

Pre-Analytic Stabilization of Bone Marrow Aspirates for Acute Myeloid Leukemia Measurable Residual Disease Testing by Spectral Flow Cytometry



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Introduction

Acute myeloid leukemia (AML) is an aggressive hematologic malignancy in which detection of measurable residual disease (MRD) after treatment roughly [halves](#) 5-year overall survival (68% vs 34%). Multiparameter flow cytometry is used in ~60% of AML MRD assessments, but it must reliably identify leukemic populations present at frequencies as low as 0.01–0.1% of total cells. ELN guidelines recommend acquiring at least 500,000–1,000,000 CD45+ events with adequate cell viability to achieve this limit of detection.

Acquiring this amount of cells depends heavily on sample quality, which is difficult to control outside specialized laboratories. With current workflows, bone marrow must be processed within 24–72 hours of collection, and even within that window, storage and transit time disproportionately degrade myeloid populations, particularly monocytic lineages, central to AML MRD. As a result, high-quality MRD testing is largely limited to centers capable of rapid processing, restricting access for community hospitals, and introducing variability that can confound results across sites.

A method for stabilizing bone marrow at collection could decouple sample acquisition from analysis, enabling community sites to collect and fix specimens locally for shipment to a central reference laboratory. This would expand access to standardized MRD testing while preventing sample degradation. We evaluated whether TokuKit fixation can stabilize bone marrow at collection, preserving the cellular populations and marker expression required for downstream spectral flow cytometric MRD analysis.

Current Teiko's TokuKit Workflow

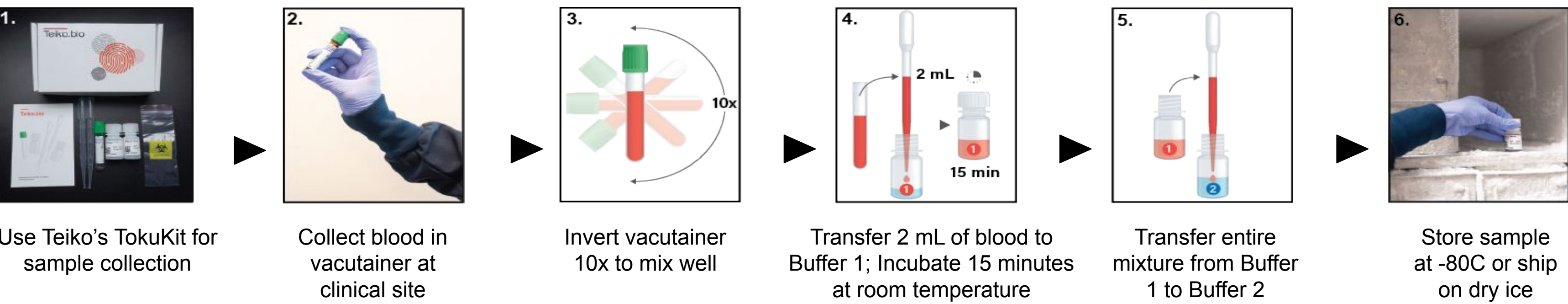
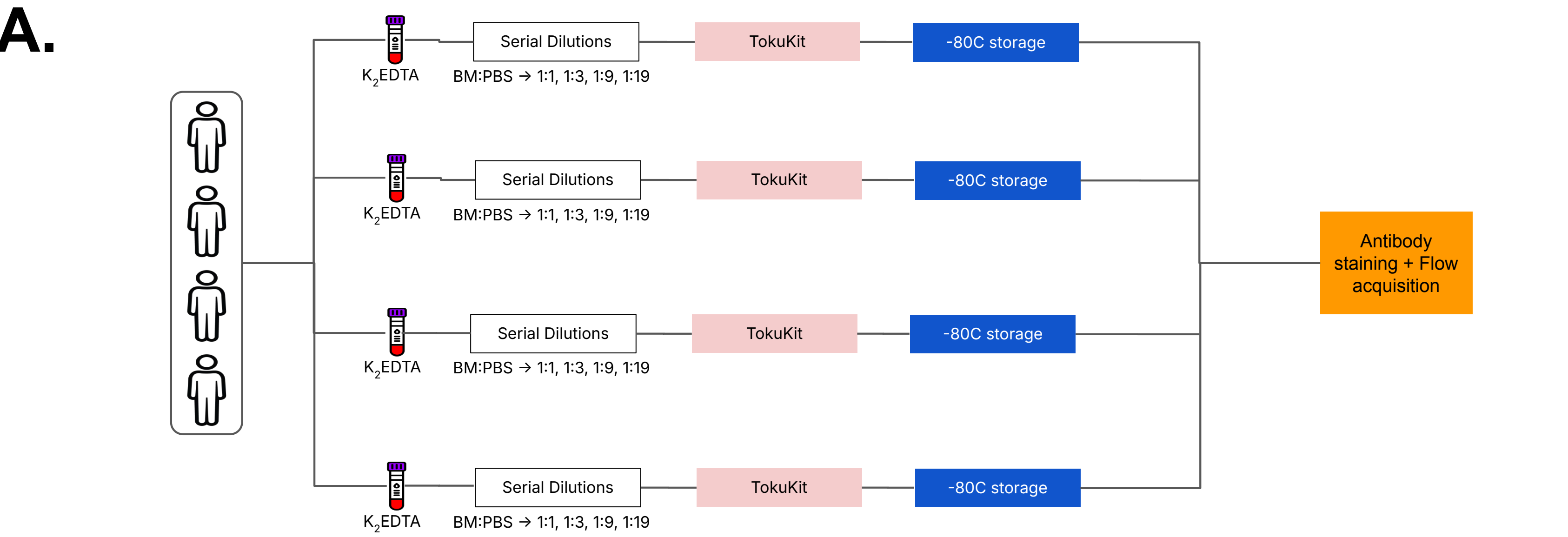


