GARDA1 (D2S44 SBA) - HARMONIZATION OF PROTOCOLS AND A COLLABORATIVE DATABASE

V.L. PASCALI and R. DOMENICI*

Gruppo Ematologi Forensi Italiani (GEFI), Dept. Forensic Medicine, Catholic University, Largo F. Vito 1, 00168, Rome, Italy; Dept. Forensic Medicine, University of Pisa.

By initiative of the Italian working group in Forensic Hemogenetics (G.E.F.I.), a collaborative experiment was promoted, aimed at standardizing protocols and procedures of Southern Blot Analysis (SBA) for addressing single locus probes (SLPs). In 1991, sixteen laboratories throughout Italy agreed to adopt the protocol proposed by the EDNAP network (1) and to produce compatible data. The procedure adopted in the experiment was also recognized as the official GEFI protocol for releasing evidentiary expertises in courtrooms.

MATERIALS AND METHODS

Each partecipating laboratory agreed to process at least 45 blood samples of random individuals and one genomic control (K562, Promega). Standard extraction procedures were used, then the DNA digestion was carried out with tenfold Hinf I excess and the separation of restriction fragments was achieved by an 0.8% submarine agarose gel electrophoresis. It was intended that all participants had to perform at least five electrophoretic run. Every gel contained the same molecular weight ladder (1Kb ladder, BRL) and the genomic control. Hybridization was carried out with freely chosen procedures, but using the same D2S44 probe batch under stringent conditions. Autoradiograms were sent to a coordinating laboratory where they were processed using a semi-automatic procedure so as to create a common database. Keys of access to the database were several error measurements reflecting intra- and inter-laboratory variance units.

RESULTS AND DISCUSSION

The general profile of frequencies emerging from the collaborative experiment is shown in Fig.1. This figure shows that, according to other Caucasian populations, Italians have two major peaks of frequency at 2.8 Kb and 4.0 Kb. The average heterozygosity of the system was 99.5%. A total of 651 individuals were typed, and most laboratories contributed more autoradiograms than the minimum figure required to comply with the experiment. All laboratories were found to share comparable measurement errors in ascribing molecular weight to bands (0.25-1.75% error in intralaboratory experiments; from

1.3 to 1.7% error in the overall experiment). Following the experiment, an electronic archive was established and distributed to all participants, who agreed to store their future data into updated versions of the archive. The archive is presently near to filing a representative group of individuals from five Italian regions.
Like other previous experiments of the kind, this initiative has shown that standardization may be achieved among a relatively large number of laboratories. Further trials will follow, dealing with creation of frequency data, harmonization of procedures and quality controls.

REFERENCES

(1) Gill et al., Forensic Science International, 53, 29-43, 1992.

Figure 1
The general profile of frequencies emerging from the present collaborative experiment is sketched in this figures. As shown two major peak of frequency are present at 2.8 and 4.0 Kb, with a 0.12 frequency value for the most common allele. The use of the proposed common protocol and the creation of a permanent archive of frequencies let the majority of Italian Laboratories involved in DNA analysis to reach a satisfactory level of standardization.