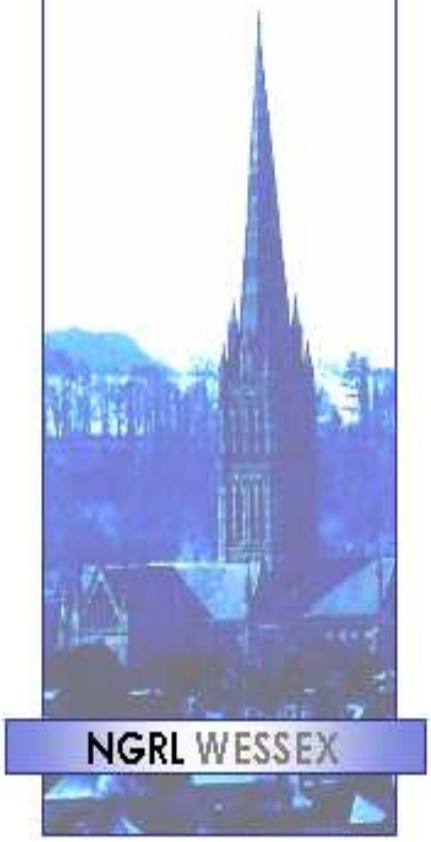
National Genetics Reference Laboratory (Wessex) & National Institute of Biological Standards and Control

NHS

Reference Reagents



Freeze Dried Cell Lines as reference materials for standardisation of *BCR-ABL* RQ-PCR methods: report of field trial evaluation 2009

March 2009

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Contributors to report writing

Name	Role	Institution
Dr Helen White	Senior Scientist	NGRL (Wessex)
Prof Nick Cross	Director	NGRL (Wessex)

Conflicting Interest Statement

The authors declare that they have no conflicting financial interests

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or by contacting

Dr Helen White
National Genetics Reference Laboratory (Wessex)
Salisbury District Hospital
Odstock Road
Salisbury
SP2 8BJ
UK

E mail: hew@soton.ac.uk
Tel: 01722 429016
Fax: 01722 338095

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SUMMARY

- An international scale (IS) for quantitative measurement of *BCR-ABL* mRNA has 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}). 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.

- Four reference standards were prepared by diluting K562 cells (b3a2 expressing cell line) into the *BCR-ABL* negative cell line HL60. Cell mixtures with four levels of *BCR-ABL* were produced and freeze dried at 1.5x10^{6} cells/ampoule.

- The aim of the field trial was to assign fixed % BCR-ABL / control gene values to each material

- The performance of the freeze dried cells was assessed by an international field trial (Jan - March 2009) that involved 10 laboratories (6 EU, 1 Canadian, 3 Asia/Australasia) using 4 different protocols and 8 different RQ-PCR platforms.

- The coefficient of variations obtained from the analysis of seventeen samples analysed in triplicate for each reference material were similar to those obtained when testing 17 aliquots of freshly prepared cell mixes suggesting that contents of the freeze dried ampoules are homogeneous.

- The mean %BCR-ABL / ABL values for the four reference materials following conversion to the IS were 0.0118, 0.1112, 1.1672 and 10.7469

- The mean %BCR-ABL / BCR values for the four reference materials following conversion to the IS were 0.0195, 0.1753, 1.6627 and 16.3129

- The mean %BCR-ABL / GUS values for the four reference materials following conversion to the IS were 0.0071, 0.0749, 0.8295 and 10.1235

- We propose that these values are adopted as the IS reference values for these materials

1. INTRODUCTION

Reverse-transcription real-time quantitative PCR (RQ-PCR) is routinely used to quantify levels of *BCR-ABL* mRNA transcripts in peripheral blood and bone marrow samples from chronic myeloid leukaemia (CML) patients. The technique can determine accurately the response to treatment and is particularly valuable for patients who have achieved complete chromosomal remission. Despite efforts to establish standardised protocols for *BCR-ABL* fusion transcript quantitation[1] there is still substantial variation in the way in which RQ-PCR for *BCR-ABL* is carried out and how results are reported in different laboratories worldwide[2]. In particular, the use of different control genes for normalisation of results means that there are several different units of measurement worldwide, e.g. *BCR-ABL/ABL*; *BCR-ABL/BCR*; *BCR-ABL/GUSB, BCR-ABL/G6PD, BCR-ABL/β2M* etc.

The CML meeting at the National Institutes of Health in Bethesda in October 2005 made several recommendations for the harmonisation of RQ-PCR for *BCR-ABL* including the use of one of three control genes (*ABL*, *BCR* or *GUSB*) [3,4]. Most importantly, a new international scale (IS) for *BCR-ABL* RQ-PCR measurements was proposed which is anchored to two key levels used in the IRIS study [5], namely a standardised baseline defined as 100% *BCR-ABL*^{IS}, and major molecular response (3 log reduction relative to the standardised baseline) defined as 0.1% *BCR-ABL*^{IS}. Laboratories interested in using the IS should derive a laboratory-specific conversion factor to relate values obtained in their laboratory to IS values. The converted value from a given laboratory should then be equivalent to an analogous converted value obtained in any other collaborating laboratory. The strength of this approach is that (i) laboratories can continue to use their existing assay conditions (provided their assay is linear on analysis of the reference samples), and (ii) that they can continue to express results according to local preferences in addition to expressing results on the international scale. The concept of the international scale is analogous to established procedures for other quantitative assays, for example the International Normalised Ratio (INR) for prothrombin time.

The original standards used for the IRIS trial are no longer available, however traceability to the IRIS scale is provided by the extensive quality control data generated by the Adelaide laboratory over a period of several years. Establishment of the IS therefore requires the alignment of local test results either directly or indirectly with those obtained in Adelaide. Currently, this can be achieved by exchange of a series of patient samples with either the Adelaide or Mannheim international reference laboratories. Although this system works well, it is very laborious and consequently only open to a limited number of laboratories at any given time. The availability of internationally accredited reference reagents should in principle help to make the IS more accessible, as well providing a more robust framework for the scale itself.

