Evaluation of a Multiplexed qPCR Assay for the Detection of BCR-ABL Fusion Transcripts on the ICE*Plex* System

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ABSTRACT

Background: Identification and monitoring of the BCR-ABL fusion oncogene has become standard of care in managing patients with CML. The majority of CML and a subset of ALL patients are shown to harbor the p210 BCR-ABL fusion transcript (b3a2 or b2a2). Other fusion transcripts such as e1a2 (p190) and e19a2 (p230) can occur in CML and ALL patients. To date, commercially available diagnostic assays primarily identify p210 transcripts. Here we evaluate an assay designed to identify multiple known transcripts of the BCR-ABL fusion gene using an automated highly multiplexed qPCR platform.

Material and Methods: Samples from patients received in the lab for the quantitative assessment of the BCR-ABL translocation using the Cepheid BCR-ABL assay on the GeneXpert, were concurrently assessed on the ICE*Plex*. Two different RNA extractions were performed using a silica column and an automated magnetic bead based technique. The total number of white cells and elution volumes were normalized. RNA was reverse transcribed and cDNA used in the subsequent multiplexed qPCR reaction on the ICE*Plex* (PrimeraDx, Mansfield, MA). Invivoscribe BCR/ABL RNA Dilution Sets for b2a2, b3a2 and e1a2 transcripts were run to evaluate the linearity of the assay.

Results: The BCR-ABL translocation was detected in a number of patients, some of which were characterized as low level positive, using the GeneXpert assay. Retesting using the ICE*Plex* system identified patients with the b3a2 and/or b2a2 transcripts in both extractions, and additional patients showed this translocation in one of the extractions only. Also, a number of patients were shown to harbor a transcript (e1a2) in at least one of the extractions used on the ICE*Plex* that is not detected using the Cepheid assay. BCR/ABL RNA Dilution sets run on the ICE*Plex* system for b2a2, b3a3 and e1a2 showed a linear regression R^2 value for calculated BCR-ABL copy number versus concentration of 0.9959, 0.9824 and 0.9747, respectively.

Conclusions: Using an automated, highly multiplexed qPCR platform, the ICE*Plex* system, to identify known transcripts of the BCR-ABL fusion gene allowed us to identify translocations detected using the Cepheid assay. The multiplexed assay also identified patients harboring a transcript not detected using commercially available assays. With further investigation into the extraction and specimen processing techniques, this method will allow the reliable detection and quantification of known BCR-ABL transcripts in patients with leukemia.

INTRODUCTION

- Identification and monitoring of the BCR-ABL fusion oncogene (Figure 1) has become standard of care in managing patients with CML.
- The majority of CML and a subset of ALL patients are shown to harbor the p210 BCR-ABL fusion transcript (b3a2 or b2a2).
- Other fusion transcripts such as e1a2 (p190) and e19a2 (p230) can occur in CML and ALL patients.
- To date, commercially available diagnostic assays primarily identify p210 transcripts.
- Here we evaluate an assay designed to identify multiple known transcripts of the BCR-ABL fusion gene using an automated highly multiplexed qPCR platform (Figure 2).

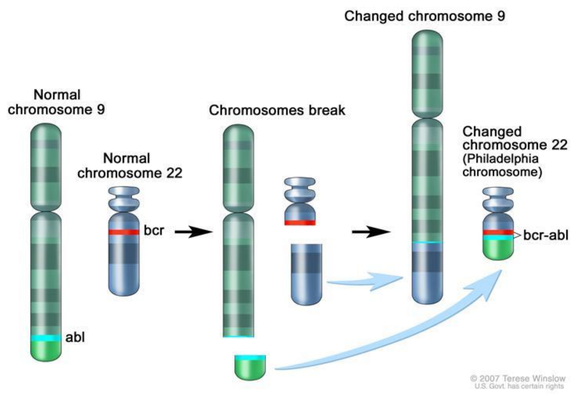

Figure 1. Graphical representation of the BCR/ABL fusion oncogene

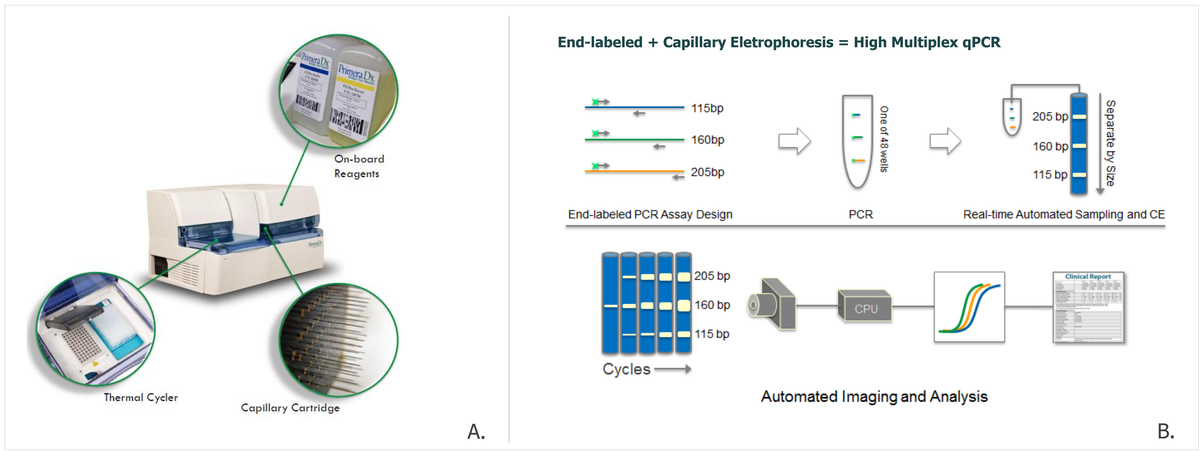

Figure 2. ICE*Plex* Techonology. **A.** Components of the ICE*Plex* System. **B.** Principles of the multiplex real-time PCR detection.

