were correctly reported by the participants.

Fifteen labs have participated in the mtDNA GEP'99 exercise. Five different samples were analysed showing seven different polymorphisms in comparison with the CRS (Cambridge Reference Sequence): two at the first hypervariable region (HV I; positions 16298C and 16304C) and five in the second hypervariable region (HV II; 195C, 263G, insertion of one C and two C's between 303 and 309 and insertion of one C between 311 and 315). A length heteroplasmy was detected in the homopolymeric track of the HV II region between positions 303–309 in sample nos. 2, 3 and 4.

Except for two labs (13%) the results of the other labs were very similar for the two hypervariable regions with all the polymorphisms detected by the majority of the participating labs. However, only four labs reported some level of length heteroplasmy in the HV II homopolymeric track. Some typographical errors were also detected as well as inconsistencies and differences in the nomenclature used. As a general conclusion, despite the increased number of participants the results remained quite satisfactory, although the detection of heteroplasmy requires a higher effort from all labs for future mtDNA exercises.

3.4. Statistical results

GEP'98 included a criminal case in which a mixed bloodstain matched with the sample from the accused. All the participants stated in their reports the sample no. 1 to be a mixture, compatible with a mixture of blood no. 2 (victim) and no. 4 (first husband).

There are 17 labs submitted statistical results, all of them (except one) using likelihood ratios (LRs). There are 13 labs reported a single LR (H1/H2) concerning the hypothesis

- H1: the crime sample no. 1 contains DNA from the victim and the suspect;
- H2: the crime sample contains DNA from the victim and an unknown person.

There are 12 of these 13 labs reported LRs>10^{10} and one lab a LR>10^5. There are three labs gave additional LRs of different scenarios.

A paternity case with two putative fathers was also included. The alleged father no. 5 was excluded by all the participants, with a number of exclusions ranging from 1 to 13, and an average of 7.5 exclusions per lab.

The alleged father no. 4 was not excluded by any of the participants and 25 labs reported the probability of paternity (W) (with an a priori value of 0.5) and the paternity index (PI).

An PI>10^6 and W>99.99% was reported by 13 labs and 13 labs gave PI values between 10^3 and 10^6. Only one lab reported an PI<10^3.

In GEP'99, a paternity case was also included. All the labs reported inclusion and gave PI and W values with an a priori value of 0.5. PI>10^{10} were reported by seven labs. The majority of the participating labs (26) reported PI values between 10^6 and 10^{10}. PI values between 10^3 and 10^6 were reported by 12 labs. Another two labs reported PI values of only 248.8 (W=99.65%) and 190.79 (W=99.47%).

In the GEP'99 trial, a theoretical paternity case was included and the frequencies of the alleles given for statistical evaluation. A number of theoretical mistakes in participants were detected and some errors in some statistical programs were also found.

3.5. Population data

The majority of the labs used their own population data for all or some of the markers, although many others used published population data from other groups or commercially available data for Caucasians.

Population data compilation is being performed by the group for nuclear DNA polymorphisms (3), mtDNA and Y chromosome polymorphisms. The results of the compilation can be seen on the group's website (http://www.usc.es/gep-isfh/).

3.6. The quality control scheme

In conjunction with the GEP'95 exercise, a proficiency testing (PT) programme was set up. This PT program is carried out simultaneously with the GEP collaborative exercises.

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.

Accreditation reports include only the markers successfully reported. There was a general agreement to only use the markers validated under the PT programme in the respective labs for forensic casework.

4. Discussion

Since 1992, the GEP-ISFG group has been distributing six samples to participants, usually by December. Data from labs is reported by May of the following year, and the results are discussed in June during the annual meeting of the GEP-ISFG group.

Each lab is given a code in order to preserve anonymity. Participants in the exercise are 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 labs increased from nine labs submitting results out of 10 participants (GEP'92), 17 labs out of 19 participants (GEP'95) to 28 labs out of 31 participants (GEP'98) and 46 labs out of 54 participants in GEP'99.

The number of DNA polymorphisms included in the exercises increased from four in GEP'92 to five in GEP'93, nine in GEP'94, 25 in GEP'95 to 52 in GEP'98 and 101 in GEP'99.

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 labs submitting results for SLP analysis continuously decreased during the last four exercises. However, the number of labs submitting results for PCR-based analysis increased continuously as did the number of STRs. The continuous increase in the number of DNA polymorphisms carried out may represent a problem in the implementation of PT programs, but it is expected that the number will remain constant due to the acceptance of kits from commercial companies and the restriction of the PT program, since only with agreement from more than two labs can the system be included into the PT program.

Results for SLPs remained within reasonable standards in all exercises. In these exercise, despite the differences in methodologies, a great uniformity of results was obtained with isolated exceptions.

Data from PCR-based polymorphisms was promising with only a few errors detected despite the high number of systems studied. The most important cause of errors was the lack of detection of intermediate alleles, however, the human factor in reading results can also be regarded as one of the major error-prone causative agent. The progress in automation will probably avoid these errors in the future.

The results in GEP'99 were slightly worse than in GEP'98 probably due to the inclusion of many new labs without experience in the trial.

Despite the difficulties of the mtDNA case, the results are also very satisfactory. There was a significant increase in the number of participants. However, the results were also better in the GEP'98 trial for mtDNA. Heteroplasmy was detected in these exercises for a few labs but a greater effort should be made regarding this.

The conformity of results achieved in the paternity case and in the criminal case, despite the different frequencies used, is a remarkable finding and a good indication of progress in statistical standardization. The theoretical paternity case included in the last trial proved to be a good exercise to detect statistical problems and software errors. This indicates that a greater effort must be made in this area.

These collaborative exercises together with the 'quality control programme' have proven to be extremely valuable and clearly improve the quality of the medico-legal expertise in forensic genetics in Iberoamerican countries.

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Appendix A.

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