Inter- and Intralaboratory Variability Among *BCR-ABL1* Monitoring Tests and Impact of PCR Platform Standardization: From the ReVEAL *BCR-ABL1* Methods Comparison Study

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INTRODUCTION

- Molecular monitoring of *BCR-ABL* transcript levels by real-time quantitative polymerase chain reaction (RQ-PCR) is the most sensitive method for measuring minimal residual disease burden for patients with chronic myeloid leukemia (CML).[1]
- Molecular response is an important aspect of successful CML therapy[1] and is included in current guidelines and recommendations published by the National Comprehensive Cancer Network (NCCN)[2] and the European LeukemiaNet (ELN).[3]
- *BCR-ABL* tests are often independently designed and developed at individual laboratories, potentially introducing sources of variation.[3,4]
- An international scale (IS) has been established to improve the reproducibility and accuracy of molecular monitoring between laboratories[5-7]; however, not all laboratories have established conversion factors that would provide for calibration of their assay to the IS.[8]

STUDY OBJECTIVES

The objectives of the ReVEAL (Reproducibility and Variability Evaluation of Assays in Leukemia) study are the following:

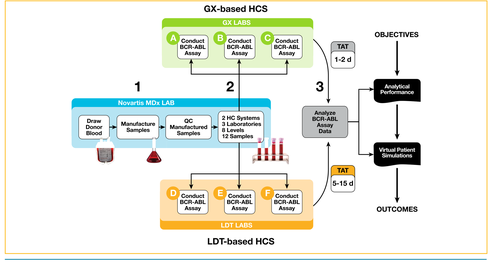
- Explore intralaboratory and interlaboratory variability of *BCR-ABL* monitoring tests by evaluating the impact of PCR platform standardization using the GeneXpert® *BCR-ABL* Assay (GX) (Cepheid, Sunnyvale, CA, USA) standardized to the IS and laboratory-developed tests (LDTs) not standardized to the IS
- Compare analytical performance of *BCR-ABL* monitoring tests in 2 health care systems in the US
  - A GX-based health care system that relies on 3 sites using an automated and IS- standardized *BCR-ABL* test
  - An LDT-based health care system relying on 3 independently developed *BCR-ABL* tests
- Compare the budget impact on GX-based health care systems vs that on LDT-based health care systems

METHODS

Study Design

- CML patient analogue samples were prepared corresponding to targeted *BCR-ABL*^{IS} ratios ranging from ~10% to ~0.01%.
- Blinded samples were sent to 6 laboratories in the US.
  - 4 rounds of testing were performed in 2 phases.
  - 3 laboratories used the GX (GX-based health care systems).
  - 3 laboratories used their own internally developed and validated *BCR-ABL* quantification assays (LDT-based health care systems).
- Results were unblinded to compare the analytical performance of the individual laboratories and the GX- and LDT-based health care systems.

Figure 1. Study Design



GX, automated GeneXpert System; HCS, health care system; LDT, laboratory-developed test; QC, quality control.

Participating Laboratories

- Clarient, a GE Healthcare Company
- Hospital of the University of Pennsylvania Molecular Pathology Laboratory
- Molecular Pathology Laboratory Network, Inc.
- Medical Genetics Laboratories, Kaiser Permanente Southern California
- Scripps Clinic Medical Laboratories
- The University of Chicago Medical Center, Molecular Diagnostics Laboratory

Study Evaluations

Phase I

- Phase I included round A on November 2, 2010, and round B on November 9, 2010 (Table 1).
- Target concentrations of K562 cells in a background of normal donor blood were selected based on an empirically determined regression of the relationship between cell concentration and *BCR-ABL*^{IS} % ratio analytical response as measured on the GX platform.
- Targeted *BCR-ABL*^{IS} levels in phase I corresponded to approximately 10.0%, 1.0%, 0.1%, and 0% (negative control).

