UKNEQAS - Molecular Diagnosis of Haematological Malignancies

BCR-ABL Quantitation Programme

Jane Holden
MDHM Programme

- 110 participants registered
- Organised into 5 sub-programmes:
  - IgH/TCR clonality testing
  - JAK2 V617F status
  - BCR-ABL and AML translocation identification (including t(8;21), t(15;17) and inv(16))
  - BCR-ABL quantitation
  - Post SCT chimerism monitoring
- 2 trials issued per year per sub-programme
BCR-ABL Quantitation Programme

- 63 participants
- K562 (M-BCR) cell-line distributed for BCR-ABL quantitative analysis – lyophilised to ensure RNA stability
- 2 trials issued
BCR-ABLQ Trial 1

- Issued to 39 participants; only 24 returned results
- 1 sample issued – 1% dilution of K562 cells in HEL cells (lyophilised)
- Participants asked to perform quantitative analysis and report M-BCR level
BCR-ABLQ Trial 1

![Bar chart showing the distribution of M-BCRQuantitative Level (%) for different number of participants for BCR control gene, β-GUS control gene, and ABL control gene. The x-axis represents the M-BCR Quantitative Level (%) ranging from 0-5 to 95-100, and the y-axis represents the number of participants. The chart displays the data for each control gene in different ranges.]
BCR-ABLQ Trial 2

- Issued to 63 participants; 55 returned results
- 3 samples issued (lyophilised)
  - 100% K562 sample (diagnostic sample)
  - ~0.4% dilution of K562 in HEL (follow-up sample)
  - 100% HEL sample (negative sample)
- Participants asked to perform quantitative analysis and report Log reduction in M-BCR at follow-up
BCR-ABLQ Trial 2

Graph to show the distribution of Log reduction results for MDHM BCR-ABLQ

Log Reduction

Number of Participants

0-0.5 0.51-1 1.01-1.5 1.51-2 2.01-2.5 2.51-3 3.01-3.5 3.51-4

Beta-2 microglobulin control gene
G6PDH control gene
BCR control gene
β-GUS control gene
ABL control gene
BCR-ABL Quantitation Results

- Variation in quantitative level due to:
  - Different in-house protocols/kits
  - Different control genes
  - Different material for standard dilutions
<table>
<thead>
<tr>
<th>Method Parameter</th>
<th>Number of Participants</th>
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<td>Control Gene:</td>
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<td>ABL</td>
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<td>G6PDH</td>
<td>6</td>
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<td>Beta-2 microglobulin</td>
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<td>β-GUS</td>
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<td>In-house Plasmids</td>
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<td>Serial dilution from K562 DNA</td>
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<tr>
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BCR-ABL Quantitation Results

- Variation in quantitative level due to:
  - Different in-house protocols/kits
  - Different control genes
  - Different material for standard dilutions
  - Different instruments and analysis software
<table>
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<td>ABI 5700</td>
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<td>BioRAD Icycler</td>
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<td>Stratagene MX3000p</td>
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</table>
UK NEQAS for Leucocyte Immunophenotyping

BCR-ABL IQ Trial 2

![Bar chart showing log reduction for different instruments: ABI 7000, ABI 7500, ABI 7900, Light Cycler, Rotor-Gene.]

Instrument

Log Reduction

0.0 0.5 1.0 1.5 2.0 2.5 3.0

ABI 7000
ABI 7500
ABI 7900
Light Cycler
Rotor-Gene
BCR-ABL Quantitation Results

- Variation in quantitative level due to:
  - Different in-house protocols/kits
  - Different control genes
  - Different material for standard dilutions
  - Different instruments and analysis software
  - RNA/cDNA quality
BCR-ABLQ Trial 2

Control gene copy number (ABL)

Number of participants

Sample 024
Sample 025
Sample 026
BCR-ABL Quantitation Results

- Control gene Ct values vary greatly indicating variation in the quality of RNA/cDNA:
  - ABL 19.93-32.08
  - G6PDH 19.12-35.95
  - β-GUS 22.26-25.76
  - B2M 24.69-25.53
  - BCR 34.98

- Several labs perform RQ-PCR with only one or two replicates

- Quantitative M-BCR level is not expressed in a standard way
BCR-ABL Q Future Work

- Decrease M-BCR level in trial samples
- Introduce scoring system – enables consistently poor performers to be identified
- Introduction of standardisation and guidelines
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UKNEQAS Genetics SAG

jlholden@btconnect.com

UK NEQAS for Leucocyte Immunophenotyping