A REVIEW OF THE COLLABORATIVE EXERCISES OF THE SPANISH AND PORTUGUESE ISFH WORKING GROUP

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1.- Introduction

The Spanish and Portuguese working group of the ISFH comprises a total of 22 Laboratories from Spain, Portugal and some South American countries. Practically all the casework in forensic genetics in Spain (15 Labs) and Portugal (5 labs) is carried out in these laboratories. Since 1990 the group have organized collaborative exercises in DNA polymorphisms with the aim of progresion in standarization and for the discussion of technical and statistical problems as a first step towards a quality control program in Spain and Portugal.

Three exercises have been carried out up to now. In all these exercises 6 bloodstains were sent to different laboratories for DNA extraction and DNA polymorphism analysis. The systems used in the first exercise (1991-1992) were two SLPs (YNH24 and MS43a) and two PCR- based systems (HLADQA1 and D1S80). HUMTH01 was added in the second exercise (1993-1994). In the last exercise (1994-1995) Polymarker, HMVWA31, HMF13A1 and HUMFES were added to the above mentioned systems. In this exercise 15 labs have participated with very satisfactory results.

The main aim now of the Spanish and Portuguese labs is now to organize a quality control program for both countries.

This quality control program is planned for December 1995 toghether with the 4th collaborative exercise of the group. For this next trial a statistical exercise has been designed in addition to the standard exercise.

These collaborative exercises together with other activities of the group (statistics, legal regulation in Spain and Portugal) have proved to be extremely valuable and have clearly improved the quality of the medical-legal work in forensic genetics in Spain and Portugal.

2.- Materials and methods

The exercise scheme

A total of six bloodstains were distributed to participants. Each bloodstain was made with whole blood placed on a cotton cloth and air dried.
Laboratories were free to use any method of electrophoresis and detection, but each laboratory was requested to inform about the methodology used in the analysis of SLP's as well as PCR-based system.
The exercises were organized once a year.
Each laboratory was given a code in order to preserve anonimate.
Participants in the exercise were supplied with a data sheet, which included methodological questions for the loci included on each exercise (primers, ladders, buffers, gel characteristics, detection system, etc.)

DNA systems included on each exercise

The number of DNA polymorphisms included increased : 4 in the first exercise, 5 in the second and 9 in the third.

1: YNH24 / Hinf I, MS43 / Hinf I, HLADQA1, D1S80

2: YNH24 / Hinf I, MS43 / Hinf I, HLADQA1, D1S80, HUMTH01

3: YNH24 / Hinf I, MS43 / Hinf I, HLADQA1, PM, D1S80, HUMTH01, HUMF13A01, HUMVWA, HUMFES

Participant laboratories

10 laboratories expressed an interest in participating in the first exercise, 16 in the second and 17 in the last exercise. 10 laboratories returned results in the first exercise, 10 in the second and 15 in the last one.

3.- Results

As the third exercise is the most significative due to the greater number of participating laboratories and DNA loci included, in this section we are going to include only the methodology used and the results obtained in this last exercise.

3.1.- PCR

HLADQ1 and PM:

The amplification and typing of the HDLA-DQA1 and PM systems were performed using the AmpliType HLA-DQA1 and PM Forensic DNA Amplification and Typing Kits (Perkin Elmer Corporation, Norwalk,CT) for all laboratories.

D1S80:

All participant laboratories performed the D1S80 amplification using the AmpliFLPD1S80 Amplification Kits (Perkin Elmer Corporation, Norwalk,CT). The typing of the PCR products was carried out by diferent electrophoretic systems.

10 laboratories used native PAGE and silver stain, 2 laboratories used SDS- PAGE and silver stain, 1 laboratory used agarose (2% Metaphor) and ethidium bromide, and 1 laboratory used Hydrolink gel and an authomatic sequencer.

All the laboratories, except one, classified the alleles by comparison with the commercial D1S80 ladder of 27 alleles (Perkin Elmer Corporation, Norwalk,CT).

STR SYSTEMS:

The amplification of STR loci was performed using different primers and detection systems depending of the laboratories. Basically, the laboratories can be classified as those that used the primers included in the GenePrint STR System (Pomega Corporation, Madison, WI, USA) or similar lab-synthesized primers and silver stain detection (10 lab.) and those that used primers of the EDNAP exercise and authomatic detection (4 lab.)

12 laboratories used denaturing PAGE (1 lab used Hydrolink) electrophoresis followed by silver stain or authomatic detection and 2 laboratories used native PAGE electrophoresis for the analysis of PCR products.

The classification of the alleles was performed with commercial ladders (GenePrint STR Systems, Promega Corporation) (5 lab.), lab-made ladders (4 lab) or internal standards (3 lab).

3.2. -Single locus probes

The fragments sizes for YNH24 and MS43 and deviation from the mean for each laboratory are shown in Table 1. In Table 2 the percentage of deviation from the mean is presented

The table can be used to determine the "bin" size needed for 2 laboratories to compare results. For example, laboratories 1 and 3 need a bin of 2,64 % to match the high molecular weight fragment (10408 y 10135 bp respectively).

