Department of Molecular Biology

UNIPATH SPECIALTY LABORATORIES

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Molecular HLA TYPING by PCR - SSP method

INTRODUCTION:

Histocompatibility testing is a specialized area of clinical laboratory science with particular relevance to transplant programs. Although entailing several different specific functions, histocompatibility testing is most often equated with the determination of the human leukocyte antigen (HLA) genotype of an individual (referred to as HLA typing)

The approaches to HLA typing have evolved over the years. This evolution has been driven by the development of technologies, allowing a more detailed and accurate assessment of an individual's HLA type and the understanding that this more detailed information has great clinical value. The clinical applications of HLA typing most commonly include (1) typing for renal transplantation as one component in the scheme to allocate deceased donor kidneys to individuals on the renal transplant waiting list because of the long-term benefit of using HLA-matched kidneys, (2) typing for hematopoietic stem cell transplantation (HSC) as it is well known that the degree of HLA match between donor and recipient has a significant influence on the risk of serious adverse events associated with this process, and (3) typing as an aid in the diagnosis of HLA-associated diseases, the best known example being the association of the HLA-B27 type with anky-losing spondylitis.

PRINCIPLE OF TEST METHOD:

At unipath specialty laboratory we use HLA SSP Typing Kits for determining HLA alleles using PCR techniques with sequence specific primers (SSP). Allele sequence-specific primer pairs are designed to selectively amplify target sequences which are specific to a single allele or group of alleles. This PCR-SSP method is based on the principle that only primers with completely matched sequences to the target sequences result in amplified products under controlled PCR conditions. The presence of amplified DNA fragment is a positive indication of the existence of allele specific sequence in the genomic DNA. On the other hand, mismatched primers do not generate amplicons. In addition to sequence specific primers, an internal control primer pair, which amplifies a conserved region of the house keeping gene, is included in every PCR reaction mix. The PCR product of the internal control primer pair serves as an indication of the integrity of PCR reaction.

We use HLA Typing Analysis Software which is gel result interpretation software that has been specially designed for users of SSP HLA typing kits. Users just indicate the positive wells on the gel-view tick box to calculate the typing result. The software also annotates the size of specific-PCR product for double confirmation at the same time.

TEST ORDER INFORMATION

TEST NAME	METHOD	SPECIMEN	REPORTING TIME
PCR HLA A B DR	PCR SSP	EDTA Blood 8 ML	72 Hours