Figure 1. Schematic of bone marrow collection and stabilization with TokuKit. Bone marrow aspirate is drawn into an anticoagulant tube, incubated with Buffer 1 for 15 minutes to lyse red blood cells and fix leukocytes, then quenched with Buffer 2 and stored at -80°C until analysis.

Method and Panel for Bone Marrow Stabilization



Marker	Fluorochrome	Clone	Manufacturer	Marker	Fluorochrome	Clone	Manufacturer
HLA-DR	cFluor V450	L243	Cytek	CD64	cFluor BYG781	10.1	Cytek
CD38	cFluor V515	HB7	Cytek	CD33	cFluor R659	P67.6	Cytek
CD4	cFluor V570	RPA-T4	Cytek	CD45RA	cFluor R720	HI100	Cytek
CD19	cFluor V605	SJ25C1	Cytek	CD15	cFluor R685	W6D3	Cytek
CD36	cFluor V670	CB38	Cytek	CD45	cFluor R840	2D1	Cytek
CD11b	cFluor B532	ICRF44	Cytek	CD34	BV421	581	BD
CD14	cFluor BYG610	M5E2	Cytek	CD56	BV711	NCAM16.2	BD
CD117	cFluor BYG667	104D2	Cytek	CD7	BV786	MT701	BD
CD123	cFluor BYG710	6H6	Cytek	CD133	PE	293C3	BD
CD13	cFluor BYG750	WM15	Cytek	CLL1/CD371	BB700	50C1	BD

Figure 2. (A) Bone marrow aspirates from four AML patients were diluted in PBS (1:1, 1:3, 1:9, 1:19) to ensure adequate fixative capacity, fixed with TokuKit at collection, stored at -80°C , and later stained with a 20-marker AML MRD panel for spectral flow cytometry. **(B)** A 20-marker AML MRD panel was used to stain samples, which were then acquired on a Cytek Aurora spectral flow cytometer.

