



Development of prototype reference reagents for *BCR-ABL* quantitative RT-PCR

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Introduction

- RQ-PCR is used routinely to quantify levels of *BCR-ABL* mRNA and thereby monitor the response of CML patients to treatment
- Despite efforts to establish standardised protocols, there is still substantial variation worldwide in the way RQ-PCR for *BCR-ABL* is undertaken
- An international scale (IS) has been established recently 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})
- Definition of the IS currently relies on relating results directly or indirectly to the Adelaide international reference laboratory
- A more robust definition of the IS requires the development of internationally accredited reference reagents

Aims

The aim of this collaborative study was to produce and assess the use of freeze dried cell lines and Armored RNA® (aRNA) constructs as candidate reference materials for the standardisation of *BCR-ABL* RQ-PCR protocols.

Summary and Conclusions

- Both freeze dried cells and aRNAs performed well in the field trials and appear to be suitable for the development of *BCR-ABL* reference reagents
- The aRNA samples worked well when directly heat lysed prior to cDNA synthesis but further optimisation is required to ensure adequate recovery of low aRNA mass input during RNA extraction.
- We are planning large scale production of the freeze dried cell lines as 'higher order' internationally accredited primary reference materials
- The aRNA constructs will undergo a further round of field trial evaluation with the aim of establishing them as secondary reference reagents.

Study participants

Asia / Australasia: Adelaide: T Hughes, S Branford, L Fletcher. Hong Kong: ESK Ma. Japan: K Miyamura. Korea: D-W Kim, H Goh, S Park. Singapore: G-F How.

Europe: Austria: D Denk, T Lion, M Neßlböck. Czech Republic: D Dvorakova, J Moravcová, J Rulcová. France: N Beaufils, J-M Cayuela, X Fund, J Gabert, F Hermitte, E Lippert, F-X Mahon, N Maroc. Finland: V Kairisto. Germany: A Hochhaus, MC Müller, H Pfeifer, O Ottmann. Greece: K Zoi. Italy: I Iacobucci, G Martinelli, F Quarantelli, G Saglio. Spain: D Colomer, MT Gómez Casares. Sweden: G Barbany, A Carpelan. UK: A Awan, N Cross, L Foroni, G Gerrard, E Gray, R Hawkins, P Matejtschuk, A Sproul, L Wang, H White.

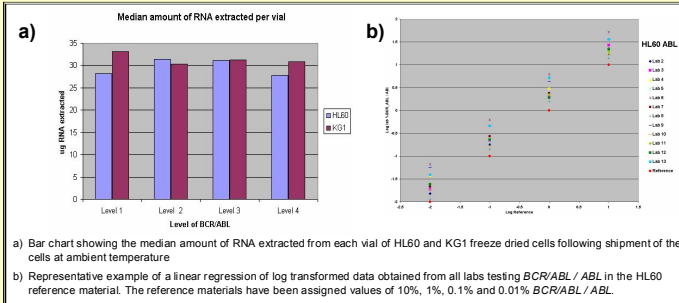
USA: Austin: J Hedges, E Labourier, C WalkerPeach. Boston: J Longtine. Houston: S Hai, D Jones. New York: YL Wang. Portland: YE Beillard, S Evonuk, C Fuller, R Press.

Freeze dried cell line field trial

Methods

- Development of the freeze dried cell lines was co-ordinated by the NGRL (Wessex) in conjunction with the NIBSC.
- An initial assessment of 30 haemopoietic cell lines, HL60 and KG1 were found to express relative levels *BCR*, *ABL* and *GUSB* that were closest to normal leukocytes and were therefore selected for further analysis.
- Eight reference standards were prepared by diluting K562 cells (b3a2 *BCR-ABL* expressing cell line) into HL60 and KG1.
- Four levels of *BCR-ABL* in each negative cell line were produced and freeze dried at 3×10^6 cells/vial. The performance of the freeze dried cells was assessed by an international field trial (June to October 2007) that involved 14 laboratories (7 EU, 4 USA, 3 Asia/Australasia) using 7 different protocols and 9 different RQ-PCR platforms.

Results



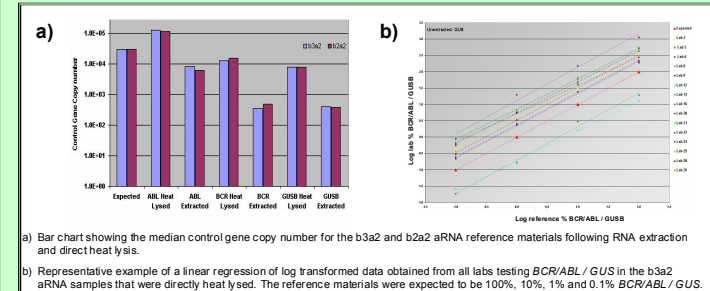
- RNA (median 30µg/vial) was successfully extracted from freeze dried cell mixtures shipped worldwide at ambient temperature.
- The median number of copies obtained for *ABL*, *BCR* and *GUSB* for the HL60 material were 1.16×10^5 , 2.60×10^5 and 1.70×10^5 respectively and for the KG1 material were 1.02×10^5 , 5.85×10^5 and 1.13×10^5 respectively.
- The coefficient of variation for % *BCR-ABL* / control gene values obtained from all labs were comparable to those obtained in the IS conversion factor rounds administered by the Mannheim laboratory using primary patient samples.
- There was no statistical difference between the CVs obtained from either cell line for any of the control genes.

Armored RNA (aRNA) field trial

Methods

- aRNA is based on bacteriophage coat protein encapsulation of specific RNA targets to form pseudo-viral particles that stabilise and protect RNA transcripts from nuclease degradation.
- aRNA is designed for use as standards and controls in molecular assays, in particular as positive controls for amplification and detection using RT-PCR.
- Development of the aRNA material was co-ordinated by the NGRL (Wessex) in conjunction with Asuragen, Inc. aRNAs were produced for b2a2 and b3a2 *BCR-ABL*, plus *BCR*, *ABL* and *GUSB*
- Nine aRNA reference standards were prepared containing 3×10^4 (Level 1), 3×10^3 (Level 2), 3×10^2 (Level 3) and 3×10^1 (Level 4) copies/ul of b3a2 or b2a2 aRNA with each control gene aRNA (*ABL*, *BCR* & *GUSB*) at 3×10^4 copies/ul.
- The performance of the aRNA samples was assessed by an international field trial (Oct - Dec 2007) that involved 29 laboratories (22 EU, 3 USA, 4 Asia/Australasia) analysing 3 different control genes on 14 different RQ-PCR platforms. aRNA samples were tested after RNA extraction or direct heat lysis.

Results



- Labs using Trizol and QIAGEN columns for RNA extraction demonstrated a median 28 and 2 fold loss of control gene copy number respectively.
- Mean % *BCR-ABL* / control gene values for extracted and heat lysed aRNA samples were not statistically different. However, for the extracted samples, 6/28 labs failed to detect level 3 or level 4 b3a2 transcripts
- r^2 values for the linear regression of the % **b3a2**/control gene data were >98% in 53% and 18% of labs for the heat lysed and extracted samples respectively.
- r^2 values for the linear regression of the % **b2a2**/control gene were >98% in 63% and 34% of labs for the heat lysed and extracted samples respectively.