Ideally, the formulation for reference reagents should be as close as possible to the usual analyte, should cover the entire analytical process, i.e. from RNA extraction to result and should be applicable to methods in use throughout the world. However it is essential that the formulation is stable over a period of several years and that it is physically possible to produce batches of sufficient size to satisfy demand over a similar period of time. It has been shown previously that good quality RNA can be extracted from freeze dried K562 cells[6] and therefore one possible solution is the use of freeze dried cell line mixtures.

The aim of this collaborative study was to produce and perform an assessment of the use of freeze dried cell line mixtures as universal reference materials. As an initial step we identified cell lines in which the relative ratio of the three recommended control genes – *BCR, ABL* and *GUSB* – was similar to that seen in normal leucocytes. We surveyed 26 haemopoietic cell lines (ACC42, Jurkat, JVM2, Loucy, MOLT3, NALM76, PEER, REH, RS4-11, T-ALL1, THP1, HeLa, MV4-11, Karpas, Caco2, ML-1, ML-2, 1E8, ALLPo, NB4, KG1, KG1a, HL60, Kasumi1, NOMO1, SKM1) in up to 4 different centres and identified KG1 and HL60 as the best candidates to take forward for detailed evaluation. Following further field trial studies the HL60 cell line was chosen for the production of the reference material (http://www.ngrl.org.uk/Wessex/downloads/pdf/NGRLW_NIBSC_BCR_ABL_1.0.pdf). This report details the preparation of four reference materials and their evaluation in an international field trial that involved 10 laboratories (6 EU, 1 Canada, 3 Asia/Australasia) using 4 different protocols and 8 different RQ-PCR platforms. The aims of this field trial were to: i)Test four different dilution levels of K562 diluted in HL60 by measuring absolute copy numbers of BCR-ABL, absolute copy numbers of control genes and the BCR-ABL/control gene ratios and ii) assign fixed % BCR-ABL / control gene values to each material.

2. MATERIALS AND METHODS

2.1 Cell lines and preparation of reference materials

The HL60 cell line was obtained from DSMZ and the K562 cell line was obtained from the Hammersmith Hospital, London, UK. All cell lines were grown by ECACC in RPMI 1640 medium supplemented with 10% fetal bovine serum (Sigma Aldrich). We produced four dilutions of K562 in HL60 that corresponded approximately to 10% (BCR/ABL 4 08/198), 1% (BCR/ABL 3 08/196), 0.1% (BCR/ABL 2 08/194) and 0.01% (BCR/ABL 1 08/192) *BCR-ABL / ABL*. Cell suspensions in ice cold 2X PBS were transferred to the National Institute for Biological Standards and Control (NIBSC) on ice. Cell suspensions were stored at 4ºC overnight with gentle stirring. The following day 3ml glass ampoules were filled with 1.5 x 10^6 cells (0.5ml) for freeze drying. The drying process took 65-67 hours and approximately 3500 vials of each level were produced.

3. FIELD TRIAL EVALUATION (26TH JAN – 6TH MARCH 2009)

Ten laboratories, who have validated IS conversion factors (appendix 1), took part in the field trial. Freeze dried cells in glass ampoules were distributed to laboratories by courier at ambient temperature. Twelve glass ampoules were supplied in three bags labelled batch 1, batch 2 and batch 3. Each bag contained 4 ampoules:

HL60 / K562 Level 1	approx 0.01% BCR/ABL	(labelled BCR/ABL 1 08/192)
HL60 / K562 Level 2	approx 0.1% BCR/ABL	(labelled BCR/ABL 2 08/194)
HL60 / K562 Level 3	approx 1.0% BCR/ABL	(labelled BCR/ABL 3 08/196)
HL60 / K562 Level 4	approx 10% BCR/ABL	(labelled BCR/ABL 4 08/198)

The cells were resuspend cells using the following protocol:

- Carefully open the glass ampoule following the instructions provided.
- Resuspend freeze dried cells in 1ml Trizol or 600µl RLT (Qiagen). Ensure that the material is fully lysed by repetitive pipetting.
- Transfer lysate to 1.5ml tube

RNA was extracted from each batch on different days using the entire lysate for each RNA extraction. Two cDNA reactions were performed for each of the extracted RNA samples from each batch using the established methods of the participating laboratories. The cDNA reaction were performed on different days, giving a total of 24 cDNA samples. Figure 1 shows the suggested order of analysis for the minimal number of reverse transcription batches. RQ-PCR for *BCR-ABL* and the control gene(s) was performed on each set of 4 cDNA samples for each batch in separate quantitative runs. Samples for *BCR-ABL* and the control gene(s) were analysed using the established methods of the participating laboratories.

The following data were recorded: date of RNA extraction, total µg of RNA, A_{260} / A_{280}, A_{260} / A_{230}, date of cDNA synthesis, final volume of cDNA reaction, volume of cDNA added to RQ-PCR, date of RQ-PCR, slope and gradient of standard curve, *BCR-ABL* transcript value (Ct value and copy number) control gene(s) transcript value (Ct value and copy number), *BCR-ABL* / control gene(s) (%) before conversion to IS, *BCR-ABL* / control gene(s) (%) converted to IS.

