Patient Name:		**CRM ID:**	
Age/DOB:		**Sample Type:**	
Sex:		**Collection Date:**	
Referring Clinician:		**Received Date:**	
Test Requested:		**Reporting Date:**	

JAK2 EXON12 MUTATION ANALYSIS BY PCR, SANGER

CLINICAL DIAGNOSIS/SYMPTOMS

diagnosis of HT with polycythemia under evaluation.

RESULTS

RESULTS	
JAK2 EXON 12	Not Detected (NEGATIVE)

CLINICAL SIGNIFICANCE

JAK2 gene mutations are important diagnostic markers for the Myeloproliferative disorder Polycythemia vera (PV). A single mutation (V617F) is found in approximately 95% of cases. Of the PV cases that are V617F- negative (approximately 5%), most are associated with mutations in a different region of the JAK2 gene within exon 12. Patients with exon 12 mutations frequently present erythrocytosis as the predominant feature, but without concurrent elevations in the megakaryocytic or granulocytic lineages as seen in V617F-positive PV.

Consequently, many exon 12 positive cases are considered clinically as Idiopathic erythrocytosis. Unlike the V617F mutation that is found in several Myeloproliferative neoplasms (PV, Essential thrombocythemia & Primary myelofibrosis), JAK2 exon 12 mutations are restricted only to cases of PV. JAK2 exon 12 mutation screening
in patients who present with a suspicion for PV that is V617F negative.

RECOMMENDATION:

1. Please correlate clinically.
2. If the above results do not correlate completely with patient phenotype, additional testing like RTPCR/NGS method based JAK2 mutation/ gene testing is highly advised based on clinician's recommendation due to high sensitivity.
3. For this report, or for assistance in locating nearby genetic counseling services, please contact the Laboratory: geneticcounselors @redcliffelabs.com, or ccsupport@redcliffelabs.com.

METHODOLOGY

Targeted sequencing and mutation analysis was performed by Polymerase Chain Reaction (PCR) followed by automated DNA sequencing of the amplicon using BigDye Terminator Chemistry on an ABI Genetic Analyzer 3500XL platform. Sequencing data were aligned to NCBI database to analyze the mutations.

COMMENT

More than 10 different sequence variations have been found in exon 12 of the *JAK2* gene, most of which are in the region between codons 536 and 544. All Mutation will be covered through this test as F537- K539delinsL H538QK539L, H538-K539delinsL, K539L, I540-E543delinsMK ,R541-E543delinsK, N542-E543del ,E543-D544del ,V536-I546dup11 ,F537-I546dup10+F547L. Results of this test must always be interpreted in the context of clinical and other relevant laboratory data such as erythropoietin level exclusion of other causes of elevated hemoglobin, and should not be used alone for a diagnosis of Polycythemia Vera which is a form of malignancy i.e Myeloproliferative disorder. Many MPD cases negative for exon 14 mutations have been observed to carry mutations in exon 12 of *JAK2*. Somatic mutations in exon 12 of *JAK2* have been found in 5% cases of suspected PV.

LIMITATIONS

- ✓ A positive result is not specific for a particular Myeloproliferative neoplasm (MPN) diagnosis and clinicopathologic correlation is necessary in all cases.
- ✓ A negative result does not exclude the presence of MPN or other neoplastic processes.
- ✓ The sensitivity of detection for Sanger sequencing is generally recognized as being approximately 15% to 20% mutant allele frequency.
- ✓ PCR is a highly sensitive technique, however inherent PCR inhibitors in the specimen result in amplification failure.

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Patient Name:		**CRM ID:**	
Age/DOB:		**Sample Type:**	
Sex:		**Collection date:**	
Referring Clinician:		**Sample Received:**	
Test Requested:	**JAK2 Gene Analysis, 2 Exon (14) By Sanger Seq.**	**Reporting date:**	

JAK-2 (Exon14) Mutation Analysis by Sanger Seq.

CLINICAL DIAGNOSIS/SYMPTOMS

diagnosis of HT with polycythemia under evaluation.

RESULTS

RESULTS	
JAK-2 (Exon 14) c.1849G>T (p.Val617Phe)	**Not Detected (NEGATIVE)**

CLINICAL SIGNIFICANCE

JAK2 V617F mutations accounts for 90% PV patients and 60% of ET or MF patients. Rare Exon 12 insertion and deletion mutations in JAK2 accounts for 2-3% of PV. For the diagnosis of PV and ET three major criteria are defined of which one is the mutation in exon 12 or 14 (V617F) of JAK2 gene for PV and mutation in either JAK2, CALR or MPL gene for ET.

PV Patients with JAK2 exon 12 mutation have younger age, increased mean hemoglobin/hematocrit, and lower WBC & platelets count at diagnosis compared to those with JAK2 V617F mutation. However both JAK2 mutations are associated with similar rates of thrombo- sis, evolution to myelofibrosis or leukemia and death.

METHODOLOGY

Targeted sequencing and mutation analysis was performed by Polymerase Chain Reaction (PCR) followed by automated DNA sequencing of the amplicon using BigDye Terminator Chemistry on an ABI Genetic Analyzer 3500XL platform. Sequencing data were aligned to NCBI database to analyze the mutations

COMMENT

Chronic myeloproliferative neoplasms (MPNs) are clonal hematopoietic stem cell malignancies characterized by excessive production of blood cells. BCR-ABL1-negative MPN frequently harbor an acquired single nucleotide mutation in JAK2 characterized as c.G1849T; p. Val617Phe (V617F) and it is a gain-of-function mutation that leads to clonal proliferation. The JAK2 V617F is present in 95% to 98% of polycythemia vera (PV), and 50% to 60% of primary myelofibrosis (PMF) and essential thrombocythemia (ET). It has also been described infrequently in other myeloid neoplasms, including chronic myelomonocytic leukemia and myelodysplastic syndrome. Diagnostic criteria for ET, MF, and PV adopted by the World Health Organization (WHO) include identification of a clonal marker, with a specific recommendation to test for the JAK2 V617F mutation in exon 14. Detection of the JAK2 V617F is useful to help establish the diagnosis of MPN and The JAK2 allele burden decreases with successful therapy, disappears in some patients, and reappears during relapse.

LIMITATIONS

- A positive result is specific for a particular Myeloproliferative neoplasm (MPN) diagnosis and clinicopathologic correlation is necessary in all cases.
- A negative result does not exclude the presence of MPN or other neoplastic processes.
- The sensitivity of detection for Sanger sequencing is generally recognized as being approximately 15% to 20% mutant allele frequency.
- PCR is a highly sensitive technique; common reasons for paradoxical results are contamination during specimen collection, selec-tion of inappropriate specimen and inherent PCR inhibitors in the sample.

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Conditions of Reporting

- Test results released pertain to the specimen submitted .
- All test results are dependent on the quality of the sample received by the Laboratory .
- Laboratory investigations are only a tool to facilitate in arriving at a diagnosis and should be clinically correlated by the Referring Physician .
- Certain tests may require further testing at additional cost for derivation of exact value. Kindly submit request within 72 hours postreporting.
- Test results may show inter laboratory variations.
- If Sample collection date is not stated on test requisition form, the current date will be printed by default as the date of collection.
- Test results are not valid for medico legal purposes.

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3. It is to be noted that variations in results may occur between different laboratories and over time, even for the same parameter for the same Customer. The assays are performed and conducted in accordance with standard procedures, and the reported outcomes are contingent on the specific individual assay methods and equipment(s) used, as well as the quality of the received specimen.

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