Phase II

- Phase II included round C on February 8, 2011, and round D on February 15, 2011 (Table 1).
- Unlike phase I, analytical response near major molecular response (MMR) was benchmarked to a specific cell ratio (9.0 K562 cells/million white blood cells [WBC]) and was independently verified at 2 separate reference laboratories.
  - Targeted MMR sample (*BCR-ABL*^{IS} = 0.1%)
    - Tested by the Department of Molecular Pathology, Centre for Cancer Biology, SA Pathology, Adelaide, Australia → *BCR-ABL*^{IS} = 0.12%
    - Tested by the Department of Pathology, Molecular Diagnostics Laboratory, University of Toronto Health Network, Toronto, Ontario, Canada → *BCR-ABL*^{IS} = 0.09%
- Samples were then prepared based on cell ratios at levels approximately one-half log below those targeted in phase I corresponding to approximately 3.16%, 0.32%, 0.03%, and 0.01%.

Table 1. Discrete Target Analyte Levels of K562 in Normal Whole Blood Assayed Across GX and LDT Health Care Systems

Level	Approx. Clinical Level	IS Log Reduction	Target BCR-ABL^{IS} Ratio	WBC Per mL Whole Blood, n	K562 Cells Per mL Blood, n	Cell Ratio (K562 Cells/ Million WBC)	Phase	Round[a]
High	CHR	1.0	10.00%	6.7	6014.0	897.6	I	A
	≥ CCyR	1.5	3.16%	8.5	3315.0	390.0	II	C
Medium	CCyR	2.0	1.00%	6.8	601.4	88.4	I	A
	≥ MMR	2.5	0.32%	5.6	218.4	39.0	II	C
Low	MMR	3.0	0.10%[b]	4.3	80.0	18.6[b]	I	B
	MR$^{3.5}$	3.5	0.03%	6.0	17.9	3.9	II	D
Very low	MR$^{4.0}$	4.0	0.01%	4.6	7.4	1.2	II	B
Negative	CMR	-	0%	3.7	0.0	0.0	I	D

CCyR, complete cytogenetic response; CHR, complete hematologic response; CMR, complete molecular response; IS, international scale; MMR, major molecular response; MR$^{3.5}$, BCR-ABL^{IS} 0.03%; MR$^{4.0}$, BCR-ABL^{IS} = 0.01%; WBC, white blood cells.

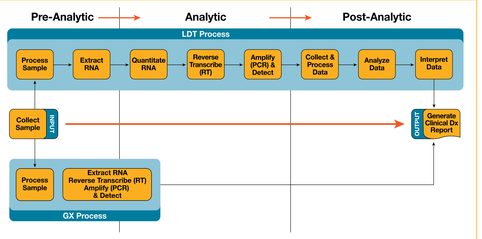
[a]Round A: November 2, 2010; Round B: November 9, 2010; Round C: February 8, 2011; Round D: February 15, 2011.

[b]A technical error in round B resulted in 33% excess concentration of K562 for the "low" analyte level, targeting MMR at *BCR-ABL*^{IS} = 0.1%. Coupled with a low WBC count in the donor blood used to manufacture samples for this analyte level, the excess K562 induced an approximately 2-fold increase in observed *BCR-ABL*^{IS}% ratio response on the GX platform, above the nominal target of *BCR-ABL*^{IS} = 0.1%. The actual K562 *BCR-ABL* target level for this sample corresponded to 0.2% (IS).

BCR-ABL Monitoring Assay Process

- GX-based health care systems
  - Samples were tested according to the manufacturer's instructions.
  - Results reported on the IS were derived by applying the manufacturer's validated conversion factor established for its CE IVD Xpert® BCR-ABL Monitor test (Cepheid, Sunnyvale, CA, USA).
- LDT-based health care systems
  - Samples were tested according to individually developed *BCR-ABL* quantification assays per the testing sites' standard operating procedures.
  - Laboratories in the LDT-based health care systems had not undertaken the process of obtaining an IS conversion factor and consequently results are reported exactly as provided by those sites, with the raw data transformed to % *BCR-ABL/ABL* ratios to facilitate data comparison.
- In both health care systems, samples underwent all preanalytic, analytic, and postanalytic workflow steps (Figure 2).