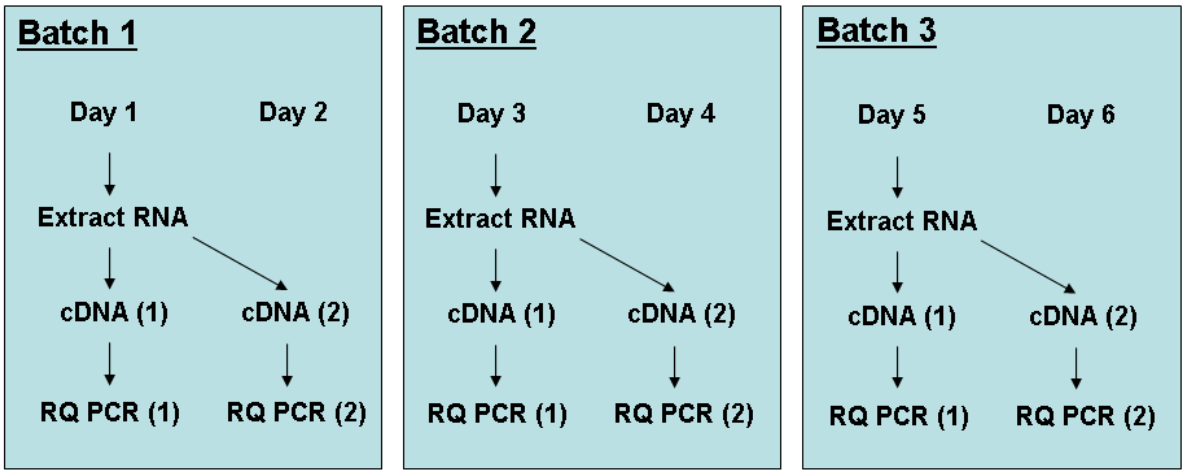


Figure 1: Schematic flow diagram showing suggested order of analysis for the minimal number of reverse transcription reactions and RQ-PCR runs.

3.1 Results of Large Field trial

10 laboratories participated in the field trial and all labs returned data; 6 participants from Europe, 1 from Canada, 1 from Korea and 2 from Australia (Appendix 1).

3.2 Methodologies used

3.2.1 Control genes analysed

Three control genes were analysed in this study: *ABL* (6 labs, all with validated CF), *BCR* (5 labs, 3 with validated CF) and *GUSB* (6 labs, 3 with validated CF). 5 labs tested the samples using more than one control gene.

3.2.2 RQ-PCR machines used

Eight different type of RQ-PCR machines were used in this study: ABI 7500 (n=3), ABI 7000 (n=1), ABI 7700 (n=1), ABI 7900 (n=1), ABI 7300 (n=1), Corbett RotorGene 6000 (n=1), Roche LightCycler 1.5 (n=1), Roche LightCycler 480 (n=1)

3.2.3 RQ-PCR methods

3.2.3.1 RNA Extraction methods

Two RNA extraction methods were used; 8 labs used Trizol (Invitrogen) and 2 used QIAGEN extraction kits.

3.2.3.2 RT-PCR method cited

Six of the participants cited Gabert et al. (2003)[1] as one of the references used as their RQ-PCR protocol. Other references cited were Branford et al. (1999)[7] and Emig et al (1999)[8]. One laboratory used an 'in house' developed assay.

3.2.3.3 RT-PCR method and primers used

All labs used random hexamers for reverse transcription.

3.3 Field trial data analysis

3.3.1 RNA Extraction

The mean and median amount of RNA extracted from each reference material is shown in table 1.

Reagent	Median µg RNA	Mean µg RNA
BCR/ABL 1 08/192	16.70	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**

Table 1: Mean and median amounts of RNA extracted for each reference material (n=10)

3.3.2 Median copy numbers obtained for control genes

The median copy number values obtained for each control gene in each lab are given in table 2. The values have not been corrected for variation in the amount of RNA extracted and used in the cDNA reaction or amount of cDNA added to the RQ-PCR and therefore the data are not comparable between labs. However, the data confirm that the number of control gene transcripts is equal to or greater than that seen in patient samples for all three control genes.

Lab ID	ABL	BCR	GUSB
Lab 1	NT	1.34E+02	NT
Lab 2	NT	NT	2.01E+05
Lab 3	1.57E+05	NT	4.60E+05
Lab 4	NT	7.45E+05	NT
Lab 5	7.12E+04	NT	1.71E+05
Lab 6	2.65E+05	4.42E+05	6.66E+05
Lab 7	5.73E+04	NT	NT
Lab 8	NT	1.14E+06	NT
Lab 9	7.93E+04	NT	2.30E+05
Lab 10	1.96E+05	1.38E+05	2.81E+05

Table 2: Median copy number values obtained for each control gene in each lab (NT = not tested).

3.3.3 Mean, standard deviation and coefficient of variation for % BCR-ABL / Control gene

The mean, standard deviation (SD) and coefficient of variation (CV) for the % *BCR-ABL* / control gene values for each reference material in each lab are shown in tables 3 (*ABL*) 4 (*BCR)* and 5 (*GUSB*). The linear regressions of the log transformed data from all labs (before and after conversion to the IS) are summarised in figures 3 (*ABL*), 4 (*BCR*) and 5 (*GUSB*).

Mean	Lab 3	Lab 5	Lab 6	Lab 7	Lab 9	Lab 10
BCR/ABL 1 08/192	0.0086	0.0161	0.0617	0.0715	0.0120	0.0238
BCR/ABL 2 08/194	0.0976	0.1285	0.3410	0.8270	0.1983	0.2185
BCR/ABL 3 08/196	1.1863	1.3316	3.0366	8.6209	2.2190	2.2352
BCR/ABL 4 08/198	13.9092	11.8597	25.3037	66.4860	23.8594	20.7830

Log 10 Mean	Lab 3	Lab 5	Lab 6	Lab 7	Lab 9	Lab 10
BCR/ABL 1 08/192	-2.0631	-1.7923	-1.2100	-1.1455	-1.9220	-1.6229
BCR/ABL 2 08/194	-1.0104	-0.8911	-0.4673	-0.0825	-0.7027	-0.6605
BCR/ABL 3 08/196	0.0742	0.1244	0.4824	0.9356	0.3461	0.3493
BCR/ABL 4 08/198	1.1433	1.0741	1.4032	1.8227	1.3777	1.3177

