Development of a BCR-ABL RT-PCR assay using the ICEPlex Platform.

Joel A. Lefferts[1], Mary C. Schwab[1], Soya S. Sam[1], Jork Nolling[2], Kyle Hart[2], Vess Diankov[2], Amy Leblang[2], Lilly Kong[2], and Gregory J. Tsongalis[1].

[1]Department of Pathology, Dartmouth-Hitchcock Medical Center, and Norris Cotton Cancer Center, Lebanon, NH, Geisel School of Medicine at Dartmouth, Hanover, NH
and [2] PrimeraDx, Inc., Mansfield, MA

ABSTRACT

Background: *BCR-ABL* fusion gene transcripts result from a translocation involving chromosomes 9 and 22 and are present in most cases of chronic myelogenous leukemia (CML) and some cases of acute lymphoblastic leukemia (ALL). *BCR-ABL* testing is performed by a variety of cytogenetic and molecular methods for diagnostic, prognostic and therapeutic monitoring purposes. Cytogenetic methods are able to detect (but not discriminate between) the various fusions but are not as analytically sensitive as molecular methods which normally detect only the most common transcripts. Therefore, clinical laboratories offer a variety of BCR-ABL tests and algorithms to accommodate all clinical scenarios requiring *BCR-ABL* analysis. The ICEPlex platform is capable of performing quantitative RT-PCR with relatively high multiplexing capabilities and is used in this study to develop a multiplexed *BCR-ABL* assay that could eliminate the need for multiple clinical *BCR-ABL* tests. As an initial step in the development of such an assay, we evaluate the qualitative performance of a multiplexed *BCR-ABL* assay on the ICEPlex platform.
Methods: A multiplexed RT-PCR assay was designed to detect nine *BCR*-ABL fusion transcripts (e13a2, e13a3, e19a2, e6a2, e8a2, e14a2, e14a3, e1a2, and e1a3) along with an *ABL* transcript. The primers for each transcript were designed to yield amplicons of different lengths to allow for the identification of transcripts by capillary electrophoresis. RNA samples were extracted from 18 whole blood samples and one bone marrow sample that were submitted for clinical testing. The 19 RNA samples were tested using the multiplexed *BCR-ABL* ICEPlex assay and the results were compared qualitatively to the GeneXpert assay (using a cut-off of 0.01% *BCR-ABL*:*ABL*).
Results: Fourteen samples were negative for BCR-ABL transcript by both assays and three samples were BCR-ABL-positive by both assays. Of the two remaining samples, one was positive by the GeneXpert assay only and one was positive by the ICEPlex assay only. The discrepant sample positive by the ICEPlex assay contained a transcript (e1a2) that the GeneXpert assay is not designed to detect. Additionally, plasmid templates representing all transcripts were amplified and produced amplicons of the predicted sizes.
Conclusions: We developed and evaluated a novel *BCR-ABL* assay capable of detecting the four most common fusion transcripts and five rare transcripts. In an initial qualitative evaluation of this assay using clinical specimens, its increased multiplexing allowed for detection of a *BCR-ABL* transcript that otherwise could have been missed. The ICEPlex assay failed to detect *BCR-ABL* transcript in one sample but it is unclear whether the sensitivity of the assay needs to be improved or if this discrepant result can be attributed to a delay of 1-2 days in processing the specimens for the ICEPlex assay. Additional studies will evaluate the quantitative performance of this assay.

INTRODUCTION

- BCR-ABL testing is commonly performed at diagnosis and to monitor patients with chronic myelogenous leukemia (CML) and a subset of patients with acute lymphoblastic leukemia (ALL).
- BCR-ABL testing is performed by a variety of cytogenetic and molecular methods for diagnostic, prognostic and therapeutic monitoring purposes.
- Quantitative RT-PCR methods used in clinical molecular laboratories are more sensitive than cytogenetic tests but are limited in the number of fusion transcripts that can be detected.

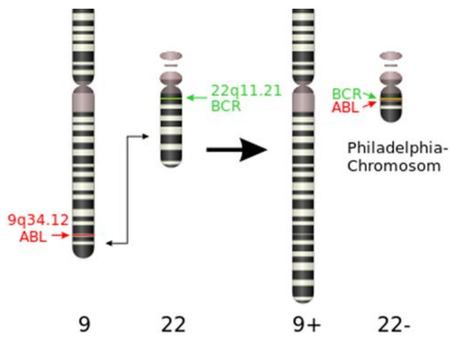

Figure 1. BCR-ABL translocation between chromosomes 9 and 22.

