



ampli set A66G MTRR ^{CE IVD} **45 tests** **cat 1302**
detection of A66G mutation of the methionine synthase reductase enzyme (MTRFR) gene

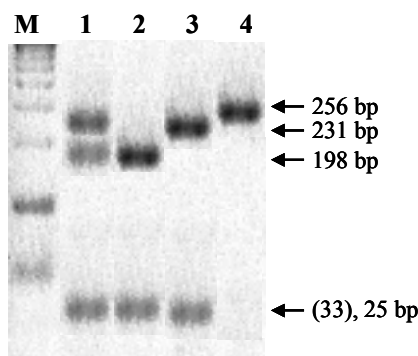
The increase of homocysteine may depend on a metabolic block of the transformation of homocysteine in cystathionine or on the unsuccessful methylation of homocysteine in methionine. The Methionine Synthase Reductase Enzyme (MTRR) is involved in the homocysteine re-methylation pathway. Fasting hyperhomocysteinemia is associated to an increased risk of vascular cerebral, peripheral and coronary diseases. Many mutations have reported in the genes involved in the homocysteine pathway, (C677T MTHFR, A1298C MTHFR). Recently, a polymorphism A66G in the MTRR enzyme was reported, which converts an isoleucine in methionine. The detection of the mutation is carried out starting with amplification using specific primers of a fragment of 256 bp, following by a restriction section due to NdeI enzyme. The mutation is confirmed by the detection of a restriction cleavage due to NdeI enzyme. The amplification product of **the normal allele is cut in 2 fragment (231 and 25 bp), whereas the mutant allele produces 3 fragment (198,33,25 bp)**. The simultaneous occurrence of this mutation and other risk factor (i.e. folic acid status), may affect the homocysteine pathway, increasing its serum level.

Principle of Assay: A) extraction of genomic DNA; B) amplification; C) enzymatic digestion; D) detection on agarose gel

Applicability: on extracted and purified genomic DNA from whole blood samples.

ANALYSIS OF RESULTS

The yield of amplification is a fragment of 256 bp. After the enzymatic digestion, the product of PCR of the normal allele is cut in 2 fragments (231 bp and 25 bp), whereas the mutated allele produces 3 fragments (198bp-33 bp-25 bp)



M) Marker ladder 100 bp.

- 1) Restriction cleavage with Nde I of a mutant heterozygous
- 3) Restriction cleavage with Nde I of a mutant homozygous
- 3) Restriction cleavage with Nde I of a normal homozygous
- 4) Not digested amplification product..

References

Gene 240 (1999) 75-88.

Mol Genet Metab. 1999 Aug;67(4):317-23.