Performance Evaluation of LymphoTrack® Clonality Assays on Ion PGM™ and Ion S5™ Platforms

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Abstract

Introduction: Detection of clonal rearrangements within the immunoglobulin (Ig) and T-cell receptor (TCR) genes in clinical specimens is used to assist in diagnosis of lymphoproliferative disease (LPD). Next-generation sequencing (NGS)-based clonality assays represent a major advance compared to the traditional capillary electrophoresis (CE)-based assays as the NGS assays can identify specific clonal sequences that can be tracked in follow-up testing.
The LymphoTrack® Dx Assays can be utilized for routine Ig/TCR clonality detection in LPD diagnosis, where multiple patient samples and all LymphoTrack Dx Assays can be multiplexed into a single sequencing run, reducing per sample testing costs without loss of sensitivity. Here we compare the performance of LymphoTrack® Dx (*IGH* (FR1/2/3), *IGK*, and *TRG*) CE-IVD assays (Ion PGM™) to results when running these tests on the Ion S5™.

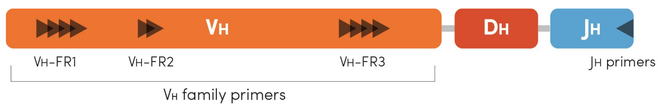
Methods: LymphoTrack® Dx Assays targeting the *IGH*, *IGK*, and *TRG* loci have been developed for the Ion systems. Consensus primers targeting V and J gene segments include specific adapter sequences and individual barcodes, thus the PCR products from multiple independent samples and multiple clonality assays can be combined and sequenced together in a single NGS run. Individual master mixes were manufactured with 12 indices. Single step PCR amplification of 50 ng DNA input was followed by pooling equimolar amounts of purified amplicons. Template preparations were performed with either the Ion OneTouch™ 2 system for Ion PGM™ or the Ion Chef™ system for Ion S5™. Fastq data from both Ion PGM™ and Ion S5™ were analyzed using LymphoTrack® Dx Software, to generate frequency distributions, determine V-J usage, identify specific sequences for top sequencing reads, and determine the somatic hypermutation rate of *IGH* FR1 amplicons. Limit of detection (LoD), limit of blank (LoB) and linearity were evaluated by testing serial dilutions of contrived samples with V-J rearrangements in tonsil. Clinical samples were used to assess the clinical performance.

Results: All LymphoTrack® Dx assays have demonstrated LoD to detect 5.0% clonality and excellent linearity for both Ion PGM™andIon S5™ systems. Great concordance between Ion PGM™ and Ion S5™ systems was demonstrated when testing clonality for all targets from subject samples.

Conclusions: This study has demonstrated that the performance of CE-IVD LymphoTrack® Dx Assays on Ion PGM™ and Ion S5™ systems is comparable. The LymphoTrack Dx Assays are undergoing full validation for use with the Ion S5™ system and will be available soon.

