Cartridge-based automated *BCR-ABL1* mRNA quantification: solving the issues of standardization, at what cost?

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ABSTRACT

Background
Molecular monitoring of chronic myeloid leukemia patients treated with tyrosine kinase inhibitors is essential for therapeutic stratification. Inter-laboratory reproducibility is, therefore, a crucial issue which requires standardization and strict alignment of *BCR-ABL1* values to the international scale. An automated cartridge-based assay (Xpert BCR-ABL Monitor™, Cepheid) had been proposed as a robust alternative to non-automated assays. This study aimed to compare inter-laboratory reproducibility of automated and non-automated quantification, the possibility of converting automated results to the international scale, and the potential economic impact of automation.

Design and Methods
One hundred and eighteen blood samples from chronic myeloid leukemia patients treated with tyrosine kinase inhibitors were prospectively analyzed in two laboratories using both automated and non-automated assays. The economic evaluation involved a micro-costing study and average costs were assessed as a function of sample throughput.

Results
Automated assays achieved similar inter-laboratory reproducibility to highly standardized non-automated assays and a short delay (≤6 h) between sampling and blood lysis had a positive impact on inter-laboratory reproducibility. Reporting automated *BCR-ABL1* ratios on the international scale was possible using a specific conversion factor which may vary with batches. Cost assessment showed that automated assays could be relevant for annual activity levels below 300 since average costs were lower than those of the non-automated assays.

Conclusions
The Xpert BCR-ABL Monitor™ assay could be appropriately used in a near-patient setting for routine quantification of e13/e14-a2 transcripts, preferably in partnership with a regional reference laboratory. However, its prognostic impact relative to non-automated quantification remains to be tested prospectively within appropriate clinical trials.

Key words: chronic myeloid leukemia, *BCR-ABL1*, real-time quantitative PCR, standardization, cost assessment.

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Introduction

Treatment response monitoring using serial analyses of *BCR-ABL1* mRNA levels by reverse transcription (RT) real-time quantitative Polymerase Chain Reaction (RQ-PCR) is essential for chronic myeloid leukemia (CML) patients treated with tyrosine kinase inhibitors (TKI). It is widely accepted that achieving major molecular response (MMR) is associated with favorable progression-free survival,[1,2] and recent data showed that molecular response at three months is predictive of MMR achievement by 24 months in patients able to tolerate imatinib 600 mg once daily.[3] It has also been proposed that patients who fail to achieve a MMR by 18 months should undergo therapy review[4,5] as should those with significant increase in *BCR-ABL1* mRNA levels.[4,6]

Standardized, accurate and reproducible molecular analyses are, therefore, essential for clinicians to refine therapeutic stratification and make clinical decisions. Inter-laboratory reproducibility is, therefore, a crucial issue that requires standardization[6] and strict alignment of *BCR-ABL1* values on the international scale (IS), as established by the International Randomized Study of Interferon and STI571 (IRIS) laboratories.[2,7,8]

Within this context, the cartridge-based microfluidic Xpert BCR-ABL Monitor™ assay developed by Cepheid, that integrates and automates RNA extraction, RT and RQ-PCR for both e13/14-a2 *BCR-ABL1* and *ABL1* mRNA on the GeneXpert® Dx System automated analyzer, might help improve inter-laboratory reproducibility.[9] Previous studies showed that this automated *BCR-ABL1* fusion detection system did indeed have similar analytical performance, including specificity, sensitivity and intra-laboratory reproducibility, to non-automated TaqMan based RT RQ-PCR assays.[9,10] Sensitivity was assessed at 1 K562 cell amongst 100,000, with a linear range of quantitation of 5 logs. Given that neither of these studies fully addressed inter-laboratory reproducibility and alignment on IS, the present study specifically focused on these aspects. A cost analysis was also carried out in order to assess the comparative economic impact of the automated *BCR-ABL1* fusion detection system and non-automated assays, with particular emphasis on the impact of annual analytical activity.

Design and Methods

Laboratories, patients, cells and pre-analytical handling

Two Paris hospital laboratories, referred to as A and B, were involved in the study. Both performed over 500 *BCR-ABL1* mRNA quantifications/year. The study involved peripheral blood samples that were harvested from 118 TKI (imatinib, dasatinib or nilotinib) treated CML patients. Informed consenting patients expressing b2/e13-a2 or b3/e14-a2 fusion transcripts were prospectively included in the study. Two series of patients were successively recruited from October 2007 to January 2008 (1st series, n = 65) and during May 2009 (2nd series, n = 53). This study was approved by the institutional review board of Saint-Louis Hospital, Paris, and registered as 2007-023.

