

TEST: HLA DQ-BETA 1 (DQB1)

PRINCIPLE:

Oligonucleotide primers (SSP sets) are designed to obtain amplification of specific alleles or groups of alleles in the test DNA. Extracted DNA is mixed with the sequence specific primers, nucleotides (Deoxynucleoside Triphosphate-dNTP's) and DNA taq Polymerase. The Polymerase Chain Reaction (PCR) is performed and DNA which completely matches the specific sequences of the primers is amplified. If the DNA does not possess the specific sequences of the primers then the DNA is not amplified. Amplification is detected by the presence or absence of amplified product on an agarose gel by electrophoresis.

PCR amplification is a cyclical process that produces large amounts of nucleic acid from small quantities of starting product. PCR mimics the natural DNA replication process in that the number of DNA molecules doubles after each cycle. The typical PCR cycle consists of three steps:

1. Denaturing the template DNA by heating to a high temperature (94-95°C), generating two single strands of DNA.
2. Annealing of the target-specific primers to the two separate strands by cooling reaction mixture to between 37-65°C.
3. Extending the annealed primers with a DNA polymerase (Taq) by warming the reaction mixture to an intermediate temperature (72°C).

The product of the reaction is a discrete double-stranded DNA molecule whose ends are determined by the set of primers used. Because the copy number of the target DNA doubles after each cycle, amplification through 25 cycles would yield about a 33 million-fold increase in specific DNA product.

The PCR-SSP method is used to type HLA-Class II alleles. Some of these alleles have been associated with certain disease processes or clinical conditions. The DQ-Beta 2 allele may be associated with recurrent miscarriages in some couples.

SPECIMEN REQUIREMENTS:

Collect three tubes of blood in EDTA. A minimum of 500 µl of buffy coat from EDTA preserved whole blood is required, 1.0 ml is optimum. Heparinized blood can not be used. Specimen should be delivered to the laboratory immediately, within 24 hours. Store or send at room temperature. Peripheral blood specimens that are clotted, have not been collected in EDTA, or frozen are not acceptable.

REFERENCES:

1. DYNAL SSP General Protocol, June 1994, DYNAL Inc., Lake Success, N.Y.
2. DYNAL DQB1 – SSP 1st Sets Product Description, Specificity and Interpretation Table, September 1994, DYNAL Inc., Lake Success, N.Y.
3. Bender, et al. Clinical Laboratory Procedure Manual, NCCLS. Code GP2-A, Vol. No. 2.

Normal Range: See lab report

Turnaround Time: 10 days