MOLECULAR PATHOLOGY

BCR-ABL p210 QUANTITATIVE TEST
Quantitative RT-PCR on the Qiagen Rotor-Gene® Q using Ipsogen BCR-ABL Mbcr IS-MMR Kits

The Indiana University Health Molecular Pathology laboratory will be changing the reagents and instrumentation for BCR-ABL p210 quantitative transcript testing to kits that convert copy number to the International Scale. These changes will be official as of January 2012.

IU Health will offer the following quantitative assays for quantitative monitoring of BCR-ABL p210, p190 transcripts:

<table>
<thead>
<tr>
<th>Assay</th>
<th>Methodology</th>
<th>Type</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCR-ABL p210 QN-IS-MMR</td>
<td>Ipsogen kits on Qiagen Rotor-Gene® Q</td>
<td>Quantitative, *relative to International Standard</td>
<td>0.002–100%; &lt;0.002 detected</td>
</tr>
<tr>
<td>BCR-ABL p190 QN</td>
<td>Ipsogen kits on Roche LightCycler® 2.0</td>
<td>Quantitative</td>
<td>0.001–100%</td>
</tr>
</tbody>
</table>

Both assays are intended for use as an aid in the management of CML/ALL individuals, including those undergoing imatinib mesylate (Gleevec) therapy. Results are to be interpreted within the context of all relevant clinical and laboratory findings.

What is different about the p210 kits?
The new kits include reference materials that allow percentage results to be converted to the International Scale, a more specific standard for determination of major molecular response (MMR) levels.

The new kits will be able to assess:
- Treatment of patients at the level of MMR with improved standardization and accuracy of BCR-ABL quantification.
- Values on the IS will allow comparison of response rates, including clinical trials for laboratories using the IS developed by the World Health Organization (WHO). IS will reduce the variation in test performance and reports from laboratories worldwide.
How will the assay changes affect clinical decisions for patients?
For p190 testing, there will be no changes.
For p210 testing, the percentage results will now be converted to the International Standard (IS). Assay performance characteristics have been evaluated by IU Health Molecular Pathology.

What differences will physicians see?
The new kits show improved accuracy now relative to the IS. Assay performance characteristics evaluated at IU Health Molecular Pathology show a range of 0.002 to 100%. We will be able to detect lower than 0.002%, but without validated quantification. Those patients will be reported as <0.002% detected. In changing reagents, methodology, and instrumentation the percentages vary most at the high positive range for the assay (> 5%). As patient transcript levels decreased, there is increased sensitivity where it is clinically significant in determining achievement of MMR.

The figure below shows a side-by-side comparison of a patient monitored over time with the p210 kits and new IS-MMR kits:

<table>
<thead>
<tr>
<th>Patient E</th>
<th>Run Number</th>
<th>Current Kit %</th>
<th>New Kit % IS</th>
<th>Difference in Log Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100.0</td>
<td>82.309</td>
<td>0.084</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>25.419</td>
<td>17.794</td>
<td>0.154</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.217</td>
<td>0.121</td>
<td>0.253</td>
<td></td>
</tr>
</tbody>
</table>

Should patients be re-baselined? Is there a conversion factor?
Patient transcript values that have been high (>5%) or not detected do not need to be re-baselined. If a physician is interested in a comparison of new transcript values for patients in the low positive range close to MMR to 5%, please contact the IU Molecular Pathology laboratory.

Additionally, due to the number of variables introduced with the changes in kits, methodology, and instrumentation there is not a consistent conversion factor.

Please note: A larger quantity of blood may be appropriate if the leukocyte count is low since we perform extraction from buffy coat.

Any questions or concerns?
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