Applied Cells

High Speed, High Recovery of Immune Cells Prepared From Whole Blood Using the MARS® Sample Preparation System

ABSTRACT

Blood immune cell flow cytometry analysis is a standard practice in both research and clinical labs. The immune cell preparation from whole blood samples normally requires multiple manual cell washing steps with centrifugation.The process can be tedious, labor intensive, time consuming, and inconsistent. We developed a new sample preparation MARS® system, using an acoustic active-microfluidic chip-wash to remove red blood cell debris, free dyes or other small particles.The MARS system automates the sample preparation for multicolor immuno-staining of white blood cells with high speed and cell recovery with minimal hands-on time.

INTRODUCTION

As a standard protocol for flow cytometry sample preparation, the whole blood is normally stained with one or a panel of fluorescent conjugated antibodies, then the red blood cells (RBCs) are lysed with a hypotonic RBC lysis buffer. Then the blood sample will go through a couple of washing steps to remove RBC debris and free dyes before being acquired by a flow cytometer. Normally this is a lengthy process with multiple steps of manual handling (Figure 1).

At Applied Cells, we developed a novel technology, using an acoustic active-microfluidic chip to wash and remove RBC debris, free dyes or other small particles. Sample and the sample wash buffer are pushed onto the microfluidic cell processing chip (CPC) simultaneously. In the flow stream on the CPC, the cells are forced into the wash buffer by the pressure generated by acoustic wave and the debris and small particles remain at the periphery. Then the cells and debris are separated and collected separately. The washed cells are ready for flow analysis (Figures 1 & 2).

OBJECTIVE

To establish a workflow using MARS wash to automate sample preparation for multicolor flow analysis

METHODS

Whole blood sample was stained with CD3 FITC, CD56 PE, CD45 PC5.5, CD4 PC7, CD19 APC, and CD8 APC/Cy7 and incubated for 15 minutes in the dark at room temperature. Then five times of the volume of the red blood cell (RBC) lysis buffer was added to the blood and incubated for 20 minutes. The lysed blood was analyzed with a flow cytometer to get the lyse no wash (LNW) sample.

Centrifugation wash procedure:
The lysed blood sample was centrifuged at 500 g for 5 minutes. Then the supernatant was discarded, and the cell pellet was resuspended in PBS. The cell suspension was then centrifuged (500 g for 5 minutes) again. The cell pellet was collected and reconstituted in PBS. The suspension was analyzed with a flow cytometer to get Centrifuge Wash sample.

MARS wash procedure:
The lysed blood from sample was passed through a 40 µm cell strainer. Then the preset MARS WASH program was used on MARS to perform wash of the sample. After running through MARS wash program, the washed sample was analyzed with a flow cytometer.

