Next-Generation Sequencing for Detection of Clonal *TRG* Gene Rearrangements Shows Improved Specificity and Positive Predictive Value Compared to Fragment Analysis Using BIOMED-2 Primers and Capillary Electrophoresis

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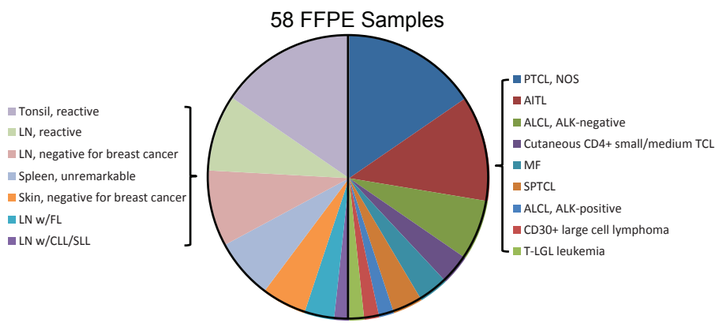
Introduction

During T cell development, somatic rearrangements of T cell receptor gamma (*TRG*) genes generate unique V-J rearrangements within each cell. Over-represented *TRG* rearrangements can be identified in the majority of T cell and some B cell malignancies, but are generally not seen in benign reactive processes. PCR-based capillary electrophoresis (PCR-CE) assays are the current gold standard for detecting clonal rearrangements. In this study, we evaluated the Invivoscribe® LymphoTrack® *TRG* clonality assay using the Illumina® MiSeq® to compare the performance of Targeted Next-Generation Sequencing (T-NGS) to the gold standard PCR-CE assay. We also sought to compare the performance of the T-NGS assay in two separate laboratories to assess inter-laboratory reproducibility.

Methods

DNA was isolated from 58 FFPE samples that had previously been evaluated in the Pathology Department at Stanford University Medical Center and evaluated by PCR-CE for *TRG* gene rearrangement (29 T cell lymphoproliferative disorders, 26 reactive tissues, and 3 lymph nodes involved by B cell lymphoma). T-NGS was performed using the Invivoscribe LymphoTrack *TRG* – MiSeq assay according to the manufacturer's instructions at Stanford University Medical Center and LabPMM®, a subsidiary of Invivoscribe. T-NGS data was analyzed by an Invivoscribe-developed bioinformatics pipeline and results were interpreted using numerical criteria, blinded to results of PCR-CE and histopathologic diagnosis.



Results

Results for the 58 samples were evaluated for sensitivity, specificity, concordance, positive predictive value (PPV), and negative predictive value (NPV). T-NGS analysis was compared between Stanford and LabPMM and showed 91% concordance. 5 cases were discordant; however, 4 of 5 cases identified many of the same clonal sequences with slight differences in frequency.

Separately, histopathologic diagnosis was considered the reference and PCR-CE was compared to T-NGS. PCR-CE as compared to T-NGS showed similar sensitivity (86% vs. 79-89%), concordance (85% vs. 90-93%), and NPV (86% vs. 83-90%). In contrast, PCR-CE showed lower specificity (83% vs. 97-100%) and PPV (83% vs. 96-100%).

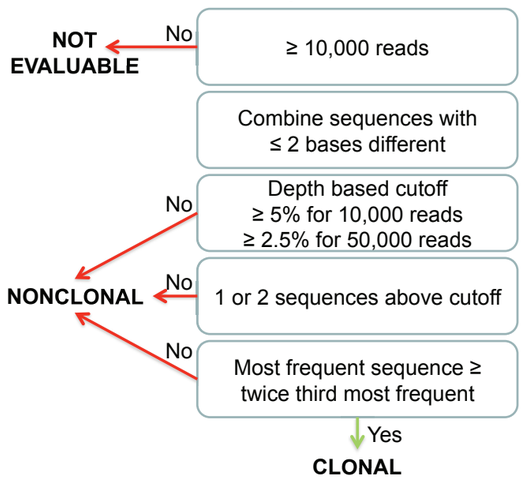
Results

LabPMM

Stanford	Positive	Negative
Positive	19	0
Negative	5	31

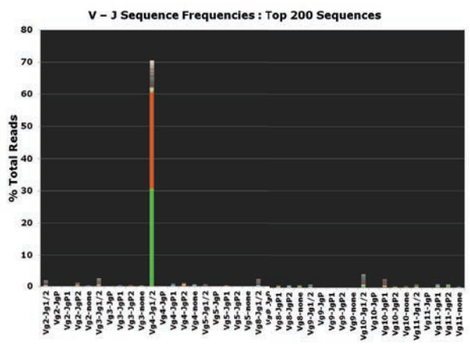
Concordance Rate = 91%

Interpretation Criteria

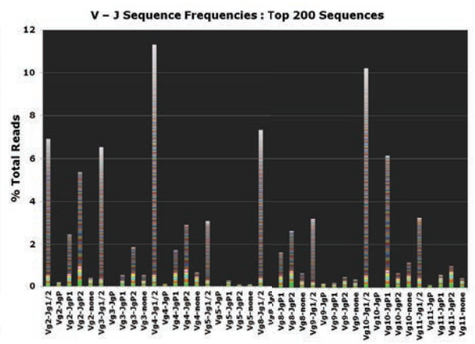


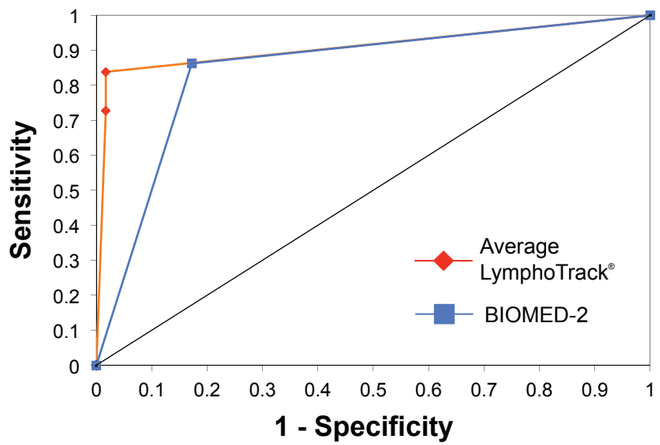
Test	Lab	Sensitivity	Specificity
LymphoTrack®	Stanford	79%	100%
	LabPMM	89%	97%
BIOMED-2		86%	83%

Sample Positive Case



Sample Negative Case





Conclusions

The Invivoscribe® LymphoTrack® *TRG* assay shows good inter-laboratory reproducibility and similar sensitivity, concordance, and NPV to PCR-CE when using histopathologic diagnosis as a reference. In contrast, T-NGS shows a higher specificity and PPV than PCR-CE. In addition, T-NGS offers the potential to follow specific clonal sequences for monitoring of minimal residual disease in T cell malignancies. Given this potential benefit and the superior assay performance demonstrated by our data, T-NGS represents an exciting advance in the diagnosis of clonal lymphoproliferative disorders.

Disclosures

MK, JP, YH, TS, and KH are employees of Invivoscribe®.

ME has received an honorarium and travel expenses from Invivoscribe®.

LymphoTrack® *TRG* Assays are for research use only (RUO). Not for use in diagnostic procedures.