UKNEQAS - Molecular Diagnosis of Haematological Malignancies

BCR-ABL Quantitation Programme

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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

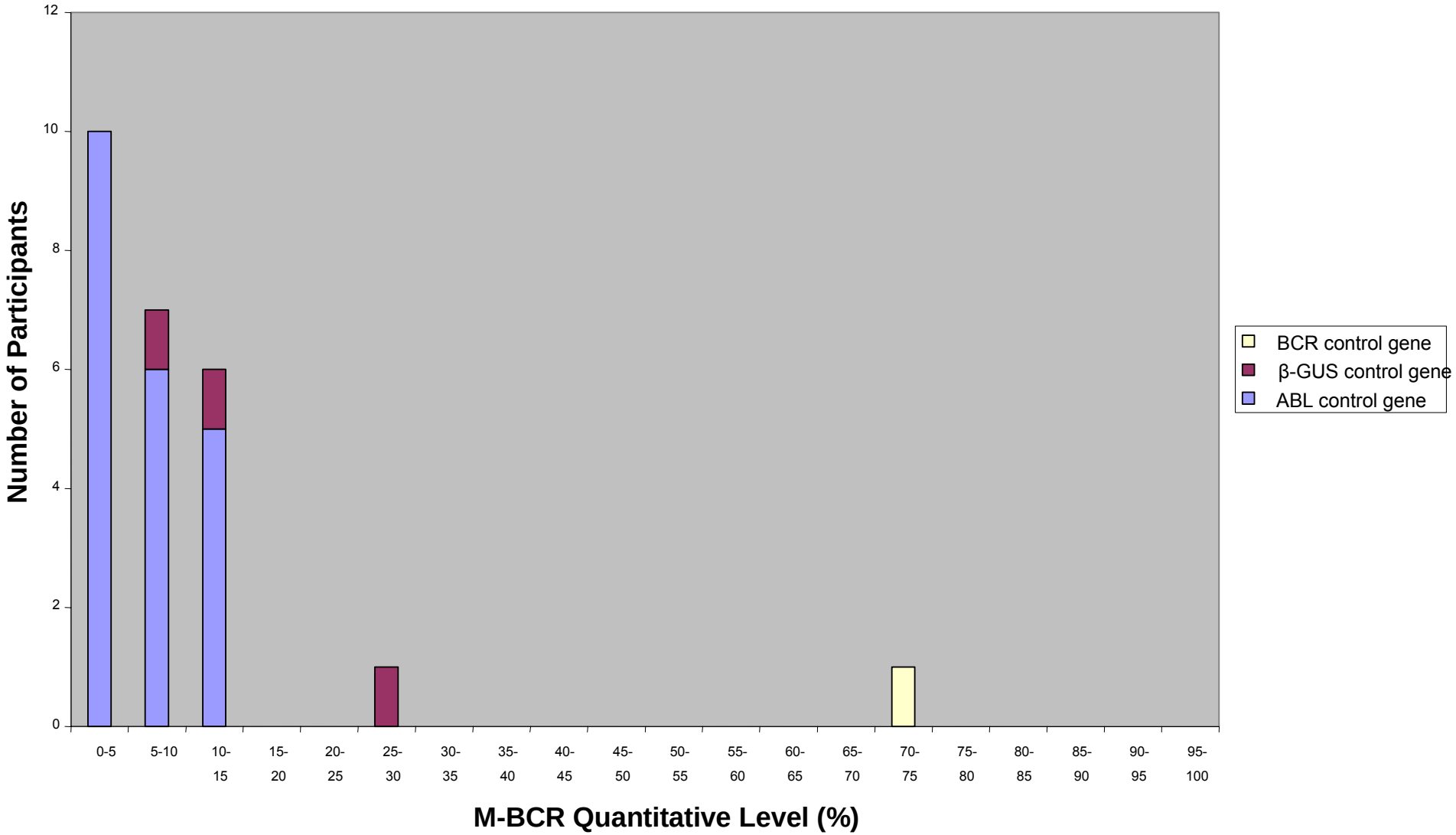
