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

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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).