Analytical Utility of a One-Step cDNA Synthesis and Real-Time PCR Assay for the Rapid Detection of Common Fusion Genes in Acute Leukemia

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Introduction

- Chromosomal structural rearrangement resulting in gene fusion is a common genetic mechanism driving hematologic malignancy.
- Several recurrent fusion genes are pathognomonic for acute leukemia and are recognized by current hematologic malignancy classification systems[1,2,3].
- Some fusion genes are associated with favorable (ie. *RUNX1::RUNX1T1, CBFB::MYH11, ETV6::RUNX1*) or unfavorable risk stratification categories in leukemia patients[4] .
- Rapid detection of some fusion genes can guide therapy management and reduce the disease-associated sequelae (ie. *PML::RARA* and treatment of acute promyelocytic leukemia (APL) with all-trans retinoic acid (ATRA) and arsenic trioxide (ATO) to reduce risk of coagulopathy-associated complications[5]).
- Practice guidelines recommend screening for *PML::RARA, CBFB::MYH11, RUNX1::RUNX1T1, KMT2A* rearrangements and *BCR::ABL1* fusion genes at diagnosis for a patient with acute myeloid leukemia[4].
- ***Objective: To evaluate the analytical performance of a one-step cDNA synthesis and real-time PCR assay to augment rapid detection of relevant fusion genes in diagnostic acute leukemia samples***

Methods

- A one-step, multiplex real time PCR (RT-PCR) kit, the Leukemia Translocation Panel for Real-Time PCR (LEUKMP kit versions 1 & 2, Entrogen Inc), was used to detect 5 fusion genes in validation and patient cohorts (refer to Figure 1)
- Detected fusion genes were verified by an independent RT-PCR[6] and by karyotype.

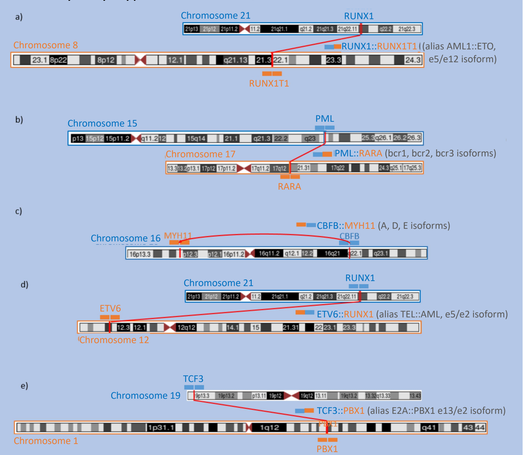


FIGURE 1: Fusion Genes Examined Using the Entrogen Leukemia Translocation Panel for Real-Time PCR. Chromosomal location of genes involved in the fusions are shown for the following fusion genes: a) RUNX1::RUNX1T1 , b) PML::RARA, c) CBFB::MYH11, d)ETV6::RUNX1 and e)TCF3::PBX1.

Results

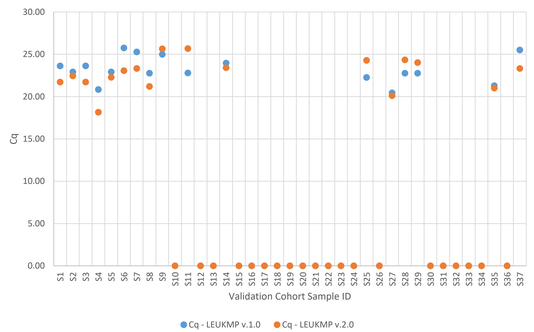


FIGURE 2: Assessment of Concordance Between Entrogen LEUKMP versions 1.0 and 2.0 for Fusion Gene Detection. Fusion gene detection using kit versions 1.0 and 2.0 were compared using 37 samples. Results from kit version 2.0 with a Cq = 0 (in orange) are overlapping results from kit version 1.0 with Cq=0 (in blue) for the same sample ID. Full concordance between kit versions 1.0 and 2.0 was noted. Mean ΔCq values for samples tested with both kits was 0.69 (range 0 to 2.88). Assay precision was noted with 100% reproducibility observed for samples re-run in the same test batch (ΔCq<1) and between test batches (ΔCq<2).

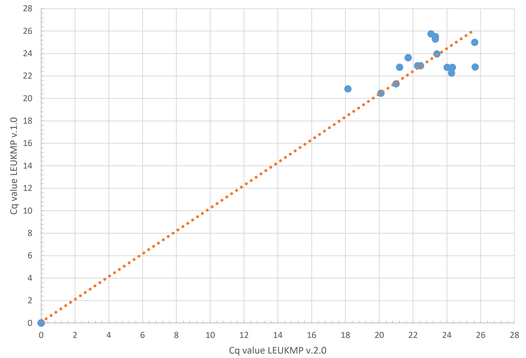
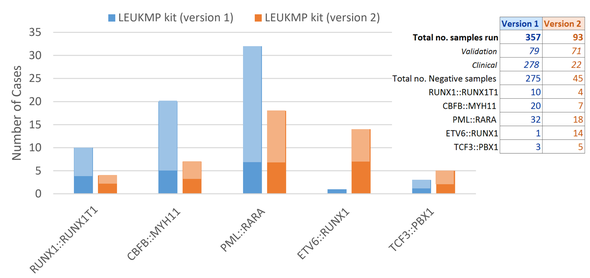


FIGURE 3: Comparison of Cq values for Samples Run on Entrogen LEUKMP versions 1.0 and 2.0. Mean ΔCq values for samples tested with both kits was 0.69 (range 0 to 2.88).