Materials and Methods

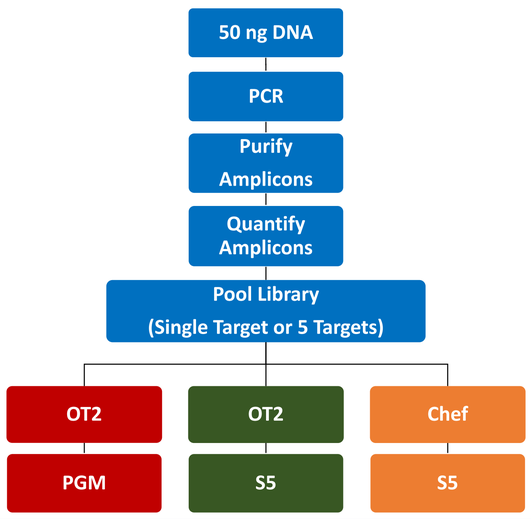
- *IGH* loci



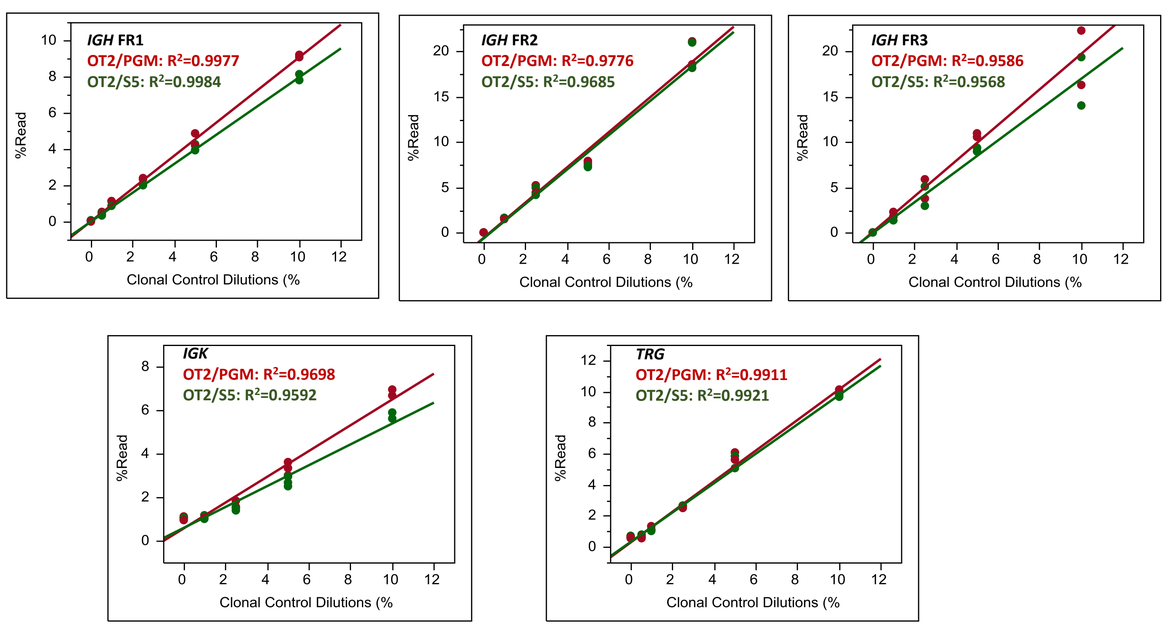
- *IGK* loci

- *TRG* loci

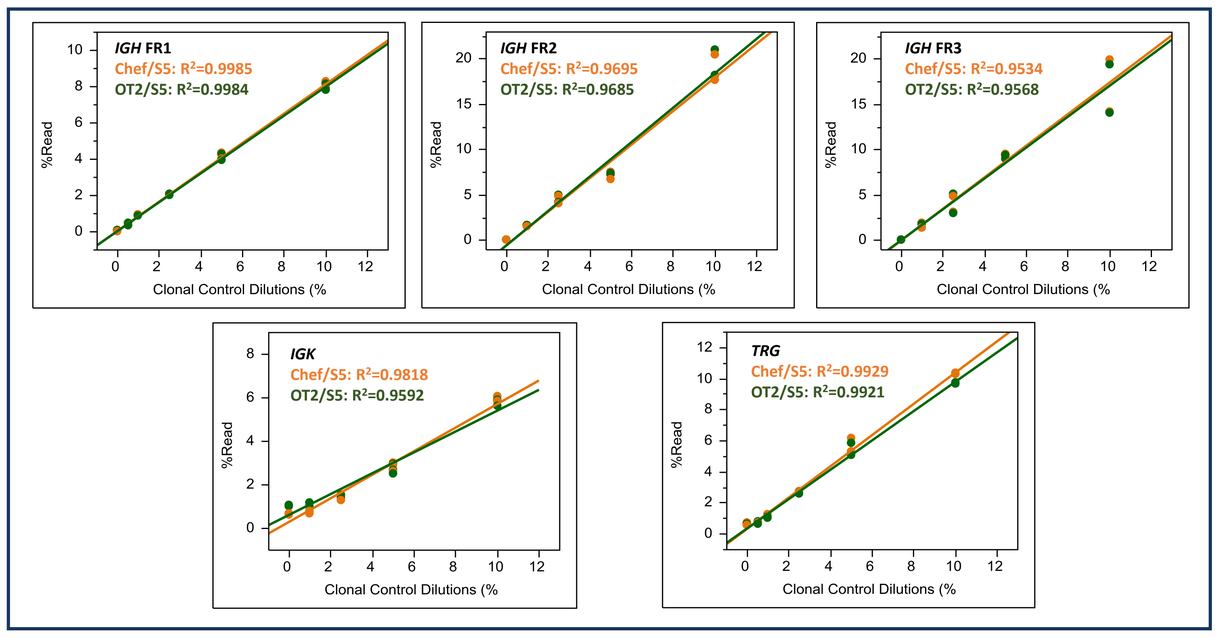
- LymphoTrack® Dx Assays for the Ion PGM™ were manufactured under cGMP standards per an ISO 13485 certified QMS. Five assays are available to target *IGH* FR1/2/3, *IGK*, and *TRG* loci, respectively. Each target consists of a one step PCR Master Mixes with 12 different indices to allow multiple samples and targets to be run on the same chip.
- Complimentary bioinformatics software package, LymphoTrack® Dx Software, was developed and validated under ISO13485 design control.
- Limit of detection (LoD), Limit of blank (LoB) and linearity were validated using clonal control DNA diluted in wild-type polyclonal (tonsil) DNA.
- 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® Dx Assays.
- The Ion PGM™ Hi-Q™ View OT2 Kit and Sequencing Kit were used for PGM runs.
- The Ion 520™ & Ion 530™ Kit-OT2 were used for OT2/S5 runs.
- The Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef were used for Chef/S5 runs.
- Workflow:



