Measuring Major and Deep Molecular Response Using Droplet Digital™ PCR (ddPCR™) Technology

The best way to assess complete molecular response (CMR) is with a highly sensitive molecular assay. The CE-IVD QXDx BCR-ABL %IS Kit elevates chronic myeloid leukemia (CML) monitoring to a new level of sensitivity (0.001%IS, MR 5.0 with four wells), precision, and reproducibility. Bio-Rad’s BCR-ABL assay delivers a scalable, reliable, and robust workflow for monitoring leukemia patients.

The assay enables:
- High precision and accuracy
  - Increased sensitivity with 2- or 4-well test per patient sample
- Absolute quantification
  - Eliminates the need for the standard curves required with RT-PCR
- Simplified and scalable workflow
  - Flexibility to process 8 to 48 samples per run
  - Flexible kit design to meet your laboratory’s throughput and workflow needs
- Standardized interpreted output
  - Direct reporting on International Scale (%IS) and molecular response (MR) values
The QXDx BCR-ABL %IS Kit is a digital PCR test that provides unparalleled reproducibility even for deep molecular responses. The digital PCR solution achieves this through absolute quantification of copies of target DNA/RNA. A PCR reaction is partitioned into 20,000 droplets. The droplets containing the target sequence are detected by fluorescence and scored as positive and those without are scored as negative. Poisson statistical analysis of positive and negative droplets yields absolute quantification of the target sequence. Unlike RT-PCR, which relies on a standard curve, sample input is the only thing contributing to minimal variability across dynamic range. The results are reported on the International Scale (IS) by using an assay-specific conversion factor determined by comparing the assay to an IS reference assay. The results are also reported as molecular response (MR) values.

The QXDx BCR-ABL %IS Kit and ddPCR technology have some inherent advantages over conventional RT-PCR. This includes scalable sensitivity with an improved LOD (1 to 2 logs) and less sensitivity to/impact by amplification efficiency compared to RT-PCR. Hence, independent labs have shown that ddPCR has obvious advantages over RT-PCR for monitoring disease burden (Jennings et al. 2014).

Digital PCR has been shown to be an accurate and highly precise method for detecting the BCR-ABL fusion gene (Cross et al. 2016). It is a lot more precise than traditional RT-PCR since it enables absolute quantification (Cross et al. 2016). The QXDx Droplet Digital PCR Systems are the premier clinical-ready platform enabling precise, sensitive, and scalable quantification of nucleic acids. The systems provide the flexibility to process 8 to 48 samples per run.

<table>
<thead>
<tr>
<th>LOQ (%IS)</th>
<th>2-Well Test</th>
<th>4-Well Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.002%IS (MR 4.7)</td>
<td>0.001%IS (MR 6.0)</td>
<td></td>
</tr>
</tbody>
</table>

"Bio-Rad’s Droplet Digital PCR System is a powerful platform for monitoring patients being treated for CML. The increased sensitivity and precision of multiplexed BCR-ABL1 measurements, as compared to qPCR, along with absolute quantification in target copies and no standard curves, lends to the rationale for ddPCR use in routine laboratory testing."

Neils Pallisgaard
Department of Pathology
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QXDx BCR-ABL %IS Kit Workflow

1. RNA Extraction
2. Reverse Transcription
3. Droplet Generation
4. Thermal Cycling
5. Droplet Reading

Clinical Performance

Correlation of the QXDx BCR-ABL %IS Kit and RT-PCR Lab Developed Test (LDT).

Analytical Data

Sensitivity and specificity of the QXDx BCR-ABL Assay.

Limit of blank (LOB) 0 0
Limit of quantitation (LOQ) 0.002%IS (MR 4.7) 0.001%IS (MR 5.0)
Limit of detection (LOD) 0.002%IS (MR 4.7) 0.001%IS (MR 5.0)

Transcript Samples Slope \( R^2 \) Value 2nd Order Fit Deviation

<table>
<thead>
<tr>
<th>Transcript</th>
<th>Samples</th>
<th>Slope</th>
<th>( R^2 ) Value</th>
<th>2nd Order Fit Deviation</th>
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</thead>
<tbody>
<tr>
<td>e13a2</td>
<td>10 (MR 0.3 to MR 4.7)</td>
<td>1.03</td>
<td>0.996</td>
<td>≤0.09 MR units</td>
</tr>
<tr>
<td>e14a2</td>
<td>10 (MR 0.3 to MR 4.7)</td>
<td>1.04</td>
<td>0.996</td>
<td>≤0.09 MR units</td>
</tr>
</tbody>
</table>

