

CD138+ Plasma Cell Enrichment from Whole Blood and Bone Marrow

ABSTRACT

CD138 expression is a hallmark of plasma cells and multiple myeloma cells, and it is considered a useful marker in plasma cells tumors diagnostics. Using our newly developed MARS® CS system (multi-physics automated reconfigurable separation) we demonstrate a cost-efficient, fast and robust workflow for isolation of CD138+ cells from whole blood and bone marrow samples within a short time. We achieved 64.5% purity with a recovery higher than 90% of CD138+ cells isolated from a blood sample, and 59% purity of cells isolated from U266-spiked bone marrow sample.

INTRODUCTION

CD138 expression is a hallmark of plasma cells and multiple myeloma cells, and it is considered a useful marker in plasma cells tumors diagnostics. Although phenotypic features of plasma cells are quite heterogeneous, depending on the disease stage, diagnosis, a patient's other biologic characteristics, studies have demonstrated the prognostic value of specific patterns of antigen expression by neoplastic plasma cells¹.

Evaluation of plasma cells in whole blood or bone marrow usually requires a density gradient separation method to isolate the mononuclear cells, typically performed using ficoll. Although it provides a good plasma cell enrichment, a density gradient methodology is very time and labor-intensive. Additionally, ficoll gradient separation accelerates the loss of antigens, including CD138, from the plasma cell surface, so the cells must be immediately stained and analyzed¹.

MARS® CS (multi-physics automated reconfigurable separation) cell separation system incorporates Applied Cells' proprietary active-microfluidics acoustics and immuno-magnetic separation technologies for efficient CD138+ plasma cell isolation from a blood and bone marrow sample. The system was designed to provide an automated, minimal hands-on workflow.

OBJECTIVE

The goal of the experiment was to enrich human CD138+ plasma cells from whole blood and bone marrow, using one of two recommended workflows for highest and most economically efficient performance. Our main goal was to achieve:

- High Recovery and High Purity
- Fast and Minimum Manipulation of the Cells
- Ease of use and within a short time

METHODS

For CD138+ cells selection, the peripheral blood and bone marrow samples were spiked with U266.

Peripheral blood cells were minimally processed before direct MARS CS magnetic separation, by a 20 min incubation with magnetic beads.

To demonstrate a 2 step workflow recommended for samples with very low endogenous plasma cells, the bone marrow sample was first lysed by incubating in RBC lysing buffer for 15 minutes at room temperature. Cells were passed through a 40um cell strainer and used in MARS® CS acoustic isolation followed by magnetic separation. A LDS751 stain was used to identify nucleated cells in flow cytometry.

RESULTS

MARS® CS system was designed to minimize time and generate automated workflows for efficient and fast cell separation. Before separation, the **spiked blood cell** sample required minimal processing, which involved 20 min incubation of the blood sample with magnetic beads (Fig. 1). After the incubation, cells were separated on the MARS CS magnetic separation module for 5 min, at a high rate of 1 mL/min.

The recovery and purity of separated cells were analyzed by staining cells with CD298 APC antibodies in the baseline sample, MARS MAG positive fraction, and a purified control sample prepared with one of our direct competitor’s protocol. The analysis revealed that using the MARS CS system CD138+ U266 plasma cells were enriched from 2.5% (spike-in concentration) to 64.5% purity, and characterized with significantly higher purity than the one obtained with a competitor’s kit, which showed 26.2% sample purity (Fig 2, MARS CS and Fig 3A).

The recovery (Fig 3B) was calculated based on CD298 APC events number recorded in 50uL of flow cytometry sample and the volume collected before and after magnetic separation. MARS CS magnetic separation recovered 69% of the spiked-in U266 Cells, while the sample processed with the competitors kit recovered 65% of cells.

