Preliminary evaluation of the GeneXpert Dx System for CML patients monitoring through the Xpert BCR-ABL Monitor™ assay: comparison with traditional RT-qPCR methods

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INTRODUCTION

The molecular signature of BCR-ABL fusion gene in chronic myeloid leukemia (CML) provides a unique tool for diagnosis and monitoring of tumor burden during therapy. The introduction of imatinib mesylate, allowing the achievement of high rates of clinical and cytogenetic remission, has revolutionized the treatment of CML patients and reinforced the fundamental role of BCR-ABL transcript levels monitoring by RT-qPCR to assess minimal residual disease. Nevertheless, many procedural aspects of this complex technique require a strong inter-laboratory optimisation and recommendations for harmonizing the different methodologies have recently been proposed.

THE GENEXPERT DX SYSTEM AND THE XPERT BCR-ABL MONITOR™ ASSAY

The Xpert BCR-ABL Monitor™, recently developed by Cepheid (Sunnyvale, CA 94089-1189), is an *In Vitro* Diagnostic assay, whose intended use is the molecular monitoring of p210 BCR-ABL transcript in peripheral blood samples of CML patients, through a fully automated platform, the GeneXpert Dx System (Cepheid). The instrument can perform nucleic acid isolation, reverse transcription and qPCR into a multi-chamber single-use disposable cartridge thanks to the integration of a quantitative real-time thermal-cycler with a software-driven cartridge processor. The analysis, that requires 200 µl of sample, is completed in less than 2 hour.

AIM OF THE STUDY

Analysis of 20 samples with the Xpert BCR-ABL Monitor assay

Comparison of the results with 2 conventional real-time PCR methods used in CML monitoring:

- a home-made method (Sybr Green I based)

- an IVD CE commercial method (TaqMan based), the M-Bcr FusionQuant kit (Ipsogen, France)

Xpert BCR-ABL Monitor™ ASSAY

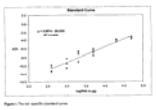
2 packages of 10 cartridges (IVD version)

same lot ID: 01401

efficiency EΔ**Ct = 10** $^{(1/slope)}$ **= 1.89**

standard curve: K562 RNA diluted in PC3 RNA

analysis volume: 20,50 or 200 µl

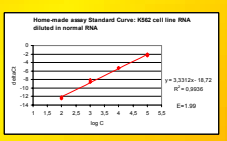


PATIENTS and SAMPLES

19 Chronic Myeloid Leukemia (CML) patients:
- 18 submitted to Glivec therapy, constantly monitored at our Division and by our Laboratory with real-time PCR
- 1 at first analysis, with CML diagnosis from another Centre
12 males and 7 females; median age: 52 (range:18-83)

Patients choice: random, according to the Laboratory reception order

Samples:
- 19 fresh peripheral blood samples (10 ml) in K_2EDTA
- 1 fresh bone marrow sample (2 ml) in K_2EDTA, from a patient also submitted to peripheral blood analysis
Samples stored max 24 h at 4°C before analysis



COMMON PRE-RTqPCR PROCEDURES

Mononuclear cells (MNCs) isolation by density gradient centrifugation with Lympholite (2 aliquotes)

RNA isolation with RNeasy Total RNA kit and QiaVac system (QIAGEN)

Qualitative and quantitative RNA evaluation by spectrophotometric analysis (Biophotometer, Eppendorf)

HOME-MADE ASSAY

Reverse transcription of 1 µg of total RNA with SuperScriptII (Invitrogen) as described in: van Dongen JJM et al. (1999) *Leukemia* 13:1901-1928

1/10 of cDNA product submitted to qPCR in 25 µl reaction volume with SybrGreen QPCR MasterMix (Eurogentec)

BCR-ABL and ABL primer sequences as described in: Gabert J et al. EAC Program (2003) *Leukemia* 17:2318-2357

GeneAmp5700(Applied Biosystems)

40 cycles (30" 95°C; 30" 60°C; 30" 72°C)

Dissociation curves analysis

IVD CE ASSAY

Reverse transcription of 1 µg of total RNA with SuperScriptII (Invitrogen) as described in: Gabert et al. (2003) *Leukemia* 17:2318-2357

1/10 of cDNA product submitted to qPCR in 25 µl reaction volume with Ipsogen M-bcr FusionQuant kit according manufacturer conditions

BCR-ABL and ABL primer and probe sequences as described in: Gabert J et al. EAC Program (2003) *Leukemia* 17:2318-2357

GeneAmp5700(Applied Biosystems)

50 cycles

16 of the 20 samples analyzed produced a valid result, while 4 (20%) were not evaluable (analysis not repeated)

	ABL			BCR-ABL			
N.	Ct	curve fit	result	Ct	curve fit	result	TEST RESULT
1	12,5	NA	FAIL	12,3	PASS	INVALID	INVALID
4	NA	NA	NO RESULT	NA	NA	NO RESULT	ERROR (aborted)
8	27,4	NA	FAIL	NA	NA	INVALID	INVALID
12	12	PASS	PASS	31,2	FAIL	INVALID	INVALID