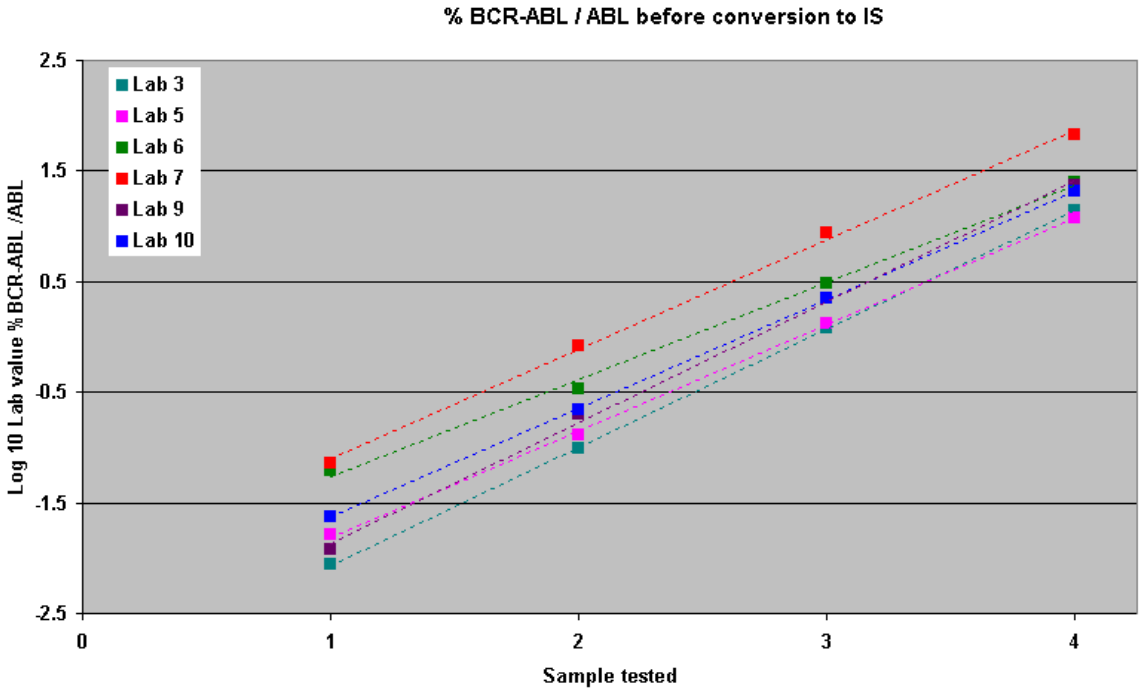
Mean IS	Lab 3	Lab 5	Lab 6	Lab 7	Lab 9	Lab 10
BCR/ABL 1 08/192	0.0076	0.0137	0.0142	0.0165	0.0041	0.0148
BCR/ABL 2 08/194	0.0857	0.1093	0.0784	0.1902	0.0676	0.1360
BCR/ABL 3 08/196	1.0416	1.1323	0.6984	1.9828	0.7567	1.3914
BCR/ABL 4 08/198	12.2123	10.0843	5.8198	15.2918	8.1360	12.9374

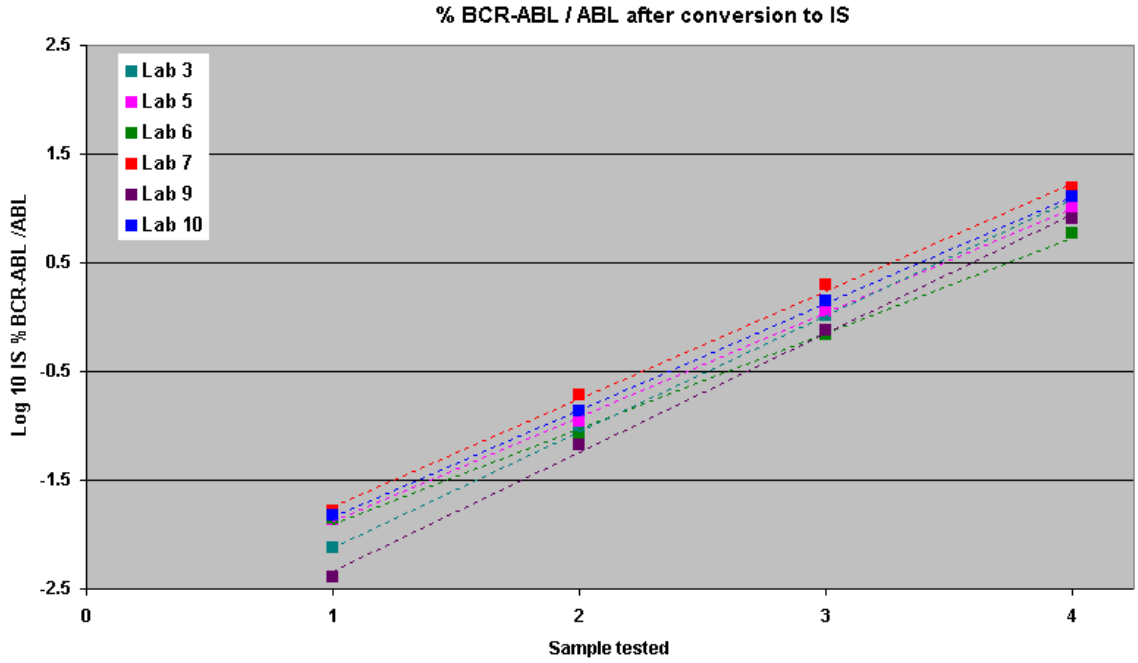
Log 10 Mean IS	Lab 3	Lab 5	Lab 6	Lab 7	Lab 9	Lab 10
BCR/ABL 1 08/192	-2.1196	-1.8626	-1.8483	-1.7838	-2.3892	-1.8287
BCR/ABL 2 08/194	-1.0669	-0.9615	-1.1055	-0.7208	-1.1700	-0.8663
BCR/ABL 3 08/196	0.0177	0.0539	-0.1559	0.2973	-0.1211	0.1435
BCR/ABL 4 08/198	1.0868	1.0036	0.7649	1.1845	0.9104	1.1118

