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Detecting Clonality in Mantle Cell Lymphoma (MCL) Subjects Using LymphoTrack® *IGH* FR1 Assays

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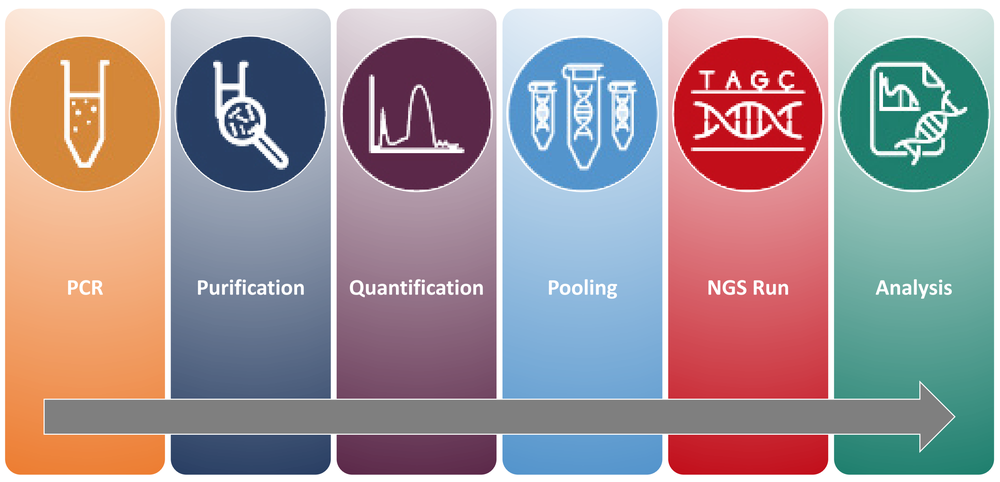
Introduction

Mantle Cell Lymphoma (MCL) is an aggressive B-cell disease and clonality based detection within the immunoglobulin heavy chain (*IGH*) rearrangement is one of the common methods to diagnose MCL. Historically, this has been achieved by PCR with detection by capillary electrophoresis or Sanger sequencing. Invivoscribe has developed *IGH* FR1 assays and software for both Illumina's MiSeq™ and NextSeq™ instruments. These one-step PCR-based assays allow sample multiplexing and simultaneous detection of clonality in subjects in a single instrument run. This pilot study presents a comparison of MCL clonality testing results from two laboratories. Invivoscribe's *IGH* FR1 assays were used to compare results using paired peripheral blood (PB) and bone marrow (BM) samples, generated on MiSeq™ and NextSeq™ instruments at Invivoscribe (IVS), and the Hospital Universitario de Salamanca (SAL) using Sanger sequencing or LymphoTrack® MiSeq sequencing.

Materials and Methods

Residual DNA from different specimen types (42 PB, 26 BM and 5 FFPE) were obtained from anonymized 48 MCL subjects that had been enrolled in a study approved by the Spanish Group of Lymphoma and Autologous Stem Cell Transplantation (clinical trial: NCT02682641, publication reference: 10.1200/JCO.21.02321). The Research Use Only (RUO) LymphoTrack® *IGH* FR1 Assay – MiSeq, and the CAP/CLIA B-cell MRD Assay on NextSeq amplify the genomic DNA using PCR primers that target the conserved V and J regions of the *IGH* genes. An illustration of the *IGH* gene is depicted below:

These PCR amplicons were then purified, quantified, and an equimolar library pool was created for MiSeq or NextSeq sequencing. The workflow for the NGS *IGH* FR1 Assays is depicted below:



Results from Invivoscribe were generated using the (RUO) LymphoTrack *IGH* FR1 – MiSeq and CAP/CLIA B-cell MRD Assay on NextSeq, while results from Salamanca lab were generated by Sanger sequencing or the CE-IVD LymphoTrack Dx *IGH* FR1 Assay – MiSeq.

