Performance Evaluation of a Novel, Rapid, Multiplexed, One-Step RT-PCR Assay for Simultaneous Detection of Common Leukemia-Associated Translocations

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INTRODUCTION

- Rapid and effective determination of the common translocations associated with acute myeloid and lymphoid leukemias is critical in order to guide treatment.
- This is especially true for acute promyelocytic leukemia (APL) where specific treatment can be initiated upon confirmation of the PML-RARA translocation.
- EntroGen Inc. has recently launched a multiplex RT-PCR assay for detection of the most common translocations seen in acute myeloid (AML) and B-lymphoid (B-ALL) leukemias.
- This assay detects 11 variants in 6 common translocations: AML1-ETO, CBFB-MYH11, MLL-AF4, TEL-AML1, E2A-PBX1, and PML-RARA.
- This assay also includes probes for the most common BCR-ABL1 translocations: b2a2, b3a2 and e1a2.

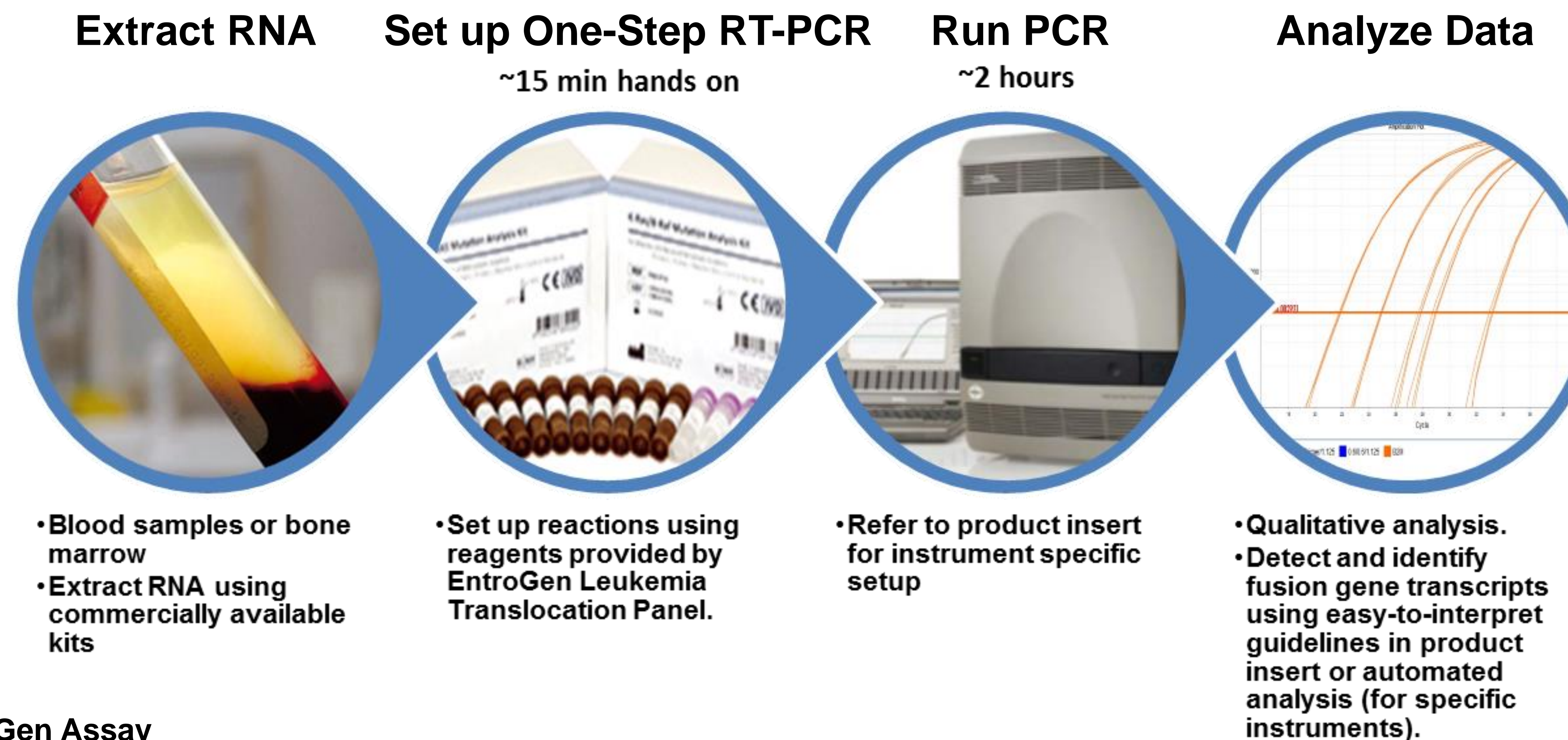
Table 1: Targets Evaluated by EntroGen Assay

DISEASE	TRANSLOCATIONS	FUSION TRANSCRIPTS
CML	t(9;22)	BCR/ABL1 (b2a2) / (b3a2)
ALL	t(9;22)	BCR/ABL1 (e1a2)
	t(1;19)	E2A/PBX1 (e13/e2)
	t(12;21)	TEL/AML1 (e5/e2)
	t(4;11)	MLL/AF4 (e9/e5) (e10/e4)
APL	t(15;17)	PML/RARα (bcr1, bcr2, bcr3)
AML	Inv 16	CBFB/MYH11 (A / D type)
	t(8;21)	AML1/ETO (e5/e12)

OBJECTIVE

❖ Objective of this study was to test the performance characteristics of this assay in a mid-sized clinical reference lab.

Figure 1: Entrogen Workflow



MATERIALS AND METHODS

- Total RNA extracted from blood (N=17) or bone marrow (N=11) in 28 patient samples (AML=15, B-ALL=5, CML=6, APL=2) and 9 synthetic fusion gene controls were tested.
- Total RNA input per reaction varied from 254-640ng (EntroGen recommendations – 200-1000ng/reaction).
- 9 synthetic fusion gene controls of known allelic frequency were diluted with HL60-derived total RNA for limit of detection studies, up to 1.1%.
- This assay comprises of 2 wells, each multiplexing 4 reactions, with *ABL1* serving as an amplification control.
- Positive samples from each well were selected and tested in ten separate reactions to evaluate well to well variability of both the control and target probes.
- Run to run variability was also evaluated by selecting a positive and negative sample to be tested on separate dates and validation runs.
- The results were compared with the Asuragen multiplex assay (discontinued by the manufacturer).

RESULTS

- There was 100% correlation between the two assays with 16 of the 28 samples showing a positive result (*AML1-ETO=3, CBFB-MYH11=3, MLL-AF4=1, TEL-AML1=2, E2A-PBX1=1, PML-RARA=2, BCR-ABL b3a2=3, BCR-ABLe1a2=1).*
- All 9 synthetic controls were detectable at 1.1% allelic frequency. All samples showed 100% correlation in terms of reproducibility and run to run variability.
- There was minimal variation of cycle threshold (CT) between runs with optimum amplification achieved at inputs around 500ng per reaction.
- The total run time for this assay was 65 minutes with average hands-on time of 15 minutes (excluding RNA extraction).

CONCLUSIONS

- The multiplexed RT-PCR assay is a rapid and cost-effective method compared to FISH panels to screen for the most common translocations seen in acute leukemias. Being a one-step RT-PCR format, it reduces hands-on time and has capability to provide fast turn-around time especially in cases suspicious for PML-RARA translocations. It also provides results for BCR-ABL1 fusions with specific breakpoint information thereby helping in triaging for specific quantitative assay(s).