E847

DETECTION OF CLONALITY IN CLINICAL SPECIMENS FROM SUSPECTED B-CELL MALIGNANCIES USING COMPREHENSIVE *IGH* (FR1/2/3) LYMPHOTRACK® MiSEQ® AND PGM® ASSAYS

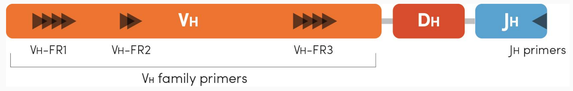
Ying Huang[1], Kasey Hutt[1], Jeff Panganiban[1], Austin Jacobsen[1], Nick Wong[1], Roshanak Bob[2], Stefano A. Pileri[3], Loris Bernard[3],Elvira Gerbino[3], Tim Stenzel[1] and Jeffrey E. Miller[1]

[1]Invivoscribe, San Diego, USA, [2] Institute for Pathodiagnostik Berlin, Germany and [3]Haematopathology Unit at European Institute of Oncology, Milan, Italy

Background

PCR-based capillary electrophoresis (PCR-CE) methods targeting immunoglobulin heavy chain (*IGH*) framework 1, 2, 3 (FR1, FR2, FR3), and joining regions (J) are historically the gold standard for clonality testing in suspected B-cell malignancies. Recently, next-generation sequencing (NGS) based approaches for immune receptor genes have been developed that improve sensitivity and identify the specific V-(D-)J DNA sequences required to track clones in follow-up testing. We developed comprehensive LymphoTrack® *IGH* (FR1, FR2, & FR3) Assays for both the Illumina® MiSeq® and ThermoFisher Scientific® Ion PGM™ platforms, which detect the vast majority of rearrangements in a single NGS run. In this pilot study, we compared the performance of both LymphoTrack® *IGH* MiSeq and PGM Assays to the IdentiClone® *IGH* PCR-CE assay by testing in over 50 anonymized, blinded clinical samples.

Materials and Methods



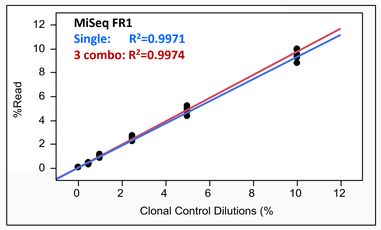
- The LymphoTrack® *IGH* FR1/2/3 Assays for the MiSeq® and Ion PGM™ were manufactured under cGMP standards and QC tested under a QSR-compliant regulatory system prior to use.
- Limit of detection (LoD), linearity, precision and reproducibility (P/R) were validated using clonal control DNA diluted in wild-type polyclonal (tonsil) DNA.
- DNA from a variety of samples (21 from peripheral blood, 1 from bone marrow aspirates, and 37 from FFPE were extracted using common extraction methods by collaborators. 59 samples were tested by all assays except that FR2 PGM tested additional 9 samples for total 68 samples.
- Libraries were prepared with amplicons generated by the LymphoTrack® *IGH* FR1/2/3 Assay optimized for each NGS platform.
- Libraries were either sequenced for each FR individually or for all FRs (*IGH* FR1/2/3) combined.
- LymphoTrack® Software - MiSeq® and 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®.

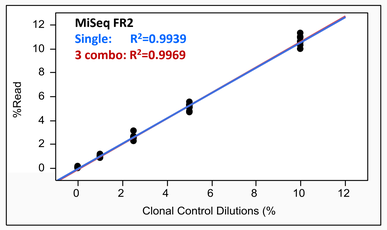
Conclusions

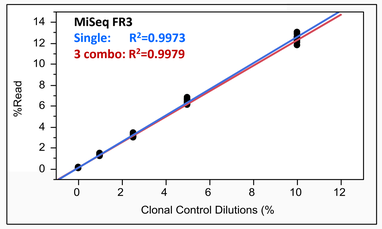
A comprehensive NGS-based LymphoTrack® *IGH* FR1/2/3 Assay was developed for both the Illumina® MiSeq® and Thermo Fisher Scientific® Ion PGM™ platforms. These assays identify clonal *IGH* V-J rearrangements and the specific clonal DNA sequences, critical for determining the SHM rate and tracking residual disease. Excellent concordance was demonstrated between these assays.

