SCIENTIFIC POSTER

Characterization of CD34+ hematopoietic stem cells from human umbilical cord blood with Cellaca PLX image cytometer.

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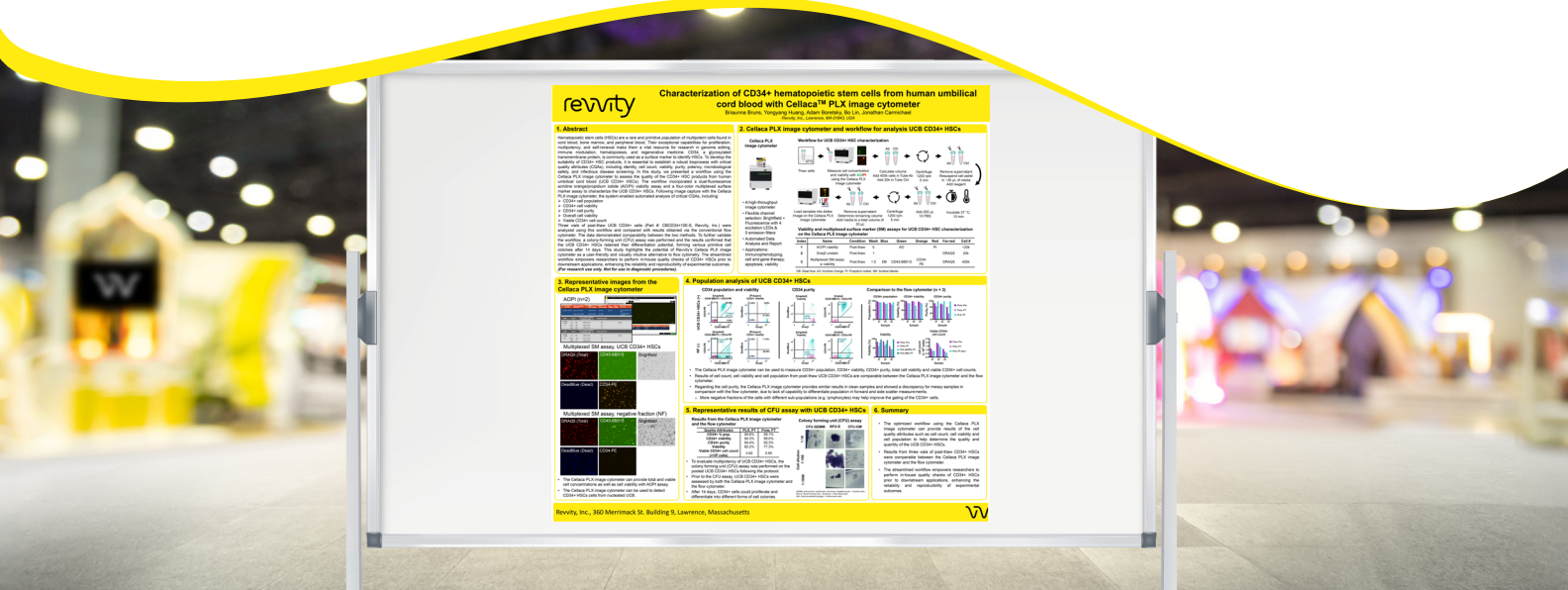
Abstract

Hematopoietic stem cells (HSCs) are a rare and primitive population of multipotent cells found in cord blood, bone marrow, and peripheral blood. Their exceptional capabilities for proliferation, multipotency, and self-renewal make them a vital resource for research in genome editing, immune modulation, hematopoiesis, and regenerative medicine. CD34, a glycosylated transmembrane protein, is commonly used as a surface marker to identify HSCs.