AML MRD Gating Scheme

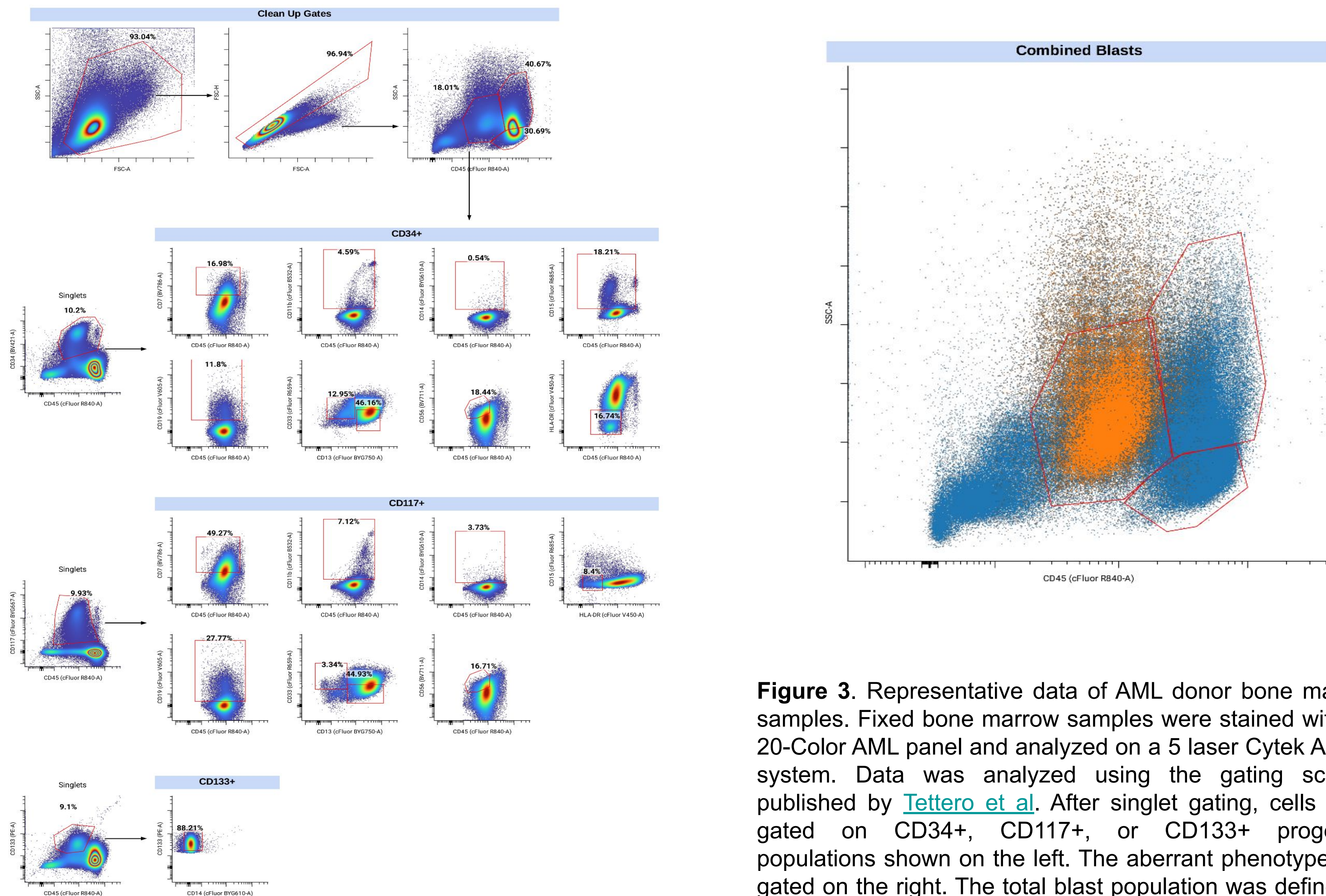


Figure 3. Representative data of AML donor bone marrow samples. Fixed bone marrow samples were stained with the 20-Color AML panel and analyzed on a 5 laser Cytek Aurora system. Data was analyzed using the gating schema published by [Tettero et al.](#) After singlet gating, cells were gated on CD34+, CD117+, or CD133+ progenitor populations shown on the left. The aberrant phenotypes are gated on the right. The total blast population was defined as the combined total of all aberrant populations.

