FNIH MRD in AML Biomarkers Consortium: Evaluation of Methods for Detection of *FLT3* Internal Tandem Duplication Measurable Residual Disease in Acute Myeloid Leukemia

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Introduction

Evidence of residual leukemia, termed measurable residual disease (MRD), in complete remission (CR) after treatment for acute myeloid leukemia (AML) is associated with adverse clinical outcomes. *FLT3* internal tandem duplication (ITD) is one of the most frequently observed mutations in AML and the persistent detection of *FLT3*-ITD in CR is highly predictive of increased incidence of relapse and death in adults with AML, with a variant allele fraction (VAF) ≥0.01% associated with the greatest risk. The FNIH MRD in AML Biomarkers Consortium is a public, private partnership aimed at advancing AML MRD testing and here benchmarked technologies for the study of *FLT3*-ITD MRD.

Materials and Methods

Figure 1. Study design

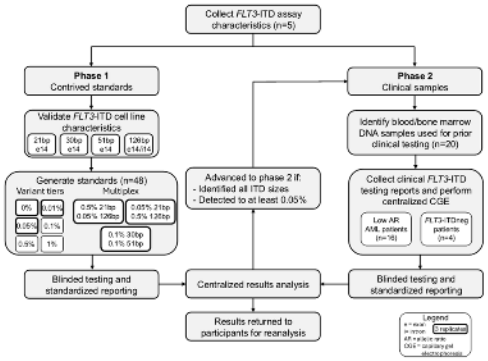


Table1. *FLT3*-ITD MRD Assay Summary

Method	Product	Availability	Sequencing Platform	Assay Input (ng)	Targeted Genomic Regions	Enrichment Strategy	Error correction	*De novo* ITD Detection	Validated Detectable ITD Size Range (bp)	Reported LOD
Company 1	Invivoscribe, Inc. *FLT3*-ITD MRD assay	CAP/CLIA Service or RUO Kit	Illumina	700	*FLT3* exon14/15	Targeted PCR	No	Yes	9-252bp	0.005%
Company 2	Sysmex Inostics AML-MRD-SEQ	CAP/CLIA Service	Illumina	132	68 gene regions	Targeted multiplex PCR	Yes, single strand UMI	No	3-114bp	0.035%
Company 3	Thermo Fisher Scientific, Inc. Oncomine Myeloid MRD DNA assay	RUO Kit	Ion Torrent	120	33 genes	Targeted multiplex PCR	Yes, single strand UMI	Yes	10-180bp	0.1-0.05%
Company 4	TwinStrand Biosciences DuplexSeq assay	RUO Kit	Illumina	500	15 genes	Hybrid capture with biotinylated oligos	Yes, duplex UMI	Yes	3-250bp	0.01%
getITD*	Loo et al, Blood 2022	Lab developed	Illumina	500	*FLT3* exon14/15	Targeted PCR	No	Yes	N/A	N/A

*modified protocol from original publication
For Research Use Only. Not for use in diagnostic procedures.

Phase 1: Singleplex *FLT3*-ITD Standards Performance

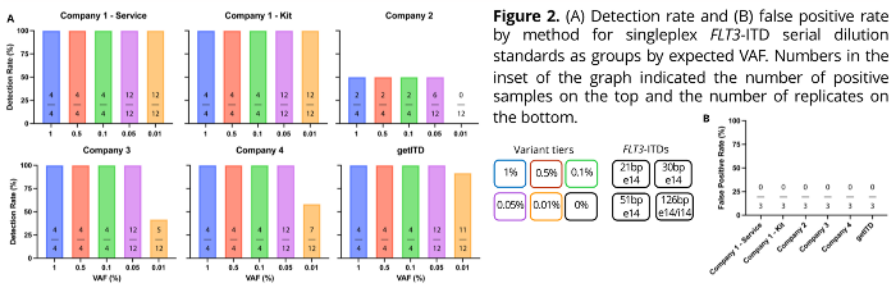
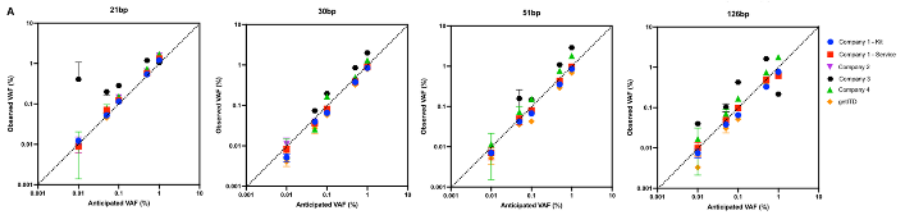


Figure 2. (A) Detection rate and (B) false positive rate by method for singleplex *FLT3*-ITD serial dilution standards as groups by expected VAF. Numbers in the inset of the graph indicated the number of positive samples on the top and the number of replicates on the bottom.