- The ICEPlex platform is capable of performing quantitative RT-PCR with relatively high multiplexing capabilities and is used in this study to develop a multiplexed BCR-ABL assay that could eliminate the need for multiple clinical BCR-ABL tests.
- As an initial step in the development of such an assay, we evaluate the qualitative performance of a multiplexed BCR-ABL assay on the ICEPlex platform.

METHOD

- A multiplexed RT-PCR assay was designed to detect nine BCR-ABL fusion transcripts (e13a2, e13a3, e19a2, e6a2, e8a2, e14a2, e14a3, e1a2, and e1a3) along with an ABL transcript (Figure 2).
- The primers for each transcript were designed to yield amplicons of different lengths to allow for the identification of transcripts by capillary electrophoresis.
- Samples: RNA extracted from 18 whole blood samples and one bone marrow sample.
- The 19 RNA samples were tested using the multiplexed BCR-ABL ICEPlex assay and the results were compared qualitatively to the GeneXpert (Cepheid) assay (cut-off of 0.01% BCR-ABL:ABL).

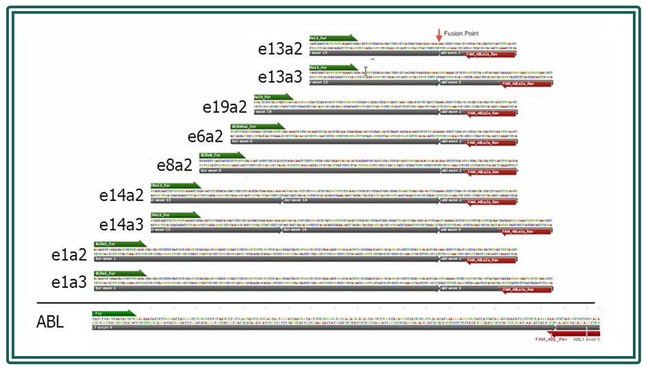

Figure 2. Nine BCR-ABL fusion transcripts and ABL transcript regions targeted by the ICEPLEX quantitative RT-PCR assay.

RESULTS

- Fourteen samples were negative for BCR-ABL transcript by both assays and three samples were BCR-ABL-positive by both assays (Figures 3 and 4).
- Of the two remaining samples, one was positive by the GeneXpert assay only and one was positive by the ICEPlex assay only.
- The discrepant sample positive by the ICEPlex assay contained a transcript (e1a2) that the GeneXpert assay is not designed to detect.*
- Plasmid templates representing all transcripts were amplified and produced amplicons of the predicted sizes.

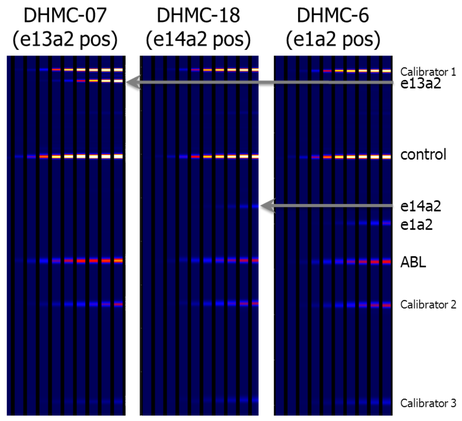

Figure 3. Depictions of real-time RT-PCR capillary electrophoresis data from three BCR-ABL positive samples.

		PrimeraDx BCR-ABL Assay	
		Positive	Negative
Cepheid BCR-ABL GeneXpert	Positive	3	1**
	Negative	1*	14

Figure 4. Comparison of GeneXpert and ICEPLEX BCR-ABL assays.

CONCLUSION

- We developed and evaluated a novel BCR-ABL assay capable of detecting the four most common fusion transcripts and five rare transcripts.
- In an initial qualitative evaluation of this assay using clinical specimens, its increased multiplexing allowed for detection of a BCR-ABL transcript missed by current methods.
- The ICEPlex assay failed to detect one low-level BCR-ABL transcript but this may be attributed to a delay of 1-2 days in processing the specimens for the ICEPlex assay.**
- Additional studies will evaluate the quantitative performance of this assay.