H024

JAK2 Exons 12, 13, 14 and 15 Mutation Analysis

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Background

JAK2 V617F mutation is detected in patients with polycythemia vera (~95%), essential thrombocythemia (~50%) and primary myelofibrosis (~50%). A small percentage of JAK2 mutation positive patients (~3.3%) contain other non-V617F mutations in exons 12 to 15. The detection of a JAK2 gene mutation aids in the specific diagnosis of a myeloproliferative neoplasm, and helps distinguish this clonal disease from a benign reactive process.

Methods

Total RNA was purified from blood, bone marrow and cell pellet specimen. The JAK2 gene region covering exons 12 to 15 was subjected to reverse-transcription coupled PCR amplification, and bi-directional sequencing to identify sequence variations. Assay's accuracy, repeatability, reproducibility, analytical sensitivity and stability were evaluated.

Results

Of the specimens tested during validation, 18 specimens and 4 synthetic RNA samples with known JAK2 results that were 100% concordant from the sequencing results in two different laboratories. Repeatability (intra-assay precision) and reproducibility (inter-assay precision) were 100%. This assay has a sensitivity to detect approximately 15% JAK2 somatic mutations. The RNA stored at -80ºC was stable for at least 118 days. The EDTA, ACD blood and heparin, EDTA bone marrow were stable for at least 6 days.

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Accuracy Validation Data

22 blood and bone marrow specimens, 4 synthetic control RNA were run. The results were 100% concordant with the original results.

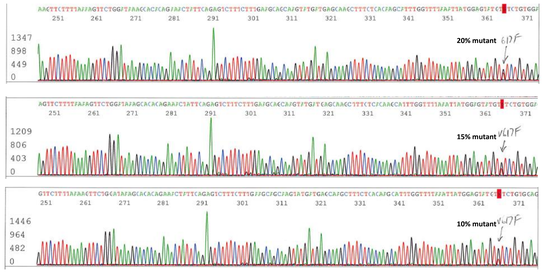
Sample barcode	Sample Type	Original Sequencing Results	Validation Sequencing Results
1710905932020	Heparin Bone Marrow	V617F	V617F
1710905945590	EDTA Blood	V617F	V617F
1710916322900	Heparin Bone Marrow	WT	WT
1710921663970	EDTA Bone Marrow	V617F	V617F
1710921675060	EDTA Blood	WT	WT
1710922004100	EDTA Blood	WT	WT
1710928638360	Heparin Bone Marrow	V617F	V617F
1710929160130	EDTA Blood	WT	WT
1710930515080	Heparin Bone Marrow	WT	WT
1710930562950	Heparin Bone Marrow	WT	WT
1710954816640	EDTA Blood	WT	WT
1710960529550	EDTA Blood	V617F	V617F
1710962159250	EDTA Blood	WT	WT
1710965261340	Heparin Bone Marrow	WT	WT
1710965261350	EDTA Blood	WT	rpt
1710975581740	Heparin Bone Marrow	WT	WT
1710984747270	EDTA Blood	c.1711G>A (p.G571S)	c.1711G>A (p.G571S)
1710991167530	Heparin Bone Marrow	V617F	V617F
1710991900380	EDTA Blood	V617F	V617F
1710991168100	Synthetic RNA	1627-1632 del6 (del E543-D544)	1627-1632 del6 (del E543-D544)
1710965600410	Synthetic RNA	1612-1620 del9insTTA (del F537-K539insL)	1612-1620 del9insTTA (del F537-K539insL)
1711070811130	Synthetic RNA	1624-1629del6 (del N542-E543)	1624-1629del6 (del N542-E543)
1711091965900	Synthetic RNA	T1639C/ G1691T/ G1711A/ T1852C/ A1933G	T1639C/ G1691T/ G1711A/ T1852C/ A1933G

Precision

Repeatability (intra-assay precision) was 100% concordant for JAK2 results using 6 positive and 5 negative specimens.

Reproducibility (inter-assay precision) was 100% concordant for JAK2 results using 6 positive and 5 negative specimens..

Detection Sensitivity



The JAK2 assay can reliably detected a 10% mutation with 5ng/ul input RNA.

The JAK2 Exons 12-15 sequencing assay has been offered as a clinical test based on the successful performance features. In a set of 5755 clinical specimens ordered for standalone testing, 11.17% having JAK2 positive result, 88.62% JAK2 negative. Among JAK2 positive specimens, 70.30% were V617F, 28% were whole exon deletion (27.53% were exon 15 deletion and 0.47% multiple exons deletion) and 1.71% were other missense variant or small indel. Results could not be obtained in 0.19% specimens. This was mainly due to RNA degradation from specimen.

In a set of 6888 JAK2 V617F negative reflex to JAK2 E12-15 testing, 2.34% were positive. In a set of 9307 JAK2 V617F negative reflex to JAK2 E12-15/CALR/MPL testing, 6.75% were positive for at least one gene. 15 JAK2 V617F were detected using JAK2 E12-15 sequencing assay from RNA while they were negative using JAK2 V617F competitive allele-specific TaqMan PCR (castPCR™) method from DNA. These findings suggest that sequencing from RNA may be a more sensitive methodology for detecting JAK2 mutations, potentially resulting from enrichment due to transcription.

Conclusions

The JAK2 Exons 12-15 sequencing assay is a robust, reproducible and sensitive assay using blood, bone marrow and cell pellet specimens for myeloproliferative neoplasm assessment.

References

1. Ma, W. et al. Mutation profile of JAK2 transcripts in patients with chronic myeloproliferative neoplasias. J Mol Diagn. 2009 Jan;11(1):49-53.
2. Catarsi, P. et al. JAK2 Exon 14 Skipping in Patients with Primary Myelofibrosis: A Minor Splice Variant Modulated by the JAK2-V617F Allele Burden. PLOS ONE. DOI:10.1371/journal.pone.0116636. Jan 24, 2015.