Evaluation of MiSelect R System: A Comparison with Flow Cytometry and/or RT-qPCR Assays for Measurable Residual Disease Monitoring in Acute Myeloid Leukemia

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Introduction and Purpose

- **Two widely used methods for measurable residual disease (MRD) detection in acute myeloid leukemia (AML) patients are multiparameter flow cytometry (MFC) and real-time quantitative PCR (RT-qPCR).**
- **MFC has limited sensitivity and requires invasive bone marrow (BM) sampling.**
- **RT-qPCR offers high sensitivity but is restricted to patients with specific gene alterations.**
- **MiSelect R is a microfluidic, image-based system that isolates and analyzes rare leukemia cells from peripheral blood (PB).**
- **The aim of this study is to develop and evaluate a MiSelect R–based MRD detection method that is non-invasive, sensitive, and applicable to a broader patient population.**

Methods

Patients & Samples

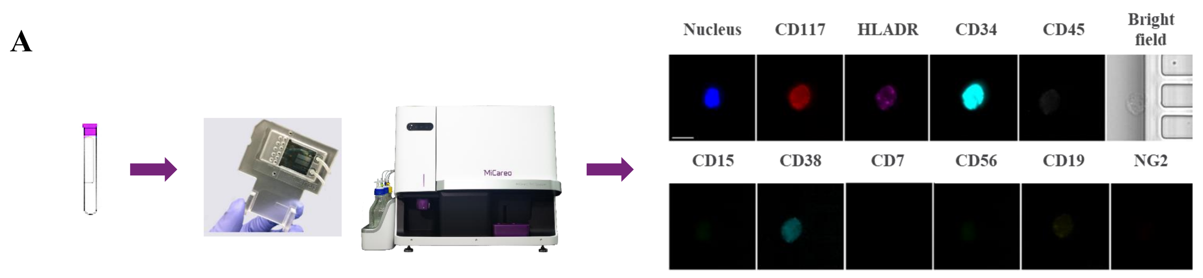
- **Patients: 44 AML patients with or without genetic alterations**
  - **Genetic alterations : *NPM1, RUNX1::RUNX1T1, CBFB::MYH11, KMT2A*-PTD, *KMT2A* rearrangement *(KMT2A*-r)**
- **Total samples : 74 paired BM and PB samples**

Criteria for MRD Positivity

- **Primary analysis :**
- **MFC (based on CGMH cut-offs) : ≥ 0.01%**
- **RT-qPCR (based on CGMH cut-offs):**
  - ***NPM1* and *KMT2A*-r : ≤ 5 log reduction**
  - ***RUNX1::RUNX1T1* and *CBFB::MYH11*: ≤ 4 log reduction**
  - ***KMT2A*-PTD: ≤ 3 log reduction**
- **MiSelect R: ≥ 4.4×10^{-7} (defined by ROC analysis)**
- **Additional analysis :**
  - **RT-qPCR (based on modifier cut-offs from Heuser et al., Blood, 2021)**
    - ***NPM1* mutations: BM: ≥ 100 copies / 10^5 *ABL1* copies; PB: ≥ 10 copies / 10^5 *ABL1* copies**
    - ***RUNX1::RUNX1T1* and *CBFB::MYH11* : ≤ 3 log reduction**
  - **MiSelect R: ≥ 7.6×10^{-7} (defined by ROC analysis)**