Two 7mL (EDTA,K3) Vacutainer® tubes (Beckton Dickinson, France) were sampled and dispatched at room temperature to both laboratories (1st series) or sent to laboratory A (2nd series). In the 1st series, blood samples underwent whole blood lysis within 24 h of sampling, but with variable delays between sampling and lysis between the two laboratories. In the 2nd series, two 0.2 mL aliquots of each sample were simultaneously subjected to the pre-analytical step of the Xpert BCR-ABL monitor™ assay. Under these experimental conditions, the delay between sampling and blood lysis was less than 6 h and was strictly identical between aliquots. One aliquot was immediately analyzed in laboratory A using the Genexpert® Dx System while the other was immediately frozen at -70°C and then sent, frozen, to laboratory B for Genexpert® Dx analysis.

BCR-ABL1 mRNA quantification

Both laboratories have a GeneXpert® Dx System (Cepheid, France) with 4 analytical positions. On receiving the samples, they were first analyzed on the GeneXpert® Dx System, starting from 200 μL as according to the manufacturer's recommendations, using lots 020.01 and 02601 for series 1 and 2, respectively. PCR efficiency provided for each lot was 92%. Samples were conditioned for RNA extraction using TRIzol® (Invitrogen, Cergy Pontoise, France), and *BCR-ABL1/ABL1* ratios were evaluated according to standard procedures routinely used in each laboratory. For both the automated and non-automated assays, results were expressed as *BCR-ABL1/ABL1* ratios and were transformed to decimal logarithmic values for comparison.

It is worth noting that both laboratories actively participated in the "Europe Against Cancer" (EAC) program and followed tightly standardized procedures at all steps of the analytical process.[11] These laboratories have also been involved since 2005 in the French national quality control program organized within the RuBIH (French National Network for Innovative Diagnostics in Onco-Hematology) network, which requires bi-annual evaluation of inter-laboratory *BCR-ABL1* reproducibility. During the period in which results were generated for the present study (2007-2009), the interlaboratory variability for RuBIH quality controls between laboratories A and B for the 14 positive (0.02% to 50%) samples undergoing non-automated quantification was maximally 2.1-fold, and none of the negative samples tested positive.

Conversion to international scale

As laboratory A is the French reference laboratory for the EUTOS program,[12] it has successfully validated an ^{IS}Conversion Factor (CF) for its routinely used non-automated assays (CF=0.48). Ratios obtained with the Xpert BCR-ABL monitor™ assay in this laboratory were, therefore, compared to IS according to the Bland and Altman comparison procedure, as described by others.[8,13,14]

Concordance between automated and non-automated quantification

Results were considered to be concordant for MMR IS when both automated and non-automated assays generated *BCR-ABL1/ABL1* values of 0.1% or less for a particular sample. Specific ^{IS}CFs for laboratory A for non-automated and automated assays were used (CF at 0.48 and 2.3, respectively). Since laboratory B had not validated its ^{IS}CF at the time of the study, concordance for MMR IS could only be assessed in laboratory A. With respect to concordance for complete molecular response (CMR),

this was achieved when *BCR-ABL1* transcripts for a given sample were undetectable by both methods. CMR was assessed in both laboratories A and B since CMR status does not depend on IS. Concordance is defined by the following ratio: number of samples attributed to a given molecular status by both methods divided by number of samples attributed to the same molecular status by either method.

Economic evaluation

The economic evaluation involved a micro-costing study conducted from a societal perspective.[15,16] Cost assessments were based on direct observation of labor times, equipment and consumables (disposables and reagents) required for quantitative assessments of *BCR-ABL1* transcripts. Cost calculations were based on purchase prices of equipment and supplies (0% and 25% price reductions were envisaged) and on cost standards for the technicians, secretary and physician involved. Following standard practice, a 5% discount rate was used to reflect the opportunity costs of investment in equipment. The overhead services costs (electricity, telephone, etc.) were provided by the Financial Services of hospital B. Cost assessments did not include rent, given the significant variability within a city, a region, or a country.

Average costs were estimated as a function of sample throughput, ranging from one sample a year to an annual activity corresponding to full-time employment of a technician involved in non-automated *BCR-ABL1* quantification, or to full-capacity use of the GeneXpert® Dx System. Two options were evaluated regarding the allocation of equipment to non-automated *BCR-ABL1* quantification. While the first option assumed that the equipment was dedicated to *BCR-ABL1* quantification, the second one simulated a polyvalent laboratory in which the equipment was increasingly assigned to *BCR-ABL1* quantification with the increase in annual activity. Similarly, with the automated assay, an extra analytical position on the GeneXpert® Dx System was assumed to be added when required (up to a maximum of 16 since it is possible to multiplex four GeneXpert® Dx Systems) because of increases in annual activity.

