Development of Synthetic External RNA Controls for Quantitative Detection of the BCR-ABL1 p190 (e1a2) Translocation

H023

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Introduction

Chronic myeloid leukemia (CML) is a disease caused by a translocation between chromosomes 9 and 22, forming the truncated Philadelphia chromosome. In this translocation event the ABL1 gene on chromosome 9 is translocated to the break point cluster region (BCR gene) on chromosome 22. Expression of the BCR-ABL1 fusion genes in myeloid cells promotes aberrant proliferation, increased cell survival, and enhanced migration and invasion through the activation of key regulatory pathways. One such translocation, called p190, occurs at exon a2 of ABL1 and exon e1 of BCR, forming a 190 kDa protein.

One method for monitoring CML treatment is measuring levels of BCR-ABL1 transcripts in peripheral blood by RT-PCR. The molecular response (MR) is the log of ratio of BCR-ABL1 to ABL1 transcripts and guides treatment. A reduction of the BCR-ABL1 to ABL1 ratio results in a higher MR demonstrating a reduction in CML; a 3-log reduction of BCR-ABL1 transcript is defined as the major molecular response (MMR), indicating the patient is responding well to therapy. Here, we describe the development of a control panel that can be used to monitor performance of the Xpert® BCR-ABL Ultra p190 assay that detects e1a2 (p190) and reports a range of p190 BCRABL1/ABL1 levels.

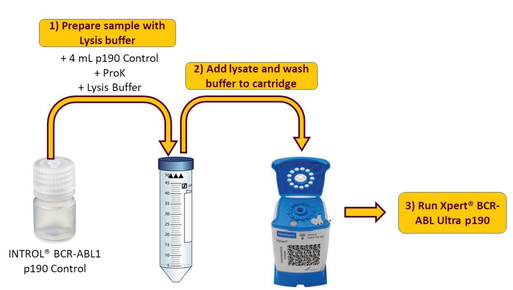
Materials and Methods

Gene segments of ABL1 and BCR were designed *in silico* to create DNA constructs and cloned to create stable frozen clone stocks. *In vitro* RNA transcripts were generated, quantified by UV spectrophotometry and formulated in a proprietary matrix that carries the RNA through extraction processes and provides stability. BCR-ABL1 and ABL1 transcripts were combined to generate a panel of 5 BCR-ABL1 e1a2 levels.

INTROL® BCR-ABL1 p190 Control Panel Component	Detection
INTROL BCR-ABL1 p190 0%	BCR-ABL p190 Not Detected
INTROL BCR-ABL1 p190 0.02%	BCR-ABL p190 Detected [0.0259]
INTROL BCR-ABL1 p190 0.1%	BCR-ABL p190 Detected [0.172%]
INTROL BCR-ABL1 p190 1%	BCR-ABL p190 Detected [1.425]
INTROL BCR-ABL1 p190 10%	BCR-ABL p190 Detected [12.8%]

Three lots of the panel were tested across 3 lots of the Xpert BCR-ABL Ultra p190 assay, over multiple days and operators to determine the linearity and reproducibility.

INTROL BCR-ABL1 p190 external controls are run just like patient blood samples. An overview of the sample preparation workflow is shown below:



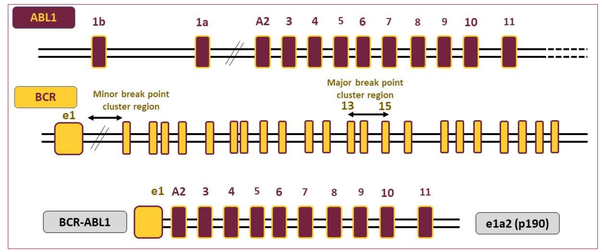


Figure 1. Gene map of ABL1 and BCR genes showing e1a2 breakpoints. The full-length human BCR activator of RhoGEF and GTPase gene (BCR) contains 23 exons spanning ~137,000 bp on chromosome 22 (6,783 nt transcript). The full-length human ABL1 gene on chromosome 9 contains 12 exons across ~174,000 bp (5,578 nt transcript). The minor break point generates the e1a2 fusion transcript which is translated into a 190 kDa protein (p190). MMQCI's synthetic controls contain WT ABL1 RNA transcript sequence and the p190 fusion RNA transcript sequence required for Cepheid's p190 assay.

