Validation of the Xpert BCR-ABL Monitor Assay Results: King Fahd Specialist Hospital Dammam Experience

Heba N. Raslan [1] and Samir Amr [2]

[1] Consultant Hematopathology, King Fahd Specialist Hospital-Dammam.
[1] National Cancer Institute, Cairo University
[2] Consultant Histopathology, King Fahd Specialist Hospital-Dammam
hebaraslan2013@gmail.com

Abstract: Objectives: King Fahd Specialist Hospital Dammam (KFSHD), a tertiary care hospital, has guidelines for BCR-ABL p210 quantitation using GeneXpert® Real Time PCR.The assay results are automatically calculated using the Cepheid International Scale (IS), mentioned in the certificate of analysis (COA) supplied with each kit. The aim of this study was to establish an IS specific for KFSHD, instead of that supplied by the manufacturer, based on thorough validation study of the assay with Mayoclinic IS, a thing which conferred more accurate interpretation of the results. Methods: A total of 29 hemato-oncology blood, bone marrow and RNA samples were run, using the GeneXpert BCR-ABL assay and validation for all samples was carried out with Mayo clinic to compare external controls to built-in controls. We cancelled the Cepheid IS and used the Mayoclinic IS factor (2.57), which is validated by its turn with the reference method (University of Adelaide). Results: All 29 results were concordant using the acceptance criterion of +/-0.5 $\log_{10}$ of the expected BCR-ABL relative quantitation. Conclusion: The Xpert BCR-ABL monitor assay provides reliable results. Replacement of the IS provided by the manufacturer with the IS calculated by our lab, and which is based upon thorough validation study, conferred more accurate results.
[Heba N. Raslan. **Validation of the Xpert BCR-ABL Monitor Assay Results: King Fahd Specialist Hospital Dammam Experience.** *J Am Sci* 2013;9(9):281-285]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 37

Key words: BCR-ABL, CML, International Scale

Introduction:

Chronic myeloid leukemia (CML) is a hematopoietic stem cell neoplasm caused by BCR-ABL fusion gene, which results in a constitutively active tyrosine kinase [(1)]. Imatinibmesylatedeveloped as a selective BCR-ABL tyrosine kinase inhibitor and became the standard first line treatment for CML patients, inducing stable minimal residual disease in the majority of patients [(2)].Imatinib induces complete cytogenetic response (CCR) in more than 80% of CML patients [(3)], of whom 11% to 27% have been shown to subsequently lose CCR [(4)]. That's why disease burden in CML patients with CCR should be routinely monitored by PCR to detect 3 or more logarithmic units reduction of BCR-ABL fusion transcripts, compared to a standardized baseline, a thing which confers major molecular response (MMR) and associates with significantly lower relapse risk and prolonged progression free survival [(5)]. Because RQ-PCR methods are not yet well standardized, relatively imprecise, have variable low-level detection limits in different laboratories, and use different techniques and various control genes, marked variation in reported BCR-ABL values is encountered [(6, 7)]. Accordingly, alignment of BCR-ABL values became necessary to achieve comparable results for the aim of using common clinical decision values, facilitating patient monitoring between clinics that use different testing laboratories and consistent interpretation of clinical research data [(8)]. An International Scale (IS) was designed by the Adelaide laboratory in Australia to replace the log reduction scale, with a defined value of MMR of 0.10% IS [(9)].

Cehpheid introduced its Gene Xpert-based assay for the identification of the BCR-ABL gene fusion from blood samples, which is a self-contained automated instrument that integrates microfluidic sample preparation with RT-PCR based real time fluorescent signal detection [(10)]. The difference in the measured BCR-ABL^{ct} (cycle threshold) and ABL^{ct} ; the delta ct ($^{\Delta ct}$) is calculated by the instrument's software and interpreted as positive, negative or invalid, and represents the ratio of the two populations of mRNAs, and ultimately the fraction of neoplastic cells present.

2. Patients and Methods:

The GeneXpert software calculates the %BCR-ABL/ABL using the following equation: %BCR-ABL/ABL IS=$E_{\Delta ct}^{(\Delta ct)}$X100Xconversion factor, where the Δct is obtained from ABL^{ct}minus BCR-ABL^{ct}.