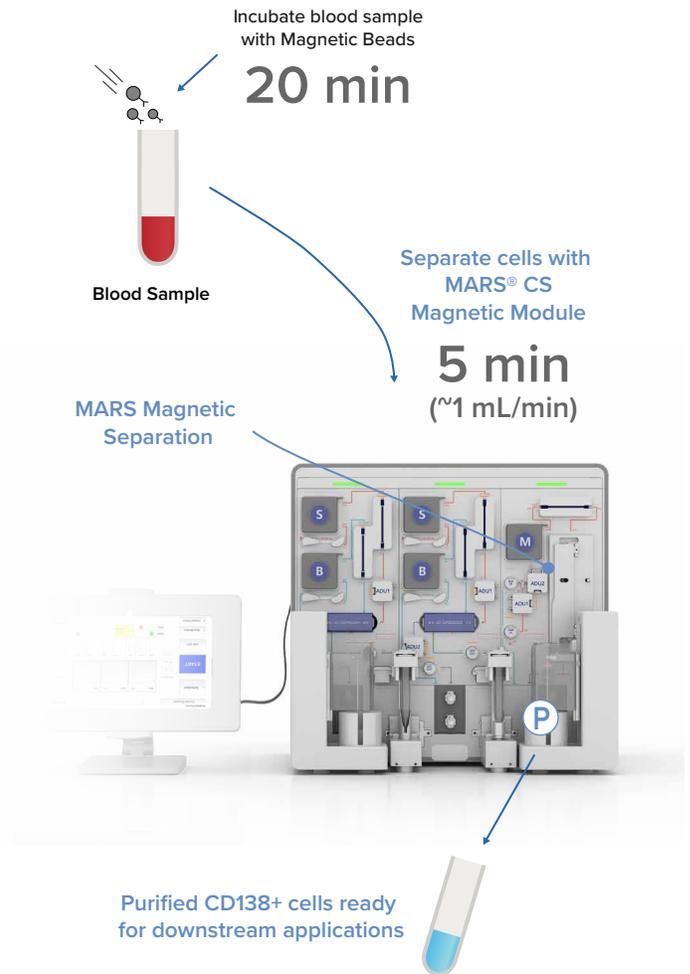


FIGURE 1. MARS® CS sample magnetic separation workflow. A 20-minute magnetic bead incubation step is performed before MARS cell isolation.

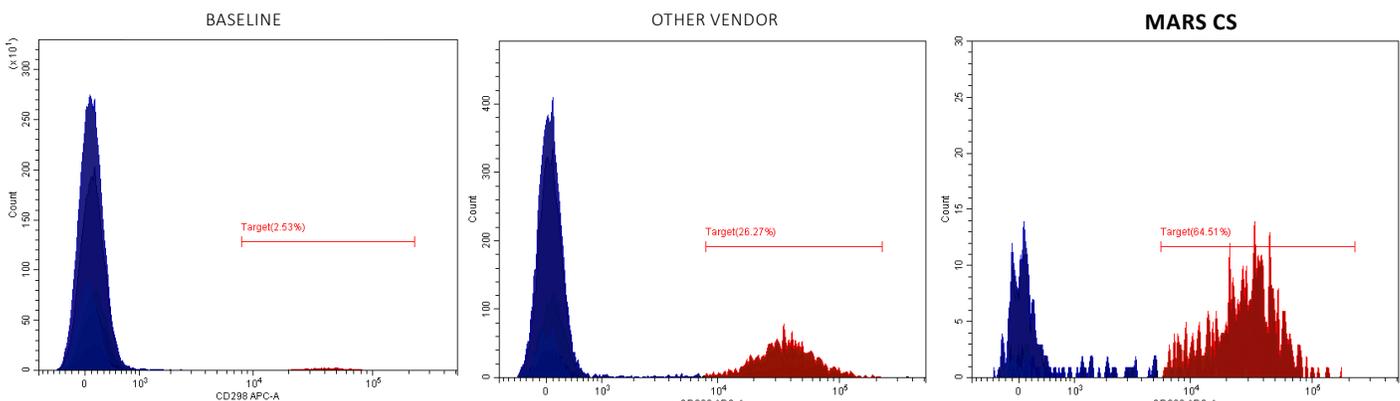


FIGURE 2. CD138+ blood cell enrichment purity data shown in histogram, U266 cells were prestained with CD298 APC.