MiSelect R MRD Assays

A



B

LAIP Assay

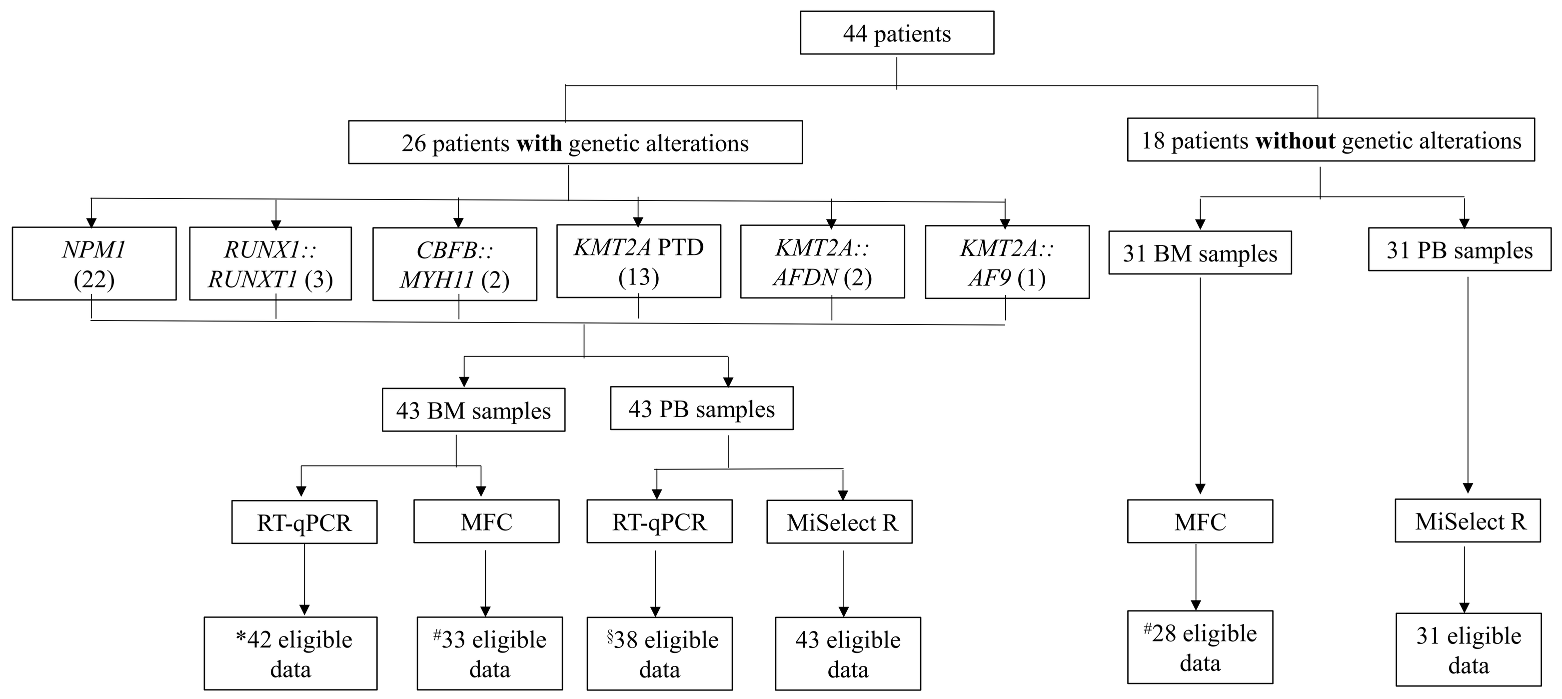
	Nucleus	FITC	PE	Qdot605	PerCP	APC	Qdot800
1st round	DAPI	CD56	CD117	CD19	CD34	HLADR	-
2nd round	DAPI	CD15	NG2	-	CD38	CD7	CD45

DfN Assay

	Nucleus	FITC	PE	Qdot605	PerCP	APC	Qdot800
1st round	DAPI	CD16	CD117	HLADR	CD34	CD71	CD14
2nd round	DAPI	CD15	CD13	CD105	CD64	CD300e	CD45

Figure 1. (A) Workflow of the MiSelect R MRD assay; (B) Panels for LAIP; (C) Panels for differentiation.

MRD Assessment Overview



Thirteen BM samples had insufficient total cell counts (< 5×10^6 cells) and thus could not be analyzed by MFC to generate eligible data.
* One BM sample had insufficient RNA quantity (*ABL1* copy number < 30,000) and were therefore ineligible for RT-qPCR analysis.
§ Five PB samples had insufficient RNA quantity (*ABL1* copy number < 30,000) and were therefore ineligible for RT-qPCR analysis.

Fig. 2 Distubution of patients in MRD assessment.

