Clonality Detection Using Next-Generation Sequencing and Capillary Electrophoresis Methods in Suspect Lymphoproliferative Samples

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Introduction

Assays used to detect clonal rearrangements within the immunoglobulin (Ig) and T-cell receptor (TCR) genes have long been used to assist in diagnosis of lymphoproliferative disease. Capillary electrophoresis (CE) based methods remain the gold standard in the majority of laboratories as they are cost effective high throughput assays. However, recently deep sequencing or next-generation sequencing (NGS)-based assays are gaining traction as they complement and supplement data from CE assays, providing both the prevalence of unique clonal Ig/TCR rearrangements as well as identifying the tumor-specific V-J DNA sequences necessary to track clonal sequence in highly sensitive residual disease testing. Here we report test results from 59 clinical samples using LymphoTrack® assays with accompanying bioinformatics using the MiSeq® and PGM NGS platforms, as well IdentiClone® assays on the ThermoFisher® 3500 capillary platform.

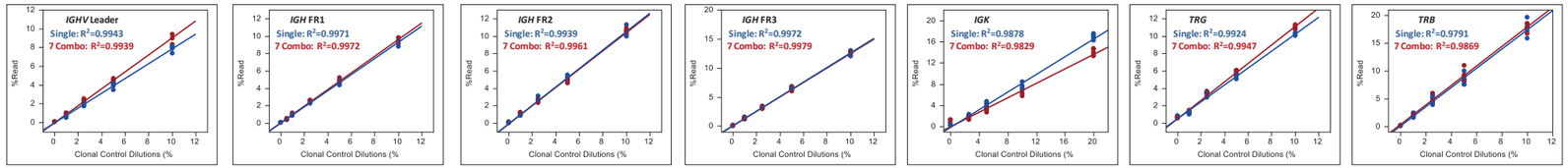
Materials and Methods

- LymphoTrack® NGS-based Assays for the MiSeq® (24 indices) and Ion PGM™ (12 indices), and IdentiClone® CE-based Assays were manufactured under cGMP standards and QC tested under a QSR-compliant regulatory system prior to use.
- Limit of detection (LoD), linearity, precision and reproducibility (P/R) of the LymphoTrack® Assays – MiSeq® and PGM™ were validated using clonal control DNA diluted in wild-type polyclonal (tonsil) DNA. Only data from MiSeq® are presented here.
- DNA from a variety of samples (peripheral blood, bone marrow aspirates, and formalin-fixed paraffin-embedded (FFPE) were extracted using common extraction methods and tested by the LymphoTrack® Assays and IdentiClone Assays.
- Single step PCR amplification of 50 ng DNA input was followed by pooling of equimolar amounts of purified amplicons. These were then loaded on the sequencing machine. NGS libraries generated from each target locus were either sequenced alone for in combination with other targets.
- LymphoTrack® Software – MiSeq®, or LymphoTrack® Software - PGM™ analyzed FASTQ data from the MiSeq® and the Ion PGM™, respectively.
- When comparing testing results, only samples that met the specimen and data acceptance criteria for both methods were evaluated.
- All statistical analyses were performed in JMP®.

Conclusions

This study demonstrated that the comprehensive NGS LymphoTrack® Assays can be utilized for routine Ig/TCR clonality detection. Furthermore, the NGS assays can identify clonal V-J rearrangements and provide the clonal DNA sequences of the tumor-specific clonotypes required to perform follow up MRD testing in order to detect and track residual disease. Combining Ig/TCR assays within one NGS run can improve the overall clonality detection rate, reduce turnaround times in busy labs, and reduce the cost of NGS-based testing.

Results: LoD, Linearity, Precision and Reproducibility for LymphoTrack® Assays - MiSeq®



Clonal Control Dilutions (%)	N	*IGHV* Leader			*IGH* FR1			*IGH* FR2			*IGH* FR3			*IGK*			*TRG*			*TRB*		
		Size (bp)	Mean % Reads	CV%	Size (bp)	Mean % Reads	CV%	Size (bp)	Mean % Reads	CV%	Size (bp)	Mean % Reads	CV%	Size (bp)	Mean % Reads	CV%	Size (bp)	Mean % Reads	CV%	Size (bp)	Mean % Reads	CV%
10	32	490	7.17	12.6	295	5.08	17.5	243	15.74	9.9	104	12.99	6.2	222	7.11	22.8	147	8.66	10.4	198	17.63	16.7
5	48	490	3.73	14.2	295	2.58	19.4	243	7.72	8.2	104	7.04	8.4	222	3.46	21.9	147	4.59	12.0	198	9.35	18.4
2.5	48	490	2.10	12.9	295	1.28	14.1	243	3.93	9.2	104	3.50	8.0	222	1.81	21.1	147	2.39	11.7	198	5.25	19.6
1	48	490	0.70	12.9	295	0.50	29.3	243	1.52	12.5	104	1.43	16.1	n/a	n/a	n/a	147	0.94	13.8	198	2.04	20.0
0	16	varies	0.04	28.2	varies	0.04	34.8	varies	0.06	26.5	104	0.07	28.6	varies	0.68	35.3	varies	0.62	40.3	varies	0.17	46.8

Results: Clinical Study between LymphoTrack® - MiSeq® , LymphoTrack® - Ion PGM™, and IdentiClone® Assays

	LymphoTrack® Assays - MiSeq®				LymphoTrack® Assays - PGM™		IdentiClone® Assays			
	IGH FR1/2/3	*IGK*	*TRG*	*TRB*	*IGH* FR1/2/3	*IGK*	*IGH* Tube A/B/C	*IGK* Tube A/B	*TRG* 2.0	*TRB* Tube A/B/C
Clonal (%)	25/59 (42%)	20/59 (34%)	16/60 (27%)	19/60 (32%)	15/40 (38%)	15/55 (27%)	26/59 (29%)	22/59 (37%)	16/60 (27%)	24/60 (40%)
Non-Clonal (%)	32/59 (54%)	36/59 (61%)	43/60 (72%)	41/60 (68%)	22/40 (55%)	35/55 (64%)	20/59 (34%)	35/59 (59%)	35/60 (58%)	20/60 (33%)

Assay	No. of Samples Met Criteria for Both Methods	No. of Samples with Indicated Result				Concordance (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
		*CE+/NGS+	CE+/NGS-	CE-/NGS+	CE-/NGS-					
MiSeq *IGH* FR1/2/3	44	24	1	1	18	95.5	96.0	94.7	96.0	94.7
MiSeq *IGK*	55	19	1	0	35	98.2	95.0	100	100	97.2
MiSeq *TRG*	51	14	2	1	34	94.1	87.5	97.1	93.3	94.4
MiSeq *TRB*	44	18	6	0	20	86.4	75.0	100	100	76.9
PGM *IGH* FR1/2/3	41	22	1	0	18	97.6	95.7	100	100	94.7
PGM *IGK*	49	15	0	0	34	100	100	100	100	100

**CE, IdentiClone® Assays, capillary electrophoresis; NGS, LymphoTrack® Assays - MiSeq®; PPV, positive predictive value; NPV, negative predictive value.*