METHOD

- Samples from patients received in the lab for the quantitative assessment of the BCR-ABL translocation using the Cepheid BCR-ABL assay on the GeneXpert, were concurrently assessed on the ICE*Plex*.
- Two different RNA extractions were performed using a silica column and an automated magnetic bead based technique. The total number of white cells and elution volumes were normalized.
- RNA was reverse transcribed and cDNA used in the subsequent multiplexed qPCR reaction on the ICE*Plex*.
- Invivoscribe BCR/ABL RNA Dilution Sets for b2a2, b3a2 and e1a2 transcripts were run to evaluate the linearity of the assay.

RESULTS

- The BCR-ABL translocation was detected in a number of patients, some of which were characterized as low level positive, using the GeneXpert assay. Retesting using the ICE*Plex* system identified patients with the b3a2 and/or b2a2 transcripts in both extractions, and additional patients showed this translocation in one of the extractions only
- A number of patients were shown to harbor a transcript (e1a2) in at least one of the extractions used on the ICE*Plex* that is not detected using the Cepheid assay
- BCR/ABL RNA Dilution sets run on the ICE*Plex* system for b2a2, b3a3 and e1a2 showed a linear regression R^2 value for calculated BCR-ABL copy number versus concentration of 0.9959, 0.9824 and 0.9747, respectively (Figure 3)

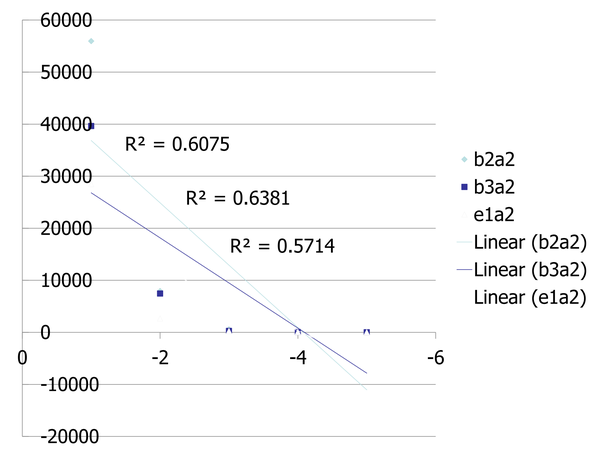

Figure 3. Linear regression of the calculated BCR-ABL copy number for the Invivoscribe RNA dilution series

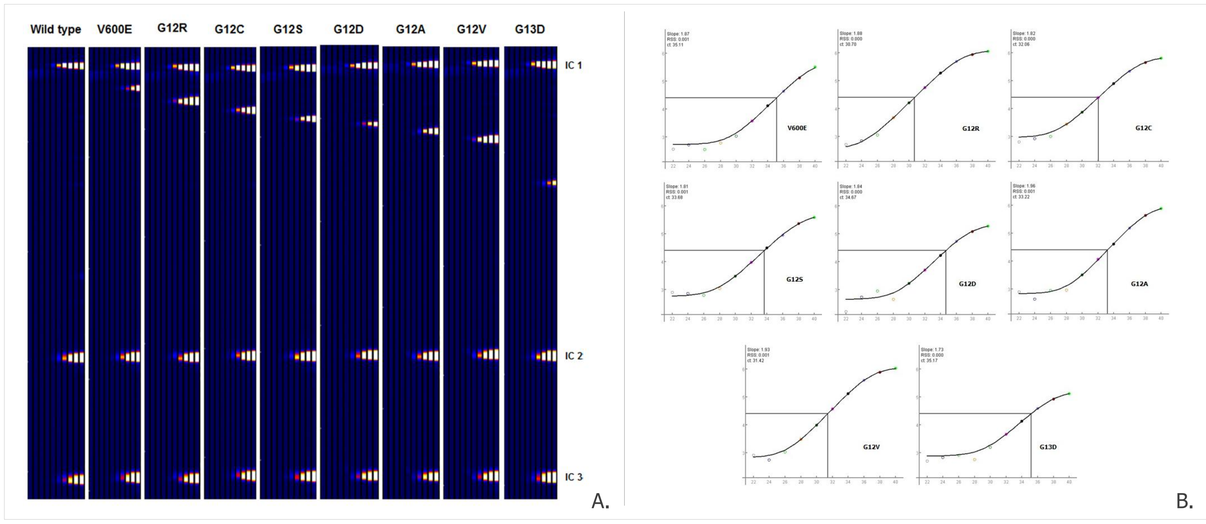

Figure 4. A graphical representation of the multiplex BCR/ABL Translocation panel (A) and amplification curve (B) visualized using the ICE*Plex* System for the eight mutations seen this sample set.

CONCLUSIONS

- Using an automated, highly multiplexed qPCR platform, the ICE*Plex* system, to identify known transcripts of the BCR-ABL fusion gene allowed us to identify translocations detected using the Cepheid assay.
- The multiplexed assay also identified patients harboring a transcript not detected using commercially available assays.
- With further investigation into the extraction and specimen processing techniques, this method will allow the reliable detection and quantification of known BCR-ABL transcripts in patients with leukemia.