Sensitivity and Specific Detection of JAK2 V617F Using Droplet Digital PCR

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Abstract

Introduction: Detection of JAK2 V617F mutation is important in the prognosis and diagnostic risk assessment of myeloproliferative neoplasms (MPNs). Methods designed to detect this variant have also been used to monitor patient response to therapy. This study evaluates the use of droplet digital PCR (ddPCR) for low level detection of JAK2 V617F in blood and bone marrow to assess disease progression and therapeutic response.

Methods: ddPCR technology incorporates end-point and emulsion based PCR techniques resulting in highly sensitive detection of somatic variants. Utilizing RainDance ddPCR technology, DNA and reagents are distributed into as many as ten million droplets per specimen allowing for direct data analysis that does not require the application of Poisson statistics. Each droplet containing a single molecule of fragmented DNA undergoes PCR amplification with fluorescently labelled probes designed to detect either wild type JAK2 sequence or the mutant V617F sequence. After amplification, droplets expressing either wild type fluorescence, variant fluorescence or no fluorescence are counted. The accompanying analysis software then quantitates the level of JAK2 V617F expression.

Results: Over 80 DNA samples from whole blood or bone marrow were tested, including 50 samples that had been previously assessed for JAK2 V617F via alternate methods, 15 samples from healthy donors, and multiple samples from individual patients to assess this method’s ability to monitor therapeutic response. In addition, a JAK2 V617F positive cell line HEL and a JAK2 wild type cell line MHI-11 were used to determine the limit of detection and limit of blank for V617F with ddPCR. This approach is capable of detecting JAK2 V617F with high specificity, sensitivity, and repeatability down to detection levels of ~0.02% mutant allele frequency (MAF). ddPCR has qualitative concordance with alternative methods including: Sanger sequencing of RNA from plasma next generation sequencing (NGS) methodologies, and qPCR; as well as quantitative concordance with NGS and qPCR. Retrospective analysis of DNA from patients with multiple time points over the course of treatment and disease reveals that ddPCR can provide a sensitive method for tracking disease course and therapeutic response.

Conclusions: ddPCR can be used to detect JAK2 V617F in the blood and bone marrow with sensitivity below 0.02% MAF for template inputs of 50 ng. This method does not require a standard curve for quantitation and has minimal non-specific background fluorescence to track low level variant detection. When additional factors such as speed, ease of use, and cost are considered, ddPCR technology provides a highly sensitive, accurate, fast, and cost effective method for detection of JAK2 V617F.

Materials & Methods

Methods:

• Quantitative real time polymerase chain reaction (qPCR) – Gold Standard method

• Limit of detection (LOD): 0.01% - 0.1% 

• Limit of blank (LOB): 0.004% - 0.034% Limits stated for at least 10,000 genomic copies per specimen

• Droplet digital polymerase chain reaction (ddPCR)

LOD: 0.02% with 50ng input (<0.001% variant with >1ng input)

LOB: 0.00002%

Material:

• HEL - V617F mutant positive cell line; MV-4-11 – wild type cell line

• DNA extracted from peripheral blood (PB) and bone marrow (BM) from 15 normal donors and over 80 specimens from patients diagnosed with PV, ET or PMF.

Results:

Fig. 1 Mass input study – Detection of JAK2 V617F by ddPCR

Table 1 Sensitivity and Specificity of ddPCR assay for Low JAK2 V617F MAF qPCR

| Patient ID | ddPCR | NGS | MSP
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>10 ng Input</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>50 ng Input</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Table 2 Decision matrix for JAK2 V617F testing

<table>
<thead>
<tr>
<th>Pat. A Myeloproliferative Disorder</th>
<th>Pat. B PV and MF converting to AML</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Marker Detected</td>
<td>40%</td>
</tr>
<tr>
<td>% Patient Chimerism</td>
<td>0%</td>
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</tbody>
</table>

Table 3 Determination of LOD - Serial dilution of HEL - V617F mutant line into MV-4-11 - V617F WT line

<table>
<thead>
<tr>
<th>Expected % HEL</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>% JAK2 V617F</td>
<td>0.1%</td>
<td>0.5%</td>
<td>1%</td>
<td>2%</td>
<td>3%</td>
<td>4%</td>
<td>5%</td>
<td>6%</td>
<td>7%</td>
<td>8%</td>
<td>9%</td>
</tr>
</tbody>
</table>

Fig. 2 Impact of tissue source on detection of JAK2 V617F by ddPCR

Fig. 3 Concordance between ddPCR and qPCR for detection of JAK2 V617F

Fig. 4 Concordance between ddPCR and qPCR for detection of JAK2 V617F

Conclusions

- ddPCR technology applied to the detection of JAK2 V617F provides a robust and reproducible assay capable of detecting the variant in both blood and bone marrow across a wide range of DNA template input levels.

- The LOD for the assay is 0.00002%.

- Th LOD for inputs of 50ng is 0.02% MAF

- Sensitivity, specificity, and calculated correlation were similar compared to qPCR

- ddPCR does not require a standard curve for quantitation of JAK2 V617F MAF

- ddPCR requires less hands on time and has lower reagent cost than qPCR

Fig. 5 Correlation between ddPCR and NGS for detection of JAK2 V617F