To develop the suitability of CD34+ HSC products, it is essential to establish a robust bioprocess with critical quality attributes (CQAs), including identity, cell count, viability, purity, potency, microbiological safety, and infectious disease screening. In this study, we presented a workflow using the Cellaca™ PLX image cytometer to assess the quality of the CD34+ HSC products from human umbilical cord blood (UCB CD34+ HSCs). The workflow incorporated a dual-fluorescence acridine orange/propidium iodide (AO/PI) viability assay and a four-color multiplexed surface marker assay to characterize the UCB CD34+ HSCs. Following image capture with the Cellaca PLX image cytometer, the system enabled automated analysis of critical CQAs, including:

- CD34+ cell population
- CD34+ cell viability
- CD34+ cell purity
- Overall cell viability
- Viable CD34+ cell count



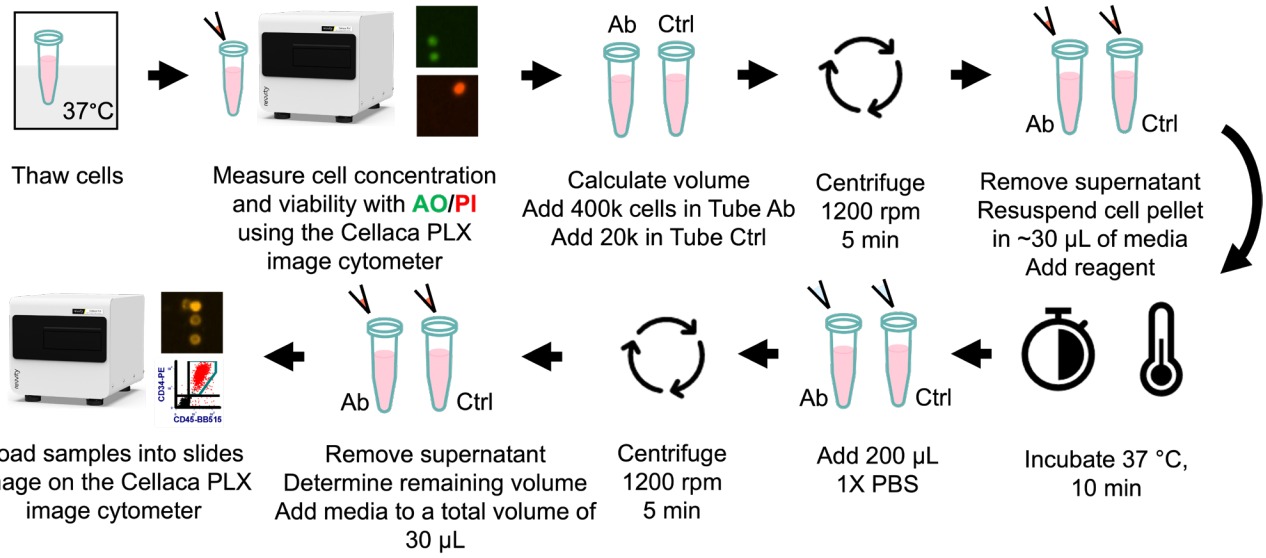
Three vials of post-thaw UCB CD34+ cells (Part #: CBCD34+1M-S, Revvity, Inc.) were analyzed using this workflow and compared with results obtained via the conventional flow cytometer. The data demonstrated comparability between the two methods. To further evaluate the workflow, a colony-forming unit (CFU) assay was performed and the results confirmed that the UCB CD34+ HSCs retained their differentiation potential, forming various primitive cell colonies after 14 days.

This study highlights the potential of Revvity's Cellaca PLX (Part #: PLX-SYS1) image cytometer as a user-friendly and visually intuitive alternative to flow cytometry. The streamlined workflow empowers researchers to perform in-house quality checks of CD34+ HSCs prior to downstream applications, enhancing the reliability and reproducibility of experimental outcomes.

Cellaca PLX image cytometer

- A high-throughput image cytometer
- Flexible channel selection: Brightfield + Fluorescence with 4 excitation LEDs & 5 emission filters
- Automated Data Analysis and Report
- Applications: Immunophenotyping, cell and gene therapy, apoptosis, viability

Workflow for UCB CD34+ HSC characterization



Viability and multiplexed surface marker (SM) assays for UCB CD34+ HSC characterization on the Cellaca PLX image cytometer

Index	Name	Condition	Wash	Blue	Green	Orange	Red	Far-red	Cell #
1	AO/PI viability	Post-thaw	0		AO		PI		~20k
2	Draq5 unstain	Post-thaw	1					DRAQ5	20k
3	Multiplexed SM assay w viability	Post-thaw	1.5	DB	CD45-BB515	CD34-PE		DRAQ5	400k

DB: Dead blue. AO: Acridine Orange. PI: Propidium Iodide. SM: Surface Marker.