The possibility of having to repeat *BCR-ABL1* quantification due to problems with sampling, RNA quality, or unexpected results was assessed using a survey addressed to the 45 laboratories performing non-automated *BCR-ABL1* quantifications within the French RuBIH network. Respondents to the survey (42%) reported repeat rates ranging from 0% to 28%, and the average rate was 10%. Since the repeated steps may concern the whole process (pre-analytical and analytical) or just the analytical phase, the assessment of additional costs due to repeated steps was based on the average 10% repeat rate applied to costs of technician time, equipment, disposables and reagents involved in both pre-analytical and analytical steps. For the Xpert BCR-ABL Monitor™ assay, the repeat rate was estimated at 5%, due to the small number of steps likely to require repetition in this automated production process.

In keeping with the general economic principle of efficient use of resources, a criterion defined by average cost stability despite variations in annual activity (reflecting, for example, variations in the demand of *BCR-ABL1* quantifications addressed to laboratories) was used as a marker for perennial laboratory activity. Specifically, annual volumes were identified for which a variation of ±50 annual *BCR-ABL1* analyses was associated with a ±1% variation in the average cost.

Results

Inter-laboratory reproducibility (1st series)

Assessment of inter-laboratory reproducibility of *BCR-ABL1* mRNA quantification using Xpert BCR-ABL Monitor™ assays or the non-automated assays was based on 65 peripheral blood samples that were sent to the two laboratories and processed within 24 hours, but with variable delays between the two laboratories. In both laboratories, the *BCR-ABL1/ABL1* ratios obtained using both automated and non-automated assays were available for 63 out of 65 samples. As shown in Figure 1, there was a high level of inter-laboratory reproducibility for both automated and non-automated assays, with correlation coefficients of 0.92 and 0.97, respectively. Correlation coefficients were, however, higher for the latter method, since there was a significant dispersion of results for lower levels of positivity, below 0.01% (-2 by $\log_{10}$ ratio, Figure 1) using the Xpert BCR-ABL Monitor™ assay.

The ratios generated by the Xpert BCR-ABL Monitor™ assay extended approximately 1.5 log lower (down to between 0.001% and 0.0001%) than those resulting from non-automated assays, which were considered to be negative ratios below 0.01% (Figure 1). The explanation for this apparent discrepancy comes from the different methods used for calculation of *BCR-ABL1/ABL1* ratios. The Xpert BCR-ABL Monitor™ assay generates this ratio from *ABL1* and *BCR-ABL1* Ct values using the delta Ct method, and a difference of up to 20 Ct between *ABL1* and *BCR-ABL1* can be obtained, providing minimum measurable ratios of 0.0001%. By contrast, non-automated assays generate ratios based on the number of copies measured, with the lowest measurable copy number being 1 plasmid copy amongst the 20000 *ABL1* copies/test, giving a theoretical lower reliable limit of sensitivity of 0.005%. As a result, *BCR-ABL1* ratios below 0.01% can be generated by the Xpert BCR-ABL Monitor™ assay as measurable, although less reproducible, values whereas ratios below 0.01% using non-automated assays are seen as discordant positive/negative results (Figure 1). In the present study, 5 of 63 were discordant positive/negative results by the automated assay, compared to 10 of 63 by the non-automated assay. These results suggest that conversion factors to IS are likely to differ for *BCR-ABL1* mRNA quantification between automated or non-automated assays.

Impact of pre-analytical delay on inter-laboratory reproducibility (2nd series)

Given the distinct inter-laboratory reproducibility for Xpert BCR-ABL Monitor™ assay for ratios below 0.1% (-1 in LogRatio, i.e. the clinically critical level defining MMR) in a setting where pre-analytical latency was variable but always less than 24 hours, a 2nd series of 53 samples was analyzed in laboratories A and B, under the same experimental conditions but with reduction of the maximal latency between sampling and lysis from 24 to six hours, and with an identical latency in both laboratories, unlike the first series. Figure 2 shows that, under these conditions, inter-laboratory reproducibility remained high down to at least 0.01% (-2 in LogRatio) and correlation coefficients for the 37 positive ratios reached 0.98, as com-

pared to the 0.92 obtained in the case of variable pre-analytical latency (up to 24 h). Linearity of reproducible quantification was extended to 5 logs, compared to 4 logs with the non-automated assay. This suggests that the positive/negative concordance but quantitative dispersion seen with low level positivity below MMR by the automated method in Figure 1 is likely to result from variable pre-analytical delays which can be transformed into quantitative reproducibility down to at least 0.01% (-2 by LogRatio) if pre-analytical delays are reduced.