Table 1. FRAGMENT SIZES (YNH24 AND MS43) AND DEVIATION FROM THE MEAN FOR EACH LABORATORY

Laboratories							Mean	SD	CV (%)
1	3	5	6	8	11	13			
10408	10135	10280	10328		10221	10511	10313,8	133,9	1,30
10166	9807	9974	10091		10221	10511	10128,3	238,7	2,36
10286	9992	10065	10015		10077	10117	10092	105,0	1,04
9870	9673	9888	9938		9938	10029	9889,3	119,5	1,21
9528	9293	9403	9297		9306	9563	9398,3	121,5	1,29
9230	9094	9261	9258		9309	9693	9307,5	202,4	2,17
9230	9173	9122	9119		9079	9178	9150,2	53,8	0,59
8522	8542	8480	8448		8463	8443	8483	40,6	0,48
7941	7980	7953	7887		7907	7981	7941,5	38,3	0,48
7659	7505	7548	7487		7497	7562	7543,	64,1	0,85
5509	5447	5493	5496	5250		5534	5454,8	104,3	1,91
5279	5211	5237	5143		4885	5238	5165,5	144,6	2,80
4999	4972	4961	4915		4990	4958	4965,8	29,6	0,60
4637	4575	4603	4591	4400		4570	4562,7	83,2	1,82
3737	3706	3701	3682	3625		3727	3696,3	40,0	1,08
3465	3458	3471	3448	3400		3464	3451	26,2	0,76
3053	3078	3068	3047	3000		3039	3047,5	27,2	0,89
3062	3039	3062	3060	3000		3040	3043,8	24,0	0,79
3034	2967	2979	2938	2925		2949	2965,3	38,8	1,31
2968	2942	2969	2976	2925		2945	2954,2	19,9	0,67
2723	2700	2732	2729	2700		2716	2716,7	14,0	0,52
2668	2650	2660	2625	2650		2635	2648	15,8	0,60
2641	2589	2614	2565	2550		2575	2589	33,5	1,30
2441	2414	2426	2426	2400		2417	2420,7	13,8	0,57
							Mean CV		**1,14**

FRAGMENT SIZES ARE RANKED IN DESCENDING ORDER

Table 2. DEVIATION FROM THE MEAN (%)

Laboratories							Range (%)
1	3	5	6	8	11	13	
0,91	-1,73	-0,33	0,14		-0,90	1,91	3,71
0,37	-3,17	-1,52	-0,37		0,91	3,78	7,18
1,92	-0,99	-0,27	-0,76		-0,15	0,25	2,94
-0,20	-2,19	-0,01	0,49		0,49	1,41	3,68
1,38	-1,12	0,05	-1,08		-0,98	1,75	2,91
-0,83	-2,29	-0,50	-0,53		0,02	4,14	6,59
0,87	0,25	-0,31	-0,34		-0,78	0,30	1,66
0,46	0,70	-0,04	-0,41		-0,24	-0,47	1,17
-0,01	0,48	0,14	-0,69		-0,43	0,50	1,19
1,54	-0,50	0,07	-0,74		-0,61	0,25	2,30
0,99	-0,14	0,70	0,75	-3,76		1,45	5,41
2,20	0,88	1,38	-0,44		-5,43	1,40	8,07
0,67	0,12	-0,10	-1,02		0,49	-0,16	1,71
1,63	0,27	0,88	0,62	-3,57		0,16	5,39
1,10	0,26	0,13	-0,39	-1,93		0,83	3,09
0,41	0,20	0,58	-0,09	-1,48		0,38	2,09
0,18	1,00	0,67	-0,02	-1,56		-0,28	2,60
0,60	-0,16	0,60	0,53	-1,44		-0,13	2,07
2,32	0,06	0,46	-0,92	-1,36		-0,55	3,73
0,47	-0,41	0,50	0,74	-0,99		-0,31	1,74
0,23	-0,61	0,56	0,45	-0,61		-0,02	1,19
0,76	0,08	0,45	-0,87	0,08		-0,49	1,64
2,01	0,00	0,97	-0,93	-1,51		-0,54	3,57
0,84	-0,28	0,22	0,22	-0,85		-0,15	1,71
Mean lab.deviation:							
0,87	**-0,39**	**0,22**	**-0,24**	**-1,58**	**-0,63**	**0,64**	**3,22**

FRAGMENT SIZES (YNH24 AND MS43) RANKED IN DESCENDING ORDER FOR EACH LABORATORY

5.- Conclusion

The number of DNA Markers used and the number of laboratories participating increased and in spite of this results remained satisfactory.

Most of the laboratories used the EDNAP electrophoresis protocol for SLP analysis and the uniformity of results is excellent.

For STRs despite the different primers and methods used for detection there is a general agreement in ladders and nomenclature and the correlation of results is also excellent. Other PCR-bases polymorphisms were studied using commercially available kits also with good results.

Only a few isolated errors were found in the last exercise. No errors were reported in the following systems : HLA-DQA1, LDLR, D7S8, GC, HUMTH01, HUMF13A01 and HUMFES.

Laboratories participating in these exercises

Cátedra de Medicina Legal y Toxicología. Granada
Cátedra de Medicina Legal, Facultad de Medicina. Alicante
Departamento de Biología Molecular, Pharma Gen S.A. Madrid
Dpto. de Medicina Legal y Toxicología. Facultad de Medicina. Universidad Complutense, Madrid
Dpto de Medicina Legal, Cátedra de Medicina Legal y Toxicología. Facultad de Medicina. Zaragoza
Dpto. de Biología Celular y C.C Morfológicas. Facultad de Medicina y Odontología. Leioa (Vizcaya)
Instituto de Medicina Legal de Coimbra (Portugal)
Instituto de Medicina Legal de Lisboa (Portugal)
Instituto de Medicina Legal do Porto (Portugal)
Instituto de Medicina Legal, Facultad de Medicina. Santiago de Compostela
Laboratorio de la Policia Científica. Lisboa (Portugal)
Laboratorio Policia País Vasco, Lab U.T.A.P, Dep. del Interior. Gobierno Vasco. Bilbao
Sección de Biología. Instituto Nacional de Toxicología. Departamento de Sevilla
Sección de Biología. Instituto Nacional de Toxicología. Departamento de Barcelona
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