A total of 29 hemato-oncology blood, bone marrow and RNA samples (27 positives and 2 negatives) were run and compared to the reference methods. All samples worked within 20 consecutive days to compare external controls to built- in controls

Xpert BCR-ABL Monitor Assay

A sample of 200 µL of peripheral blood, bone marrow aspirate, was lysed according to the

manufacturer's protocol. Lysates were added to GeneXpert cartridges (Cepheid) and analyzed on the GeneXpert instrument. The automated procedure is as follows: (1) purification of RNA using nucleic acid purification beads, (2) reverse transcription of RNA, and (3) quantitative real-time nested polymerase chain reaction of complementary DNA. Wild-type ABL transcripts served as an internal control. For patient specimens, the blood or bone marrow aspirate was lysed immediately on receipt of the sample and run on the GeneXpert instrument. Total turnaround time was less than 2.5 hours.

Calculation of % BCR-ABL to ABL Transcript

For positive specimens, the % *BCR-ABL* to *ABL* transcript was calculated by the following equation: % *BCR-ABL*/*ABL* = $E\Delta\ Ct^{\Delta Ct}$; where *Ct* is the cycle threshold, $E\Delta Ct$ is the efficiency of the *BCR-ABL* to *ABL* RQ-PCR reaction for a given lot of reagent, and $\Delta Ct = ABL\ Ct - BCR\text{-}ABL\ Ct$. For specimens that were negative for the *BCR-ABL* transcript, the detection limit was calculated as follows: %*BCR-ABL*/*ABL* detection limit = $E\Delta Ct^{ABL\ Ct}$.

After thorough validation study of the assay with Mayaoclinic, which by its turn validates its results with the reference method of the University of Adelaide, King Fahd Specialist Hospital-Dammam (KFSHD) molecular diagnostic lab decided to cancel the Cepheid International Scale which is mentioned in the certificate of analysis (COA) provided with each kit and to use instead the Mayoclinic IS of 2.57. We calculated our results according to the equation: **X= (Y/Z)*N**

Where:

X = KFSHD % BCR-ABL/ABL IS

Y = Xpert % BCR-ABL/ABL IS

Z = Cepheid COA Conversion Factor

N = Mayo Clinic Conversion Factor

3. Results:

Accuracy:

A total of 29 hemato-oncology blood,bone marrow and RNA samples (27 positives and 2 negatives) were run and compared to the reference methods. All samples worked within 20 consecutive days to compare external controls to built- in controls. All results were concordant as shown in table (1):

Table 1: Results of 29 CML patients validated with Mayoclinic

No.	MR#	Lab #	REF. %	Log	Xpert%	Log	Log Diff	Status
1	60655	2011260609	13.30	1.12	9.57	0.98	0.14	Valid
2	30935	2011294132	0.00	0.00	0.00	0.00	0.00	Valid
3	48895	2011285748	16.95	1.23	12.98	1.11	0.12	Valid
4	56361	2011260089	42.83	1.63	23.40	1.37	0.26	Valid
5	54375	2011303252	4.21	0.62	3.19	0.50	0.12	Valid
6	45888	2011306153	0.10	-1.00	0.03	-1.47	0.47	Valid
7	56227	20125865	0.03	-1.60	0.03	-1.47	-0.13	Valid
8	62882	20127506	23.00	1.36	17.45	1.24	0.12	Valid
9	63569	20127149	39.00	1.59	25.53	1.41	0.18	Valid
10	21504	20128956	13.00	1.11	12.13	1.08	0.03	Valid
11	32963	201214281	0.02	-1.70	0.02	-1.72	0.02	Valid
12	58785	201214689	0.20	-0.70	0.07	-1.15	0.45	Valid
13	58428	201214690	0.60	-0.22	0.60	-0.22	0.00	Valid
14	10978	201215468	0.06	-1.22	0.05	-1.27	0.05	Valid
15	58540	2011259554	29.08	1.46	27.66	1.44	0.02	Valid
16	62346	2011256888	100.00	2.00	34.04	1.53	0.47	Valid
17	56361	201247824	82.00	1.91	70.21	1.85	0.07	Valid
18	31058	201250260	0.01	-2.00	0.01	-2.00	0.00	Valid
19	62186	201250008	6.30	0.80	7.23	0.86	-0.06	Valid
20	64709	201257024	100.00	2.00	95.74	1.98	0.02	Valid
21	64742	201257169	54.00	1.73	31.91	1.50	0.23	Valid
22	58082	201277574	36.81	1.57	20.64	1.31	0.25	Valid
23	64483	201283987	0.40	-0.39	0.28	-0.56	0.16	Valid
24	48895	201285184	14.16	1.15	25.53	1.41	-0.26	Valid
25	51248	201286222	0.80	-0.10	0.53	-0.27	0.18	Valid
26	60975	20122075	0.00	0.00	0.00	0.00	0.00	Valid
27	CAP01	MRD-2011 A	97.04	1.99	100.00	2.00	-0.01	Valid
28	CAP03		0.02	-1.70	0.02	-1.63	-0.07	Valid
29	CAP04	MRD-2011 B	87.00	1.94	80.85	1.91	0.03	Valid

All results are concordant using the acceptance criterion of +/- 0.5 $\log_{10}$ of the expected BCR/ABL relative quantitation.

