Ultra-sensitive molecular MRD by flow cytometry using superRCA mutation assay for NPM1 positive AML patients

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BACKGROUND

Analyzing measurable residual disease (MRD) in acute myeloid leukemia (AML) with mutated *NPM1* is vital for assessing treatment response and early relapse detection. While reverse transcription quantitative PCR (RT-qPCR) is the gold standard for MRD detection, DNA-based methods offer benefits like better sample stability. This study evaluates the DNA-based superRCA *NPM1* mutation detection assay (Rarity Bioscience, Uppsala) by comparing it with targeted deep sequencing (deep seq) and RT-qPCR.

METHOD

Samples (n=86; 55 bone marrow, 31 blood) from AML patients with NPM1 type A mutation were analyzed using superRCA and compared to deep sequencing and RT-qPCR of *NPM1 Mut A*. Samples were analyzed in 96-well plates using an automated benchtop platform. Readout was performed using DxFLEX with a plate adapter.

The superRCA assay uses rolling-circle amplification (RCA) and padlock probes to convert DNA into fluorescent particles detectable by flow cytometry. Mutant allele frequency (MAF) is determined by enumerating particles originating from mutant and wild-type DNA.

LoD/LLOQ for superRCA was assessed per CLS EP17-A2 guidelines, using the non-parametric classical approach. The deep seq assay had a Limit of Blank (LoB) of 0.001% and Lower Limit of Quantification (LLoQ) of 0.02% using 100 ng input. For RT-qPCR, LoB and LLoQ were 15 copies of mutated transcripts. **RT-qPCR was used as the gold standard reference**.

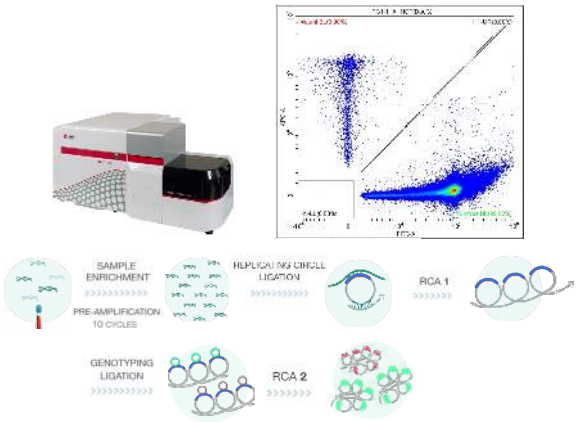

Figure 1. Instrumentation setup, Opentron OT2 and Beckman Coulter and molecular method description. RCA = Rolling Circle Amplification.

RESULTS

Analytical performance superRCA

The superRCA assay had an **LoB = 0.0007%** and **LLoQ = 0.0047%** using 660 ng DNA input (Figure 3). Detectable signal below LLoQ was defined at >LoB and > five mutant events. Linearity showed an R^2 = 0.99965 (Figure 2).

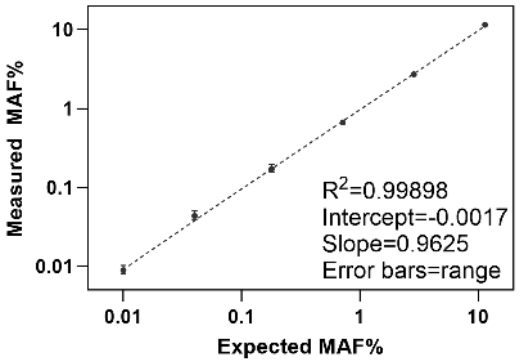

Figure 2. Linearity of superRCA *NPM1 MutA*, three replicates per dilution point.

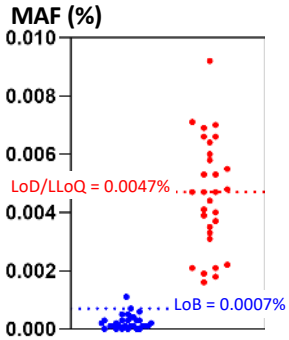

Figure 3. LoB and LoD/LLoQ analysis using 30 replicates each (CLSI EP-17 A2)

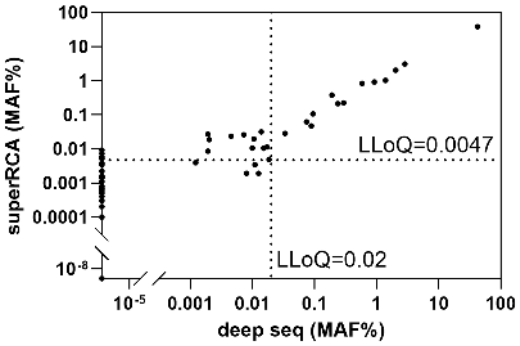

Figure 4. Correlation of superRCA and deep seq NGS, lines indicating the respective Lower Limit of Quantification

Method comparison

Analysis of patient samples showed a significant correlation between superRCA and the DNA based method deep seq NGS (spearman, r_s **=0.9720, p<0.0001**) for samples above the LLoQ of deep seq (Figure 4).

Comparison against the RNA based reference method RT-qPCR demonstrated a significant correlation (spearman, r_s**=0.8656, p<0.0001,** Figure 5). For sensitivity and specificity of the superRCA assay, see Table 1.

Table 1. Sensitivity and specificity of superRCA compared to RT-qPCR when using the LLoQ as cut-off, as well as when considering all samples with detectable signals

Cut-off	LLoQ	Detectable signals
Sensitivity TP/(TP+FN)	64%	89%
Specificitiy TN/(TN+FP)	97%	84%
PPV	97%	87%
NPV	70%	87%

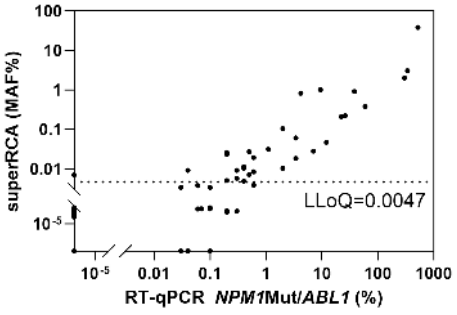

Figure 5. Correlation of superRCA and the reference method RT-qPCR

CONCLUSION

The superRCA *NPM1* assay has strong potential for ultra-sensitive DNA-based molecular MRD analysis using conventional flow cytometry, with high sensitivity and specificity when compared to RNA-based molecular MRD analysis.