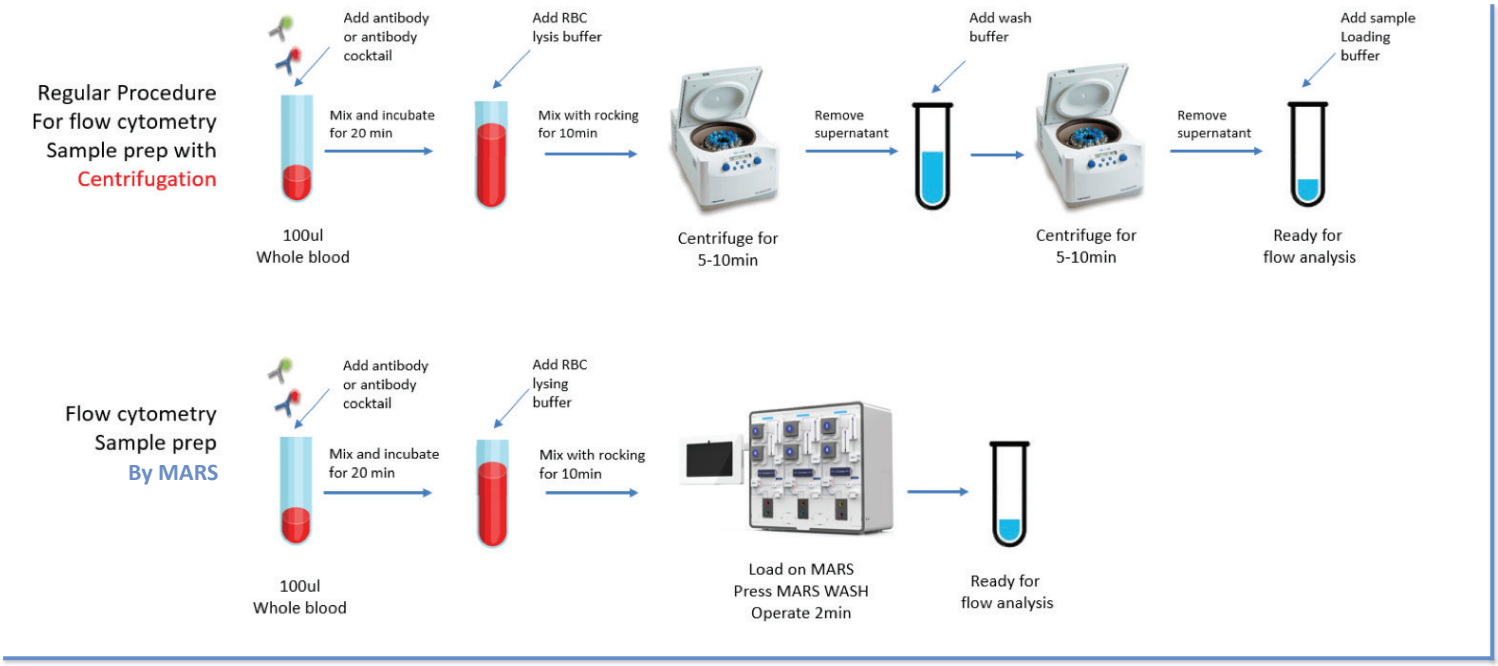


Figure 1. Workflow for sample preparation using centrifugation or MARS for flow cytometry cell analysis.

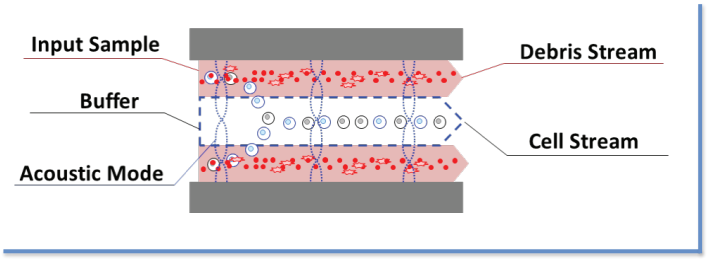


Figure 2. Principal of CPC acoustic cell separation.

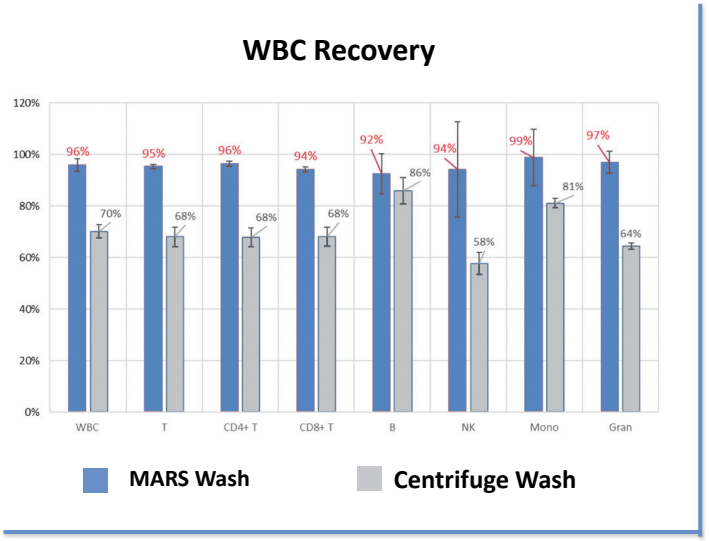


Figure 3. WBC recovery of MARS Wash and Centrifuge Wash after RBC lysis.

For both MARS and centrifugation wash protocols, each condition was performed in triplicates.

To assess the viability of the cells after centrifugation or MARS wash, the samples were stained with a cell viability dye, 7-AAD, for 5 minutes before running on the flow cytometer.

RESULTS

The MARS wash requires no human intervention once the blood is stained and loaded onto MARS. On the other hand, the conventional cell wash with centrifugation requires multiple steps of human intervention.

MARS wash yielded greater than 90% cell recovery of all white blood cell (WBC) populations, which is much higher than the cell recovery from the centrifugation wash (around 60% as an average) (Figure 3). The percentage of WBC subpopulations remained almost the same with this MARS-wash process comparing with LNW or centrifugation wash protocols (Figure 4). And the cell viability study further demonstrated MARS wash did not reduce cell viability (Figure 5). MARS wash also showed significant superiority in debris removal comparing with centrifuge wash (Figure 6).