Primary Analysis

- RT-qPCR showed 91.8% concordance between BM and PB across 37 paired samples, supporting the use of PB as a reliable and less invasive alternative for MRD monitoring (**Table 1**).
- The RT-qPCR (BM) results of CGMH were used as the clinical reference standard. The receiver operating characteristic (ROC) analysis yielded an area under the curve (AUC) of 0.75, indicating a moderate discriminative performance to differentiate MRD-positive from MRD-negative cases. Based on the maximum Youden's J index, the optimal cut-off value for MiSelect R was determined to be 4.4×10^{-7} (**Figure 3**).
- The detection rates of MiSelect R-MRD in PB samples were significantly higher than those of MFC-MRD in BM samples (**Figure 4**).
- MiSelect R (PB) demonstrated moderate agreement with RT-qPCR (BM) (**Table 2**) and RT-qPCR (PB) (**Table 3**) while showed a superior sensitivity to MFC (**Table 4**).

Table 1. Comparison of MRD assessment between BM and PB samples by RT-qPCR

RT-qPCR (PB) \ RT-qPCR (BM)	Positive	Negative	Total	Percent Agreement (95% CI)
Positive	29	0	29	PPA: 90.6% (75.0 – 98.0%)
Negative	3	5	8	NPA: 100% (47.8- 100%)
Total	32	5	37	

Sensitivity: 90.6% **Specificity**: 100% **Concordance**: 91.8% **Cohen's Kappa**: 0.72

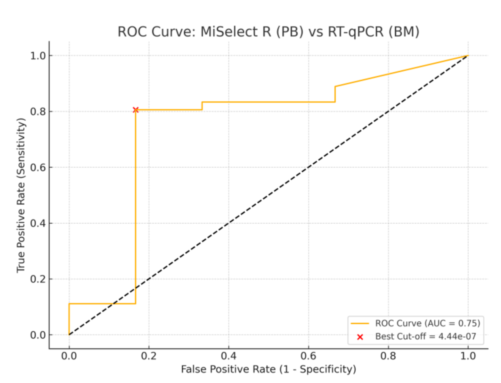


Figure 3. ROC analysis of MiSelect R (PB) for MRD detection using CGMH cut-offs as reference.

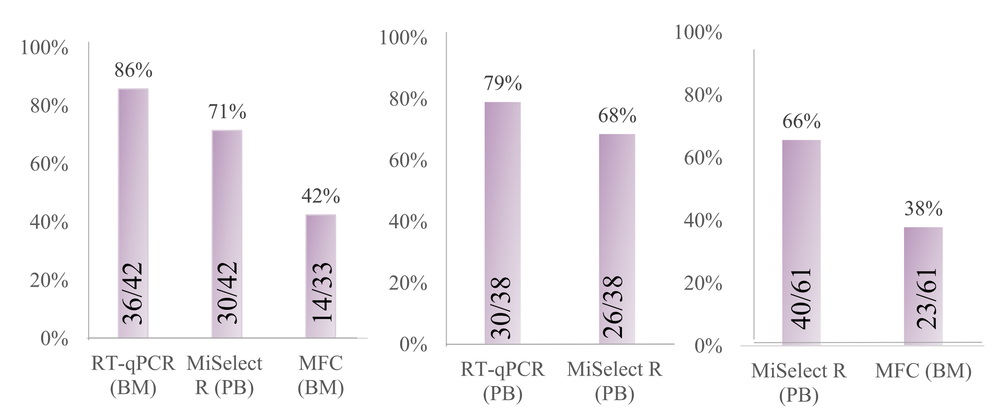


Figure 4. Comparison of MRD detection rates by RT-qPCR, MiSelect R and MFC in BM and PB samples.