Figure 2. *BCR-ABL* Monitoring Assay Process



- Qualitative results were reported for every sample and data were assigned into 1 of the following 4 categories:
  - Positive (POS; LDT and GX): analytical result confirming presence of *BCR-ABL* transcript in a sample at levels that permitted quantitation expressed as a ratio (*BCR-ABL/ABL*)
  - Nonquantifiable positive (NQP; LDT only): analytical result confirming presence of *BCR-ABL* transcript in a sample at levels that could not be quantitated as a ratio (*BCR-ABL/ABL*) (ie, below limit of quantitation for the assay)
  - Negative (NEG; LDT and GX): analytical result that did not detect the presence of *BCR-ABL* transcript in a sample in the context of a valid *ABL* signal (ie, below limit of detection for the assay)
  - Failure (FAIL; LDT and GX): permanent and unrecoverable loss of clinical diagnostic data due to failure(s) during execution of preanalytical, analytical, and/or postanalytical sample processing

Statistical Analysis

- The Wilcoxon test was used to determine whether the distribution of measurements of 2 laboratories was identical.
- The Levene test (with median) was used to determine whether the variances between 2 laboratories was identical.
- Fisher exact test was used to test if distribution of qualitative results were identical for 2 laboratories.
- A significance level $\alpha = 0.0018 = 0.05/(9 * 3)$ was used, given 9 pairwise laboratory comparisons, with 3 tests each, per the Bonferroni correction method to adjust for multiplicity.

RESULTS

Table 2. Summary of Results for Each Laboratory by Target *BCR-ABL* Levels

HCS	Lab	Target BCR-ABL^{IS} Ratio							
		10.00%	3.16%	1.00%	0.32%	0.10%[a]	0.03%	0.01%	Negative Control
		Median of POS Results (Range)[b]							
GX (IS)	Lab A	9.6 (3.8-11)	3.3 (0.17-4.7)	0.82 (0.47-1.2)	0.36 (0.25-0.85)	0.23 (0.13-0.41)	0.02 (0.0004-0.05) 2 NEG	0.006 (0.001-0.03) 8 NEG	12 NEG
	Lab B	9.9 (7.0-14)	2.3 (0.12-3.7)	0.99 (0.75-1.6)	0.34 (0.11-0.56)	0.19 (0.05-0.30)	0.02 (0.0003-0.08) 3 NEG	0.003 (0.001-0.004) 9 NEG	11 NEG 1 FAIL
	Lab C	10 (8.5-13)	3.7 (2.4-6.1)	1.1 (0.61-1.6)	0.42 (0.04-0.80)	0.19 (0.09-0.42)	0.03 (0.003-0.14) 1 NEG	0.006 (0.002-0.01) 8 NEG	12 NEG
LDT	Lab D	20 (16-27)	8.6 (4.8-10)	1.8 (1.3-2.5)	1.15 (0.84-1.6)	0.51 (0.22-0.77)	0.05 (0.04-0.11) 1 NQP	0.02 (0.02-0.02) 11 NQP	11 NEG 1 NQP
	Lab E	2.8 (1.6-3.9)	1.7 (0.22-2.5)	0.23 (0.21-0.30)	0.18 (0.06-0.31) 1 NEG	0.05 (0.01-0.13)	0.008 (0.002-0.01) 1 NEG 5 NQP	0.01 (0.01-0.01) 7 NEG 4 NQP	12 NEG
	Lab F	29 (21-35)	9.40 (8.2-12)	2.1 (1.2-4.7)	0.90 (0.02-1.6) 1 FAIL	1.1 (0.65-2.1) 1 FAIL	0.12 (0.11-0.17) 8 FAIL	0.01 (0.003-0.03) 4 FAIL	0.009 (0.006-0.03) 7 NEG

FAIL, failure; GX, automated GeneXpert System; HCS, health care system; IS, international scale; LDT, laboratory-developed tests; NEG, negative; NQP, nonquantifiable positive; POS, positive.

[a]During panel manufacturing, the actual K562 *BCR-ABL* target level for this sample corresponded to 0.2% (IS).

[b]Median and range of positive results; 12 replicate measurements per level per laboratory.

