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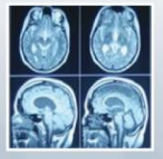


November 8, 2016

CLINICAL PRACTICE CHANGE

Change in Methodology of Janus Kinase 2 (JAK2) V617F Mutation Testing

Effective: Immediately

What is changing?
The test methodology will now output both a qualitative (positive/negative) and quantitative (% Mutant DNA) result. The limit of detection of this assay has been measured in our laboratory to detect 0.01% Mutant DNA.

How this is different from previous testing?
Previously, JAK2 testing was limited to a qualitative result (positive/negative) with a semi-quantitative limit of detection of approximately 5% Mutant DNA.

Why the methodology is changing?
The scientific literature indicates that positive levels of mutant DNA above 1% are diagnostic for **myeloproliferative neoplasm (MPN)** in the correct clinical context. For a full discussion, see Background, below.

Recommendations:
JAK2 V617F mutation testing, in concert with testing for BCR-ABL1 fusion gene, is an appropriate screening methodology for MPN in patients who present with a persistent elevation in one or more myeloid cell lineages (erythroid, granulocytic, or thrombocytic). Other testing, such as sequencing for calreticulin, MPL, and JAK2 exon 12 mutations, are of limited diagnostic yield outside of specific patient subpopulations, and are not suitable for general screening. These more specialized tests should only be ordered in the context of a Clinical Haematology or Haematopathology consultation.

Please direct any questions on the new JAK2 assay to the Hematopathology Molecular Service:
Dr. Arshad Ahsanuddin (aahsanuddin@dsmanitoba.ca, pager: 204-932-0562) or
Dr. Michel Nasr (mnasr@dsmanitoba.ca, pager: 204-932-1219)

Background:
Mutations of the Janus Kinase 2 (*JAK2*) gene have been have been widely recognized as being positively associated with *BCR-ABL1*-negative myeloproliferative neoplasms (MPN's) since the V617F mutation was described in 2005[1-4]. The vast majority of the clinically-significant mutations are found in codon 617, resulting in the replacement of the amino acid valine with phenylalanine [V617F, alias JAK2 NM_004972.3:c.1849G>T(p.Val617Phe)]. This mutation is present in approximately 96% of Polycythemia Vera (PV) cases, 55% of Essential Thrombocytosis (ET) cases, and 65% of Primary Myelofibrosis (PMF) cases. Therefore testing for BCR-ABL1 fusion gene and

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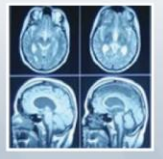


JAK2 V617F mutation are recommended in patients with a strong suspicion of MPN. Because JAK2 mutations can be found in disease states other than MPN, results should be interpreted in context with the patient's clinical and laboratory presentation. If clinically indicated, consultation with the Clinical Haematology or Haematopathology services is recommended prior to ordering the test. Due to proprietary restrictions on JAK2 molecular testing, the Haematopathology Molecular Service will be shifting this test to a new methodology, based on an assay kit designed by Qiagen Marseilles (formerly Ipsogen), which acquired the worldwide license on such testing in 2006. The test will quantitatively measure copy numbers of JAK2 wild-type (i.e. normal) and V617F mutant genes, and express their relationship as % Mutant DNA (i.e. Mutant JAK2 DNA/Total JAK2 DNA). The analytical sensitivity of this assay has been measured in our laboratory to be less than 0.01% Mutant DNA.

Recent laboratory guidelines[5] suggest that positive results of greater than 1% Mutant JAK2 DNA are clinically significant in association with a myeloproliferative phenotype (significant and persistent elevation in haemoglobin, granulocytes, or platelets). If JAK2 results for a specific patient are positive but fall below 1% Mutant DNA, then caution must be exercised, as very low levels of JAK2 V617F mutant DNA (usually < 0.1%) have been detected in healthy individuals[6,7].
If clinical suspicion is high for ET or PMF, a negative JAK2 V617F result should prompt consideration for subsequent testing to detect clonal mutations of Calreticulin. This testing is currently not performed in our laboratory, and will be referred out to a reference lab until such time as we can perform these assays in-house.
Other MPN-associated genetic abnormalities, such as JAK2 exon 12 and MPL exon 10 mutations, are far less common, therefore diagnostic yield is limited. We do not recommend these tests for screening purposes. Specialized testing for Calreticulin, JAK2 exon 12, and MPL exon 10 mutations will only be considered in the context of a formal Clinical Haematology or Haematopathology consultation.

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