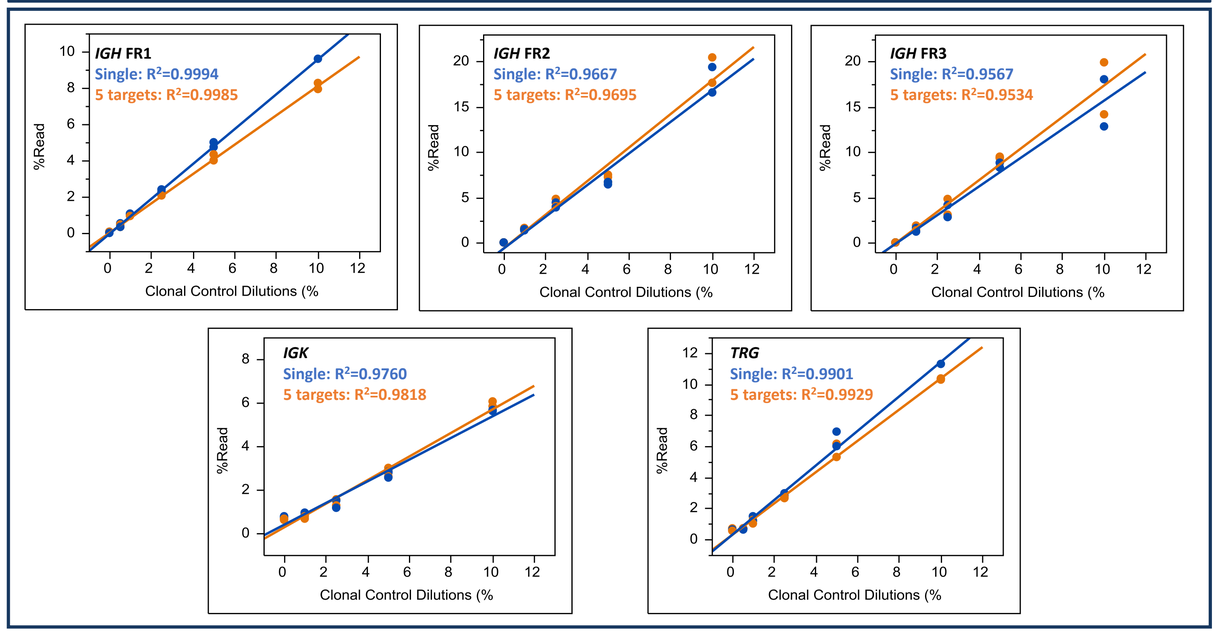
Results: OT2/PGM and OT2/S5 Comparison



Results: Chef/S5 and OT2/S5 Comparison



Results: Single and 5 Targets on Chef/S5



Results: OT2/PGM and Chef/S5 for Clinical Samples*

PCR MM Id	Sample Type	Total reads		Length		Vg		Jg		Top %reads	
		S5	PGM	S5	PGM	S5	PGM	S5	PGM	S5	PGM
id01	BM	125,836	65,573	135	135	Vg8	Vg8	Jg1/2	Jg1/2	22.22	22.95
id02	PB	128,593	67,483	145	145	Vg9	Vg9	JgP	JgP	0.97	0.82
id03	BM	121,253	61,727	153	144	Vg10	Vg10	JgP1	Jg1/2	0.42	1.06
id04	PB	158,328	78,668	152	152	Vg9	Vg9	JgP1	JgP1	13.44	12.81
id07	PB	157,917	78,141	142	142	Vg10	Vg10	Jg1/2	Jg1/2	9.83	10.18
id08	BM	145,657	70,777	138	142	Vg8	Vg3	Jg1/2	Jg1/2	0.92	1.03
id09	BM	142,184	71,685	151	151	Vg10	Vg10	JgP1	JgP1	3.16	3.01
id10	BM	179,240	86,093	161	161	Vg3	Vg3	JgP2	JgP2	4.35	4.69
id11	PB	149,402	76,159	149	149	Vg3	Vg3	JgP2	JgP2	6.84	6.97
id12	PB	124,543	64,658	135	135	Vg8	Vg8	Jg1/2	Jg1/2	2.58	2.73
id13	Pos control	191,660	92,662	147	147	Vg11	Vg11	Jg1/2	Jg1/2	7.09	7.17
id14	Neg control	181,054	88,382	147	144	Vg10	Vg10	JgP1	Jg1/2	0.42	0.37

**TRG samples as an example.*

Run Time and Cost Comparison

	OT2/PGM	OT2/S5	*Chef/S5
Hands on time	2 hr	1.1 hr	0.3 hr
Instrument time per run	16.5 hr	11.5 hr	17.5 hr
Reagents cost per sequencing run	$935	$1,202	$1,189
Reagents cost per sample (12 samples/run)	$78	$100	$99
Reagents cost per sample (12 samples x 5 targets/run)	$16	$20	$20

** Ion Chef can do 2 runs per setup.*

Conclusions

- Excellent correlation was observed when comparing the performance of LymphoTrack® Dx Assays on Ion PGM™ and Ion S5™ systems.
- The per sample testing costs can be significantly reduced and TATs improved by combining multiple samples from up to 5 LymphoTrack Dx PGM/S5 Assays into a single sequencing run.
- The provided bioinformatics software sorts the complex PGM- or S5-NGS data for easy analysis and visualization of individual samples.
- The Ion S5™ can produce 2x to 5x more reads with less hands-on time than the Ion PGM™.
- With the LymphoTrack® Dx Assays, the same reagents and workflow can be utilized for subject Ig/TCR clonality testing and for tracking of clonal populations. The LymphoTrack Dx Assays are undergoing full validation for use with the Ion S5™ system and will be available soon.

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