Table 3. Statistical Comparisons of Results of All Laboratories

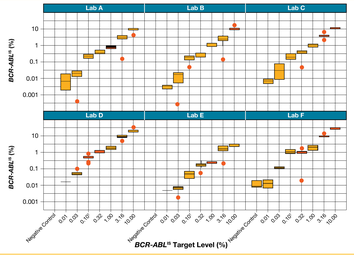
	Wilcoxon	Levene	Fisher
GX Laboratories			
Lab A vs Lab B	0.043	0.69	1
Lab A vs Lab C	0.16	0.3	1
Lab B vs Lab C	0.015	0.72	0.98
LDT Laboratories			
Lab D vs Lab E	1.7 x 10^{-10}[a]	0.0072	0.0077
Lab D vs Lab F	5 x 10^{-7}*	0.21	0.0026[a]
Lab E vs Lab F	7.1 x 10^{-10}[a]	0.00082[a]	0.0002[a]
GX[a] vs LDT Laboratories			
GX Labs vs Lab D	0.00077*	0.016	1.8 x 10^{-3}[a]
GX Labs vs Lab E	4.3 x 10^{-7}[a]	0.011	0.0002[a]
GX Labs vs Lab F	5.5 x 10^{-7}[a]	0.22	2.9 x 10^{-3}[a]

*Significant difference [$\alpha = 0.0018 = 0.05/(9 * 3)$]; [a]P values for GX laboratories combined vs LDT laboratories using all rounds.

[a]GX, automated GeneXpert System; LDT, laboratory-developed tests.

- Ratio of median measurements for each target level was < 2 for the GX sites and a range of ~10 to 22 for the LDT sites.
- None of the 3 GX laboratory comparisons were significant for Wilcoxon, Levene, or Fisher exact tests on level $\alpha = 0.0018$.
- Mean measurement levels for all LDT laboratories differed significantly for all levels (tested by Wilcoxon).

Figure 3. Results for Each *BCR-ABL* Target Level by Laboratory



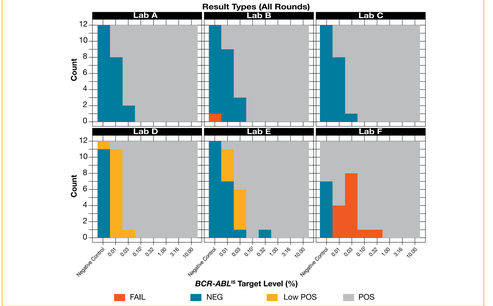
[a]During panel manufacturing, the actual K562 BCR-ABL target level for this sample corresponded to 0.2% (IS).

- Overall, a high level of agreement was observed among laboratories using the GX system standardized to the IS at each of the analyte levels tested.

Qualitative Sample Results by Laboratory

- A subset of matched replicate samples with low *BCR-ABL* levels were reported as positive but not quantifiable by LDT laboratories (21 of 48 replicates).
  - Low *BCR-ABL* levels were positive but not quantifiable in laboratories D and E.
  - Laboratory F reported half of the replicates for low-level samples as failures (12/24) because of a malfunctioning instrument and all others as positives (12/24).
- A subset of samples with low levels of *BCR-ABL* (below MMR) was reported as negative by the GX system (31/72 replicates).
- Qualitative sample results are shown in Figure 4.

Figure 4. Qualitative Sample Results by Laboratory



[a]During panel manufacturing, the actual K562 *BCR-ABL* target level for this sample corresponded to 0.2% (IS).

