

JAK2 c.1849G>T (V617F) Mutation Quantification by Real-Time PCR

FOR QUANTITATIVE ASSESSMENT OF THE JAK2 C.1849G>T (V617F) MUTATION IN MYELOPROLIFERATIVE DISORDERS: POLYCYTHEMIA VERA, ESSENTIAL THROMBOCYTHEMIA, AND IDIOPATHIC MYELOFIBROSIS

Test Highlights

- Rapid and sensitive allele-specific quantitative detection of mutation
- Simple sample type: granulocytes isolated from peripheral blood

Clinical Background

- The human myeloproliferative disorders (MPD) are acquired clonal hematologic malignancies which include polycythemia vera (PV), essential thrombocythemia (ET), idiopathic myelofibrosis (IMF), and chronic myeloid leukemia (CML). This heterogeneous group of disorders is believed to result from the clonal proliferation of cells following a somatic pathogenetic event.
- The primary characteristics of PV and ET are the increased production of red blood cells and platelets, leading to the clinical manifestation of thrombosis or hemorrhage. Further, patients suffering from PV or ET can develop IMF, which is characterized by bone marrow fibrosis, cytopenia, and splenomegaly and has an average survival of less than five years. Additionally, these diseases may also transform to acute leukemia either directly or soon after they first transform to IMF.
- In 2005, four independent research groups identified a single acquired mutation in the Janus Kinase 2 (*JAK2*) gene on chromosome 9 that had a high incidence of occurrence in patients with PV, ET, or IMF.¹⁻⁴ The point mutation in *JAK2* alters codon 617 from a valine to a phenylalanine. This amino acid alteration in the JH2 domain of *JAK2* causes a constitutive activation of the tyrosine kinase, which is believed to confer erythropoietin hypersensitivity and erythropoietin-independent survival to the myeloid stem cell.
- Greater than 80 percent of PV and 40 percent of ET and IMF cases used in the recent studies harbored the *JAK2* point mutation. The detection of the *JAK2* c.1849G>T (V617F) mutation provides a reliable discrimination from other types of congenital, acquired, and idiopathic polycythemic disorders; unexplained elevated platelet counts; and marrow fibrosis of uncertain origin.⁴ The proportion of the mutant allele is highly variable, and as was shown in a recent publication that homozygosity resulted from the loss-of-heterozygosity of chromosome 9p in approximately 30 percent of PV patients.²
- Recent evidence indicates that that some complications of MPD, such as the degree of marrow fibrosis, thrombotic tendencies, and overall survival, correlate with overall proportion of the mutant allele in circulating clonal cells (granulocytes).

Indications for Use

Patients who might have PV, ET, or IMF, those with idiopathic polycythemic disorders, patients with unexplained elevation platelet counts, and marrow fibrosis of uncertain origin. Also patients who previously were determined positive for the *JAK2* c.1849G>T (V617F) mutation and a quantitative assessment of mutation burden is desired.

Interpretation

- A positive result quantifies the mutant allele (T) relative to the level of the normal allele (G) at nucleotide position 1849 within the *JAK2* gene.
- A negative result does not rule out the presence of a *JAK2* c.1849G>T (V617F) mutation nor the possibility of diagnosis of PV, ET, or IMF. The mutation has been correlated to disease state in >80 percent PV and 50 percent IMF and ET patients.

Limitations

The limit of quantification for the assay is approximately 1/1000 cells harboring the mutation. Limit of detection of the mutation is 1/10,000 cells. The mutation must exist within the granulocyte population to be detected.

Methodology

Real-time polymerase chain reaction (PCR)

References

1. James C, Ugo V, Le Couedic JP, et al. A unique clonal *JAK2* mutation leading to constitutive signaling causes polycythemia vera. *Nature* 2005;434:1144-1148.
2. Kralovics R, Guan Y, Prchal JT. Acquired uniparental disomy of chromosome 9p is a frequent stem cell defect in polycythemia vera. *Exp Hematol* 2002;30:229-236.
3. Levine RL, Wadleigh M, Cools J, et al. Activating mutation in the tyrosine kinase *JAK2* in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell* 2005;7:387-397.
4. Nussenzveig RH, Swierczek SI, Jelinek J, et al. Polycythemia vera is not initiated by *JAK2V617F* mutation. *Exp Hematol* 2007;35:32-38.

Test Information

0040168 *JAK2* Gene, V617F Mutation, Quantitation

For specific collection, transport, and testing information, refer to the ARUP Web site at www.aruplab.com.