A SENSITIVE NGS ASSAY TO DETECT MEASURABLE RESIDUAL DISEASE (MRD) IN B-CELL LYMPHOPROLIFERATIVE DISEASES

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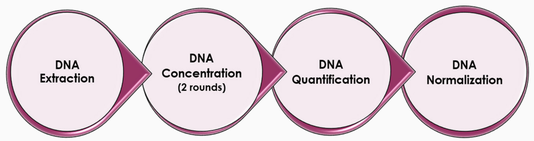
Background

MRD assessment in B-cell lymphoproliferative diseases has proven utility in assessing response to therapy, refining treatment and predicting clinical outcome in patients previously treated with a combination of treatments (Garcia-Marco et al. Haematologica 2019; Pui et al. Leukemia 2017). Next-generation sequencing (NGS) based approaches for MRD detection have provided high sensitivity and specificity to identify and track measurable residual disease using clonal immunoglobulin heavy chain (*IGH*) gene rearrangements (Rawstron, Leukemia, 2016). Here, we report a sensitive NGS-based B-cell MRD Assay to detect MRD in B-cell malignancies such as acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), multiple myeloma (MM) and mantle cell lymphoma (MCL) with *IGH* FR1 and FR3.

Methods

Sample Prep



- **Sample Input volume:** ~1-2 mL
- **Specimen type**: Bone Marrow Aspirates
- **Two methods for DNA extraction**
  - Manual using QIAmp DNA Blood Midi Kit
  - Automated using the Kingfisher Flex

Assay Workflow

- **Sample DNA Input:** 0.5 µg to 20 µg
- **MRD Positive & Negative Controls:** Run controls
- **MRD Internal Controls:** Quantitative Controls (Spiked into samples and Positive Control)

Results: Carryover and Crossover Contamination

- The clonal sequence of a sample is searched in a previous run for carryover analysis and within the same run for crossover contamination. No two samples should have the same clonal sequence in a previous or within the same run.
- If target reads of a sample is greater than minimum threshold reads , a Z-score is calculated based on target reads, average rate and standard deviation to calculate the probability (p-value) for a sequence to be detected.
  - If p-value < 0.05 then the sample is considered detected, it's a true positive
  - If p-value ≥ 0.05 then the sample is considered not detected, and signal is a false positive.

Study	Read Cutoff (MRD Detection)	Minimum Threshold Reads	Rate 95% CL ± 2 sd	Average Rate
Crossover	≥ 2 reads	≥165,160	1.21E-05	1.11E-06
Carryover	≥ 2 reads	≥ 19,730	1.01E-04	7.91E-06

Results: LOB, LOD, and LOQ

- **LOB Results**: No clonal sequence was detected in 10 healthy bone marrow samples tested at 0.5 µg and 20 µg (N= 180 per master mix lot) with both *IGH* FR1 and FR3 gene targets. Thus, LOB was zero.

***IGH* FR1- LOD/LOQ Results**

DNA Input (µg)	Clonal cell estimates LOD [95% CI]	Clonal MRD Frequency LOD [95% CI]	Clonal cell estimates LOQ [95% CI]	Calculated (Clonal) MRD Frequency LOQ [95% CI]
0.5	7.0 [4.9 - 12.1]	9.1E-05 [6.4E-05 - 1.6E-04]	5.4 [3.8 - 8.5]	7.0E-05 [4.9E-05 - 1.1E-04]
2	4.7 [3.5 - 7.7]	1.5E-05 [1.1E-05 - 2.5E-05]	4.6 [2.6 - 7.9]	1.5E-05 [8.5E-06 - 2.6E-05]
20	5.4 [4.0 - 8.5]	1.8E-06 [1.3E-06 - 2.7E-06]	7.3 [5.4 - 9.2]	2.4E-06 [1.7E-06 - 3.0E-06]

- LOD is highest value at 20 µg : 7.0 cells which is equivalent to 2.3 x10^{-6}
- LOQ is highest value at 20 µg: 7.3 cells which is equivalent to 2.4 x10^{-6}

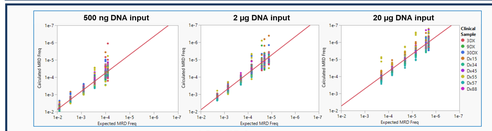
***IGH* FR3 - LOD/LOQ Results**

DNA Input (µg)	Clonal cell estimates LOD [95% CI]	Clonal MRD Frequency LOD [95% CI]	Clonal cell estimates LOQ [95% CI]	Calculated (Clonal) MRD Frequency LOQ [95% CI]
0.5	3.9 [3.0-7.2]	5.11E-05 [3.9E-05 – 9.4E-05]	6.4 [5.1-8.0]	8.3E-05[6.6E-05-1.0E-04]
2	4.7 [3.7-7.4]	1.54E-05[1.2E-05 - 2.4E-05]	12.1 [5.2-28.2]	3.9E-05[1.7E-05-9.2E-05]
20	5.7 [4.2-10.1]	1.84E-06[1.4-06 – 3.3E-06]	51.4*[6.7-396.9]	1.7E-05[2.2E-06-1.3E-04]

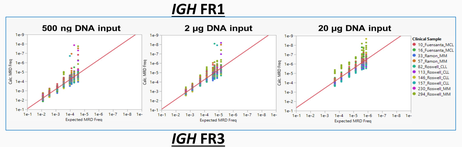
- LOD is highest value at 20 µg : 7.0 cells which is equivalent to 2.3 x10^{-6}
- LOQ is highest value at 20 µg: 51.4 cells which is equivalent to 1.7 x10^{-5}