BCR-ABL Quantitation Programme

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

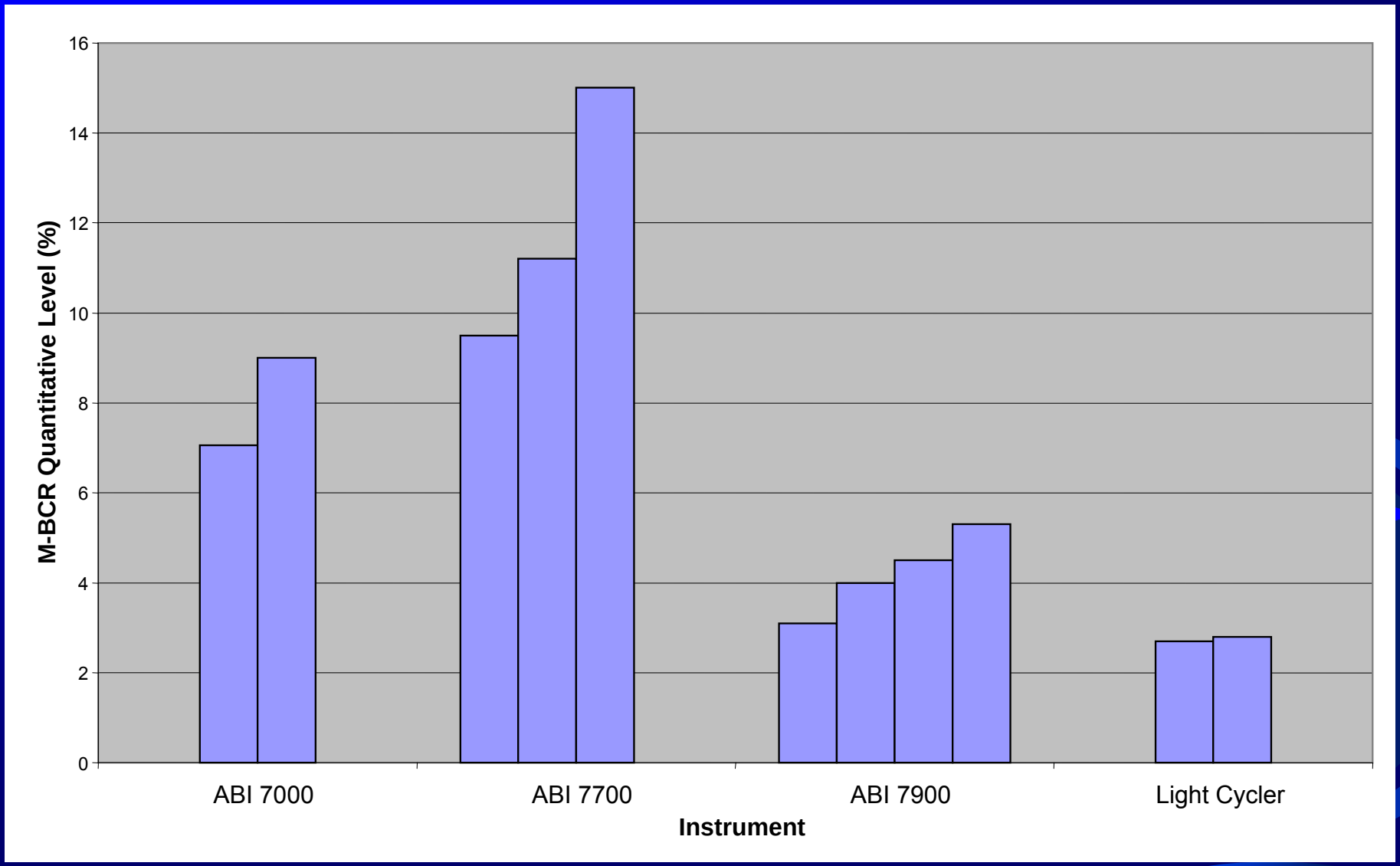
BCR-ABLQ Trial 1

- 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



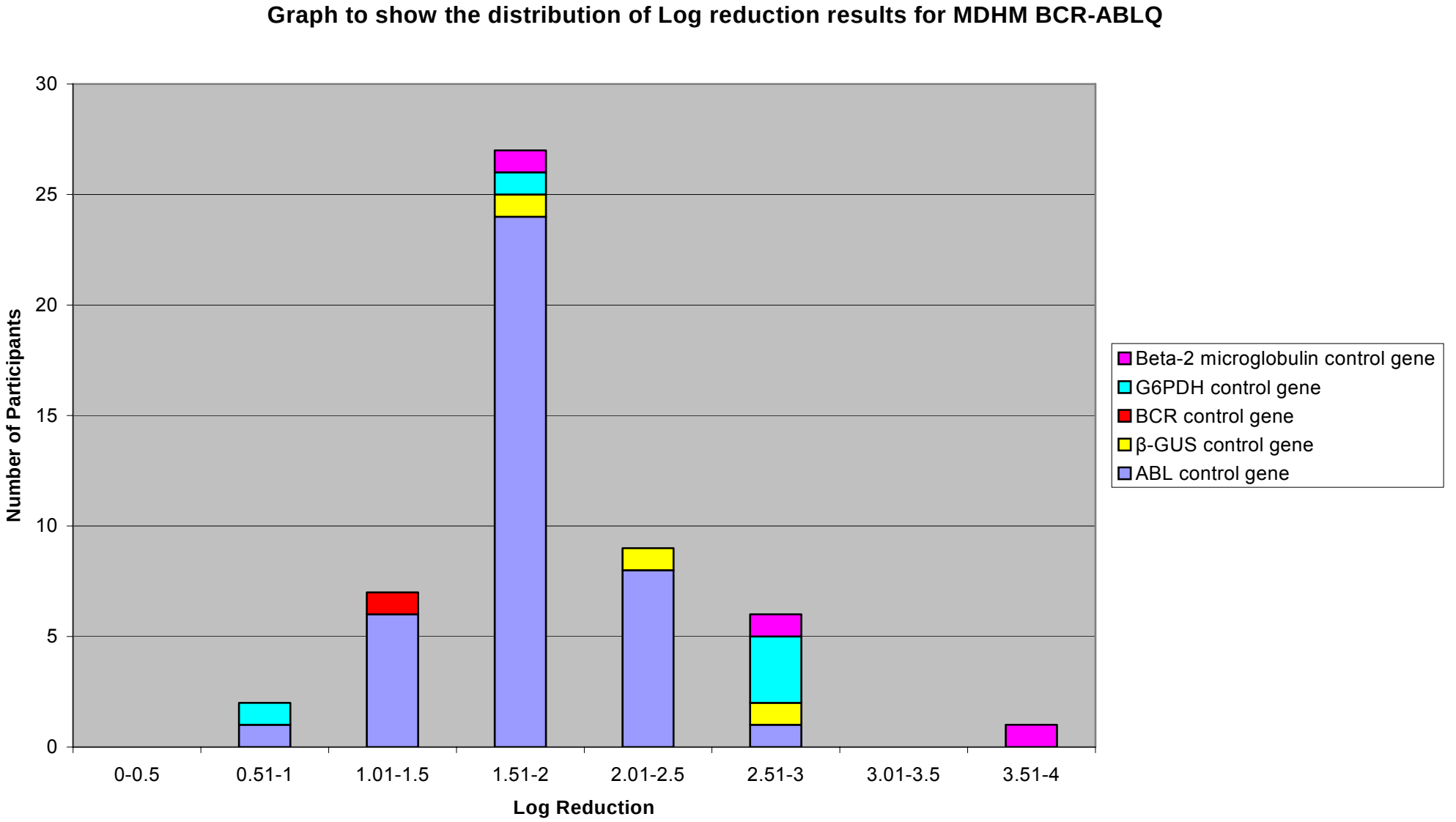
BCR-ABLQ Trial 1



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



BCR-ABL Quantitation Results

- Variation in quantitative level due to:
  - Different in-house protocols/kits
  - Different control genes
  - Different material for standard dilutions

