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Second DNA recommendations

1991 report concerning recommendations of the DNA commission of the International Society for Forensic Haemogenetics relating to the use of DNA polymorphisms

"The time has come" the Walrus said "to speak of many things": Lewis Carroll.

1. Introduction

In 1989 the ISFH published its first recommendations concerning the application of DNA investigations to forensic science (Forensic Sci Int, 1989, 43: 109–111; Vox Sang, 1989, 57:276–277; Biotech-Forum 1989, 6:111–112). It was obvious, even at the time of publishing, that these guidelines would need revision and updating because of the rapidly changing situation with regards to DNA technology. Since then the DNA commission of the ISFH has met twice with the aim of improving the recommendations so that they encompass the new developments in this field. This report is concerned primarily with the detection of DNA polymorphisms by restriction fragment length polymorphism (RFLP) analysis. It also contains general recommendations applicable to all DNA polymorphism analysis.

2. Definitions of genetic systems and documentation

A genetic locus is defined by a segment of unique DNA sequence that occupies a specific position on a chromosome. Genetic polymorphism at the DNA level can generally be divided into two categories:

a) polymorphism in sequence resulting from nucleotide base substitutions, and

b) polymorphism in sequence resulting from insertions or deletions of a nucleotide or nucleotides.

Among the most informative DNA polymorphisms for identification purposes are insertion/deletion polymorphisms containing variable numbers of tandem repeat (VNTR) sequences.

2.1 Definition of systems and alleles

a) DNA polymorphism detection systems can be divided operationally into two groups: single locus systems (SLS) and multi-locus systems (MLS). With the latter, polymorphisms at multiple loci are detected simultaneously.

b) A DNA single locus system is defined by the designation of a genetic locus and the information needed to detect allelic variation at that locus. RFLP systems are defined by the probe and restriction enzymes used for the typing. Systems based on amplification by the polymerase chain reaction (PCR) are defined by the sequences of the PCR primers and the method used for detecting sequence polymorphism.

c) At VNTR loci "alleles" are defined by DNA fragments of discrete length, which are inherited in agreement with a formal genetic model. They are usually represented by one or two restriction fragments of a given size generated by the use of one enzyme (or two enzymes in double digestions) and detected with one probe.

d) VNTR "allele" designation should be preferentially in kilobase size, but other methods can be applied if proven to be more appropriate (e.g. Rf, molecular weight or allele number).

e) Multi-locus probes recognize genomic sequences which are usually distributed throughout the entire genome. Multi-locus typing gives a pattern of bands, the so-called "DNA-fingerprint". Although the pattern of bands (restriction fragments) is genetically determined, it is not possible to specify the genetic locus from which each band originates. Accordingly, the bands in a multi-locus pattern cannot be defined explicitly by a formal genetic model.

2.2 General requirements

It is generally recommended that any method used should be based on an established protocol.

2.2.1 Requirements relating to genetics

a) DNA polymorphisms should be defined by family and population studies. At least 500 meioses and an adequate population sample should have been tested and published, before a polymorphism can be introduced into paternity testing.

b) The chromosomal localisation and linkage data to other polymorphisms used in paternity testing should be available. This information should be documented in the publications of the International Human Gene Mapping Workshop.

c) For RFLP systems, the description must include: information on the probe and restriction enzyme and information on the size of constant and variable fragments. The description must further include sufficient data to define the locus, proof of Mendelian inheritance, "allele" or haplotype frequencies, frequencies of mutations and/or recombinations and a check using a suitable statistical procedure that the population is not out of genetic equilibrium.

d) Collaboration and exchange of data should be encouraged to establish the usefulness of a system and comparability of data.