*LOQ is 14.4 cells which is equivalent 4.7 x10^{-6} *when removing data for one sample that was an outlier*

Results: Linearity



***IGH* FR1**



***IGH* FR3**

DNA Input (µg)	Log-transformed Fit Slope	Intercept	R^2
0.5	1.06	-0.005	0.91
2	1.06	0.021	0.88
20	1.01	-0.542	0.85

DNA Input (µg)	Log-transformed Fit Slope	Intercept	R^2
0.5	1.05	-0.017	0.80
2	1.06	-0.020	0.82
20	1.05	-0.588	0.74*

R^2 = 0.85 when removing data for one sample that was an outlier

- MRD Detection is Linear for spanning the whole detection range of the *IGH* FR1 and FR3 MRD Assay (8.0E-03 to 2.6E-06)

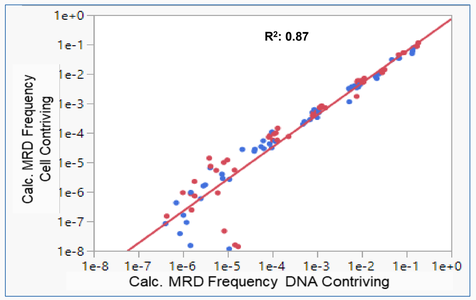
Results: Clinical Accuracy

- 10 Healthy bone marrow and 20 contrived MRD clinical samples (CLL, MM, and MCL) were tested with *IGH* FR1 and FR3 MRD Assay.
- The clonal sequence detected at baseline level with clonality assay (reference method) was used to track in contrived MRD clinical samples.
- Positive Percent agreement (PPA) and Negative Percent Agreement (NPA) was 100% for both *IGH* FR1 and FR3 gene targets.

N =220		*IGH* FR1 Detected	*IGH* FR1 Not Detected
FR1 Clonality Assay	Clonal	20	0
	Non-Clonal	0	200

N =220		*IGH* FR3 Detected	*IGH* FR3 Not Detected
FR3 Clonality Assay	Clonal	20	0
	Non-Clonal	0	200

Results: Cell vs DNA Contriving



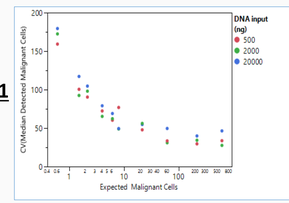
Transformed Fit to Log	
Slope	Intercept
1.09	- 0.32

- Study was conducted with two Multiple Myeloma cell lines – U-266 and LP-1 contrived in healthy bone marrow background that covered dynamic range from 2.0E-01 to 3.0E-07.
- MRD detection of cell and DNA contrived samples was observed to be equivalent with R^2 of 0.87 and slope of 1.09 in regards to calculated (calc.) MRD Frequency (aka Clonal MRD Frequency)

Results: Precision

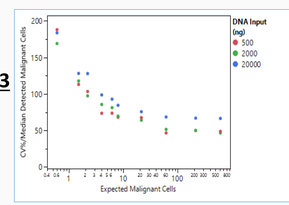
Sadler's Precision Profile

***IGH* FR1**



N (Total data points)	%CV per Malignant Cell Level 216	216	216	216	216	216	216	216	216	216
Malignant Cell Input	615.4	215.4	61.5	21.5	8.0	6.2	4.0	2.2	1.5	0.6
DNA Input	12.9	10.0	14.3	10.4	0.0	5.3	13.3	0.0	22.6	24.1
Operator	3.4	0.0	0.0	0.0	5.1	0.0	0.0	0.0	0.0	3.8
MM Lot/Instrument /Run/Day	0.0	0.5	0.0	0.0	7.7	2.0	9.4	0.0	4.6	0.0
Random Error	35.4	34.3	37.2	52.9	59.0	63.6	71.4	97.2	102.3	173.8
Precision (Total Variation)	37.8	35.8	39.8	53.9	59.2	63.8	72.7	97.2	104.7	175.5

***IGH* FR3**



N (Total data points)	%CV per Malignant Cell Level 216	216	216	216	216	216	216	216	216	216
Malignant Cell Input	615.4	215.4	61.5	21.5	8.0	6.2	4.0	2.2	1.5	0.6
DNA Input	25.8	30.5	30.4	19.5	30.8	26.5	26.4	24.6	25.9	30.2
Operator	13.3	14.7	0.0	7.0	13.1	16.3	0.0	0.0	6.5	0.0
MM Lot/Instrument /Run/Day	10.7	11.1	9.4	8.6	14.6	0.0	0.0	0.0	0.0	35.1
Random Error	41.9	44.2	46.2	60.3	64.0	72.5	78.3	100.6	110.4	178.4
Precision (Total Variation)	52.1	56.8	56.1	64.3	73.6	78.9	82.6	103.6	113.6	184.4

- Study was conducted with two master mix lot, 2 operators, 2 instruments, 3 DNA inputs with 9 clinical samples (CLL, MM and MCL).
- CV% decreases with increase in Clonal Malignant cell level

Conclusions

- *IGH* FR1 exhibited a LoB at 0, LoD at 2.3E-06, LoQ at 2.4E-06, good linearity with R^2>0.88 across 4.5 logs and precision with %CV ranging from 27.6% to 63.5% at the LoD.
- *IGH* FR3 exhibited a LoB at 0, LoD at 1.8E-06, LoQ at 1.7E-05, good linearity with R^2>0.74 across 4.5 logs and precision with %CV ranging from 46.6% to 89.4% at the LoD.
- The B-cell MRD Assay demonstrated excellent clinical performance; yielding 100% agreement in an accuracy study that included 20 clinical specimens (CLL, MM and MCL) and 10 healthy donors.

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