Representative images from the Cellaca PLX image cytometer

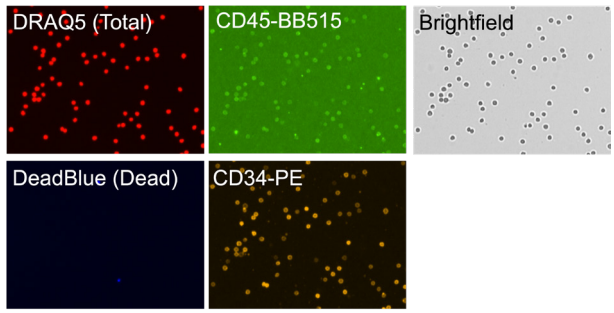
AO/PI viability

Sample	Avg concentration (cells/mL)	Stdev Concentration	%CV Concentration	Avg Viability	Stdev Viability	Warnings
110724-1.AOPI	Live: 0.81 x10^6	0.181 x10^6	22.31%	80.1%		% CV for group is Too high
	Total: 1.01 x10^6	0.213 x10^6	21.19%			% CV for group is Too high

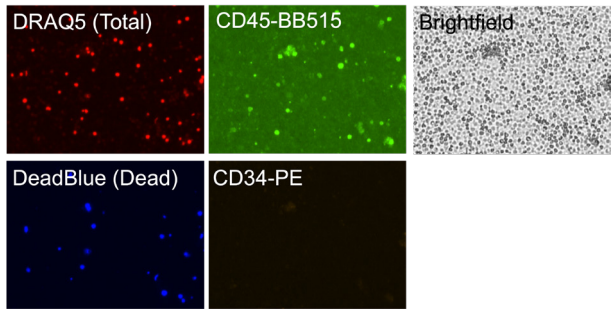
Well	Count		Concentration (cells/mL) at Dilution Factor: 2	Mean Size (um)	
110724-1.AOPI A1	Live:	455	0.938 x10^6	6.9	80.9%
	Dead:	109	0.225 x10^6	8.0	
	Total:	564	1.160 x10^6	7.1	

Well	Count		Concentration (cells/mL) at Dilution Factor: 2	Mean Size (um)	
110724-1.AOPI B1	Live:	331	0.682 x10^6	7.1	79.4%
	Dead:	86	0.177 x10^6	8.0	
	Total:	417	0.859 x10^6	7.3	

Multiplexed surface marker assay, UCB CD34+ HSCs

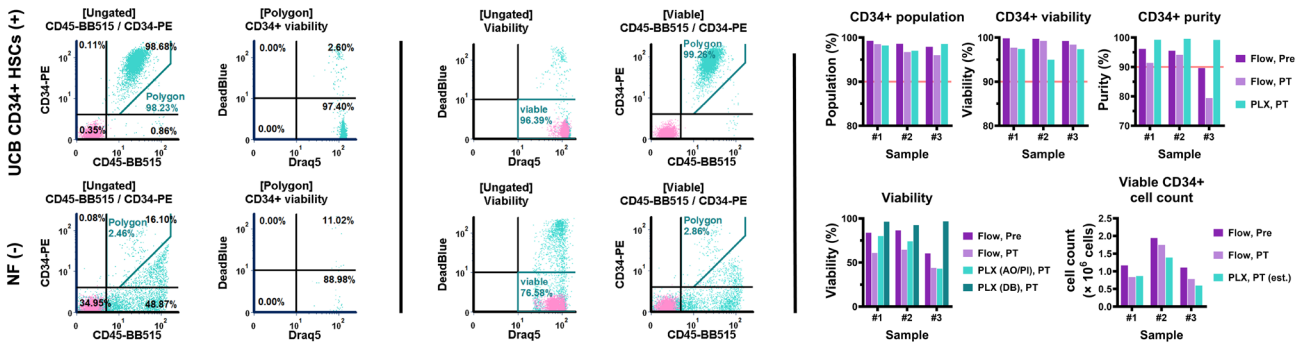


Multiplexed surface marker assay, negative fraction (NF)



- The Cellaca PLX image cytometer can provide total and viable cell concentrations as well as cell viability with AO/PI viability assay.
- The Cellaca PLX image cytometer can be used to detect CD34+ HSCs cells from nucleated UCB.

