# ampli set ApoB CE IVD 45 test cat 1354

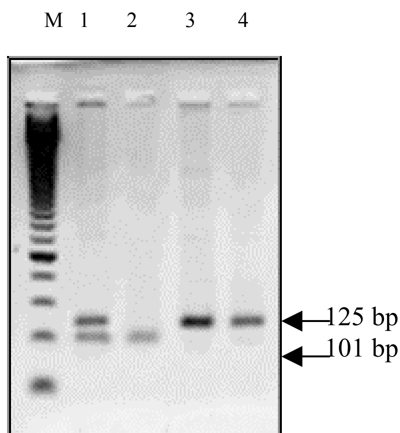
## detection of R3500Q polymorphism in the promoter of the gene Apoliprotein B (ApoB)

Apo-lipoproteins , as proteic components of plasmatic lipoproteins, play a main function in the lipid metabolism .Apo-lipoprotein B-100 (apoB-100) is the main protein component of low density lipoproteins, lipoproteins which carry almost two third of all the plasmatic cholesterol . ApoB -100 is necessary for the construction, secretion and metabolism of the lipoproteins. Particularly, the high affinity interaction between LDL and the receptor of LDL ( located on the surface of almost all human cells) occurs through apoB-100, that is the physiological ligand responsible of the regulation of plasmatic LDL-cholesterol (LDL-C) levels. The gene encoding for apoB-100 ( protein of 4563 aa) is located on the short arm of chromosome 2 and is made of 28 introns and 28 exons. Many puntiform mutation have been reported in the putative binding domain of apoB-100 and the LDL receptor .The first mutation reported , the more frequent, is the substitution of a base, from CGG to CAG, at the nucleotide 10708 in exon 26. This mutation leads to an amino acid substitution , in the codon 3500, Arginin in Glutamin (R3500Q) that changes the structure of the binding domain of apob-10 decreasing the affinity to the LDL receptor( 3-9% of normal state) . The mutation R3500Q is responsible of “family defective apoliproteinB-100”, an autosomal disorder related to high plasmatic concentration of total cholesterol and LDL-C. The mutation R3500Q has a frequency between 1:500 and 1:700 in Caucasian population. The detection of the mutation G10708A is performed with amplification with specific primers for the exon 26 of the apoB gene.The yield is a fragment of 125 bp cut by the enzyme *MspI*. The presence of mutation is confirmed by the loss of a restriction site. The yield of PCR of the normal allele 10708G produce, after enzymatic digestion, two fragments of 101 bp and 24 bp, whereas the mutant allele 10708A isn’t cut and remains a fragment of 125 bp.

**Principle of method**: 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 125bp. The next restriction section made by the *MspI* enzyme can be done the following results:




M) Marker 50 bp ladder
1)Heterozygosis R3500Q
2) Wild Type ( control DNA)
3) Homozygosis R3500Q
4) Undigested PCR product

| Wild type subject | Heterozygote subject | Homozygote subject |
|---|---|---|
| Absence of mutation | Mutation R3500Q on an allele | Mutation R3500Q on both the alleles |
| 2 fragments | 3 fragments | 1 fragment |
| | **125 bp** | **125 bp** |
| **101 bp** | **101 bp** | |
| 24 bp | 24 bp | |


**References**
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