Evaluation of BCR/ABL1 Quant Assay (RUO)* for the Quantitative Detection of e1a2, b2a2, and b3a2 Fusion Transcripts

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SUMMARY

• BCR/ABL1 Quant is a multiplex assay with a streamlined workflow for the detection of ALL1 and BCR/ABL1 fusion transcripts.
• Multiplexing and compatibility with capillary electrophoresis enables the detection and identification of 3 BCR/ABL1 fusion transcripts: e1a2, b3a2, and b2a2.
• Inclusion of Armored RNA QuantTM Norm enables internal assay calibration for absolute quantification of BCR/ABL1 copy number.
• Assay validation at GenPath (BRL) increased the laboratory throughput and improved results reporting by including e1a2 data, percent ratio, and normalized copy number.

INTRODUCTION

The Philadelphia translocation, t(9;22), which juxtaposes the ABL1 (tyrosine kinase) and BCR (b2a2) genes, is the hallmark of chronic myeloid leukemia (CML). Fusion transcripts corresponding to the BCR/ABL1 (b2a2) product are present in 95% of CML patients. About 90% of children with acute lymphoblastic leukemia (ALL) and 20 to 30% of adult ALL also carry BCR/ABL1, most often in t(9;22). This molecular signpost provides a tool for initial diagnosis, risk-stratified patient management, and monitoring of residual disease and potential recurrence during targeted therapies with kinase inhibitors.

Asuragen’s BCR/ABL1 Quant (RUO) is a reagent system for simultaneous detection of three BCR/ABL1 fusion transcripts (e1a2, b2a2, b3a2). Armored RNA Quant (RUO) (Armored Norm) is included to facilitate the quantitation of e1a2 and b2a2, the fusion products of BCR/ABL1. This combination with targeted multiplex technology and the resulting PCR products are compatible with capillary electrophoresis (CE) for the quantification and subsequent confirmation of the fusion transcript staining intensity (e1a2, b2a2, or b3a2) by size fractionation. Previous studies showed that the assay had the required analytical sensitivity and linearity for targeted therapy monitoring. The objectives of this study were to further evaluate the performance of the assay for the quantification of e1a2 and the reporting of absolute copy number of BCR/ABL1 fusion transcripts.

MATERIALS & METHODS

Peripheral blood or bone marrow from leukemia patients were collected at the point of standard clinical care and following a protocol approved by University of Texas MD Anderson Cancer Center Institutional Review Board. RNA was isolated from white blood cells and enriched for RNA using the RNeasy Mini Kit (Qiagen). The BCR/ABL1 QUANT (RUO) assay was performed using 8 µL of aliquoted total RNA following the packaged instructions (Qiagen). The InterAssay Control RNA (IC-9600) was added to each sample and serially diluted to provide a range of measured RNA copy numbers. The assay was performed following the packaged instructions (Qiagen) and using the Molecular Diagnostic System (MDS) for quantitative assessment of the fusion transcript copy number. The assay was performed on an ABI 7500 Real-Time PCR system. The target copy number and normalized copy number were calculated using the MDS. The results were reported as mean percent copy numbers for each sample.

RESULTS

Figure 1. Data Processing and Reporting Workflow. For each pair of samples, the ABI 7500 real-time PCR machine was used to process the four different samples. The results were then analyzed using the MDS software and normalized copy number was calculated for each sample. The data were then analyzed using the MDS software and normalized copy number was calculated for each sample. The results were then analyzed using the MDS software and normalized copy number was calculated for each sample. The results were then analyzed using the MDS software and normalized copy number was calculated for each sample.

Figure 2. Determination of the Reference Assay (RUO) and Reference Assay Copy Number (TNS). For each pair of samples, the ABI 7500 real-time PCR machine was used to process the four different samples. The results were then analyzed using the MDS software and normalized copy number was calculated for each sample. The results were then analyzed using the MDS software and normalized copy number was calculated for each sample. The results were then analyzed using the MDS software and normalized copy number was calculated for each sample. The results were then analyzed using the MDS software and normalized copy number was calculated for each sample.

CONCLUSIONS

The BCR/ABL1 Quant assay (RUO) has the advantage of both quantifying and distinguishing e1a2, b2a2, and b3a2 fusion transcripts in a single reaction. Streamlined reagent formulation, multiplex assay format, and inclusion of Armored RNA Quant external calibrator improve the workflow and increase the number of specimens that can be tested per run. Co-detection of ABL1 and inclusion of an exogenous AQR process control enable reporting of BCR/ABL1 to 1:1 ratios for standardization to the International Scale as well as reporting of absolute BCR/ABL1 copy number. This format may contribute to enhanced patient care by preventing inaccurate results from potential inter-specimen or inter-run variability and by enabling inter-laboratory comparisons for different quantitative BCR/ABL1 testing and reporting methods.

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• BCR/ABL1 Quant Assay: Asuragen and GenPath.


For further information, contact Frank Buccino at R&D at rdu@genpath.com; or call 888-GENPATH.