Population analysis of UCB CD34+ HSCs



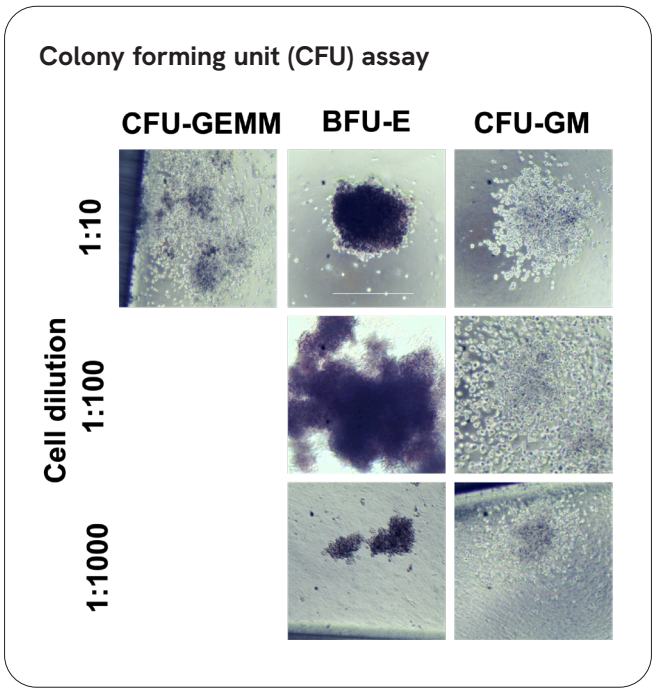
- The Cellaca PLX image cytometer can be used to measure CD34+ population, CD34+ viability, CD34+ purity, total cell viability and viable CD34+ cell counts.
- Results of cell count, cell viability and cell population from post-thaw UCB CD34+ HSCs are comparable between the Cellaca PLX image cytometer and the flow cytometer.
- Regarding the cell purity, the Cellaca PLX image cytometer provides similar results in clean samples and showed a discrepancy for messy samples in comparison with the flow cytometer, due to lack of capability to differentiate population in forward and side scatter measurements.
  - More negative fractions of the cells with different sub-populations (e.g. lymphocytes) may help improve the gating of the CD34+ cells.

Representative results of CFU assay with UCB CD34+ HSCs

Results from the Cellaca PLX image cytometer and the flow cytometer

Quality attributes	PLX, Post thaw	Flow, Post thaw
CD34+ % population	98.6%	99.1%
CD34+ viability	94.3%	98.0%
CD34+ purity	99.4%	90.3%
Viability	85.2%	77.3%
Viable CD34+ cell count (×10⁶ cells)	0.62	0.65

- A colony forming unit (CFU) assay was performed using a pooled UCB CD34+ sample to evaluate potency.
- Prior to the CFU assay, UCB CD34+ HSCs were assessed by both the Cellaca PLX image cytometer and the flow cytometer.
- After 14 days, CD34+ cells could proliferate and differentiate into different forms of cell colonies.



CFU-GEMM: colony-forming unit - granulocyte, erythrocyte, macrophage and megakaryocyte

BFU-E: burst-forming unit - erythroid

CFU-GM: colony-forming unit - granulocyte, macrophage

Summary

- The optimized workflow using the Cellaca PLX image cytometer can provide results of the cell quality attributes such as cell count, cell viability and cell population to help determine the quality and quantity of the UCB CD34+ HSCs.
- Results from three vials of post-thaw CD34+ HSCs were comparable between the Cellaca PLX image cytometer and the flow cytometer.
- The streamlined workflow empowers researchers to perform in-house quality checks of CD34+ HSCs prior to downstream applications, enhancing the reliability and reproducibility of experimental outcomes.