	Version 1	Version 2
Total no. samples run	**357**	**93**
Validation	*79*	*71*
Clinical	*278*	*22*
Total no. Negative samples	275	45
RUNX1::RUNX1T1	10	4
CBFB::MYH11	20	7
PML::RARA	32	18
ETV6::RUNX1	1	14
TCF3::PBX1	3	5

FIGURE 4: Distribution of Fusion Genes Detected. Fusion genes detected in cases and validations are shown. Positive cases in the validation cohort are represented in the dark blue and orange, while positive cases from consecutive leukemia cases are shown in light blue and orange. The breakdown of the total number of samples tested in the validation cohort is also provided.

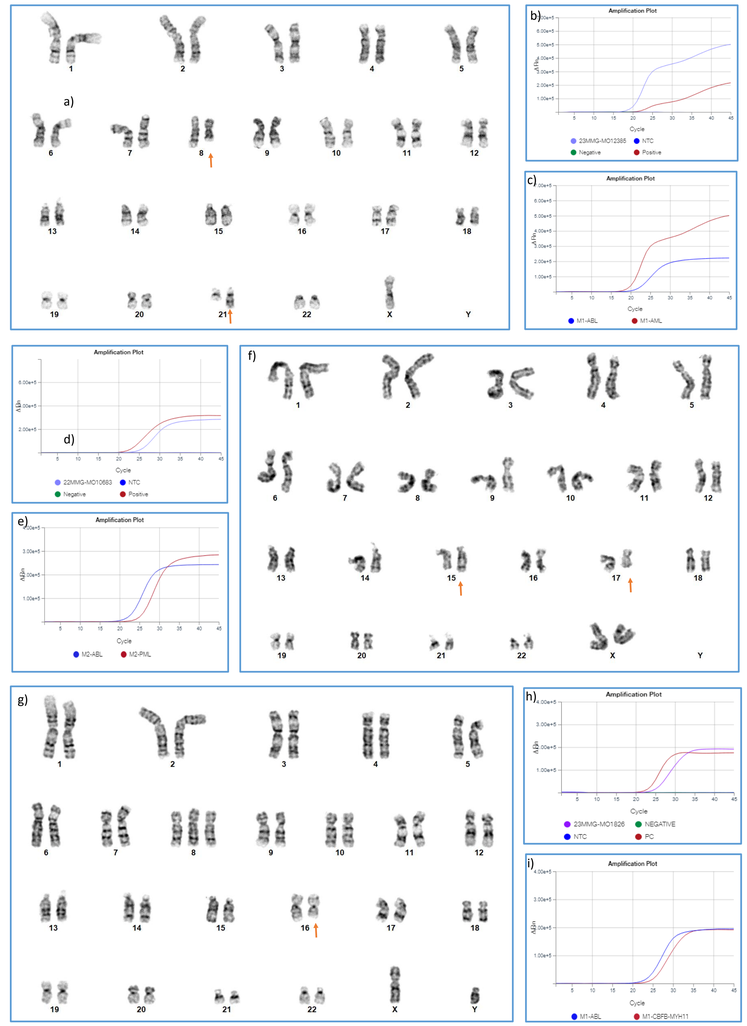
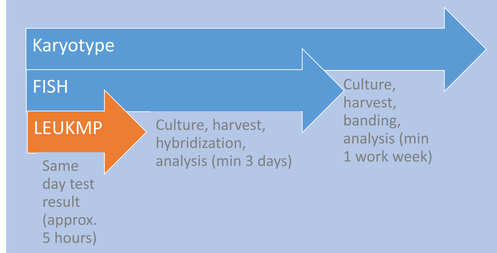


FIGURE 5: Comparison of Real-Time PCR and Karyotype Test Results. a) Karyotype results for Case A. The arrows indicate a t(8;21)(q22;q22) chromosome rearrangement. **b)** RUNX1::RUNX1T1 RT-PCR amplification plots for Case A (light blue), a negative control (green), no template control (dark blue) and a positive control (red). **c)** Case A RT-PCR result for RUNX1::RUNX1T1 and ABL1 (amplification control). **d)** PML::RARA RT-PCR amplification plots for Case B (light blue), a negative control (green), no template control (dark blue) and a positive control (red). **e)** Case B RT-PCR result for PML::RARA and ABL1. **f)** Karyotype results for Case B. The arrows indicate a t(15;17)(q24;q21) chromosome rearrangement. **g)** Karyotype result with an inv(16)(p13.1q22) indicated by the arrow. **h)** CBFB::MYH11 RT-PCR amplification plots for Case C (purple), a negative control (green), no template control (dark blue) and a positive control (red). **i)** Case C RT-PCR result for CBFB::MYH11 and ABL1. Full concordance of the LEUKMP assay with cytogenetic karyotype findings was observed in 300 consecutive samples from newly diagnosed acute myeloid leukemia patients (300/300 samples; 100% concordance).

Fusion Gene Testing Workflow



- Following stabilization and extraction of RNA, the assay could be performed and analyzed within a few hours, thus enabling same-day turnaround of test results.
- The LEUKMP panel has been incorporated into the laboratory leukemia testing workflow to supplement other cytogenetic testing.

Conclusion

The EntroGen Leukemia Translocation Panel enables accurate detection of a panel of five fusion genes common in acute leukemia. Evaluation of analytic performance criteria indicates that this assay can facilitate acute leukemia investigations through accurate, same-day detection of targeted fusion gene transcripts in RNA from bone marrow and peripheral blood.

References

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