Novel Buoyancy Based Cell Selection: X-BACS™ Technology

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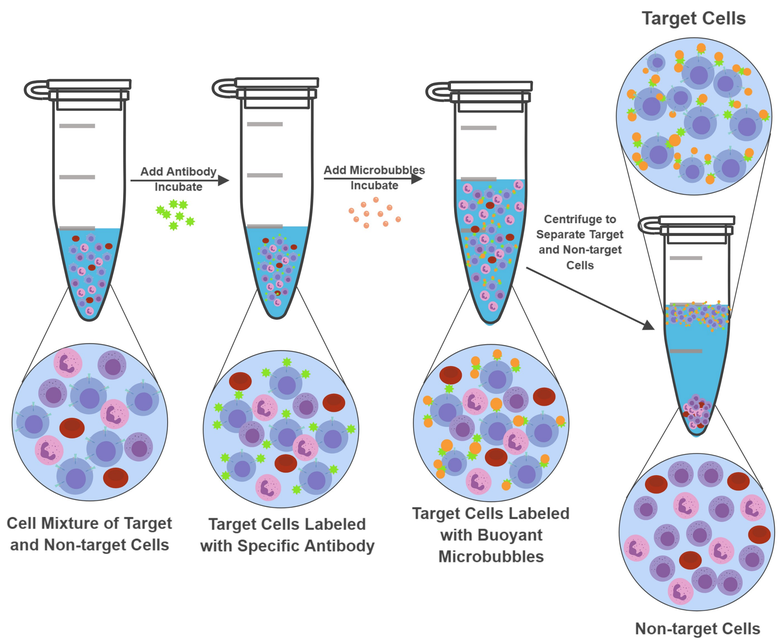
Introduction

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



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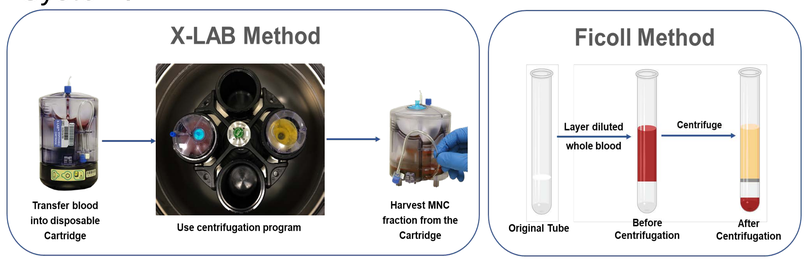
Materials and Methods

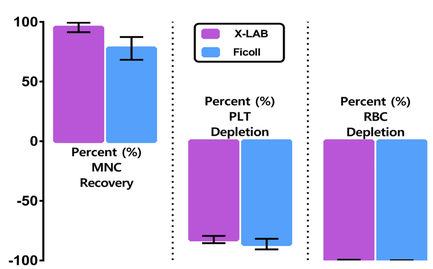
Peripheral Blood (PB) units were purchased from BloodSource, Mather, CA. MNC fractions were prepared using both Ficoll-Hypaque density gradient centrifugation or, as an alternative, the X-LAB™ System.



Both MNC fractions were used to select CD3⁺ T-Cells using BACS technology. MNC fractions were incubated with 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

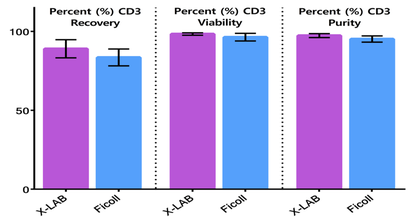
Whole blood units (n=4) were split among three (3) independent users and each recovered MNCs using both Ficoll-Hypaque gradient centrifugation as well as with the X-LAB System.



	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.

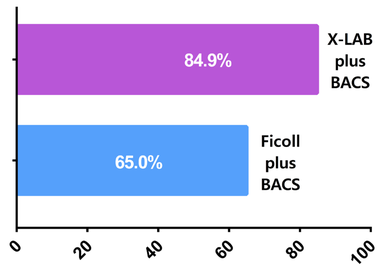
CD3⁺ T-cells were selected from both MNC fractions using the X-BACS System. A mean CD3⁺ recovery of 83.5%, 96.3% viability and 95.1% purity was obtained from Ficoll MNC fractions while a mean CD3⁺ recovery of 89.0%, 98.3% viability and 97.3% purity was obtained from X-LAB MNC fractions.



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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