Linearity/ Reportable range:

Linearity was verified by testing prepared BCR-ABL Mbcr fusion gene serial dilutions (100%, 10%, 1%, 0.1% and 0.01%) using MRD A 2011 CAP01 Sample. All results were within the putative acceptable criteria (0.5 Log difference) and the established linearity is 0.01-100% BCR/ABL to ABL (Table 2).

Table 2: Verification of linearity of the assay

n	Sample ID	Target	Result	Acceptable Range	
				low	high
1	100%	2	2.00	1.5	2.5
2	10%	1	0.98	0.5	1.5
3	1%	0	-0.22	-0.5	0.5
4	0.1%	-1	-1.07	-1.5	-0.5
5	0.01%	-2	-1.99	-2.5	-1.5

Precision:

Precision has been validated using 100% and 0.01% CAP samples 01 and 03 respectively.
100% and 0.01% samples were run in triplicate for two consecutive days.
All results were with Coefficient of variability(CV)< 5% (Table3)

Table 3: Precision of results with CV < 5%

n	Date	100%	0.01%
1	01/04/2012	2.02	-2.19
2		2.06	-2.05
3		2.00	-2.06
4	02/04/2012	2.01	-2.06
5		1.98	-1.99
6		1.86	-1.92

Limit of Detection (LOD):

We achieved 0.001% LOD, by diluting the CAP06 sample (0.01) 1:10, and testing it in four replicates, all results were positive (Detected) as shown in table (4).

Table 4: Limit of detection of the assay

LOD	Ratio %			
	Average	S.D	Positive/Total	confidence interval
0.001%	0.0009 %	0.0001	4/4	>95%

Recovery / Interferences:

Known BCR/ABL RNA Negative (0%) samples were selected and spiked with known positive t(12;21), t(15;17) RNA samples.

No interference of t(12;21) or t(15;17) RNA was detected.

Clinical Performance Criteria:

The clinical sensitivity and specificity of the test are 100% (Table 1). The 2x2 contingency table below shows the agreement of BCR-ABL quantitative results between KFSHD and reference method showing our lab sensitivity and specificity to be 100% (table 5).

Table 5: Comparison of BCR/ABL *p*210 detection assay at KFSHD and the reference method*:

		Reference * BCR ABL Quant		
		Detected	Not Detected	Total
KFSH-D BCR-ABL Quant	Detected	27	0	27
	Not Detected	0	2	2
	Total	27	2	29

* Reference method: Mayo Clinic Laboratories and Ipsogen results.
Diagnostic Specificity = 1
Diagnostic Sensitivity = 1

4. Discussion:

During imatinibmesylate therapy, more than 70% of patients achieve CCR [(11)]. A major molecular response exceeding 3 logs of BCR-ABL transcript reduction from the reference baseline in RT-PCR studies associates with excellent progression-free survival [(121,13)]. However, a minority of such patients relapse [(14)]. The predominant mechanism of imatinib resistance is point mutations in the BCR-ABL kinase domain, which impair optimal imatinib binding to its target [(15-17)].Thus sensitive monitoring of minimal residual disease is of major importance and early recognition of patients at higher risk of relapse could trigger more intense follow up and help expedite necessary changes to therapeutic strategies, including dose modification or shifting to second line kinase inhibitors [(18,19)]. RT-PCR is by far the most sensitive technique for monitoring CML patient response, in addition to the feasibility of being performed on peripheral blood samples, being therefore less invasive than techniques that require bone marrow aspirate [(20)]. Increasing levels of BCR-ABL transcripts may indicate loss of response to imatinib or relapse after transplantation [(21, 22)].

There was a clear need for standardization of measurement of BCR-ABL transcripts by RT-PCR by different laboratories. The IRIS trial established a standard baseline for measurement (100% BCR-ABL on the international scale) and a major molecular response (MMR, good response to therapy) was defined as a 3 log reduction in the amount of BCR-ABL (0.1% BCR-ABL on the international scale). Laboratories wishing to align their results with the international scale could do so by exchanging samples with the Adelaide laboratory, Australia, and by this process, conversion factors are established for different laboratories [(18)].

