Establishment of the 1st World Health Organization International Genetic Reference Panel for quantitation of BCR-ABL mRNA

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Introduction

- Estimation of *BCR-ABL* mRNA levels is an important indicator of therapeutic response for patients with chronic myeloid leukemia and Philadelphia-chromosome positive acute lymphoblastic leukemia, but there is substantial variation in the real time quantitative PCR (RQ-PCR) methodology employed, what control gene is used for normalisation and how results are reported
- An international scale (IS) has recently been established that is anchored to two key points defined in the IRIS trial: a common baseline (100% *BCR-ABL*^{IS}) and major molecular response (0.1% *BCR-ABL*^{IS})
- To help propagate and improve the accessibility of the IS we sought to develop internationally accredited *BCR-ABL* reference reagents.
- Following an evaluation of candidate cell lines, the aim of the international collaborative study was to manufacture and evaluate a reference material panel comprising four different dilution levels of freeze dried preparations of K562 cells diluted in HL60 cells and assign fixed % *BCR-ABL* / *control gene* IS values to each material

Methods

Development of materials was co-ordinated by the UK National Genetics Reference Laboratory (Wessex) in conjunction with the National Institute for Biological Standards and Control

- Four dilutions of K562 in HL60 corresponding approximately to 10%IS (*BCR/ABL* 4 08/198), 1%IS (*BCR/ABL* 3 08/196), 0.1%IS (*BCR/ABL* 2 08/194) and 0.01%IS (*BCR/ABL* 1 08/192) *BCR-ABL / ABL* were prepared.
- 3ml glass ampoules were filled with 1.5 x 10^6 cells (0.5ml) for freeze drying and approximately 3500 vials of each material were produced
- Field trial evaluation of the reagents was performed by 10 laboratories with stable and validated conversion factors (6 European Union, 1 Canada, 3 Asia/Australasia) RNA was extracted from each material and, following reverse transcription, cDNA was tested in lab specific qPCR assays.
- Transcript copy numbers for *BCR-ABL* and the three control genes were used to calculate %*BCR-ABL*/*ABL*, %*BCR-ABL*/*BCR* and %*BCR-ABL*/*GUSB*.
- The mean % values for each material were then converted to the IS by multiplying the %*BCR-ABL*/control gene value by the established CF for each laboratory.

Results

Reagent	Median µg RNA	Mean µg RNA
BCR/ABL 1 08/192	16.79	14.86
BCR/ABL 2 08/194	16.66	14.34
BCR/ABL 3 08/196	15.40	14.16
BCR/ABL 4 08/198	18.99	17.42
Combined	**17.11**	**15.19**

NIBSC Code	08/192 BCR-ABL 1	08/194 BCR-ABL 2	08/196 BCR-ABL 3	08/198 BCR-ABL 4
Date filled	04.09.08	04.09.08	04.09.08	04.09.08
CV of the fill (%) (n=132-144)	0.19	0.19	0.28	0.22
Residual moisture after lyophilisation (%) (n=12)	0.4701	0.4208	0.4833	0.4760
Mean dry weight (g) (n=6)	0.0115	0.0115	0.0114	0.0112
Mean residual oxygen % (n=12)	0.20	0.28	0.29	0.43
No of ampoules available	3614	3467	3389	3558
Presentation	Sealed, glass 3mL DIN ampoules			
Excipient	2X phosphate buffered saline			
Address of facility where material was processed	NIBSC, Potters Bar, Herts, UK			
Present custodian	NIBSC, Potters Bar, Herts, UK			
Storage temperature	-20°C			

Figure 1: RNA Extraction and product summaries for the four reference materials. The median and mean quantity of RNA extracted from each vial (1.5 x 10^6 cells / vial) was 17.11µg and 15.19µg respectively (n=30). The product summaries fulfilled the requirements of the WHO for establishment of the materials as an international genetics reference panel