Results

Figure 2. Linearity across 3 manufactured control lots. Lot A (n = 50 total); 0.02% (n = 12), 0.1% (n=14), 1% (n = 12), 10% (n=12). Lot B (n = 56 total; all BCR-ABL-positive levels n = 14 each). Lot C (n = 48 total); all BCR-ABL-positive levels n = 12 each). Testing was performed across 3 Xpert cartridge lots which were provided by Cepheid.

Figure 3. Descriptive statistics of the 3 external control lots. Averages and Standard Deviations of ABL and BCR-ABL Ct values: the assay reports out a Ct for both ABL and BCR-ABL p190. The assay software uses the delta Ct (ABL Ct – BCR-ABL Ct) to calculate the %IS of the control sample. Out of a total 195 Xpert® BCR-ABL Ultra p190 runs, there were 3 invalid runs. Of 192 valid runs, 100% had correct results with reported % ratio values within expected limits of assays performance. The %IS ratios are manufactured by mixing ABL and BCR-ABL synthetic RNA transcripts at different ratios. All controls have the same concentration of ABL transcript. ABL Cts and BCR-ABL Cts from all 3 control lots across 5 levels had standard deviations of < 0.75. Control lots demonstrated low variability for both ABL and BCR-ABL Cts as calculated by %CV (SD/mean Ct). The %CV of Cts across all lots and levels was 5% for ABL and 2.7% for BCR-ABL p190.

		Average ABL Ct			Standard Deviation			%CV		
	Component	Lot A	Lot B	Lot C	Lot A	Lot B	Lot C	Lot A	Lot B	Lot C
ABL	p190 0%	11.2	11.2	11.4	0.6177	0.5484	0.6293	5.5%	4.9%	5.5%
	p190 0.02%	11.4	11.4	11.6	0.5213	0.5789	0.5901	4.6%	5.1%	5.1%
	p190 0.1%	11.5	11.5	11.7	0.7371	0.5413	0.4562	6.4%	4.7%	3.9%
	p190 1.0%	11.3	11.6	11.4	0.5712	0.4653	0.7137	5.1%	4.0%	6.3%
	p190 10%	11.3	11.6	11.5	0.5694	0.5398	0.5638	5.0%	4.7%	4.9%

		Average BCR-ABL Ct			Standard Deviation			%CV		
	Component	Lot A	Lot B	Lot C	Lot A	Lot B	Lot C	Lot A	Lot B	Lot C
p190 BCR-ABL	p190 0%	0.0	0.0	0.0	0.0000	0.0000	0.0000	N/A	N/A	N/A
	p190 0.02%	24.1	23.9	23.8	0.5975	0.5611	0.6329	2.5%	2.3%	2.7%
	p190 0.1%	21.5	21.1	21.4	0.6847	0.4462	0.4852	3.2%	2.1%	2.3%
	p190 1.0%	18.2	18.1	18.2	0.5242	0.4599	0.5630	2.9%	2.5%	3.1%
	p190 10%	15.0	15.0	15.1	0.4316	0.5421	0.4309	2.9%	3.6%	2.9%

Control	Min Fold difference from Average	Lot A Min %IS	Lot A Avg %IS	Lot A Max %IS	Max Fold difference from Average
p190 0.02%	***2.1***	0.010	***0.021***	0.033	***1.6***
p190 0.1%	***1.9***	0.078	***0.150***	0.240	***1.6***
p190 1.0%	***1.4***	0.89	***1.22***	1.71	***1.4***
p190 10%	***1.3***	9.39	***12.01***	16.65	***1.4***