Reporting GeneXpert® Dx System results on the International Scale

Thirty-one samples from the first series with ratios greater than 0.01% by the GeneXpert® Dx System were used for calculation of the IS conversion factor.[8,12] *BCR-ABL1/ABL1* ratios obtained in laboratory A using non-automated assays were multiplied by the IS conversion factor assessed by the EUTOS procedure[12] (^{IS}CF=0.48) and were then compared to those obtained in the same laboratory with the Xpert BCR-ABL Monitor™ assay. Figure 3A shows that the ratios resulting from the Xpert BCR-ABL Monitor™ assay were systematically lower than the IS ratios, indicating a reproducible bias over the entire measurement domain ranging from 50% down to 0.01%. The regression curve was Y = 0.998 X – 0.357 with R^2 = 0.90. This consistent bias allowed calculation of a conversion factor for alignment on the IS of ratios obtained with the Xpert BCR-ABL Monitor™ assay. Using the Bland Altman method,[8] the mean bias between the Log_{10}Ratios relative to the IS was -0.357, corresponding to an ^{IS}CF of 2.3, expressed in fold change. The differences observed were distributed within a 95% confidence interval of ± 3.1, expressed as fold change (Figure 3B). Validation of this conversion factor was performed using an independent series of 19 samples from the 2nd series with ^{IS}ratios greater than 0.01%. Figures 3C and 3D show that applying this conversion factor to Xpert BCR-ABL Monitor™ assay ratios greatly improved their alignment on the IS. The remaining mean bias was assessed at 1.2 fold greater instead of 2.3 fold lower than IS ratios and a 95% confidence interval was calculated as a ± 2.7 fold change (Figure 3D).

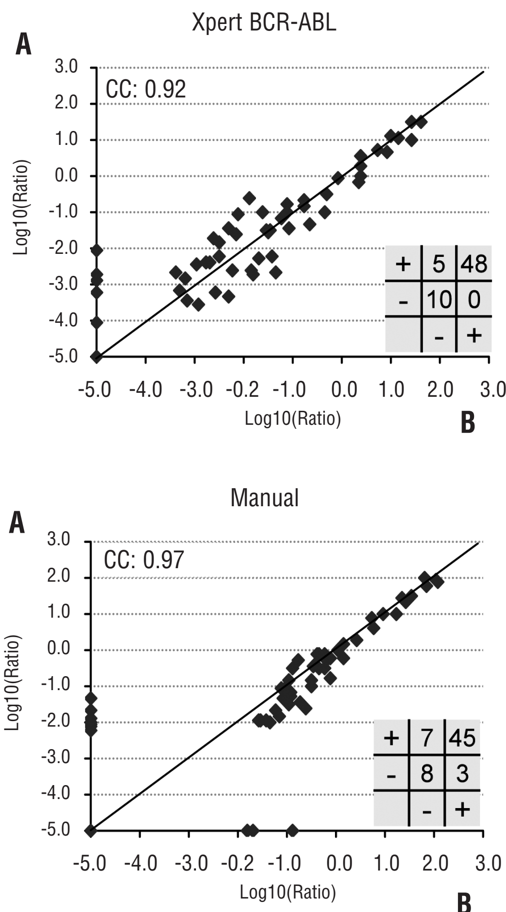

Figure 1. Inter-laboratory correlations for Xpert BCR-ABL Monitor™ and non-automated methods, respectively. Bias plots were generated from the 63 Log_{10}(Ratio) values obtained in site A and B by automated Xpert BCR-ABL Monitor™ and non-automated methods (1st series). In each plot Log_{10}(Ratios) are depicted as dots. Negative ratios are reported as -5. Equality lines are indicated. Number of negative and positive ratios are indicated in a table at the bottom right corner of each plot. CC = correlation coefficients calculated for ratios found to be positive in both laboratories.

Concordance between methods (1st series)

Concordance between automated and non-automated quantification was assessed using the data generated from the first series of samples, for both MMR IS and CMR. In laboratory A, 35 and 39 samples out of 63 were measured at or below 0.1% IS by both or either methods, respectively, giving a 90% concordance rate (Table 1). All 4 discordant samples gave values below 0.1% with the Xpert BCR-ABL Monitor™ assay and between 0.15 and 0.3% with the non-automated assay. Concordances for CMR reached 62% (8 of 13) and 71% (12 of 17) in laboratories A and B, respectively (Table 1). Discordant results corresponded to samples measured positive either with automated (n=6, median=0.003%, 0.001%-0.006% range) or non-automated quantification (n=4, median=0.008%, 0.004%-0.06% range). These data show comparable sensitivity of the two techniques with positive/negative discrepancies only involving low level positivity, always below MMR.