Results: LoD, Linearity, Precision and Reproducibility

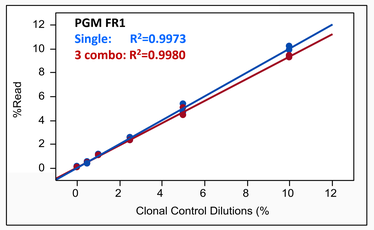
Clonal Control Dilutions (%)	N	MiSeq® *IGH* FR1			MiSeq® *IGH* FR2			MiSeq® *IGH* FR3		
		Size (bp)	Mean % Reads	CV%	Size (bp)	Mean % Reads	CV%	Size (bp)	Mean % Reads	CV%
10	32	295	5.08	17.5	243	15.74	9.9	104	12.99	6.2
5	48	295	2.58	19.4	243	7.72	8.2	104	7.04	8.4
2.5	48	295	1.28	14.1	243	3.93	9.2	104	3.50	8.0
1	48	295	0.50	29.3	243	1.52	12.5	104	1.43	16.1
0	16	varies	0.04	34.8	varies	0.06	26.5	104	0.07	28.6

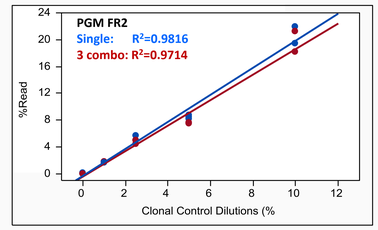


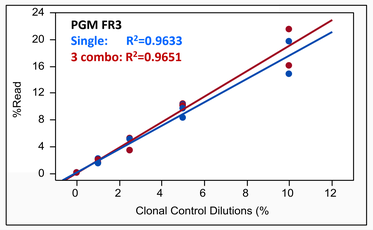




Clonal Control Dilutions (%)	N	Ion PGM™ *IGH* FR1			Ion PGM™ *IGH* FR2			Ion PGM™ *IGH* FR3		
		Size (bp)	Mean % Reads	CV%	Size (bp)	Mean % Reads	CV%	Size (bp)	Mean % Reads	CV%
10	32	295	10.86	4.7	243	19.81	6.9	104	17.98	19.6
5	32	295	5.20	6.7	243	8.80	13.0	104	10.70	9.3
1	16	295	0.97	12.4	243	1.91	14.2	104	2.14	13.1
0	16	varies	0.057	35.1	varies	0.05	45.9	varies	0.08	37.5







Results: Clinical Study between *IGH* MiSeq® and Ion PGM™ Assays

	MiSeq® *IGH* Assays				Ion PGM™ *IGH* Assays				IdentiClone *IGH* Assays			
	FR1	FR2	FR3	FR1/2/3	FR1	FR2	FR3	FR1/2/3	Tube A (FR1)	Tube B (FR2)	Tube C (FR3)	Tube A/B/C (FR1/2/3)
Clonal (%)	18/59 (31%)	16/59 (27%)	22/59 (37%)	25/59 (42%)	17/54 (32%)	18/68 (27%)	15/40 (38%)	15/40 (38%)	18/59 (31%)	21/59 (36%)	15/40 (38%)	26/59 (29%)
Non-Clonal (%)	37/59 (63%)	41/59 (70%)	36/59 (61%)	32/59 (54%)	32/54 (59%)	27/68 (40%)	24/40 (60%)	22/40 (55%)	21/59 (36%)	21/59 (36%)	24/40 (60%)	20/59 (34%)

		IdentiClone IGH Tube A/B/C	
		Clonal	Non-Clonal
MiSeq® FR1/2/3	Clonal	24	1
	Non-Clonal	1	18

		IdentiClone IGH Tube A/B/C	
		Clonal	Non-Clonal
PGM® FR1/2/3	Clonal	22	0
	Non-Clonal	1	18

		MiSeq FR1/2/3	
		Clonal	Non-Clonal
PGM® FR1/2/3	Clonal	22	0
	Non-Clonal	0	32

	MiSeq FR1/2/3 vs. IdentiClone Tube A/B/C	PGM FR1/2/3 vs. IdentiClone Tube A/B/C	PGM FR1/2/3 vs. MiSeq FR1/2/3
Concordance (%)	95.5	97.6	100
Sensitivity (%)	96.0	95.7	100
Specificity (%)	94.7	100	100
PPV (%)	96.0	100	100
NPV (%)	94.7	94.7	100

EHA, June 22–26 2017, Madrid, Spain