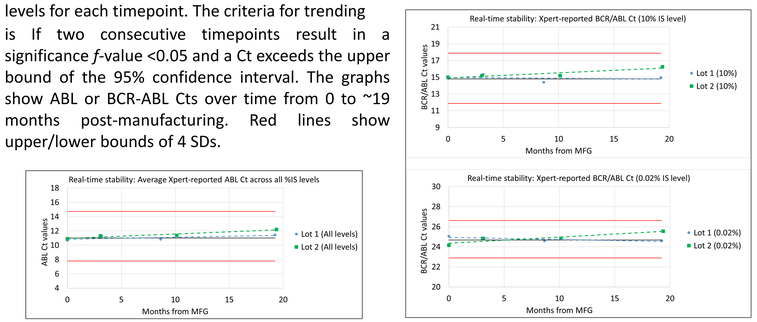
Control	Min Fold difference from Average	Lot B Min %IS	Lot B Avg %IS	Lot B Max %IS	Max Fold difference from Average
p190 0.02%	***1.9***	0.014	***0.026***	0.035	***1.3***
p190 0.1%	***1.4***	0.14	***0.190***	0.250	***1.3***
p190 1.0%	***1.5***	1.06	***1.61***	2.17	***1.3***
p190 10%	***1.2***	11.43	***14.00***	20.12	***1.4***

Control	Min Fold difference from Average	Lot C Min %IS	Lot C Avg %IS	Lot C Max %IS	Max Fold difference from Average
p190 0.02%	***2.8***	0.011	***0.031***	0.061	***2.0***
p190 0.1%	***1.6***	0.11	***0.180***	0.270	***1.5***
p190 1.0%	***1.4***	0.97	***1.38***	2.01	***1.5***
p190 10%	***1.3***	9.67	***12.15***	14.29	***1.2***

Figure 4. Averages of %IS values and their minimum/maximum fold-differences. The three MMQCI control lots show average %IS values near their respective targeted ranges (0.02, 0.1, 1.0, and 10%). Minimum and maximum %IS values observed during testing are listed within the tables and were used to calculate the fold-differences from the average %IS. All fold differences are within 2 for 0.1-10% and within 3 for 0.02%. The GeneXpert reports %IS values for p190-positive samples based on the calculation below. Calculation of %IS by the GeneXpert system takes into account the delta Ct and cartridge-specific efficiencies and scaling factors.

$\%IS = E_{\Delta Ct}^{(\Delta Ct)} \times 100 \times$ Scaling Factor (SF)	
%IS	International Scale (standardizes reporting of BCR-ABL RNA levels)
ΔCt	ABL Ct minus BCR-ABL p190 Ct
$E_{\Delta Ct}$	Cartridge lot-specific assay Efficiency
SF	Scaling Factor specific to the cartridge lot

Figure 5. Real-time stability studies demonstrate >12 months stability when stored at -20°C. Real-time stability was measured for historical controls with a similar formulation. To assess stability of ABL Cts over time, samples across all %IS levels were run in duplicate. For BCR-ABL, duplicate samples were run for all p190-positive levels. Linear regression was performed on the average Cts for all %IS levels for each timepoint. The criteria for trending is If two consecutive timepoints result in a significance *f*-value <0.05 and a Ct exceeds the upper bound of the 95% confidence interval. The graphs show ABL or BCR-ABL Cts over time from 0 to ~19 months post-manufacturing. Red lines show upper/lower bounds of 4 SDs.



Conclusions

- The synthetic INTROL BCR-ABL1 p190 Control Panel (C183) demonstrated reproducible and robust performance when tested on Xpert BCR-ABL Ultra p190 assay. These controls are beneficial for routine monitoring of p190 BCRABL1/ABL1 assay performance.
- BCR-ABL1 p190 controls are stable for over 1 year when stored at -20°C.

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