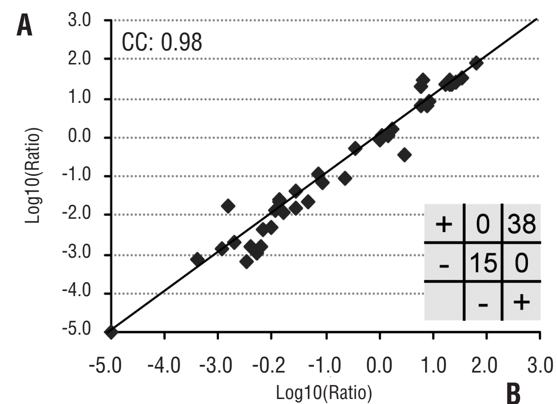

Figure 2. Inter-laboratory correlations for Xpert BCR-ABL Monitor™ assay, with fixed pre-analytical delays below 6 h. The settings guaranteeing identical delays prior to analysis in both laboratories are described in *Design and Methods*: 53 samples were analyzed, 15 of which were negative and 38 positive (2nd series).

Economic evaluation

Since both the Xpert BCR-ABL Monitor™ and standardized non-automated quantification showed similar inter-laboratory reproducibility, the cost-effectiveness analysis was reduced to a cost-minimization analysis based on data collected through direct observation of labor, equipment and supplies involved. The employment of a full-time technician for non-automated *BCR-ABL1* quantification led to an estimated maximum of 2,514 assays per year. In comparison, the time necessary for a quantitative assessment of e13/e14-a2 *BCR-ABL1* transcripts using Xpert BCR-ABL Monitor™ assay was estimated at 3.28 hours (including 2.25 h machine time), leading to an estimated maximum of 3,012 assays per year (consistent with a GeneXpert Dx System with 4 analytical positions).

Figure 4A shows that using the GeneXpert Dx System allowed stabilization of average costs at lower levels of activity compared to the non-automated assay. For annual activity levels over 300 or 600, depending on whether the equipment was assumed to be shared or dedicated to the activity, the average costs of automated assay were, however, systematically higher than those of the non-automated assay. Stability of average costs was seen with annual activity levels over 600 for the automated method, over 1,200 for the non-automated method with shared equipment and at least 1,800 with dedicated equipment (Figure 4A). The corresponding average costs are given in Table 2 with the relative contribution to costs from labor, equipment and supplies. Hypotheses regarding price reductions (0% or 25%) on equipment and supplies had an impact on average costs that was less marked for non-automated than for automated assay (Figure 4A and B). Hypotheses on repeat rates (plus or minus 5%), technician time (10% increase due to the possibility of hazards in production), allocation of equipment (in the case of a polyvalent laboratory), service overheads (20% variation in costs) or discount rates used for the calculation of annual values of equipment (0%, 5% or 8%) all had a low impact on the average costs (ranging from 0.4% to 4%; *data not shown*).

Table 1. Concordance for MMR IS and CMR between automated and non-automated methods. Data obtained with the first series of 63 samples are cited.

	Either methods	Xpert BCR-ABL ™ assay	Non-automated method	Both methods (% of concordance)
Number of samples with ratio ≤ 0.1% (MMR IS) in laboratory A*	39	39	35	35 (90)
Number of samples found negative (CMR) in laboratory A	13	10	11	8 (62)
Number of samples found negative (CMR) in laboratory B	17	14	15	12 (71)

* *CFs of 2.3 and 0.48 were applied to ratios generated by automated and non-automated methods, respectively.*