2.2.2 Requirements for RFLP methodology and standardization

a) Intactness of the individual genomic DNA before restriction enzyme digestion and complete digestion of the DNA should be assured by appropriate control experiments. It is recognized that there are situations where this may not always be possible (e.g. stain work). Under these circumstances analysis can still be carried out but the results should be interpreted with caution.

b) Alleles are detected by means of conventional Southern blot analysis or comparable methods.

c) A human control DNA of known allele composition should be included on each gel. Commercially available cell lines (eg K 562) or other control human DNA are considered to be suitable.

d) Size markers with discrete fragments of known size should span and flank the entire range of the DNA system being tested. The size marker must have an adequate number of fragments and should be placed at regular intervals to correct for possible distortions across the gel. The number of bands and the spacing of bands cannot be precisely stated and must be left to the discretion of the operator depending on the system in question. If a fragment lies outside the standard ladder this cannot be assigned an accurate size but should simply be coded as lying above or below a particular fragment size.

e) No particular method of fragment size measurement is recommended. However, for RFLPs automatic methods would be preferred to manual methods due to their precision and reproducibility but for systems with discrete alleles visual methods can be more convenient.

2.2.3 Establishment of a population data base

Each laboratory should construct its own data base for appropriate local populations. Such data bases should be composed of not less than 100 individuals.

The population sample should be representative of the relevant local population(s). Data bases for different local populations should not be merged until it can be demonstrated that it is statistically acceptable to do so. Raw data on RFLP fragment sizes should designate band sizes to at least 10 bp resolution

3. Recommendations for paternity testing

3.1 Single locus systems

3.1.1 Mutation

a) The mutation rate for single locus systems should be known.

b) Systems with high mutation rates should not be used routinely and require special considerations. Mismatches possibly due to mutational events must be adequately addressed.

3.1.2 Matching criteria

A match is considered to occur when genetic types cannot be distinguished.

a) Comparison of specimens and conclusions concerning matches in paternity cases can be made by (purely) visual comparison (side-to-side and/or co-electrophoresis). In addition, a numerical evaluation is recommended and is essential when comparison is made from different gels.

b) The initial conclusion obtained by visual comparison of bands may be confirmed by numerical methods such as Bayes, sliding or fixed windows as long as the prerequisites and limitations of each method are taken into account. By definition 2 bands to be compared can also be called a match if they fall within the limits of the match-window.

c) The significance of an inconclusive or borderline determination may be estimated by the Bayesian approach using correlation and standard deviation of the band measurements in combination with rehybridisation using other probes.

3.1.3 Fragment frequency

a) A discrete allele system can be unambiguously resolved by comparison with suitable ladders of known fragment lengths.

b) If the fragment distribution is quasi-continuous the frequency of a single fragment can be estimated according to the predefined criteria for matching, but will vary depending on the method used (i.e. floating or fixed bins).

c) Under normal circumstances it is essential to estimate the frequency of a given fragment size. This requires the application of predefined criteria which must be at least equal to or greater than the criteria for a match (e.g. if matching window = $\pm 3 \times$ sigma, the corresponding bin for frequency estimation must be at least the same or greater).

d) For small data bases with less than 200 individuals consideration should be given to the measurement errors of the frequency estimate.

e) Care must be taken that the population is truly representative to eliminate sampling error.

f) Bayesian approaches based only on observed fragment sizes may also be used.

g) It would be preferable to give the correct result concerning the fragment frequency. As this is not possible the best alternative is to give a conservative estimate.

In the sliding window approach and its variations, the centre of the window is the point corresponding to the actual measured fragment size. In the fixed bin approach the actual measured size of the fragment falls somewhere within the bin. Conservatism is achieved by moving into adjacent bins if these provide a higher frequency or creating larger bins. Binning is based on the rungs of molecular weight ladders, or on natural valleys in a fragment distribution curve.

h) Phenotype frequencies from several systems can be combined by multiplication unless it has been proven by appropriate statistical testing that there is disequilibrium between systems. At present there is no evidence of disequilibrium between the single locus systems in common use.

3.1.4 Compliance to Hardy-Weinberg equilibrium expectation

a) Population data bases should be checked using suitable statistical procedures for deviations from the norm. However, potential artefacts exist which can influence the results of such tests so that apparent significant deviations may also be due to technical problems and should be investigated further.

b) Estimation of profile (band) frequencies should be tested for dependence. If non-independence is demonstrated then frequency estimates incorporating 2 or more probes cannot be calculated by multiplication of genotype frequencies. Either haplotype frequencies should be quoted or a suitable statistical analysis used in which it has been demonstrated that it is not necessary to make the assumption of independence.

c) Obvious deviations from the expectations must be adequately addressed and taken into account for the interpretation of results.