TokuKit Enables Marker Retention up to 3 months

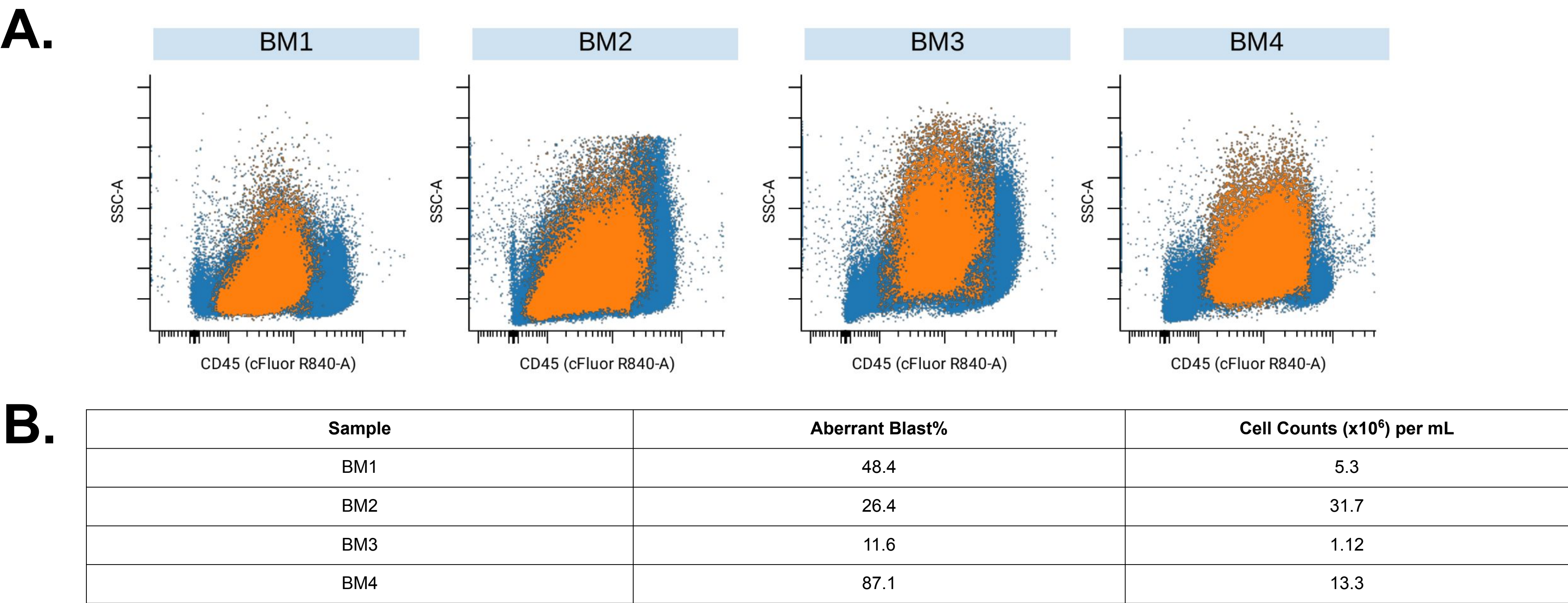


Figure 4. (A) Flow plots illustrating AML bone marrow TokuKit samples that were frozen at -80°C . The aberrant blast population is highlighted in orange. **(B)** Table illustrates blast and cell counts of bone marrow samples.

Conclusions and Future Directions

- TokuKit fixation successfully stabilizes bone marrow samples at collection up to 3 months, enabling delayed and centralized analysis, and supporting future use in baseline and post-treatment sample banking.
- Blast populations were identified and preserved across fixed samples, supporting feasibility for AML MRD applications.
- These findings support the feasibility of decoupling sample collection from analysis, potentially expanding MRD testing access to community hospitals.
- Optimal gating strategy for the 20-marker panel is still being refined; future work will finalize gating and validate marker expression concordance between fixed and fresh samples across a larger cohort.