NA= NOT AVAILABLE

QUALITATIVE RESULTS

VALID SAMPLES	16
NEGATIVE WITH ALL	2
NEGATIVE WITH BOTH RTqPCR METHODS/ POSITIVE WITH GENEXPERT	2
NEGATIVE WITH ONE RTqPCR METHODS/ POSITIVE WITH GENEXPERT	2
POSITIVE WITH ALL	10

	BCR-ABL/RATIO		
	GENEXPERT	HOME-MADE	IPSOGEN
PERIPHERAL BLOOD	0,00252	0,00491	0,00169
BONE MARROW	0,00055	0,0004	0,00016

	ABL Ct						
	N	Mean	Sd	Ct <13	13-13,99	14-16,99	>17
All	19	13,88	3,55	11	5	1	2
Valid samples only	16	13,24	1,46	9	5	1	1

Considering the valid samplesonly, 56,3% has ABL Ct values < 13 and 87,5% <14; the sample with a Ct > 17 has been evaluated with a volume of 20 ul (Valid ABL Ct range with the Xpert BCR-ABL Monitor assay = 12-18)

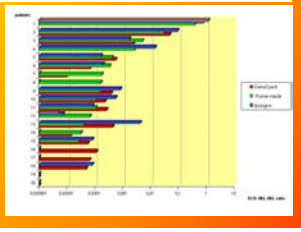
Cell number comparison

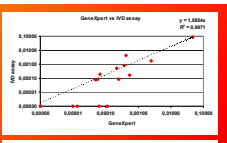
				GeneXpert		RT-qPCR		
sample n.	WBC x10^3/µl	neutrophil %	volume µl	analyzed cell n. x10^6	isolated MNC n. x10^6	tot RNA µg	estimated cell n. x10^6 in RT
1	50,64	89,7	50	2,53	15,00	8,06	1,86
2	3,71	34,8	200	0,74	10,89	10,26	1,06
3	4,41	56,7	200	0,88	8,59	11,75	0,73
4	7,34	59,8	200	1,47	13,28	18,23	0,73
5*	na	na	50	na	15	33,69	0,45
6	3,63	38,2	200	0,73	10,10	12,85	0,79
7	5,38	62,3	200	1,08	9,13	12,28	0,74
8	na	na	200	na	10	19,06	0,52
9	5,52	70	200	1,10	7,45	11,21	0,66
10	5,48	46	200	1,10	13,32	14,34	0,93
11	5,74	52,1	200	1,15	12,37	19,09	0,65
12	5,47	39,4	200	1,09	14,92	16,60	0,90
13	5,6	46,8	200	1,12	13,41	9,96	1,35
14	5,55	60,2	200	1,11	9,94	15,40	0,65
15	4,82	47,3	200	0,96	11,43	6,81	1,68
16	6,03	53	200	1,21	12,75	13,15	0,97
17	7,93	69,3	200	1,59	10,96	6,48	1,69
18	7,12	63,1	200	1,42	11,82	11,29	1,05
19	5,12	58,1	200	1,02	9,65	13,66	0,71
20	5,72	44,2	20	1,14	14,36	19,31	0,74
mean	8,07	55,06		1,19	11,72	14,17	0,94
sd	10,68	13,47		0,40	2,26	6,04	0,40
min	3,63	34,80		0,73	7,45	6,48	0,45
max	50,64	89,70		2,53	15,00	33,69	1,86

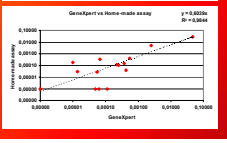
* bone marrow sample

Sample comparison with the 3 methods and absolute copy number detected by the IVD assay

	Copy n.	
Sample	ABL	BCR/ABL
1	6145,47	4673,67
2	3751,25	333,64
3	4125,05	6,99
4	3970,27	56,02
5	4480,15	0,73
6	1429,64	0,28
7	836,11	0,00
8	3609,79	0,00
9	3965,76	3,18
10	5856,67	3,12
11	10117,73	0,84
12	8385,75	0,06
13	9125,33	37,38
14	5549,18	0,00
15	4793,95	0,38
16	462,09	0,00
17	2939,11	0,00
18	4031,87	0,32
19	9205,26	0,00
20	3725,89	0,00







CONCLUSIONS

The preliminary data here collected may suggest that, even if more extensive studies are undoubtedly needed, the Xpert BCR-ABL Monitor™ assay combined with the GeneXpert Dx System, thanks also to its user-friendliness, could offer a valid alternative to traditional RT-qPCR methods, allowing a better standardization of the molecular techniques used for CML monitoring. Some technical issues, such as the possibility of use of other sample type (bone marrow, isolated cells, RNA), the analysis of sample with high WBC count and high transcripts level, should be improved in the near-future, not forgetting economical considerations, such as the costs of possible sample repetitions.