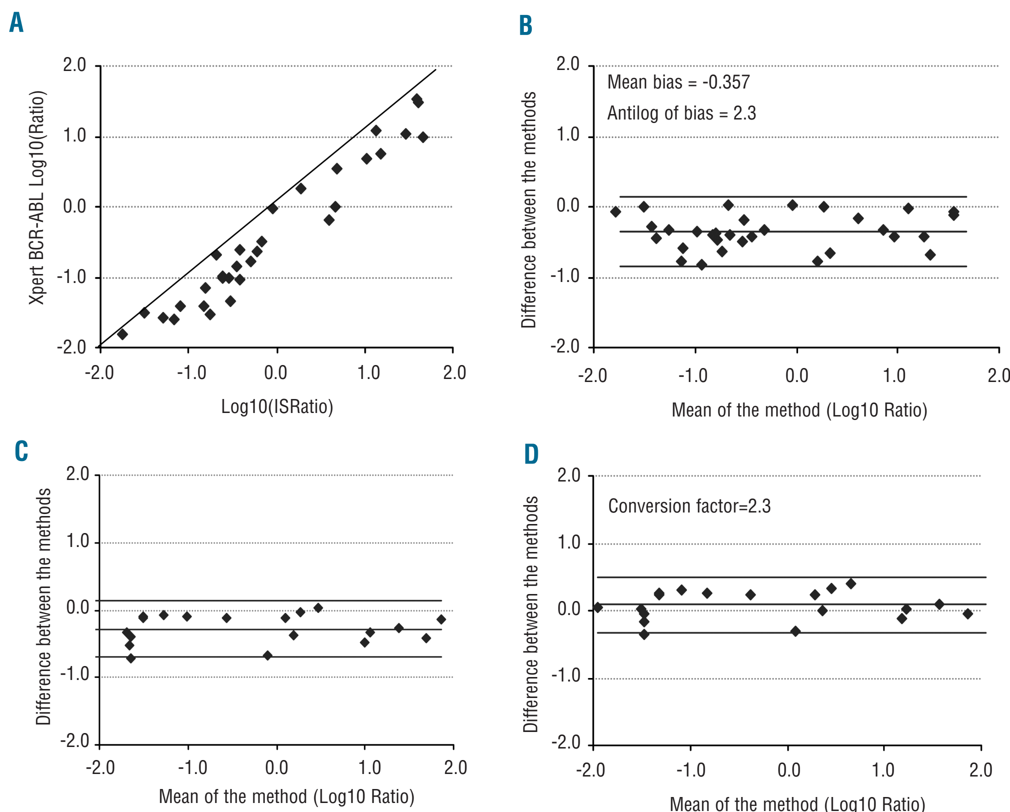

Figure 3. Conversion of GeneXpert® Dx System ratios to IS. ISCF calculation (A, B). Thirty-one samples from the 1st series with ratio > 0.01% were used. (A) Visual inspection of the data showed that ratios generated by the Xpert BCR-ABL Monitor™ assay were systematically lower than those generated by the standard method and expressed on IS, indicating a consistent bias between the methods. Equality line is indicated as a straight line. (B) Bias plots of the same data showed that ratios generated by Xpert BCR-ABL Monitor™ assay were, on average, 2.3-fold lower than IS ratios. Validation of ISCF calculated for automated Xpert BCR-ABL Monitor™ assay (C, D). Nineteen samples from the 2nd series with ratio > 0.01% were used. Data were plotted before (C) and after (D) conversion of GeneXpert ratios to the IS (x 2.3). Mean: horizontal median line, 95% confident interval: upper and lower horizontal lines.

A price reduction of 25% on equipment and 60% on cartridges would align the costs of automated and non-automated assay, also at stabilized annual activity levels.

Discussion

Previous published studies showed that the limit of detection using the cartridge-based Xpert BCR-ABL Monitor™ assay run on the GeneXpert® Dx System assessed in K562 cell spiked blood samples and intra-laboratory reproducibility were similar to those reported for non-automated RT-PCR-based assay with approximate sensitivities of 1 K562 cell in 100,000 and a 5 log range of linear quantification.[9,10] We, therefore, chose not to repeat these experiments but to assess reproducibility on patient samples, analyzed in duplicate in two EAC standardized laboratories, with variable pre-analytical times which were systematically less than 24 hours.

The prospective study presented here shows that the automated assay allows similar inter-laboratory reproducibility to standardized non-automated assays even in highly harmonized laboratories applying EAC guidelines and that *BCR-ABL1/ABL1* ratios can be converted to the international scale by applying an ISCF. Moreover, it shows that despite an apparently lower inter-laboratory reproducibility for ratios around and below 0.1% for the Xpert BCR-ABL Monitor™ assay, agreement between methods remained high, once corrected to the ISCF.

Reducing the latency between sampling and cell lysis to less than six hours improved inter-laboratory reproducibility to levels at least similar, if not superior, to that of highly standardized non-automated assays. Under these experimental conditions, high inter-laboratory reproducibility was also achieved down to 0.01%, which is the required level for accurate measurement of MMR. At these low levels, positive/negative discordances were relatively frequent when using non-automated quantification and there was no evidence based on patient samples with low level of residual disease that automated quantification was less sensitive than non-automated quantification. The 5 log range of linearity for automated quantification (Figure 2) is in line with that previously described.[9,10]

Reproducible results at these low levels of minimal residual disease were significantly more frequently obtained when pre-analytical steps were reduced to six hours or less, compared to 24 hours. We emphasize that this situation may only apply to patients undergoing treatment with TKI, in which case the samples remain in contact with the TKI during the pre-analytical phase. A similar impact of pre-analytical delay may not apply to patients not being treated with TKI, such as in the stem cell transplantation setting. The impact of pre-analytical delay, however, merits further investigation since, if these