Linear regression of the QXDx BCR-ABL Assay vs. qPCR. 34 clinical patient samples. \( y = 1.0332x – 0.0722, R^2 = 0.976. \)

Precision — Minimal Variability across Dynamic Range of %IS and MR Value

QXDx BCR-ABL %IS Kit precision data — patient and control samples. Precision: \( n > 100 \) samples were verified as SD ≤ 0.25.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Target MR</th>
<th>( n )</th>
<th>Mean MR Level</th>
<th>SD</th>
<th>%CV</th>
<th>Target % BCR-ABL</th>
<th>( n )</th>
<th>Mean %IS Level</th>
<th>SD</th>
<th>%CV</th>
<th>% BCR-ABL Total Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR 1</td>
<td>1</td>
<td>108</td>
<td>1.37</td>
<td>0.035</td>
<td>2.533</td>
<td>10</td>
<td>108</td>
<td>4.28</td>
<td>0.29</td>
<td>6.98</td>
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<tr>
<td>MR 2</td>
<td>2</td>
<td>108</td>
<td>2.44</td>
<td>0.049</td>
<td>2.004</td>
<td>1</td>
<td>108</td>
<td>0.37</td>
<td>0.037</td>
<td>10.3</td>
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<tr>
<td>MR 2.5</td>
<td>2.5</td>
<td>108</td>
<td>2.77</td>
<td>0.05</td>
<td>1.804</td>
<td>0.32</td>
<td>108</td>
<td>0.17</td>
<td>0.019</td>
<td>11.1</td>
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<tr>
<td>MR 3</td>
<td>3</td>
<td>108</td>
<td>3.28</td>
<td>0.081</td>
<td>2.462</td>
<td>0.10</td>
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<td>0.009</td>
<td>17.9</td>
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<td>3.60</td>
<td>0.103</td>
<td>2.861</td>
<td>0.03</td>
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<td>4.10</td>
<td>0.166</td>
<td>4.044</td>
<td>0.01</td>
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<td>0.0085</td>
<td>0.008</td>
<td>37.0</td>
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<tr>
<td>Cell line control 1</td>
<td>&lt;1.0</td>
<td>108</td>
<td>0.70</td>
<td>0.007</td>
<td>1.063</td>
<td>&gt;10</td>
<td>108</td>
<td>20.13</td>
<td>0.77</td>
<td>3.82</td>
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<tr>
<td>Cell line control 2</td>
<td>4.7</td>
<td>108</td>
<td>4.63</td>
<td>0.250</td>
<td>5.420</td>
<td>0</td>
<td>108</td>
<td>0.0025</td>
<td>0.0017</td>
<td>70.7</td>
<td></td>
</tr>
</tbody>
</table>

SD, standard deviation.
References
Cross NO et al. (2016). Development and evaluation of a secondary reference panel for BCR-ABL1 quantification on the International Scale. Leukemia 30, 1,844–1,852.

Ordering Information
Catalog # Description
12006134 QXDx BCR-ABL %IS Kit, CE-IVD*, 192 reactions (96 samples)

Materials needed but not provided
Reagents and Consumables
12001921 ddPCR Dx Consumable Pack*, 192 reactions
12001922 ddPCR* Dx AutoDG* Consumable Pack, 480 reactions
12002526 ddPCR Dx Droplet Reader Oil Pack*, 784 reactions

Instruments
17002229 QX200™ AutoDG Droplet Digital PCR Dx System*
17000034 QX200 Droplet Digital PCR Dx System*
12001045 QX200 Droplet Reader, IVD*
12001630 QX200 Automated Droplet Generator, IVD*
12001049 QX200 Droplet Generator, IVD*
10026368 QuantaSoft* Software
1814000 PXI™ PCR Plate Sealer

* Thermal cycler, with the following specifications:
  - Accuracy: ± 0.2°C
  - Uniformity: ± 0.4°C well-to-well within 10 sec
  - Adjustable ramp capability with required ramp rate: up to 2°C/sec
  - Temperature range: 0–100°C

* These products are CE Marked IVD and have not been submitted to U.S. FDA and are not available in the U.S. market.

Note: Bio-Rad is in the process of updating IVD product names to include QXDx. Visit bio-rad.com/web/QXDxNameUpdate for more detailed information about product name updates. Current product is labeled as QX200.

Visit bio-rad.com/web/CEIVD/BCR-ABL for more information.

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