SD	Lab 3	Lab 5	Lab 6	Lab 7	Lab 9	Lab 10
BCR/ABL 1 08/192	0.0037	0.0120	0.0097	0.0232	0.0056	0.0096
BCR/ABL 2 08/194	0.0189	0.0141	0.0218	0.2190	0.0473	0.0818
BCR/ABL 3 08/196	0.2118	0.1688	0.0734	0.8593	0.3550	0.4995
BCR/ABL 4 08/198	1.2875	1.8706	0.7137	7.9925	6.9429	3.0605

CV	Lab 3	Lab 5	Lab 6	Lab 7	Lab 9	Lab 10
BCR/ABL 1 08/192	42.7216	74.3631	15.7977	32.4352	46.6353	40.0975
BCR/ABL 2 08/194	19.3601	10.9946	6.3898	26.4822	23.8553	37.4123
BCR/ABL 3 08/196	17.8522	12.6745	2.4173	9.9676	15.9963	22.3450
BCR/ABL 4 08/198	9.2565	15.7728	2.8207	12.0213	29.0993	14.7259

Table 3: Mean, Log 10 Mean, Mean IS, Log 10 Mean IS, SD and CV values for ***%BCR-ABL* / ABL** for each laboratory for the HL60 / K562 reference materials.





Mean IS Value	Median IS Value
0.0118	0.0140
0.1112	0.0975
1.1672	1.0869
10.7469	11.1483

Figure 2: Summary of the linear regressions of the log transformed data obtained from all labs testing *BCR-ABL / ABL* for the 4 reference materials (Sample 1; BCR/ABL 1 08/192, Sample 2; BCR/ABL 2 08/194, Sample 3; BCR/ABL 3 08/196 and Sample 4 BCR/ABL 4 08/198). Regression plots are shown for data before and after conversion to the IS.

Mean	Lab 1	Lab 4	Lab 6	Lab 8	Lab 10
BCR/ABL 1 08/192	0.0016	0.0167	0.0373	0.0167	0.0260
BCR/ABL 2 08/194	0.0115	0.1799	0.1915	0.1431	0.2408
BCR/ABL 3 08/196	0.0876	1.8261	1.7285	1.4058	2.7437
BCR/ABL 4 08/198	1.0354	17.8138	16.9756	12.4605	28.4188

Log 10 Mean	Lab 1	Lab 4	Lab 6	Lab 8	Lab 10
BCR/ABL 1 08/192	-2.7951	-1.7777	-1.4281	-1.7779	-1.5842
BCR/ABL 2 08/194	-1.9411	-0.7449	-0.7178	-0.8443	-0.6184
BCR/ABL 3 08/196	-1.0575	0.2615	0.2377	0.1479	0.4383
BCR/ABL 4 08/198	0.0151	1.2508	1.2298	1.0955	1.4536

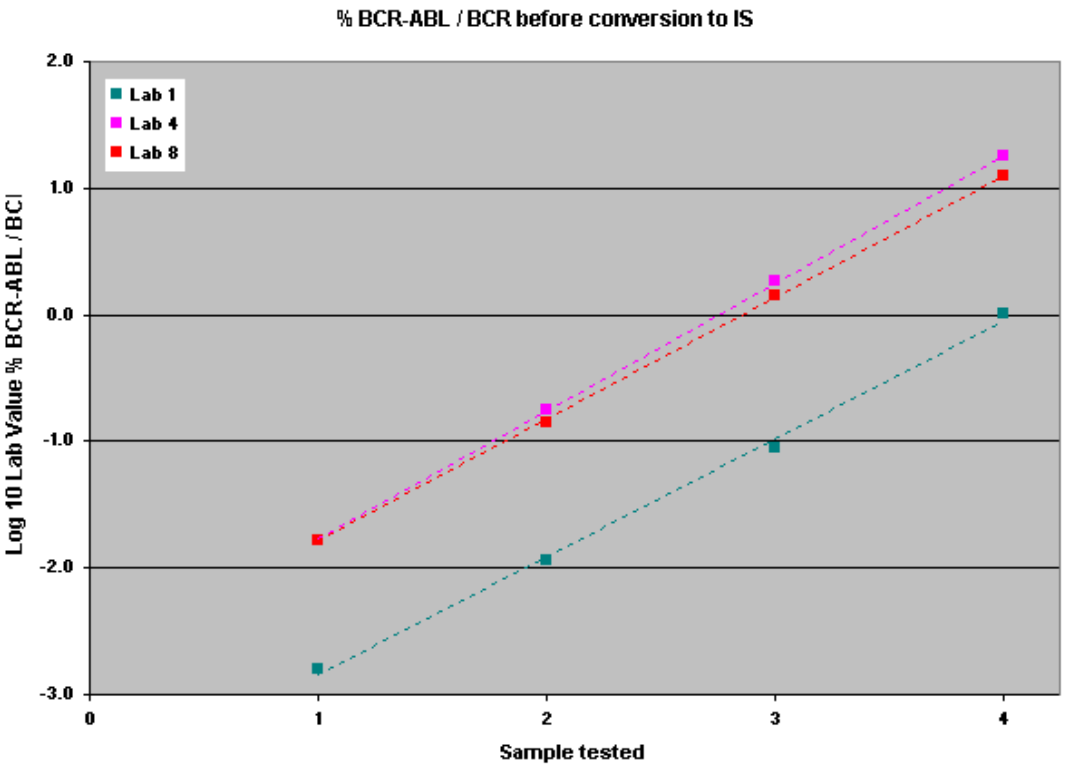
Mean IS	Lab 1	Lab 4	Lab 6	Lab 8	Lab 10
BCR/ABL 1 08/192	0.0164	0.0214		0.0208	
BCR/ABL 2 08/194	0.1168	0.2303		0.1789	
BCR/ABL 3 08/196	0.8936	2.3374		1.7573	
BCR/ABL 4 08/198	10.5613	22.8017		15.5756	