GeneXpert introduced by Cepheid identifies leukemia cells harboring the BCR-ABL gene fusion through a self-contained automated instrument which integrates microfluidic sample preparation with RT-PCR based real time fluorescent signal detection [(10)]. KFSHD uses the Cepheid GeneXpert BCR-ABL fusion detection system as an alternative to current clinical diagnostic tools for CML patients monitoring for minimal residual disease. To align the results of any laboratory using the GeneXpert system, the system automatically calculates the BCR-ABL copies and multiplies them by the Cepheid international scale, which is stated in the certificate of analysis provided with each kit.

Our experience in KFSHD, which was gained after thorough validation studies of our results with Mayoclinic, revealed that better validation of the our results were obtained when we cancelled the Cepheid international scale and multiplied the results by the Mayoclinic factor, which is equal to 2.57. It is noteworthy that Mayoclinic validates its results with the reference laboratory, which is Adelaide laboratory in Australia.

Conclusion:

Standardization of RT-PCR results of BCR-ABL copies detected by various laboratories throughout the world is of utmost importance for the alignment of the results. Comparable results are mandatory for facilitating patient monitoring between clinics that use different testing laboratories and consistent interpretation of clinical research data. KFSHD experience with the Cepheid fully automated BCR-ABL detection system revealed better alignment of results after cancelling the Cepheid international scale and applying our own calculated one.

References:

1. Daley GQ, Van Etten RA, Baltimore D. Induction of chronic myelogenous leukemia in mice by the p210bcr/abl gene of the Philadelphia chromosome. Science 1990;247:824-30.
2. Baccarani M., Saglio G., Goldman J, Hochhaus A, Simonsson B, Applebaum F. Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European leukemiaNet. Blood 2006;108:1809-20.
3. Drucker BJ, Guilhot F, O'Brien SG, Gathman I, Kantarjan H, Gatterman N, Deininger M, Silver R, Goldman J, Stone R, Cervantes S, Hochhaus A, Powel B. Five year follow up of patients receiving imatinib for chronic myeloid leukemia. N Engl j Med 2006;355:2408-17.
4. Iacobucci I, Saglio G, Rosti G, Testoni N, Pane F, Amabile M, Poerio A, Soverini S, Bassi S, Cilloni D, Bassan R, Breccia M, Lauria F, Izzo B, Merante S, Frassoni F, Paolini S, Montefusco E, Baccarani M, Martinelli G; GIMEMA Working Party on Chronic Myeloid Leukemia. Achieving a major molecular response at the time of a complete cytogenetic response (CCgR) predicts a better duration of CCgR in imatinib treated chronic myeloid leukemia patients. Clin Cancer Res 2006;12:3037-42.
5. Ludan T, Juvonen V, Mueller M, Mustjoki S, Lakkala T, Kairisto V, Hochhaus A, Knuutila S, Porkka k. Comparison of bone marrow high mitotic index metaphase fluorescence in situ hybridization to peripheral blood and bone marrow real time quantitative polymerase chain reaction on the International Scale for detecting residual disease in chronic myeloid leukemia. Hematologica 2008; 93(2):178-85.
6. Jobbagy Z, van Atta R, Murphy K, Eshleman J, Gocke C. Evaluation of the Cepheid GeneXpert BCR-ABL assay. J MolDiag 2007; 9(2):220-7.
7. Zhang T, Greiner S, Nwachukwu B, Wei C, Lipton J, Kamel-Reid S. Inter-laboratory comparison of

