

TEST: DETECTION OF THE PLASMINOGEN ACTIVATOR INHIBITOR-1 (PAI-1) 4G/5G GENE POLYMORPHISM BY PCR

PRINCIPLE

Plasminogen activator inhibitor-1 (PAI-1) is an essential regulatory component of the fibrinolytic pathway. It binds to tissue plasminogen activator and inhibits conversion of plasminogen to plasmin, which leads to decreased fibrinolysis. A single base deletion at nucleotide 675 (4G/5G) from the start of the promoter region of *PAI-1* gene is responsible for higher levels of PAI-1 protein, due to high levels of transcription. Individuals with 4G/5G (heterozygous) or 4G/4G (homozygous) genotypes have elevated levels of PAI-1, which can suppress fibrinolysis and cause an increased risk of thrombosis. PAI-1 levels may be associated with pathogenetic mechanisms of myocardial infarction, coronary artery disease and ischemic stroke. Additionally, women with pregnancy complications, such as pre-eclampsia, stillbirth and recurrent spontaneous miscarriages, were shown to be homozygous (4G/4G) for the *PAI-1* gene.

The PAI-1 4G/5G polymorphism can be identified by specific amplification of a 163-bp DNA fragment of the promoter region of the *PAI-1* gene, surrounding nucleotide 675, by PCR. The amplification product is digested with Bsl-1 restriction enzyme which yields four fragments of 107, 74, 56 and 34 bp for mutant alleles which can then be visualized by gel electrophoresis.

SPECIMEN COLLECTION AND PREPARATION:

Collect 10ml blood by standard venipuncture techniques in lavender top EDTA tubes. Specimens should be delivered to the lab immediately or stored overnight at room temperature. Shipment to the laboratory should be by the same day or overnight, at room temperature. Peripheral blood specimens that are clotted, frozen or have not been collected in EDTA tubes are not acceptable.

METHOD: Polymerase chain reaction (PCR)

REFERENCES:

1. Doggen CJ, Bertina R, Manger C et al. *Thromb Haemost* 1999;82:115-120
2. Buchholz T, Lohse P, Rogenhofer N et al. *Hum Reprod* 2003;18:2473-77
3. Dossenbach-Glaninger A et al. *Clinical Chemistry* 2003;49:1081-86

Normal Range: Reported as Normal, Heterozygous or Homozygous

Normal	74, 56, 34 bp
Heterozygous	107, 74, 56, 34 bp
Homozygous	107, 56 bp

Turnaround time: Two Weeks