Table 4. Summary of Results by Health Care System

	Median of POS Results (Range)[a]						
	10.00%	3.16%	1.00%	0.32%	0.10%[b]	0.03%	0.01%	Negative control
GX-based HCS	9.9 (3.8-14)	3.5 (0.12-6.1)	0.94 (0.47-1.6)	0.35 (0.04-0.85)	0.21 (0.05-0.42)	0.02 (0.0003-0.14) 6 NEG	0.004 (0.001-0.03) 25 NEG	35 NEG 1 FAIL
LDT-based HCS	20 (1.6-35)	8.3 (0.22-12)	1.5 (0.21-4.7)	0.90 (0.02-1.6) 1 FAIL 1 NEG	0.5 (0.01-2.1) 1 FAIL	0.05 (0.002-0.17) 1 NEG 6 NQP 8 FAIL	0.01 (0.003-0.03) 7 NEG 15 NQP 4 FAIL	0.009 (0.006-0.03) 30 NEG 1 NQP

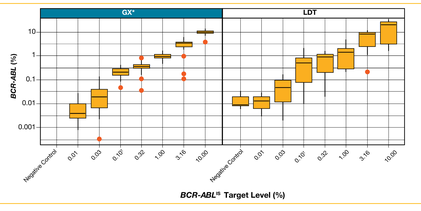
FAIL, failure; HCS, health care system; GX, automated GeneXpert System; LDT, laboratory-developed tests; NEG, negative; NQP, nonquantifiable positive; POS, positive.

[a]Median and range of positive results; 36 replicate measurements per level per health care system.

[b]During panel manufacturing, the actual K562 *BCR-ABL* target level for this sample corresponded to 0.2% (IS).

- A 10-fold variation existed among samples in the LDT-based health care systems; fewer differences were observed for the IS standardized and automated GX-based health care systems.

Figure 5. Results for Each *BCR-ABL* Target Level by Health Care Systems



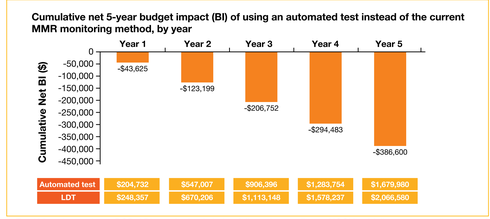
*Results reported per the IS.; [a]During panel manufacturing, the actual K562 BCR-ABL target level for this sample corresponded to 0.2% (IS).

- Considerable overlap was observed among different *BCR-ABL* levels in the samples tested within an LDT-based health care system.
- Overlap was generally not observed for the GX-based health care systems (with the exception of the *BCR-ABL*^{IS} = 0.01% and 0.03% samples).

Economic Impact

- A model to estimate the budget impact with an automated *BCR-ABL* monitoring test compared with an LDT was developed for newly diagnosed patients with CML in the United States.
- The full details and assumptions of this model have been previously reported.[8]
  - The model used best available data.
  - Epidemiology data were combined with workflow costs based on a survey of US laboratories.
  - A testing algorithm based on NCCN[9] guidelines was used to capture a number of different monitoring tests for CML, including determination of MMR.

Figure 6. Cost Savings Over 5 Years With an Automated *BCR-ABL* Monitoring Test vs an LDT



- For 100 patients whose *BCR-ABL* monitoring is being followed according to NCCN guidelines, an overall savings of $43,625 could be achieved in 1 year and $386,600 over 5 years when implementing an automated test.

CONCLUSIONS

- **Statistical evaluation of the GX results demonstrated no significant differences across the 3 testing sites.**
- **Results significantly varied among the 3 laboratories each using a unique LDT. Although within a laboratory most LDT results were reproducible, some LDTs consistently under- or over-reported *BCR-ABL* transcript levels with respect to the IS.**
- **Variability in the LDT-based health care systems was considerably higher than in the GX-based health care systems at levels near or above MMR.**
- **Automated *BCR-ABL* testing not only reduces interlaboratory variability, but represents an estimated $387,000 cost-saving alternative vs LDTs over a 5-year period.**
- **These results underscore the need for PCR standardization and suggest that automated platform standardization and adoption of the IS can improve *BCR-ABL* molecular monitoring for patients with CML.**
- **Interlaboratory variation and differences in *BCR-ABL* ratios may impact clinical disease management in patients with CML.**
  - **An erroneous assignment of MMR may prevent guideline-recommended follow-up to investigate potential problems, such as patient noncompliance or possible early emergence of TKI-resistant disease.**
  - **An erroneous report indicating MMR has not been achieved could trigger unnecessary follow-up, such as additional bone marrow aspiration and biopsy for cytogenetics or repeated PCR testing.**

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