

TEST: DETECTION OF THE METHYLENE-TETRAHYDROFOLATE REDUCTASE (MTHFR) A1298C GENE POLYMORPHISM BY PCR

PRINCIPLE

5,10-methylenetetrahydrofolate reductase (MTHFR) is an enzyme that acts as a substrate in the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a critical step in the homocysteine pathway. Mutations in the *MHTFR* gene cause elevated levels of homocysteine, which are, subsequently, a risk factor for venous and arterial thrombosis. The most common mutation in the *MTHFR* gene is the alanine-to-valine substitution at nucleotide 677 (C677T), which leads to a defective enzyme. The second most common mutation is the glutamate-to-alanine substitution at nucleotide 1298 (A1298C), which also leads to reduced enzymatic activity. MTHRF gene polymorphisms have been associated with vascular diseases, pregnancy complications (such as recurrent spontaneous abortions) and malformations in fetal development. Women who are heterozygous in both *MTHFR* mutations are in increased risk of developing pregnancy complications.

The MHTFR A1298C polymorphism can be identified by specific amplification of a 163-bp DNA fragment of *MTHFR* gene, by PCR. The amplification product is digested with *MboII* restriction enzyme which yields four fragments of 84, 56, 31 and 30 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. Skibola C, Smith M, Kane E et al. *PNAS* 1999;96:12810-12815
2. Friedman G, Goldsmith N et al. *J Nutr* 1999;129:1656-1661

REPORTING RESULTS:

Digestion of the 163 bp amplified fragment from the wild type *MHTFR* gene with *MboII* produces four fragments of 84, 56, 31 and 30 bp. The results are reported as follows:

<u>Results</u>	<u>Base Pairs</u>
Normal (1298AA)	56, 31, 30
Heterozygous (1298AC)	84, 56, 31, 30
Homozygous (1298CC)	84, 31, 30

Turnaround time: Two Weeks