chronic myeloid leukemia minimal residual disease moinitoring: summary and recommendations. J MolDiag 2007;9:421-30.
8. Branford S, Cross N, Hochhaus A, Radich j, Saglio G, Kaeda j, Goldman j, Hughes T. Rationale for the recommendations for harmonizing current methodology for detecting BCR-ABL transcripts in patients with chronic myeloid leukemia. Leukemia 2006;20:1925-30.
9. Branford S, Fletcher L, Cross N, Muller M, Hochhaus A, Kim D, Radich J, Saglio G, Pane F, Kamel-Reid S, Wang Y, Press R, Lynch K, Rudzki Z, Goldman J, Hughes T. Desirable performance for BCR-ABL measurement on an international reporting scale to allow consistent interpretation of individual patient response and comparison of response rates between clinical trials. Blood 2008; 112:3330-8.
10. Raja S, Ching J, Hughes S, Chang R, Wong W, McMillan W, Gooding W, McCarty K, Chestney M, Luketich J, Godfrey T. Technology for automated, rapid and quantitative PCR or reverse transcription-PCR clinical testing. ClinChem 2005;51P882-90.
11. O'Brien S, Guilhot E, Larsons R, Gathman I, Baccarani M, Cervantes F, Cornelissen J, Fischer T, Hochhaus J, Hughes T, Lechner k. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med 2003;348:994-1004.
12. Hughes T, Kaeda J, Branford S, Rudzki Z, Hochhaus A, Hensley M,Gathmann I, Bolton A, van Hoomissen I, Goklman J, Radich j. Frequency of major molecular responses to imatinib or interferonalfa plus cytarabine in newly diagnosed chronic myeloid leukemia. N Engl J Med 2003; 349:1423-32.
13. Wang L, Knight K, Lucas C, Clark R. The role of serial BCR-ABL transcript monitoring in predicting the emergence of BCR-ABL kinase mutations in imatinib-treated patients with chronic myeloid leukemia. Leukemia 2003;17:1707-12.
14. Press RD, Galderisi C, Yang R, Rempfer C, Willis SG, Mauro MJ, Druker BJ, Deininger M.A half-log increase in BCR-ABL RNA predicts relapse in patients with chronic myeloid leukemia with an imatinib-induced complete cytogenetic response. Clin Cancer Res 2007;13:6136-43.
15. Roy L, Guilhot J, Krahnke T,Guerci-Bresler A, Druker BJ, Larson RA, O'Brien S, So C, Massimini G, Guilhot F. Survival advantage from imatinib compared to the combination interferon-α plus cytarabine in chronic phase CML: historical comparison between two phase III trials. Blood 2006;108:1478-84.
16. Marin D, Kaeda J, Szydlo R, Saunders S, Fleming A, Howard J, Andreasson C, Bua M, Olavarria E, Rahemtulla A, Dazzi F, Kanfer E, Goldman J, Apperley J. Monitoring patients in complete cytogenetic remission after treatment of CML in chronic phase with imatinib: patterns of residual leukemia and prognostic factors for cytogenetic relapse. Leukemia 2005;19:507-12.
17. Kantarjan H, Cortes J, O'Brien S, Luthra R, Giles F, Verstovsek S, Faderl S, Thomas D, Garcia-Manero G, Rios MB, Shan J, Jones D, Talpaz M. Long-term survival benefit and improved complete cytogenetic and molecular response rates withjimatinibmesylate in Philadelphia chromosome-positive chronic-phase chronic myeloid leukemia after failure of interferon-α. Blood 2004;104:1979-88.
18. Kantarjan H, Giles F, Wunderle L, Bhalla K, O'Brien S, Wassmann B, Tanaka C, Manley P, Rae P, Mietlowski W, Bochinski K, Hochhaus A, Griffin J, Hoelzer D, Albitar M, Dugan M, Cortes J, Alland L, Ottmann O. Nilotinib in imatinib-resistant CML and Philadelphia chromosome-positive ALL. N Engl J Med 2006;354:2542-51.
19. Talpaz M, Shah N, Kantarjian H, Donato N, Nicoll J, Paquette R, Cortes J, O'Brien S, Nicaise C, Bleickardt E, Blackwood-Chirchir A, Iyer V, Chen T, Huang F, Decillis A, Sawyers C. Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. N Engl J Med 2006; 354:2531-41.
20. Metcalfe P, Gray E, Hawkins R, Rigsby P, A proposed 1st WHO international genetic reference panel for the quantitation of BCR-ABL translocation by RQ-PCR, NIBSC code 09/138. Expert committee on biological standardization. Geneva 2009
21. Olavarria E, Kanfer E, Szydlo R, Kaeda J, Rezvani K, Cwynarski K, Pocock C, Dazzi F, Craddock C, Apperley JF, Cross NC, Goldman J.Early detection of BCR-ABL transcripts by quantitative reverse transcriptase polymerase chain reaction predicts outcome after allogenic stem cell transplantation for chronic myeloid leukemia. Blood 2001;97:1560-5.
22. Kanatrjaian H,Talpaz M, Cortes J, O'Brien S, Faderl S, Thomas D, Giles F, Rios MB, Shan J, Arlinghaus R. Quantitative polymerase chain reaction monitoring of BCR-ABL during therapy with imatinibmesylate (ST1571; gleevec) in chronic-phase chronic myelogenous leukemia. Clin Cancer Res 2003;9:160-6.