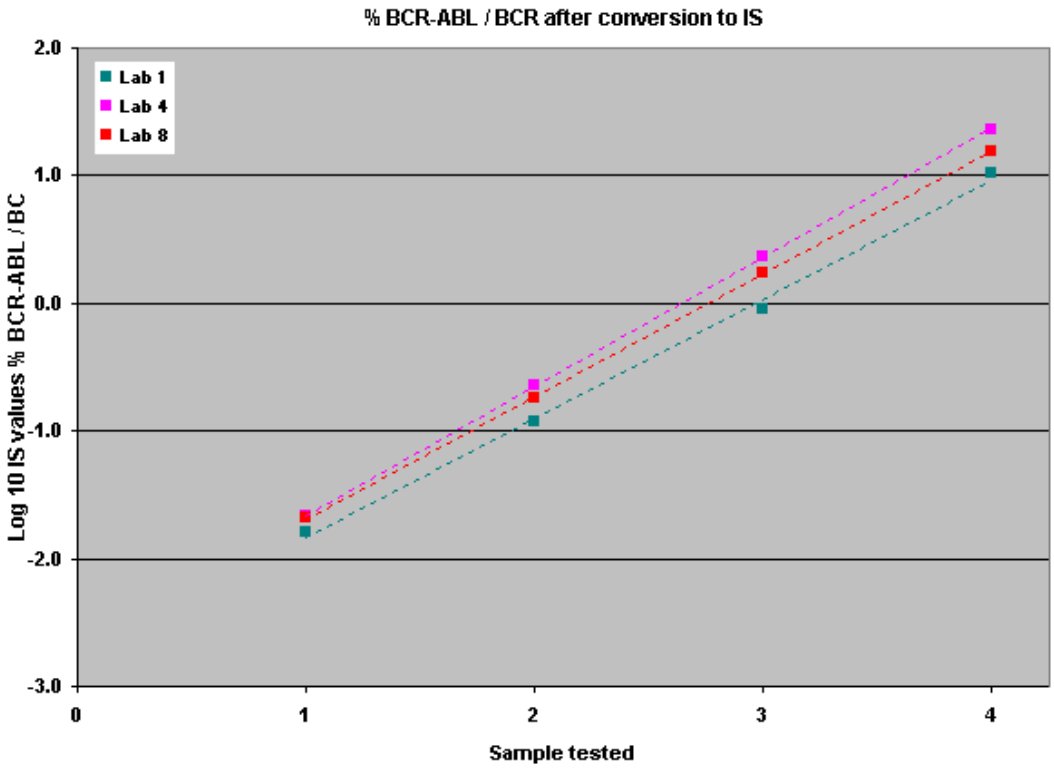
Log 10 Mean IS	Lab 1	Lab 4	Lab 6	Lab 8	Lab 10
BCR/ABL 1 08/192	-1.7865	-1.6705		-1.6810	
BCR/ABL 2 08/194	-0.9325	-0.6377		-0.7474	
BCR/ABL 3 08/196	-0.0489	0.3687		0.2448	
BCR/ABL 4 08/198	1.0237	1.3580		1.1924	

SD	Lab 1	Lab 4	Lab 6	Lab 8	Lab 10
BCR/ABL 1 08/192	0.0011	0.0051	0.0076	0.0059	0.0070
BCR/ABL 2 08/194	0.0029	0.0573	0.0155	0.0361	0.0445
BCR/ABL 3 08/196	0.0220	0.5794	0.0962	0.2913	0.3986
BCR/ABL 4 08/198	0.1363	6.5382	0.7564	6.1754	4.6916

CV	Lab 1	Lab 4	Lab 6	Lab 8	Lab 10
BCR/ABL 1 08/192	67.2156	30.5044	20.3541	35.5514	26.8568
BCR/ABL 2 08/194	25.5523	31.8234	8.0898	25.2317	18.4648
BCR/ABL 3 08/196	25.1497	31.7304	5.5668	20.7235	14.5288
BCR/ABL 4 08/198	13.1598	36.7030	4.4558	49.5595	16.5086

Table 4: Mean, Log 10 Mean, Mean IS, Log 10 Mean IS, SD and CV values for ***%BCR-ABL / BCR*** for each laboratory for the HL60 / K562 reference materials.





Mean IS Value	Median IS Value
0.0195	0.0208
0.1753	0.1789
1.6627	1.7573
16.3129	15.5756

Figure 3: Summary of the linear regressions of the log transformed data obtained from all labs testing *BCR-ABL / BCR* for the 4 reference materials (Sample 1; BCR/ABL 1 08/192, Sample 2; BCR/ABL 2 08/194, Sample 3; BCR/ABL 3 08/196 and Sample 4 BCR/ABL 4 08/198). Regression plots are shown for data before and after conversion to the IS.