Table 2. Comparison of MRD assessment between RT-qPCR (BM) and MiSelect R (PB)

MiSelect R (PB) \ RT-qPCR (BM)	Positive	Negative	Total	Percent Agreement (95% CI)
Positive	29	1	30	PPA: 80.6% (64.0 – 91.8%)
Negative	7	5	12	NPA: 83.3% (35.9 – 99.6%)
Total	36	6	42	

Sensitivity: 80.6% **Specificity**: 83.3% **Concordance**: 81.0% **Cohen's Kappa**: 0.45

Table 3. Comparison of MRD assessment between RT-qPCR (PB) and MiSelect R (PB)

MiSelect R (PB) \ RT-qPCR (PB)	Positive	Negative	Total	Percent Agreement (95% CI)
Positive	24	2	26	PPA : 80% (61.4 – 92.3%)
Negative	6	6	12	NPA : 75% (34.9 – 96.8%)
Total	30	8	38	

Sensitivity: 80.0% **Specificity**: 75.0% **Concordance**: 78.9% **Cohen's Kappa**: 0.47

Table 4. Comparison of MRD assessment between MiSelect R (PB) and MFC (BM)

MFC (BM) \ MiSelect R (PB)	Positive	Negative	Total	Percent Agreement (95% CI)
Positive	22	1	23	PPA : 47.8.% (32.9 - 61.3%)
Negative	24	14	38	NPA : 93.3% (61.8 – 99.8%)
Total	46	15	61	

Sensitivity: 47.8% **Specificity**: 93.3% **Concordance**: 59.0% **Cohen's Kappa**: 0.27

Additional Analysis

- ROC analysis was conducted only in *NPM1* mutations, *RUNX1::RUNX1T1* and *CBFB::MYH11* using RT-qPCR (BM) results based on the modifier cut-offs as the standard comparator. The AUC was 0.73. The optimal cut-off value for MiSelect R was determined to be 7.6×10^{-7} (**Figure 5**).
- MiSelect R (PB) demonstrated moderate agreement with RT-qPCR (BM) (**Table 5**) and RT-qPCR (PB) (**Table 6**).

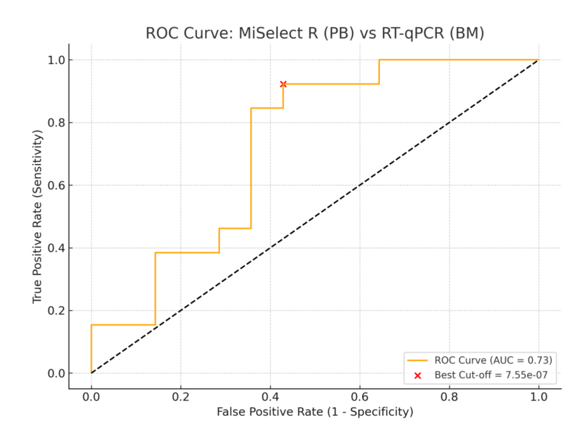


Figure 5. ROC analysis of MiSelect R (PB) for MRD detection using modifier cut-offs as reference.

Table 5. Comparison of MRD assessment between RT-qPCR (BM) and MiSelect R (PB)

MiSelect R (PB) \ RT-qPCR (BM)	Positive	Negative	Total	Percent Agreement (95% CI)
Positive	14	4	18	PPA : 93.3% (68.1-99.8%)
Negative	1	8	9	NPA : 66.7% (34.9 – 90.1%)
Total	15	12	27	

Sensitivity: 93.3% **Specificity**: 66.7% **Concordance**: 81.5% **Cohen's Kappa**: 0.61

Table 6. Comparison of MRD assessment between RT-qPCR (PB) and MiSelect R (PB)

MiSelect R (PB) \ RT-qPCR (PB)	Positive	Negative	Total	Percent Agreement (95% CI)
Positive	12	3	15	PPA : 85.7% (57.2 – 98.2%)
Negative	2	6	8	NPA : 66.7% (29.9 - 92.5%)
Total	14	9	23	

Sensitivity: 85.7% **Specificity**: 66.7% **Concordance**: 78.3% **Cohen's Kappa**: 0.53

Conclusions

- **MiSelect R (PB) demonstrates higher sensitivity than MFC (BM) and comparable performance to RT-qPCR (BM and PB).**
- **As a non-invasive tool, MiSelect R combines high sensitivity and broadly applicability, supporting its value for MRD monitoring in AML.**
- **Future studies may help further support its generalizability across broader clinical contexts.**