Development and evaluation of a quantitative multiplex assay for the harmonization of BCR-ABL1 measurement on the international reporting scale

Review category: 632. Chronic Myeloid Leukemia – Therapy [*including diagnosis*, prognosis, therapy, epidemiology, complications, follow-up, quality of life, and socioeconomic aspects. For comparative trials of treatment regimens versus transplantation, see categories 731-733.]

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Introduction: Sensitive and standardized methods are required for the monitoring of BCR-ABL1 fusion transcripts in chronic myeloid leukemia (CML) patients treated with tyrosine kinase inhibitors (TKI). Large multi-site studies have led to establishment of the International Scale (IS) for consistent interpretation of individual patient response and comparison between patients and laboratories. On this scale, the baseline of BCR-ABL1 (b2a2 or b3a2) to control gene ratio is defined as 100% IS with a 3-log reduction indicative of major molecular response (MMR; 0.1% IS or lower). Growing evidence indicates that the e1a2 variant is associated with inferior outcome and higher risk of relapse. It is therefore also important to closely monitor e1a2 expression levels during the course of TKI treatment. The objective of this study was to evaluate the use of a single multiplex assay (BCR/ABL1 Quant™, for Research Use Only) for the quantitative and qualitative detection of e1a2, b2a2, and b3a2 fusion transcripts and to compare its performance against existing laboratory-developed tests (LDT) and the IS. **Methods:** Total RNA was reverse transcribed and then amplified by multiplex, real-time quantitative PCR on the ABI 7500 or ABI 7500 Fast Dx system using target-specific primers for e1a2, b2a2, b3a2 and ABL1 as an endogenous control. Four-point standard curves were generated in each run using the included multiplex Armored RNA Quant® (ARQ) Calibrators. Fusion transcript identity was determined by capillary electrophoresis. Archived total RNA samples from two independent sites were purified from white blood cells enriched from bone marrow or whole blood specimens with the respective laboratory validated extraction methods and tested with each site's LDT. Establishment and validation of a conversion factor (CF) was performed as described in Branford et al. (Blood, 2008) by exchanging representative total RNA samples with the IMVS lab (Australia). **Results:** Analytical performance assessed using cell line dilutions and synthetic in vitro transcript RNA showed a limit of quantitation of 10-15 copies of BCR-ABL1 per PCR reaction and a limit of detection of 2-5 copies. The linear dynamic range of the assay reached the equivalent of at least one translocation-positive cell in 100,000 normal cells. Analysis of 115 residual clinical specimens showed an excellent correlation between each LDT and the BCR/ABL1 Quant assay for the quantitative measurement of BCR-ABL1 to ABL1 ratio (paired correlation of 0.97 for both LDTs). There was 100% agreement for the qualitative detection of major and minor fusion transcripts. The assay was compared to an IS reference laboratory by testing 20 specimens covering ratios of about 0.005 to 10%. The mean bias between methods corresponded to a CF of 1.04 (BCR/ABL1 Quant measured % ratio x 1.04 = IS % ratio). Fourteen months later the process was repeated with an independent lot of reagents. The difference between methods was again minimal (CF = 0.96) validating that % ratio obtained with the BCR/ABL1 Quant assay are comparable to the IS. Overall, the 95% limit of agreement between methods was less than plus or minus 5 fold and 87.5% of the specimens were correctly classified in terms of MMR status (above or below 0.1% IS). **Conclusions:** The BCR/ABL1 Quant assay has the advantage of both quantifying in a single reaction and distinguishing fusion transcripts e1a2, b2a2, and b3a2 . Our results indicate that the assay also has the performance characteristics required for reporting on the IS. Validation of this multiplex assay format may therefore improve laboratories' workflow and facilitate harmonization of BCR-ABL1 quantitative measurement, increasing adherence to the current recommendations for reporting on the IS.

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