Results: Comparison of Sample Types and Instruments

Sample Name	MiSeq				NextSeq	
	PB (n=23)		BM (n=25)		BM (n=25)	
	Result	V-J	Result	V-J	Result	V-J
1DX	Clonal	V3-J4	Clonal	V3-J4	Clonal	V3-J4
3DX	Clonal	V3-J4	Clonal	V3-J4	Clonal	V3-J4
4DX	Clonal	V1-J4	Clonal	V1-J4	Clonal	V1-J4
6DX	Clonal	V3-J2	Clonal	V3-J2	Clonal	V3-J2
7DX	Clonal	V4-J6	Clonal	V4-J6	Clonal	V4-J6
8DX	Clonal	V4-J3	Clonal	V4-J3	Clonal	V4-J3
9DX	Clonal	V3-J4	Clonal	V3-J4	Clonal	V3-J4
10DX	Clonal	V3-J3	Clonal	V3-J3	Clonal	V3-J3
11DX	N/A*	N/A*	Non-Clonal	V3-J4	Non-Clonal	V4-J6
12DX	Clonal	V3-J4	Clonal	V3-J4	Clonal	V3-J4
13DX	Clonal	V3-J4	Clonal	V3-J4	Clonal	V3-J4
15DX	N/A*	N/A*	Clonal	V1-J6	Clonal	V1-J6
16DX	Clonal	V3-J4	Clonal	V3-J4	Clonal	V3-J4
19DX	Clonal	V3-J3	Clonal	V3-J3	Clonal	V3-J3
23DX	Clonal	V3-J1	Clonal	V3-J1	Clonal	V3-J1
24DX	Clonal	V4-J4	Clonal	V4-J4	Clonal	V4-J4
30DX	Clonal	V5-J4	Clonal	V5-J4	Clonal	V5-J4
32DX	Clonal	V3-J4	Clonal	V3-J4	Clonal	V3-J4
33DX	Clonal	V4-J4	Clonal	V4-J4	Clonal	V4-J4
35DX	Clonal	V3-J4	Clonal	V3-J4	Clonal	V4-J6
36DX	Clonal	V4-J4	Clonal	V4-J4	Clonal	V4-J4
42DX	Clonal	V2-J3	Clonal	V2-J3	Clonal	V2-J3
44DX	Clonal	V1-J4	Clonal	V1-J4	Clonal	V1-J4
47DX	Clonal	V1-J5	Clonal	V1-J5	Clonal	V1-J5
48DX	Clonal	V3-J3	Clonal	V3-J3	Clonal	V3-J3

*PB samples were not available to test

Results: Comparison of Sample Type (PB and BM) Sequences

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PB_MiSeq    GCCTCTGGATTCCCCTTTAGTATCTATTGGATGAATTGGGTCCGCCAGGCTCCGGGGAGG   60
BM_MiSeq    GCCTCTGGATTCCCCTTTAGTATCTATTGGATGAATTGGGTCCGCCAGGCTCCGGGGAGG   60
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PB_MiSeq    GGGCTGGAGTGGGTGGCCAACATCAACCAAGATGGAAGTGAGAAATACTATATGGACTCT   120
BM_MiSeq    GGGCTGGAGTGGGTGGCCAACATCAACCAAGATGGAAGTGAGAAATACTATATGGACTCT   120
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PB_MiSeq    GTGAAGGGCCGATTCACCATCTCCAGAGACAACACCAAGAACTCACAGTATCTGGAAATG   180
BM_MiSeq    GTGAAGGGCCGATTCACCATCTCCAGAGACAACACCAAGAACTCACAGTATCTGGAAATG   180
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PB_MiSeq    GACAGCCTGAGAGCCGAAGACACGGCCGTATATTACTGTACGAGAACATCTGATAGTAGT   240
BM_MiSeq    GACAGCCTGAGAGCCGAAGACACGGCCGTATATTACTGTACGAGAACATCTGATAGTAGT   240
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PB_MiSeq    GATCATCACCGCCCATCTGACTACTGGGGCCAGGGAACCCT 281
BM_MiSeq    GATCATCACCGCCCATCTGACTACTGGGGCCAGGGAACCCT 281
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Results: Comparison of Laboratories