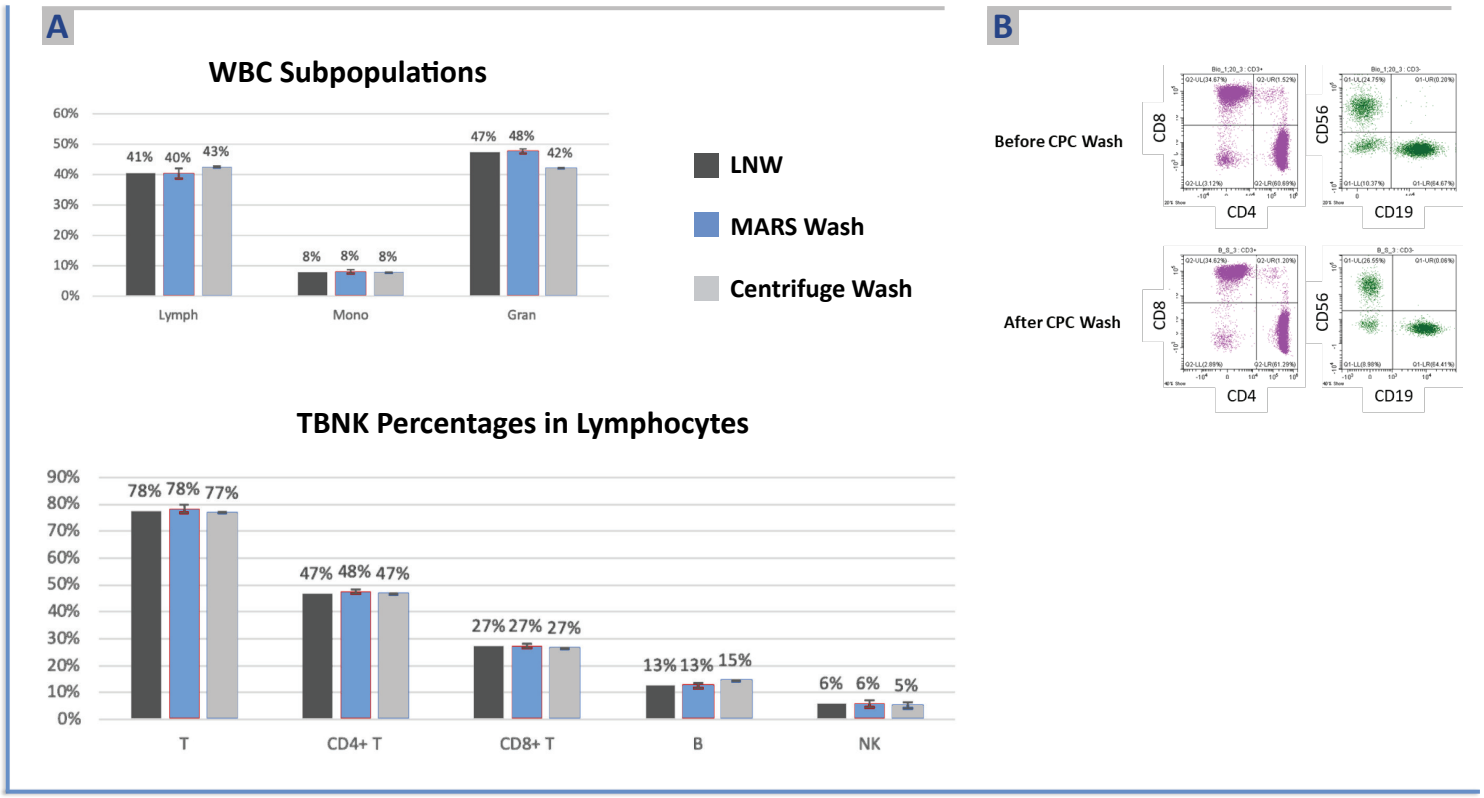


Figure 4. 4A Percentage of WBC subpopulations from LNW, MARS Wash, and Centrifuge Wash sample preparations were very closed or the same. Each experiment was repeated three times (N=3). **4B** showed representative flow cytometry dot plots of the T cell, B cell, NK cell populations.

