Novel Buoyancy Based Cell Selection: X-BACS™ Technology

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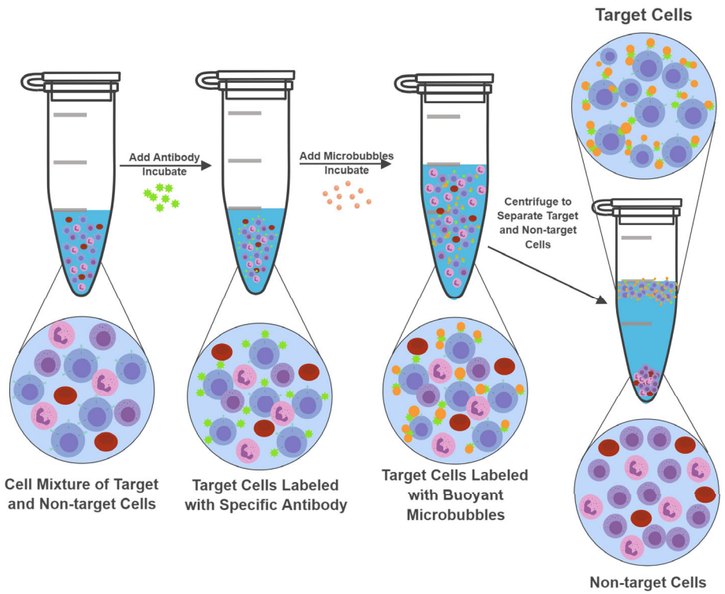
Introduction

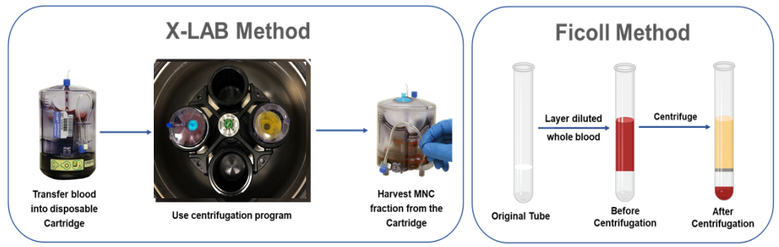
New developments in cell biology and genetic engineering have revolutionized the field of medicine with the greatest impact observed in the field of Immuno-oncology. Efficient isolation of desired cell populations is the cornerstone in the development of manufacturing and quality control processes for emerging cell therapies. We have developed a buoyancy-based cell selection method that isolates desired cell populations from a mixture of cell types, such as mononuclear cell (MNC) fraction preparations from whole blood. Using this method, we have efficiently recovered highly pure populations of T-cells (95%) with good yield (85%). The method is simple to execute with standard laboratory equipment.

Principle of Operation



Materials and Methods

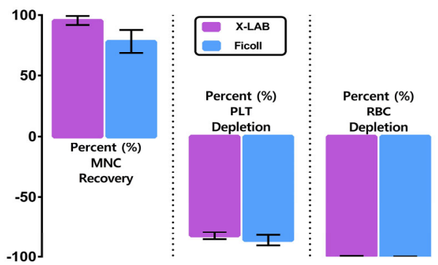
Peripheral Blood (PB) units were purchased from BloodSource. MNC fractions were prepared using both Ficoll-Hypaque density gradient centrifugation or, as an alternative, the X-LAB® System.



Both MNC fractions were used to select $CD3^+$ T-Cells using BACS technology. MNC fractions were incubated with anti-CD3 antibody for 30 minutes, followed by microbubble reagent for 20 minutes. Following incubation, the target and non-target fractions were separated using centrifugation.

Results

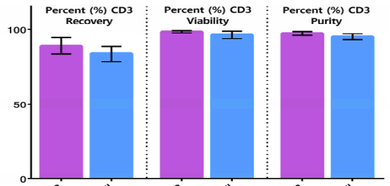
Whole blood units (n=4) were split among three (3) independent users, and each recovered MNCs using both Ficoll-Hypaque gradient centrifugation, as well as with the X-LAB System.



	X-LAB System			Ficoll Method		
	MNC (%) Recovery	PLT (%) Depletion	RBC (%) Depletion	MNC (%) Recovery	PLT (%) Depletion	RBC (%) Depletion
Mean	95.4	82.4	99.5	77.8	86.2	99.9
Std. Deviation	4.0	3.1	0.1	9.5	4.4	0.1
Std. Error of Mean	1.1	0.9	0.04	1.6	0.7	0.01
Lower 95% CI of mean	92.8	80.5	99.4	74.6	84.7	99.8
Upper 95% CI of mean	97.9	84.4	99.6	81.0	87.7	99.9

Figure 1. Comparative cell recoveries and depletions obtained using X-LAB Technology and Ficoll method.

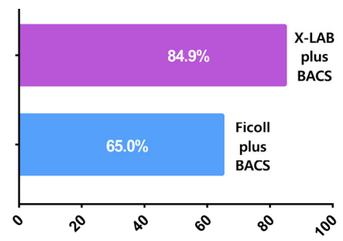
$CD3^+$ T-cells were selected from both MNC fractions using the X-BACS System. A mean $CD3^+$ recovery of 83.5%, 96.3% viability, and 95.1% purity was obtained from Ficoll MNC fractions, while a mean $CD3^+$ recovery of 89.0%, 98.3% viability, and 97.3% purity was obtained from X-LAB MNC fractions.



BACS Data	CD3 (%) Recovery		CD3 (%) Viability		CD3 (%) Purity	
	Mean	SD	Mean	SD	Mean	SD
X-LAB MNC	89.0	5.7	98.3	0.8	97.3	1.2
Ficoll MNC	83.5	5.3	96.3	2.4	95.1	2.0
Combined	86.3	6.2	97.3	2.0	96.3	2.0

Figure 2. Comparative $CD3^+$ selection data obtained from MNC fractions prepared with X-LAB Technology and Ficoll method.

The overall percent (%) $CD3^+$ cell recovery was calculated by multiplying percent (%) MNC recovery with percent (%) $CD3^+$ BACS recovery. In summary, better $CD3^+$ selection was obtained using the X-LAB System followed by BACS compared to Ficoll method followed by BACS.



Overall Percent (%) $CD3^+$ Cell Recovery

Figure 3. Comparative percent (%) $CD3^+$ recovery data from starting material, whole blood, to purified $CD3^+$ T-cells.

Conclusions

We have developed a buoyancy-based cell selection method that provides an efficient means of isolating a highly pure population of $CD3^+$ T-cells (>95%) with good yield (>85%) and excellent viability (>95%).

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