Sample Name	Sample Type	IVS		SAL	
		MiSeq/NextSeq (n=41)		Sanger/NGS (n=41)	
		Result	V-J	Result	V-J
1DX	PB/BM	Clonal	V3-J4	Clonal	V3-J4
2DX	PB	Clonal	V4-J4	Clonal	V4-J4
3DX	PB/BM	Clonal	V3-J4	Clonal	V3-J4
4DX	PB/BM	Clonal	V1-J4	Clonal	V1-J4
6DX	PB/BM	Clonal	V3-J2	Clonal	V3-J2
8DX	PB/BM	Clonal	V4-J3	Clonal	V4-J3
9DX	PB/BM	Clonal	V3-J4	Clonal	V3-J4
10DX	PB/BM	Clonal	V3-J3	Clonal	V3-J3
11DX	BM	Non-Clonal	V3-J4	Clonal	V4-J6
12DX	PB/BM	Clonal	V3-J4	Clonal	V3-J4
13DX	PB/BM	Clonal	V3-J4	Clonal	V3-J4
14DX	FFPE	Clonal	V3-J5	Clonal	V3-J5
16DX	PB/BM	Clonal	V3-J4	Clonal	V3-J4
17DX	PB	Clonal	V3-J6	Clonal	V3-J6
18DX	PB	Clonal	V3-J4	Clonal	V3-J4
19DX	PB/BM	Clonal	V3-J3	Clonal	V3-J3
20DX	PB	Clonal	V3-J5	Clonal	V3-J5
21DX	PB	Clonal	V3-J4	Clonal	V3-J4
22DX	PB	Clonal	V3-J6	Clonal	V3-J6
23DX	BM	Clonal	V3-J1	Clonal	V3-J1
24DX	PB/BM	Clonal	V4-J4	Clonal	V4-J4
25DX	PB	Clonal	V4-J4	Clonal	V4-J4
27DX	PB	Clonal	V4-J6	Clonal	V4-J6
28DX	PB	Clonal	V3-J4	Clonal	V3-J4
29DX	PB	Clonal	V3-J6	Clonal	V3-J6
30DX	PB	Clonal	V5-J4	Clonal	V5-J4
32DX	PB/BM	Clonal	V3-J4	Clonal	V3-J4
33DX	PB/BM	Clonal	V4-J4	Clonal	V4-J4
35DX	PB/BM	Clonal	V3-J4	Clonal	V4-J6
36DX	PB/BM	Clonal	V4-J4	Clonal	V4-J4
37DX	PB	Clonal	V3-J4	Clonal	V3-J4
38DX	PB	Non-Clonal	V3-J4	Non-Clonal	N/A*
39DX	PB	Clonal	V3-J4	Clonal	V3-J4
40DX	PB	Clonal	V4-J4	Clonal	V4-J4
41DX	PB	Clonal	V3-J6	Clonal	V3-J6
42DX	PB	Clonal	V2-J3	Clonal	V2-J3
43DX	PB	Clonal	V4-J6	Clonal	V4-J6
44DX	PB/BM	Clonal	V1-J4	Clonal	V1-J4
46DX	PB	Clonal	V1-J6	Clonal	V1-J6
47DX	PB/BM	Clonal	V1-J5	Clonal	V1-J5
48DX	PB/BM	Clonal	V3-J3	Clonal	V3-J3

*Only Sanger Sequencing performed, therefore no V-J rearrangements are available

Conclusions

Both the LymphoTrack *IGH* FR1 Assay – MiSeq and the CAP/CLIA B-cell MRD Assay on NextSeq, reliably identified clonal *IGH* V-J rearrangements in MCL subjects.

Excellent concordance (100%), was observed between paired PB and BM specimen types, and across MiSeq and NextSeq platforms (100%). Concordance in results between international test centers was 95.1%.

These results demonstrate that various *IGH* FR1 NGS assays, run both on different sequencing platforms and in different clinical laboratories, can reliably detect clonal rearrangements testing either PB or BM from MCL subjects.

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