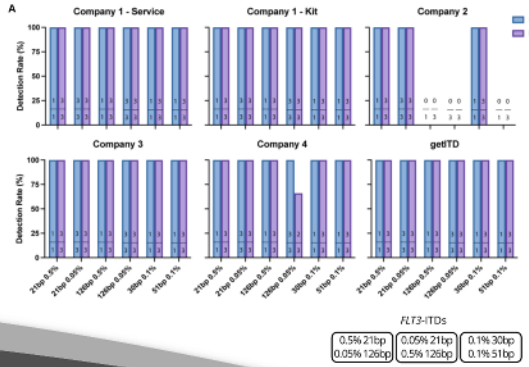


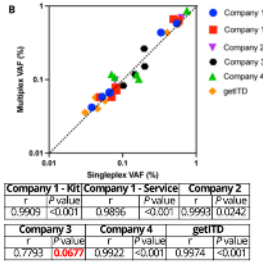
B

FLT3-ITD size (bp)	Company 1 – Kit		Company 1 - Service		Company 2		Company 3		Company 4		getITD	
	r	*P* value	r	*P* value	r	*P* value	r	*P* value	r	*P* value	r	*P* value
21	0.9989	<0.001	0.9977	<0.001	0.9956	<0.001	0.8362	0.078	0.9981	<0.001	0.9999	<0.001
30	0.9986	<0.001	0.9974	<0.001	0.9939	<0.001	0.9973	<0.001	0.9908	0.001	0.9965	<0.001
51	0.9961	<0.001	0.9982	<0.001	N/A	N/A	0.9919	<0.001	0.9973	<0.001	0.9967	<0.001
126	0.9976	<0.001	0.9626	0.009	N/A	N/A	0.2509	0.684	0.9965	<0.001	0.9983	<0.001

Figure 3. (A) The anticipated versus observed VAF of the *FLT3*-ITD variants of interest from contrived standards is plotted for each assay evaluated, with a value of equivalence line displayed as a dotted line. (B) The Pearson correlation coefficient (r) and and *P* value for each comparison is displayed in the table.

Phase 1: Multiplex *FLT3*-ITD Standards Performance



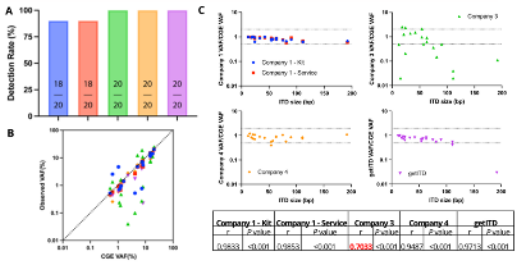


Company 1 - Kit		Company 1 - Service		Company 2	
r	*P* value	r	*P* value	r	*P* value
0.9909	<0.001	0.9896	<0.001	0.9993	0.0242

Company 3		Company 4		getITD	
r	*P* value	r	*P* value	r	*P* value
0.7793	0.0677	0.9922	<0.001	0.9974	<0.001

Figure 4. (A) Detection rate and (B) anticipated versus observed VAF of the *FLT3*-ITD variants of interest from multiplexed contrived standards for each assay evaluated, with a value of equivalence line displayed as a dotted line and the Pearson correlation coefficient (r) and and *P* value for each comparison.

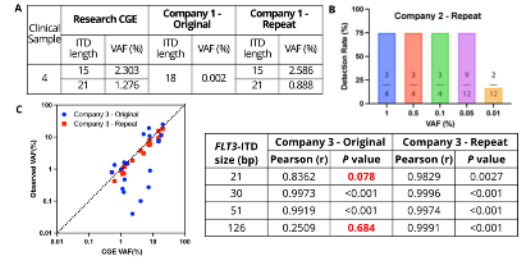
Phase 2: Clinical *FLT3*-ITD Sample Performance



Company 1 - Kit		Company 1 - Service		Company 3		Company 4		getITD	
r	*P* value	r	*P* value	r	*P* value	r	*P* value	r	*P* value
0.9833	<0.001	0.9853	<0.001	0.7035	<0.001	0.9487	<0.001	0.9713	<0.001

Figure 5. (A) Detection rate and CGE versus observed VAF by (B) method and (C) ITD size in clinical samples for each assay evaluated.

Result Re-analysis



Clinical Sample	Research CGE		Company 1 - Original		Company 1 - Repeat	
	ITD length	VAF (%)	ITD length	VAF (%)	ITD length	VAF (%)
4	15	2.303	18	0.002	15	2.586
	21	1.276			21	0.888

FLT3-ITD size (bp)	Company 3 - Original		Company 3 - Repeat	
	Pearson (r)	*P* value	Pearson (r)	*P* value
21	0.8362	0.078	0.9829	0.0027
30	0.9973	<0.001	0.9996	<0.001
51	0.9919	<0.001	0.9974	<0.001
126	0.2509	0.684	0.9991	<0.001

Figure 6. (A) Company 1 missing ITDs in one Phase 2 sample were complex indel variants filtered out by the reporting software. (B) Company 2 missing ITDs in Phase 1 were due to primer design and requirement for prior knowledge of the variant of interest in the variant calling algorithm. (C) Modifications to the Company 3 bioinformatics pipeline improved *FLT3*-ITD VAF calling accuracy.

Conclusions

- *FLT3*-ITD MRD assessment is increasingly critical for risk stratification and management of AML.
- Study of 5 NGS-based technologies (kit, service, lab developed) for *FLT3*-ITD MRD evaluation showed the ability of multiple strategies to detect and quantify ITDs down to a relevant level (0.01-0.05% VAF).
- Multiple factors, both technical and bioinformatic, can impact assay performance and should be carefully evaluated before implementation.
- This study demonstrates the power of collaborative efforts like the FNIH MRD in AML Biomarkers Consortium to advance research discoveries to the clinic.

Acknowledgements

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Questions?
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