Mean	Lab 2	Lab 3	Lab 5	Lab 6	Lab 9	Lab 10
BCR/ABL 1 08/192	0.0138	0.0036	0.0050	0.0245	0.0047	0.0141
BCR/ABL 2 08/194	0.1354	0.0315	0.0493	0.1325	0.0837	0.1192
BCR/ABL 3 08/196	1.5174	0.3740	0.4747	1.1538	0.8164	1.3341
BCR/ABL 4 08/198	16.6688	4.9804	4.8942	11.2312	10.9180	13.7997

Log 10 Mean	Lab 2	Lab 3	Lab 5	Lab 6	Lab 9	Lab 10
BCR/ABL 1 08/192	-1.8603	-2.4481	-2.2991	-1.6108	-2.3234	-1.8522
BCR/ABL 2 08/194	-0.8685	-1.5011	-1.3075	-0.8777	-1.0774	-0.9238
BCR/ABL 3 08/196	0.1811	-0.4271	-0.3236	0.0621	-0.0881	0.1252
BCR/ABL 4 08/198	1.2219	0.6973	0.6897	1.0504	1.0381	1.1399

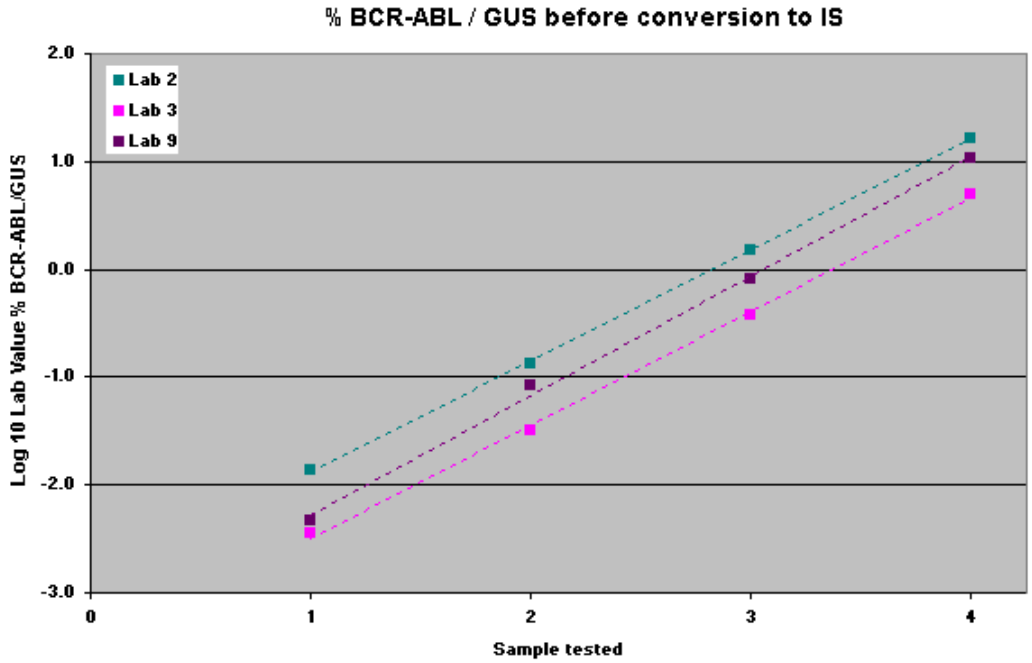
Mean IS	Lab 2	Lab 3	Lab 5	Lab 6	Lab 9	Lab 10
BCR/ABL 1 08/192	0.0109	0.0076			0.0028	
BCR/ABL 2 08/194	0.1069	0.0675			0.0502	
BCR/ABL 3 08/196	1.1981	0.8004			0.4898	
BCR/ABL 4 08/198	13.1616	10.6580			6.5508	

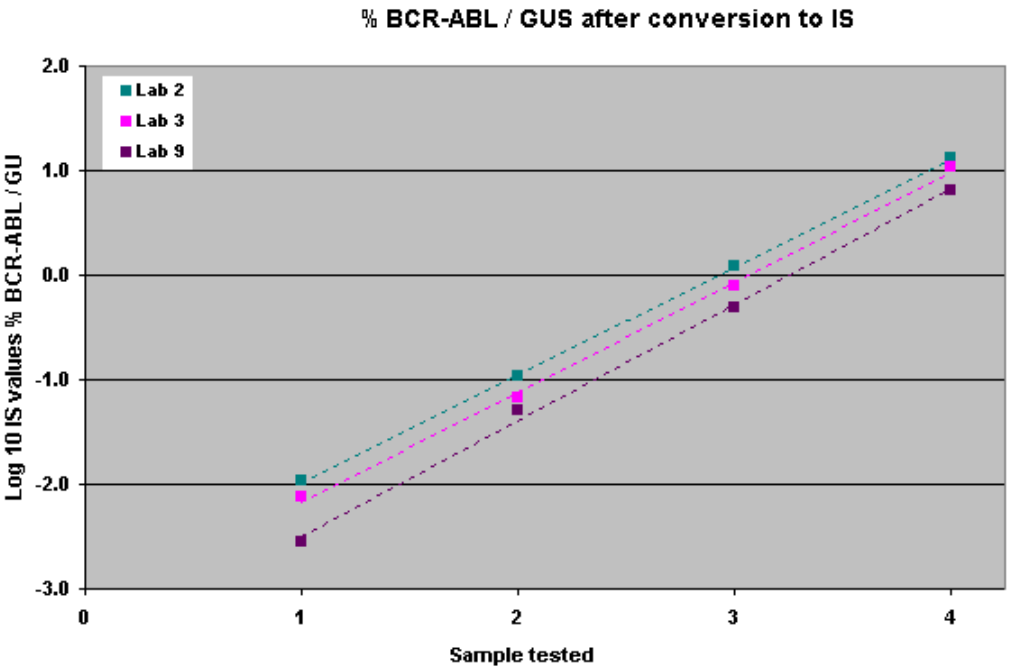
Log 10 Mean IS	Lab 2	Lab 3	Lab 5	Lab 6	Lab 9	Lab 10
BCR/ABL 1 08/192	-1.9629	-2.1177			-2.5453	
BCR/ABL 2 08/194	-0.9711	-1.1707			-1.2993	
BCR/ABL 3 08/196	0.0785	-0.0967			-0.3100	
BCR/ABL 4 08/198	1.1193	1.0277			0.8163	

