

TEST: 5,10-METHYLENETETRAHYDROFOLATE REDUCTASE GENE MUTATION (MTHFR)

PRINCIPLE:

Severe 5,10-methylenetetrahydrofolate reductase (MTHFR) deficiency is an autosomal recessive disorder and the most common inborn error of folate metabolism. MTHFR polymorphism is the result of a missense mutation at nucleotide position 677, C \square T substitution, in the MTHFR gene. This mutation replaces Ala677 (GCC) with Val (GTC), thereby creating an *HinfI* site. Homozygosity or heterozygosity for the MTHFR mutation result in reduced enzymatic activity and decreased synthesis of 5-methyltetrahydrofolate, the primary methyl donor in the conversion of homocysteine to methionine, which causes hyperhomocysteinemia, homocystinuria and hypomethioninemia. Increased plasma levels of homocysteine are risk factors for venous and arterial thrombosis as well as cardiovascular and neurologic diseases. Hyperhomocysteinemia has been reported in women with recurrent spontaneous abortion, thus MTHFR polymorphism may be a risk factor for unexplained pregnancy loss. Underlying maternal vascular disease augments the risk for preeclampsia, one of the most common and serious complications of pregnancy. The MTHFR mutation is more prevalent in women with severe preeclampsia and increases the risk associated with the FV Leiden mutation for deep-vein thrombosis. Mutation in MTHFR gene is rather common and can be detected by molecular analysis. Specific amplification of a 198-bp DNA fragment of the MTHFR gene surrounding nucleotide 677 by PCR followed by digestion with *HinfI* yields two fragments of 175- and 23-bp for mutant alleles which can be visualized by gel electrophoresis.

SPECIMEN REQUIREMENTS:

Collect **two to three tubes of blood (lavender) with EDTA**. Heparinized blood cannot 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.

METHOD: Polymerase Chain Reaction (PCR)

REFERENCES:

1. Frosst, P., H. J. Blom, R. Milos, P. Goyette, C. A. Sheppard, R. G. Matthews, G. J. H. Boers, M. den Heijer, L. A. J. Kluijtmans, L. P. van den Heuvel, and R. Rozen. 1995 A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nature Gen.* 10:111-113.
2. Grandone, E., M. Margaglione, D. Colaizzo, G. Cappucci, D. Paladini, P. Martinelli, S. Montanaro, G. Pavone, G. Di Minno. 1997 Factor V Leiden, C T MTHFR polymorphism and genetic susceptibility to preeclampsia. *Thromb Haemost* 77:1052-1054.
3. Kupferminc, M. J., A. Eldor, N. Steinman, A. Many, A. Bar-Am, A. Jaffa, G. Fait, J. B. Lessing. 1999 Increased frequency of genetic thrombophilia in women with complications of pregnancy. *N. Engl. J. Med.* 340:9-13.

Normal Range: Reported as MTHFR Normal , MTHFR Heterozygous, or MTHFR Homozygous

MTHFR Normal – 198-bp fragment

MTHFR Heterozygous – 198-, 175- & 23-bp fragments

MTHFR Homozygous – 175- & 23-bp fragments

Turnaround time: Two weeks