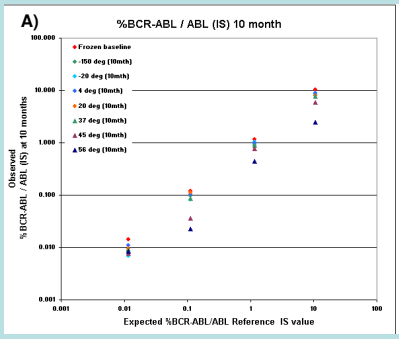
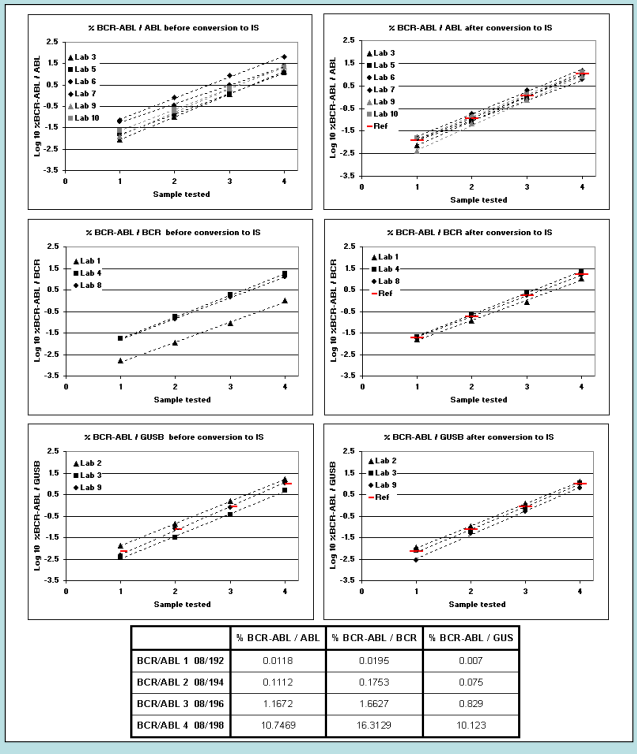


Figure 2: Accelerated degradation study. A) %*BCR-ABL*/ ABL IS at 10 months post fill. The *BCR-ABL*/*ABL* ratios per fixed volume of cDNA for each of the four reference materials are shown after storage at the designated temperatures for 10 months. **B) Mean total copy number of *ABL*, *BCR* and *GUSB* control gene transcripts** per fixed volume of cDNA for the four reference materials after storage at the designated temperatures for 10 months. These data demonstrate that the materials are acceptable for analysis if stored at or below +37°C for 10 months



	% BCR-ABL / ABL	% BCR-ABL / BCR	% BCR-ABL / GUS
BCR/ABL 1 08/192	0.0118	0.0195	0.007
BCR/ABL 2 08/194	0.1112	0.1753	0.075
BCR/ABL 3 08/196	1.1672	1.6627	0.829
BCR/ABL 4 08/198	10.7469	16.3129	10.123

Figure 3: Linear regression plots of log transformed %*BCR-ABL*/ *control gene*. (Sample 1; BCR/ABL 1 08/192, Sample 2; BCR/ABL 2 08/194, Sample 3; BCR/ABL 3 08/196 & Sample 4 BCR/ABL 4 08/198). Regression plots are shown for each control gene before and after conversion to the IS. The red lines indicated the IS reference values assigned for each material as designated in the table. The assigned IS values were slightly different for each control gene as the relative level of expression of these three genes in HL60 cells is not identical to that seen in peripheral blood leukocytes.

Summary

This study indicates that the freeze dried materials are suitable for use as reference materials for the quantitation of *BCR-ABL* mRNA (b3a2 transcript) using RQ-PCR. The materials were approved as the 1st International Genetic Reference Panel quantitation of *BCR-ABL* translocation by RQ-PCR by the Expert Committee on Biological Standardization of the World Health Organization in November 2009 and are available from NIBSC (www.nibsc.ac.uk) for the calibration of secondary reference reagents.

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