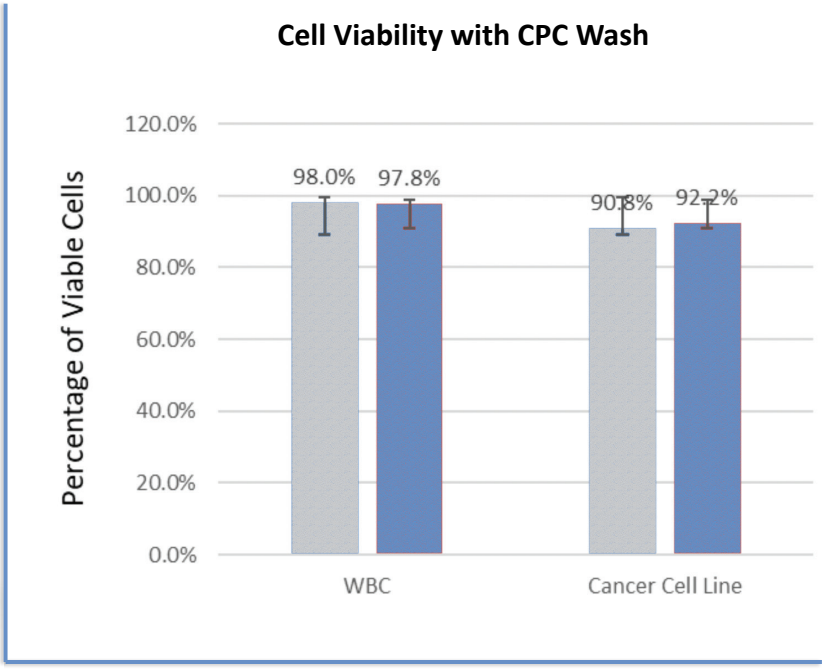
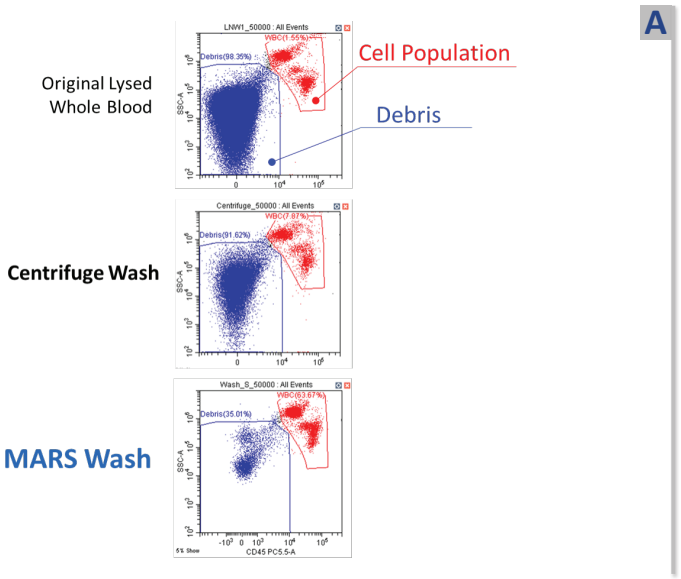


Figure 5. Cell viabilities before and after MARS wash



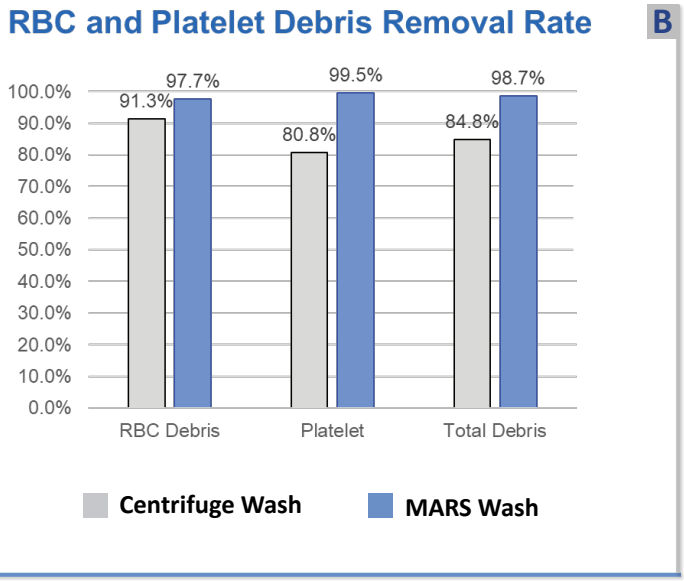


Figure 6. Debris removal by MARS wash or centrifugation wash. Dot plots of 10 µL blood samples were shown in **6A**. The threshold was significantly reduced for flow cytometer sample acquisition to visualize debris. Percentage of debris removal is shown in **6B**.

DISCUSSION

In a conventional flow lab, sample preparation for multicolor immune-staining assay, researchers normally rely on manual operations with centrifugation to remove cell debris and free dyes from staining. The process is tedious, labor intensive, and prone to significant cell loss. At Applied Cells, we developed an acoustic activate-microfluidic technology to automate cell wash process. Comparing with current cell wash with centrifugation, the MARS wash achieves significantly superior performance than standard centrifugation cell wash, with higher cell recovery, and greater debris removal with high consistency.

CONCLUSIONS

The MARS system automates blood sample processing with minimum human intervention, which could significantly reduce humor error. The minimized hands-on time will potentially cut down labor cost in research and clinical labs. The MARS systems could potentially be a low-cost option for sample preparation automation in a flow cytometry lab with high performance.

3375 Scott Blvd, Suite 102
Santa Clara, CA 95054
+1 650•539•4318

www.appliedcells.com
info@appliedcells.com