CERTIFIED SOCIETY
UNI EN ISO 9001, UNI EN ISO 13485

IDENTIFICATION OF POLYMORPHISMS OF GENE CALR

(Type 1, 52bp del e Type 2, 5 bp ins) in Real time PCR

AMPLI set CALR Cat. n. 1.009RT

Janus kinase (JAK) is a family of non-receptor tyrosine kinase able to transduce the signals mediated by cytokine through the metabolic pathway JAK-Stat. This kinase family has been named as "just another kinase - JAK" 1 & 2 (because these kinases are only two among the many found by a PCR experiment). They are also called "Janus kinase" because of the roman god Janus who had two faces. The JAK have two domains almost identical able to transfer phosphate: one shows kinase activity while the other one rules it. The JAK/STAT pathway is involved in the regulation of the cells' response to the cytokines and to the growth factors. The kinase Janus proteins (JKAs) and the Signal transducer and Activators of Transcription (STATs) translate the signal generated by the cytokines' activity and growth factors in an intracellular response, caused by the activity of the activated STAT able to modify the genetic expression. Even if the STATs proteins were found to be Janus kinase's target, studies have shown that some kind of stimuli can trigger them regardless of JAKs. The pathway has an important role in the main decisions regarding the cells' destiny regulating the proliferation process, differentiation and apoptosis. The common mutations of JAK2 gene (point mutation V617F in exon 14 and the ones in exon 12) have been found in over 97% patients with polycythemia vera (PV); the mutation JAK2V617F and mutation MPL (Proto-Oncogene, Thrombopoietin Receptor), usually in the codon 515, are present in 60-65% of patients with Essential Thrombocythaemia (ET) and Primary Myelofibrosis (PMF). In December 2013 two groups of researchers (Tony Green and Robert Kralovics) described simultaneously new mutations in the calreticulin gene (CALR) (Klampfl T et al, 2013; Nangalia J et al, 2013). These mutations are present in about 20% of patients with ET and PMF and they are expressed exclusively in subjects non-mutated for JAK2 and MPL (by definition the CALR mutations are absent in PV). The CALR gene, on chromosome 19, codes a multifunctional protein (its activity is known as molecular chaperone: it binds calcium ions and it regulates its accumulation in the endoplasmic reticulum and controls the proteins' and glycoproteins' folding) with many cellular localizations (it can be found in the endoplasmic reticulum, in the cytosol, on the cytoplasmic membrane and in the nucleus; the calreticulin function in these compartments is not yet clear, although if this protein is expressed on the cell membrane may promote the cell phagocytosis). The CALR mutations are heterogeneous, but Type 1 (deletion of 52 bp) and Type 2 (insertion of 5 bp) represent 80-85% of cases. They are located on exon 9 only and they give rise to a frameshift that will generate a protein with a new C-terminal portion, common in all the mutations known so far. The changing of sequence in the C-terminal portion of the protein could alter the stability and the intracellular functions. Anyhow actually there are not scientific reliable information. Some data, however, showed that the expression of the mutated calreticulin in the cell lines can lead to hyper-sensitivity and proliferative independence from cytokines activating the JAK/STAT pathway, as suggested by the increased level of phosphorylated STAT5 and by the anti-proliferative effect showed, in vitro, by the fedratinib (JAK2 inhibitor). The close association of the CALR mutation with essential thrombocythaemia and myelofibrosis justifies this molecule to be a major diagnostic criteria in the WHO classification (Tefferi A et al, 2014).

Principle of method:) genomic DNA extraction B) amplification and revelation by Real-Time PCR instrument
Applicability: on extracted and purified genomic DNA from whole blood.
Number of Test: 25.

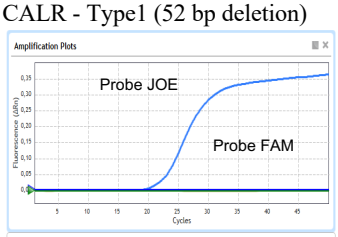
REAGENTS AND STORAGE

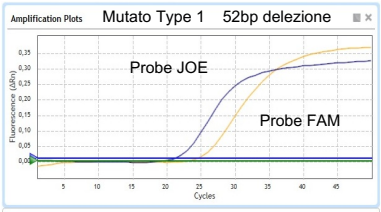
AMPLIFICATION	
PCR mix 5X	-20°C in the dark
Internal control 20X	-20°C in the dark
Primer-probe mix CALR INS 5 bp 20X	-20°C in the dark
Primer-probe mix CALR DEL 52 bp 20 X	-20°C in the dark
Control CALR INS 5 bp	-20°C
Control CALR DEL 52 bp	-20°C
H_2O sterile	-20°C

Stability: over 18 months if correctly stored.

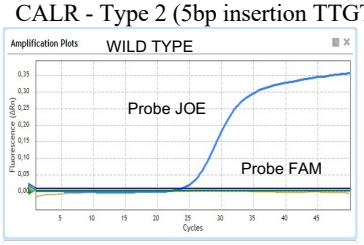
ANALYSIS OF RESULTS

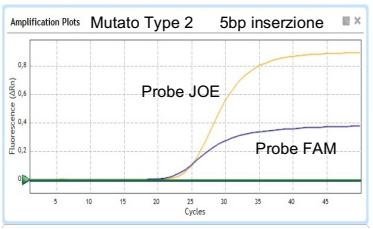
CALR - Type1 (52 bp deletion)



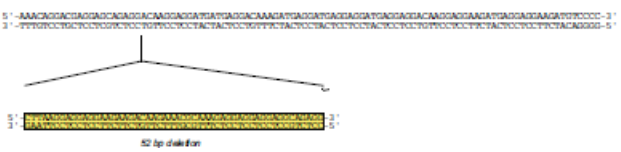


CALR - Type 2 (5bp insertion TTGTC)

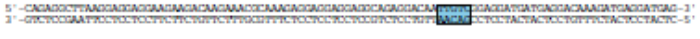




CALR - Type1 (52 bp deletion)



CALR- Type 2 (5bp insertion TTGTC)



References:
Klampfl T, Gisslinger H, Harutyunyan AS, et al New England Journal of Medicine 2013;369:2379-90.
Nangalia J, Massie CE, Baxter EJ, et al. New England Journal of Medicine 2013;369:2391-405.
Tefferi A, Thiele J, Vannucchi AM, Barbui T. Leukemia 2014 Jan 20.