3.2 Multi-locus systems

a) Mutation rates must be known, but it should be recognized that rates of 10^{-2} and higher can occur.

b) Band sizes and/or band patterns should be scored in an objective manner.

c) All questions of independence, allelism and linkage disequilibrium need to be addressed and used in a conservative way if included in biostatistical calculations.

d) Calculated probabilities on the statistical basis for paternity are at present still under discussion. Probabilities must be based on full genetic and biostatistical analysis as outlined in (c) otherwise only a verbal opinion on exclusion or non-exclusion should be given.

3.3 Conclusions

Paternity testing with conventional techniques is a well established procedure for producing evidence in court cases, and can continue to be used either alone or in combination with DNA polymorphisms. Providing that DNA systems have been suitably and adequately scrutinised there is no reason why DNA should not be used alone.

4. Concerns for identity testing

4.1 Specific requirements for the application in criminal investigations

In this section some specific requirements are listed with regard to the analysis of stains; however, it is stressed that many (but not all) of the requirements discussed previously are also relevant under this heading. The sections referring to family studies and to mutation rates are not applicable except in those cases where identity testing entails testing of family members such as in missing person cases.

4.2 Somatic stability

The application of DNA analysis in criminal investigations is mainly concerned with the comparison of genetic types obtained from a reference blood sample with those obtained from an evidentiary body fluid or stain. The stain may be a deposit of blood, semen, vaginal fluid, saliva or even a smear of tissue. Also the analysis of hairs, in particular hair roots, may be undertaken. The system used should therefore be shown to be somatically stable: that is, tissue specific modifications to DNA (such as methylation) must be shown not to affect genetic typing determinations.

4.3 Band matching and statistical interpretation

4.3.1 Genetic typing systems yielding discrete genetic types

Typing results should be interpreted according to standards established for blood group and protein genetic markers.

4.3.2 RFLP typing systems

In addition to the factors mentioned under paternity testing it is recognized that the reproducibility of any method may lead to the imprecise alignment of bands which are nevertheless considered to match (for definition see 3.1.2). Furthermore in stain analysis interference from substrates or degradation of the DNA can cause minor variations in band position again leading to some distortion. Such positional variations must be shown to be within the expected experimental variation and wherever possible the statistical assessment should be correspondingly adjusted. A record of the analysis, the associated results and the method of the statistical evaluation should be readily available for examination by a second independant analyst.

4.4 Analysis of semen-contaminated vaginal swabs

When differential extraction of vaginal swabs is carried out the supernatant generally containing mainly female DNA may also be tested, as it can provide a useful internal control.

5. Quality assurance

5.1 Intra-lab quality assurance

Quality assurance for the individual laboratory is essentially covered under points 2.2 and 3 of this report. For RFLP typing systems intra-assay measurement precision (i.e. inter- and intragel) within one laboratory should be evaluated and used as the basis of any statistical calculations, in determination of match window and allelic frequencies.

5.2. Minimum requirements for inter-lab comparisons

If it is required to pool or compare data from two separate laboratories, the exchange of a relatively small number (e.g. 20) of samples between laboratories allows some assessment of the ability to reproduce typing results. For RFLP systems, samples spanning the full range of allele fragments are required in order to obtain information as to whether or not results correlate between two laboratories. An adequate number of samples needs to be exchanged between laboratories if databases are to be combined or compared and it is necessary to determine by experiment how much variation exists between the laboratories over the whole range of fragment sizes.

5.3 Blind trials

Laboratories should participate in appropriate inter-laboratory trials and aim to achieve consensus results. Each laboratory should operate in its own sphere using its own database so long as the required standards have been obtained but to compare results with other laboratories the recommended criteria should have been met.

The DNA Commission consisted of the Executive Committee of the International Society for Forensic Haemogenetics (B. Brinkmann, R. Bütler, P. Lincoln, W. R. Mayr, U. Rossi) and coopted external experts (W. Bär, M. Baur, B. Budowle, R. Fimmers, P. Gill, J. Morris, S. Rand, Ch. Rittner, G. Sensabaugh)