DETECTION OF CYP1A2*1C and CYP1A2*1F POLYMORPHISMS OF CYP1A2 GENE using Real-Time PCR.

AMPLI -CYP1A2 Real-Time Cat.n.2.048RT

The CYP1A cytochrome is a member of P-450 cytochromes family, it is a liver enzyme highly polymorphic and it is responsible for 5-10% of the drugs currently use in clinical applications, the most important of which are clozapine, imipramine, caffeine, fluvoxamine, paracetamol, phenacetin, theophylline and tacrine. CYP1A2 is also involved in metabolic activation of some aromatic amines and, therefore, has a key role in carcinogenesis by chemical toxins, as the one found in cigarettes. Some studies on the metabolism CYP1A2-dependent on caffeine or phenacetin have been showing that the enzyme is expressed on different levels in the liver in different subjects, suggesting a polymorphic control of enzymatic activity.
There is a considerable variability in the CYP1A2 metabolic activity due to genetic, environmental factors and drugs interactions. CYP1A2 can be induced or inhibited by many drugs and food-drugs interactions. This prevents the metabolism of active substances present simultaneously, like theophylline and caffeine, responsible of collateral effects on nervous system and cardiac stimulations. On the contrary, smoke does induce the CYP1A2 activation, causing an increased metabolism of CYP1A2 substrates with consequent pharmacologic response to therapeutic sub-dosage. Variation in the level of CYP1A2 activity can cause an increasing or decreasing in the capability of substrates activation. The CYP1A2 gene can show polymorphisms making it less or more active to drugs, resulting in inter-individual differences. The CYP1A2*1A allele leads to a normal enzymatic activity; homozygous subjects for this allele show a normal induction of this enzyme. As regards of CYP1A2*1A allele two important polymorphisms have been showed, they cause functional variations in the CYP1A2 enzymatic activity: CYP1A2*1C allele, result of a point mutation (-3860 G>A), it is associated to a decreased metabolic activity compared to the normal allele CYP1A2*1A; the CYP1A2*1F allele, result of a point mutation (-163 C>A) it is associated to an increased induction, especially in the smokers. The CYP1A2 genotypes distribution is as following: 1F/*1F ~ 46 %; *1A/*1F ~ 44%; *1A/*1A ~ 10%, showing that an increasing in the inducibility represents the most common phenotype.
The ampli kit CYP1A2 Real Time allows the detection of the allele CYP1A2*1° polymorphisms with Real Time PCR using specific primers and florescent primers to reveal gene mutations. The Real-Time PCR, compared to other techniques (sequencing, RFLP, etc), provides the mutation detection even when only a small percentage of cells is present (sensibility 1-2% of mutated cells, specificity 99%).

Principle of method: A) extraction of genomic DNA B) amplification C) detection using real time PCR instrument
Applicability: Genomic DNA extracted and purified from whole blood samples

Number of reactions: 50.

Stability: over 18 months if correctly stored

REAGENTS AND STORAGE

REAGENTS	STORAGE
Mix PCR 2X	+4°C
H2O RNase/DNase Free	-20°C
Primer-Probe Mix CYP1A2*1c	-20°C (in the dark)
Primer-Probe Mix CYP1A2*1f	-20°C (in the dark)
Wilde Type control CYP1A2*1c	+4°C
Heterozygous control CYP1A2*1c	+4°C
Wilde Type control CYP1A2*1f	+4°C
Heterozygous control CYP1A2*1f	+4°C
Mutated Homo.control CYP1A2*1f	+4°C

References

Frequency of two Genetic Polymorphisms of CYP1A2 Gene in Iranian Population
Maryam Sadat et al.2014

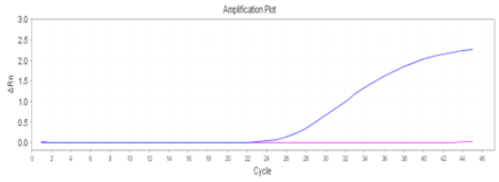
ANALYSIS OF RESULTS

The results analysis will be carried out by a specific program (ALLELIC DISCRIMINATION) previously set.

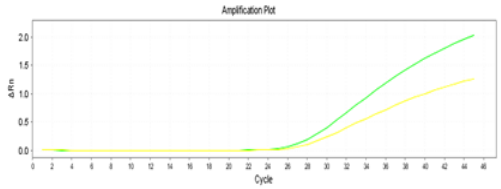
Anywhere it is useful analyzing the amplification plots, in order to check the amplification reaction.

Below an exemplification of an ALLELIC DISCRIMINATION graph of an heterozygous and an homozygous sample.

CYP1A2*1a/1a Wilde Type sample



CYP1A2*1c/1a heterozygous sample



CYP1A2*1f/1f mutated homozygous sample