SD	Lab 2	Lab 3	Lab 5	Lab 6	Lab 9	Lab 10
BCR/ABL 1 08/192	0.0043	0.0025	0.0023	0.0040	0.0025	0.0047
BCR/ABL 2 08/194	0.0220	0.0068	0.0264	0.0066	0.0356	0.0331
BCR/ABL 3 08/196	0.1665	0.0982	0.2350	0.0276	0.2377	0.2624
BCR/ABL 4 08/198	3.0550	0.6007	2.3728	0.1794	6.1061	2.4782

CV	Lab 2	Lab 3	Lab 5	Lab 6	Lab 9	Lab 10
BCR/ABL 1 08/192	31.4776	70.7647	45.4162	16.4089	52.1911	33.5693
BCR/ABL 2 08/194	16.2440	21.4722	53.5099	4.9773	42.5233	27.7338
BCR/ABL 3 08/196	10.9735	26.2433	49.5028	2.3960	29.1159	19.6684
BCR/ABL 4 08/198	18.3275	12.0620	48.4815	1.5976	55.9269	17.9586

Table 5: Mean, Log 10 Mean, Mean IS, Log 10 Mean IS, SD and CV values for ***%BCR-ABL / GUS*** for each laboratory for the HL60 / K562 reference materials.





Mean IS Value	Median IS Value
0.0071	0.0076
0.0749	0.0675
0.8295	0.8004
10.1235	10.6580

Figure 4: Summary of the linear regressions of the log transformed data obtained from all labs testing *BCR-ABL / GUS* for the 4 reference materials (Sample 1; BCR/ABL 1 08/192, Sample 2; BCR/ABL 2 08/194, Sample 3; BCR/ABL 3 08/196 and Sample 4 BCR/ABL 4 08/198). Regression plots are shown for data before and after conversion to the IS.

3.4 Homogeneity testing

To assess the homogeneity of the material distributed into the glass ampoules, 17 vials (randomly picked from each freeze fry fill) of each reference material were tested in triplicate in the same RQ-PCR run. 17 samples of freshly made material (i.e. not freeze dried) were also tested in triplicate. The coefficient of variation (CV) for each reference material tested in triplicate in the same run is shown in table 6. The CV of the fresh material is similar to that of the freeze dried samples suggesting that contents of the freeze dried ampoules are homogeneous.

	%BCR-ABL / ABL	%BCR-ABL / BCR	%BCR-ABL / GUS
BCR/ABL 1 08/192	23.31	21.10	18.92
BCR/ABL 2 08/194	12.84	15.74	19.57
BCR/ABL 3 08/196	9.07	13.14	13.21
BCR/ABL 4 08/198	14.23	11.61	16.02
Level 1 Fresh	10.31	14.52	14.52
Level 4 Fresh	13.31	19.20	13.93

Table 6: Coefficient of variation for triplicate analysis of 17 vials of freeze dried cells of each reference material and 17 aliquots of freshly prepared cell line mixtures (level 1 and 4 only)

4. CONCLUSIONS

The aim of the field trial was to assign fixed % BCR-ABL / control gene values to each material

The mean %BCR-ABL/control gene values obtained following conversion to the international scale are shown in table 7. We propose that these international scale values should be assigned to this batch of material.

	% 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

Table 7: The mean %BCR-ABL/control gene values obtained for each reference material following conversion to the international scale.

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Appendix A: Field Trial Participants

Asia:

Dr Harriet Goh, Prof. Dong-Wook Kim, Sahee Park, Catholic Research Institute of Medical Science, The Catholic University of Korea, #505 Banpo-dong, Seocho-gu, Seoul 137-701, REPUBLIC OF KOREA

Australia:

Linda Fletcher, Dr Susan Branford, and Prof Tim Hughes, Division of Molecular Pathology, Institute of Medical and Veterinary Science, Frome Road, Adelaide, SOUTH AUSTRALIA 5000

Dr Giuliana Romeo, Molecular Haematology Lab, Haematology Dept, PathWest Laboratory Medicine, Royal Perth Hospital, Level 2 North Block, Wellington Street, Perth, WESTERN AUSTRALIA 6000

Canada:

Suzanne Kamel-Reid, Institution Department of Pathology, The University Health Network, 200 Elizabeth St. , 11 Eaton North, Room 213, Toronto, Ontario, CANADA

Europe:

Hakim El Housni Erasme hospital, Medical Genetics Dept, 808 Route de Lennik, Batiment C Niveau 5, Local CS-111, 1070 Brussels, BELGIUM

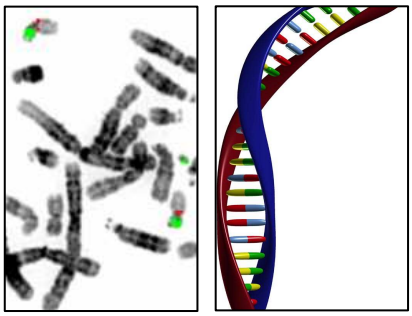
Jiri Mayer and Dana Dvorakova University Hospital Brno, Center of Molecular Biology and Gene Therapy, Cernopolni 9, 625 00 Brno, CZECH REPUBLIC

Prof Andreas Hochhaus and Dr Martin C. Mueller, Wissenschaftliches Labor, III. Medizinische Klinik, Wiesbadener Str. 7-11, D-68305 Mannheim, GERMANY

Stephen Langabeer and Mireille Crampe, Cancer Molecular Diagnostics, Central Pathology Laboratory, St. James's Hospital, Dublin 8, REPUBLIC OF IRELAND

Hans Ehrencrona, Klinisk Genetic, Rudbecklaboratoriet, Akademiska Sjukhuset, S-751 85 Uppsala, SWEDEN

Dr Helen White and Dr Feng Lin, National Genetics Reference Laboratory (Wessex), Salisbury District Hospital, Salisbury, Wiltshire, SP2 8BJ, UNITED KINGDOM



National Genetics Reference Laboratory (Wessex)
Salisbury District Hospital
Salisbury SP2 8BJ, UK

www.ngrl.org.uk/Wessex