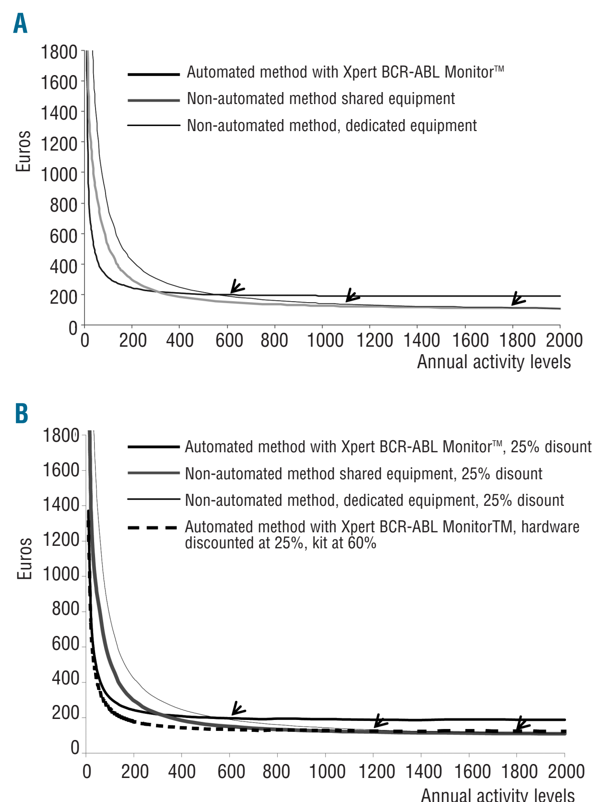

Figure 4. Average costs as a function of annual activity levels of *BCR-ABL1* quantification performed using the Xpert BCR-ABL Monitor™ assay or the non-automated quantitative *BCR-ABL1* on shared equipment or a dedicated *BCR-ABL1* only platform. (A) No price discount was applied to equipment and supplies. Minimum annual activity levels consistent with average cost stability is indicated by arrows. (B) A 25% price discount was applied to equipment and supplies. Note: Cells, RNA or cDNA cannot be conserved for banking purposes when automated quantitative *BCR-ABL1* is performed. Thus, in order to compare automated and non-automated assays, the conservation of products relating to non-automated assays were not included in the costing.

Table 2. Average costs of quantitative assessments of *BCR-ABL1* transcripts associated with annual volumes consistent with average cost stability (25% price reduction applied on equipment and supplies).

Annual activity Allocation of equipment	Non-automated 1800 dedicated	 1200 Shared (polyvalent lab)	Automated 600 dedicated
Reception and cell retrieval	45.3 €	47.0 €	n.a.
Reception and blood sample preparation	n.a.	n.a.	16.2 €
RNA extraction	4.5 €	4.3 €	n.a.
Reverse transcription	5.4 €	6.3 €	n.a.
RQ-PCR for *ABL1*	18.1 €	19.0 €	n.a.
RQ-PCR for *BCR-ABL1*	22.0 €	23.4 €	n.a.
Xpert BCR-ABL Monitor™ assay	n.a.	n.a.	157.1 €
Final analysis and sending of results	12.6 €	13.2 €	15.3
Overhead services	4.7 €	6.3 €	8.6 €
Average cost	**112.5 €**	**119.5 €**	**197.2 €**
Labor	50.0%	51.7%	20.0%
Equipment	16.9%	14.3%	5.6%
Laboratory material	1.4%	1.2%	0.1%
Reagent	22.7%	22.4%	69.6%
Disposables	5.1%	5.1%	0.3%
Overhead services	3.9%	5.3%	4.4%

Note : n.a. for not applicable.

results are confirmed within the context of prospective clinical trials, delays of even 24 hours, necessary for transfer to reference laboratories, may reduce inter-laboratory reproducibility, thus favoring near-patient testing.

Although systematically lower than IS values, the Xpert BCR-ABL Monitor™ ratios showed a consistent bias over the whole measurement range and could, therefore, be converted to IS by applying a 2.3 ^{IS}CF. This 2.3 ^{IS}CF was subsequently applied to an independent series of samples, resulting in a remaining bias of 1.2 fold (95% confidence interval: ± 2.7 fold). This level of corrected performance is consistent with requirements recommended by Branford *et al.* as a guarantee of reproducible assessment of molecular response status between laboratories.[8] In fact, the level of concordance between methods for MMR IS was measured at 90% in laboratory A. This level of concordance between the two methods is close to that reported by Branford *et al.* for group 1 laboratories.[8] Concordances for CMR were comparable between the two laboratories and positive/negative discordant results were only observed among samples with positivity below 0.1%, in line with previously published data.[9,10] These data suggest that there is no significant bias in achieving CMR and its impact on therapeutic decisions, such as stopping TKI.

