

Current and Emerging Technology for HLA Typing

Derek Middleton

N. Ireland Histocompatibility and Immunogenetics Laboratory, Belfast, N. Ireland

Abstract

Many techniques based on polymerase chain reaction amplification have replaced the traditional serological technique in the Histocompatibility laboratory. In some tests the specificity is provided in the PCR amplification whereas in others all alleles will be amplified and the specificity is obtained in a second step. The choice of method will depend on a number of factors including time needed to provide result, resolution required and number of samples being processed. New methods or variations of existing methods are continually being developed. However the cost of equipment makes it difficult for a laboratory to decide on which methods to use.

The many methods based on polymerase chain reaction (PCR) amplification available for the detection of HLA alleles can be categorised into three types: those methods that generate a product containing internally located polymorphisms that are identified by a second technique see (a); those in which the polymorphism is identified directly as part of the PCR process although there are post-amplification steps see (b); those analysis in which different mutations generate specific conformation changes (heteroduplexes) see (c). The choice of method in a laboratory will depend on a number of factors; clinical urgency, resolution, sample numbers, budget, equipment availability, staff skills.

In Histocompatibility laboratories today the following methods are those most commonly used:

(a) Sequence specific oligonucleotide probes (SSOP). The main difference in SSOP methods is the use of a forward (individual DNA samples on a membrane/microtitre plate and each probe added separately to each membrane/plate) or reverse method (SSOP probes immobilised and one individual's DNA added to all the probes). With the latter technique (all probes to work under same conditions) and equipment required (to immobilise probes on membrane or other solid structure) it is beyond the capabilities of most laboratories to make reverse SSOP systems and this format is usually supplied commercially. The reverse approach is intended for single sample use within a short time period. In con-

trast, the forward approach requires a much longer time scale, but can accommodate large numbers of samples. One other difference in SSOP methods is whether to use one amplification and use a battery of probes to cover all polymorphisms or to rely on a tiered method whereby a first round amplification followed by probing will give resolution to the allele lineage level (i.e. 2 digits e.g. *02) and further group specific amplification and probing leads to allele determination.

Sequence based typing whereby the alleles are sequenced after amplification. On many occasions, the use of group specific amplification simplifies this sequencing technique, with only one allele from a heterozygous test sample amplified at a given time. However when this is not possible computer software based on specific nucleotide positions known to be polymorphic, is required for interpretation.

(b) Sequence specific primers (SSP). In this method under defined conditions only completely matched primers take part in amplification. Using large numbers of pairs of primers to identify specific sequences will result in presence or absence of a PCR product, which is detected with ethidium bromide staining under UV light after agarose gel electrophoresis. Positive control primers to amplify conserved regions of DNA are included in each amplification. A variation in SSP, Taqman, uses a probe (increased in length relative to length of primers favouring hybridisation of probe if target of interest is

present), prior to annealing of primers. The probe contains a reporter and a quencher fluorescent dye. Extension of primers by Taq polymerase leads to cleavage of the probe, separation of the reporter and quencher molecules and a resultant increase in fluorescence. The Taq polymerase does not cleave free probe.

(c) Heteroduplexes form because a percentage of coding strands may hybridise to non-coding strands of different alleles and vice versa. This double stranded DNA will thus be mismatched in certain regions leading to alteration in conformation of the DNA molecule, which is detected by migration in non-denaturing gels. A variation of this method reverse strand conformational analysis (RSCA) uses a known reference DNA with one strand fluorescently labelled during amplification. The amplified reference DNA is mixed with the amplified DNA of the sample being tested and the duplexes separated by polyacrylamide gel electrophoresis in a sequencer the duplexes travelling the same distance over different times. Only those duplexes with the fluorescent labelled strand of the reference are detected.

Several problems are common to many of the techniques. The vast increase in the number of HLA alleles (at April 2002 there were 243 HLA-A locus alleles, 478 B alleles, 115 C alleles and 307 DRB1 alleles) means methods need to be continually updated. One tactic to circumvent this problem is for a laboratory to know the alleles and their frequencies, which are present in the populations they are testing. For instance when we typed 1,000 individuals representative of the Caucasian population of Northern Ireland we found that only 30, 50 and 23 alleles from the HLA-A, -B, -C loci respectively were present, representing less than 13% of known HLA class I alleles. Recently to help with this difficulty a web-site www.allelefreqencies.net has been

established to record the frequency of alleles from HLA loci and other immunogenetic loci in different world-wide populations.

A further problem is that several alleles are not expressed. Once the mutations causing the non-expression are discovered they can be tested for, but DNA-based typing methods alone will not determine new examples of these so-called null alleles. Fortunately the frequency of the null alleles is less than 0.1% in most populations. Similarly many of the tests, set up for testing of existing polymorphisms will not detect new alleles. However strange patterns of results in SSP, SSOP etc will make the investigator aware of an undiscovered allele.

In the future many of the techniques now evolving to ascertain single nucleotide polymorphisms (SNP) will be applied in the HLA field e.g. wave technology, mass spectrometry, arrays. Many methods will become automated. The advent of capillary sequencers has made HLA typing by sequencing more economical and gives a quicker and larger throughput.

New technology is now being applied to the detection of HLA antibodies. Previously this was usually performed by the microlymphocytotoxicity test, which has been the standard for 30 years. At present technology using beads with various fluorescent dyes and elisa tests are becoming popular and the next step will be the introduction of beads with only one HLA antigen rather than beads with all HLA antigens of an individual present.

Laboratories will come to rely more and more on techniques supplied by commercial companies for both allele and antibody detection rather than in-house methods.