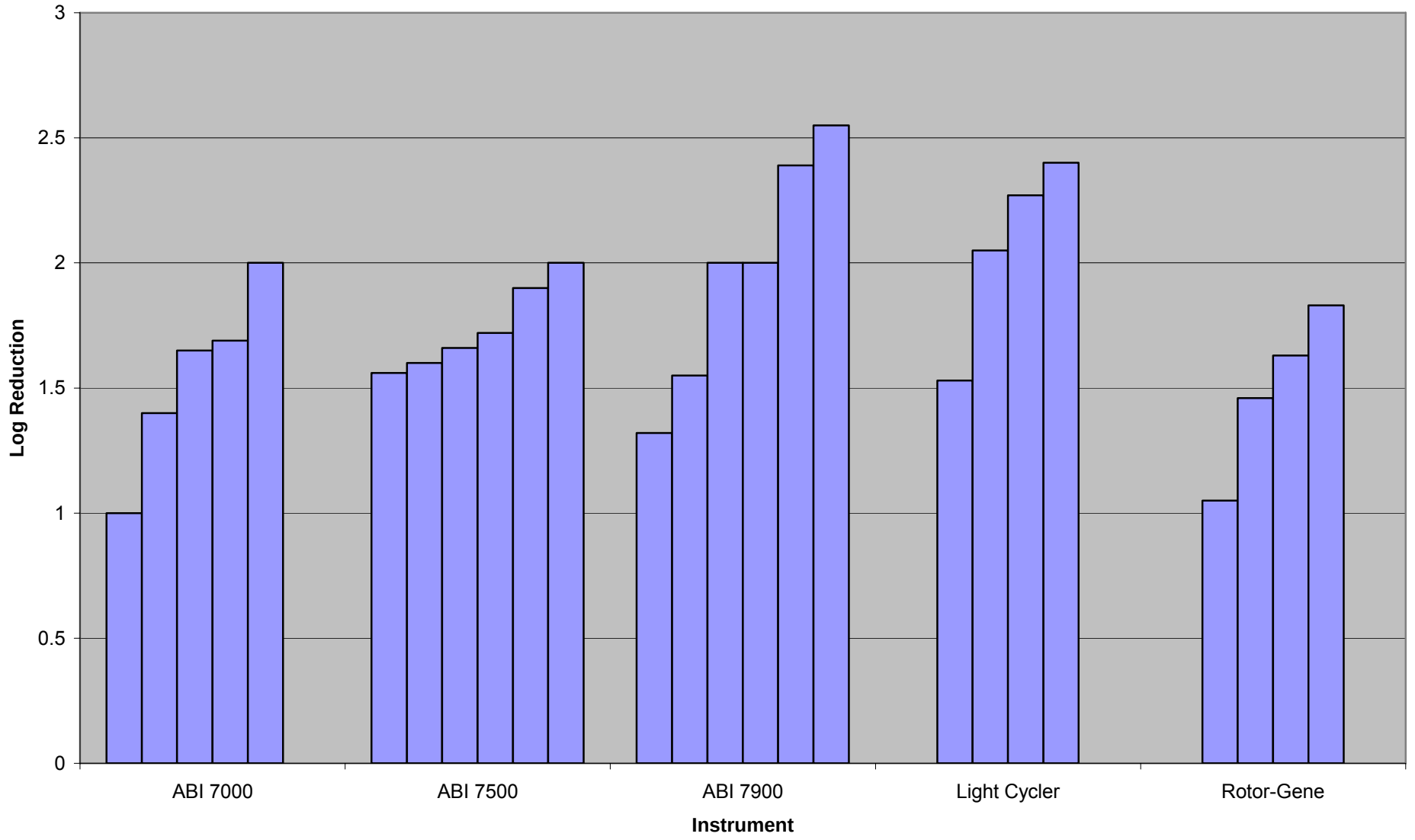
Method Parameter	Number of Participants
Control Gene:	
ABL	42
G6PDH	6
Beta-2 microglobulin	3
β-GUS	3
BCR	1
Material for Standard Dilutions:	
Commercial Plasmids (Ipsogen)	32
In-house Plasmids	13
Serial dilution from K562 DNA	3
No standards used	4
No info provided	3

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

Instrument	Number of Participants
ABI 5700	1
ABI 7000	6
ABI 7300	2
ABI 7500	10
ABI 7700	4
ABI 7900	8
Roche Light Cycler	15
Rotor-Gene	4
Cepheid Smartcycler	1
BioRAD Icycler	1
Stratagene MX3000p	2