The Xpert BCR-ABL Monitor™ assay can, therefore, be used to efficiently measure *BCR-ABL1* mRNA levels in virtually all peripheral blood samples taken from TKI treated CML patients expressing e13/e14-a2 *BCR-ABL1* fusion transcripts and produce inter-laboratory reproducible ratios that can be expressed on IS. Once converted to the IS, the data presented here suggest that comparable sensitivity can be obtained with automated and non-automated standardized *BCR-ABL1* quantification at levels down to 0.001%, if pre-analytical delays are reduced to less than six hours. It is, however, worth noting that conversion factors may vary between Xpert BCR-ABL Monitor™ assay batches, and needs to be re-evaluated for each new lot/batch of assays. This aspect must be evaluated in collaboration with the suppliers.

Given the significant reduction in the complexity of analysis, it is likely that automated quantification will provide more reliable inter-laboratory results between non-standardized laboratories than the two EAC laboratories which participated in this study. Indeed, comparison of results with a non-standardized laboratory which used different techniques and house-keeping gene generated, as expected, different quantitative results which could not be normalized using the non-automated method, but comparable results using the Xpert BCR-ABL Monitor™ assay (*data not shown*).

Economic evaluation was based on an estimated duration (approximately 3.5 h) for the Xpert BCR-ABL Monitor™ assay, close to previously published estimations[9] and substantially lower than that of non-automated assays. We are not aware of other published studies involving economic evaluation of *BCR-ABL1* mRNA quantification, but our cost assessments showed that using the Xpert BCR-ABL Monitor™ assay could be economically relevant for annual activity levels below 300. At these levels, average costs remained lower than those of non-automated quantification, even on shared platforms where equipment costs are optimized. For higher activity levels, automated quantification was associated, in the absence of significant price reductions on cartridges, with higher average costs than those of non-automated assay, even in the less realistic scenario where the equipment was entirely dedicated to *BCR-ABL1* quantification.

Annual activity is likely to increase as the number of patients benefitting from TKI maintenance increases. From an economic perspective, the Xpert BCR-ABL Monitor™ assay is not optimal for diagnostic screening in order to exclude *BCR-ABL1*, since it will not detect variant fusion transcripts. This option would require that all negative results (about 90% in a diagnostic setting) be confirmed using non-automated assays in order to detect the approximately 1% of patients with *BCR-ABL1* fusion transcripts other than e13-a2/e14-a2. Providing rapid identification of e13a2/e14a2 transcripts using the Xpert BCR-ABL Monitor™ assay would then be obtained at much higher overall unit cost and could lead to longer delays in providing results. If the automated assay is to be used in this setting, such use should be restricted to cases with a very high index of suspicion.

Overall, the present study showed that the Xpert BCR-ABL Monitor™ assay could be appropriately used to provide reproducible results for CML patients treated with TKI in a near-patient testing setting. This would allow shorter delays between sampling and blood lysis, with a positive impact on inter-laboratory reproducibility, more rapid (same day) transmission of results, and lower average costs for laboratories with annual activity levels below 300 follow-up samples. Rapid, near-patient follow up of CML patients with classical *BCR-ABL1* transcripts treated with TKI could be carried out in local laboratories using the Xpert BCR-ABL Monitor™ assay, but should be undertaken within a partnership with a reference center performing more specialized molecular analyses, including TKI domain mutations detection, quantification of *BCR-ABL1* fusion transcripts other than e13-a2/e14-a2, and follow up of patients following stem cell transplantation. Obviously, automated *BCR-ABL1* quantification needs to be undertaken in respect of state-of-the-art management of quality control, training, interpretation of results and record keeping.

Approximately 700 individuals with CML are diagnosed in France every year;[17] not all are treated within prospective clinical trials. Clinical guidelines recommend quarterly follow-up testing for patients treated with TKI.[4,5] With approximately 20,000 *BCR-ABL1* analyses performed in France in 2009 (population 66m), reproducibility and costs are crucial issues. Therefore, therapeutic stratifying cut-off values must be reproducibly generated for patients treated with TKI, whether within a clinical trial or not. While it is likely that patients in trials will undergo molecular monitoring in reference centers, the data presented here, applicable to the vast majority of CML patients, could encourage rational provision of health resources and the establishment of appropriate partnerships between local and reference molecular diagnostic laboratories. This would optimize molecular monitoring and use of TKI.

Authorship and Disclosures

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