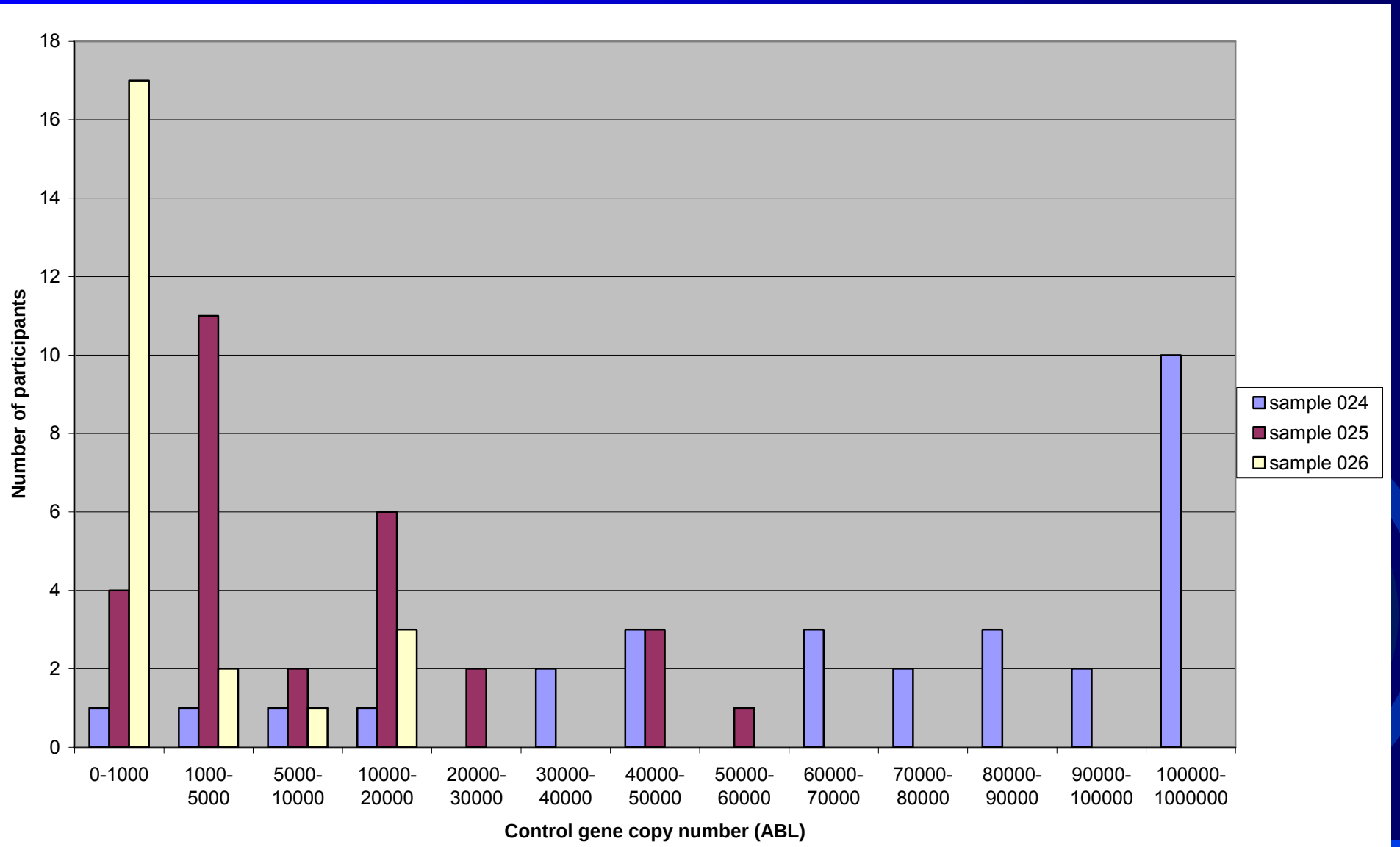
BCR-ABLQ Trial 2



BCR-ABL Quantitation Results

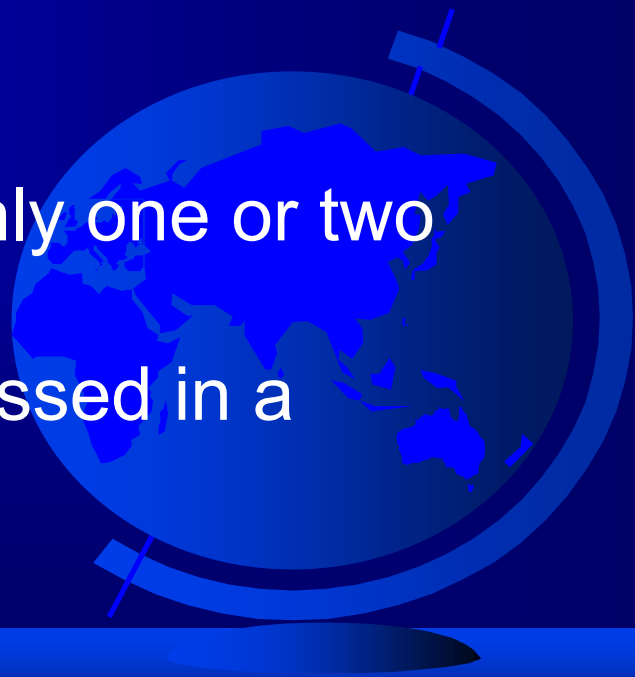
- Variation in quantitative level due to:
  - Different in-house protocols/kits
  - Different control genes
  - Different material for standard dilutions
  - Different instruments and analysis software
  - RNA/cDNA quality

BCR-ABLQ Trial 2



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

Acknowledgements

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