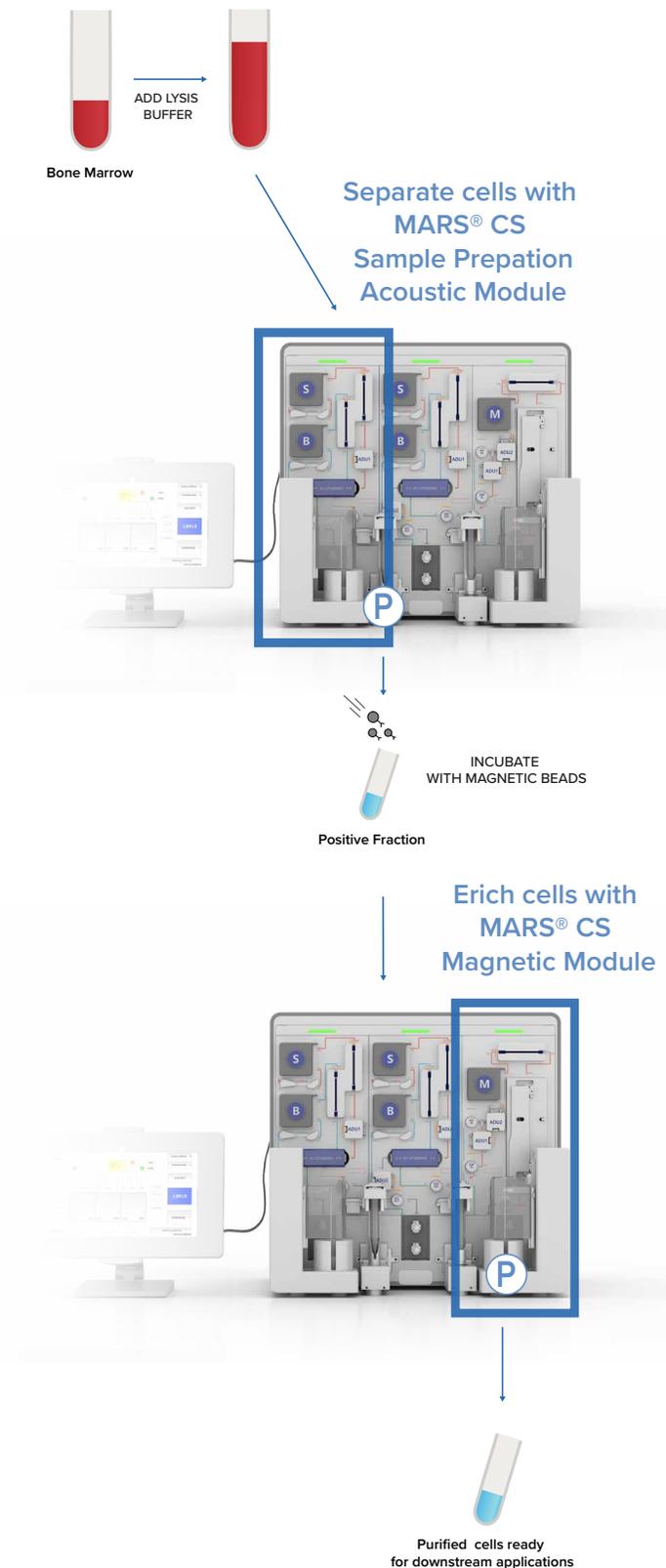


FIGURE 4. MARS® CS sample CPC-wash without beads, followed by magnetic separation workflow ensuring cell selection with high purity.

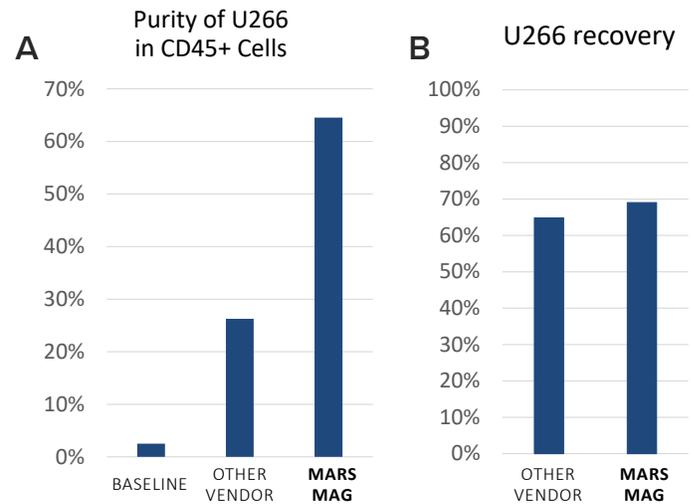


FIGURE 3. Analysis of the purity of CD138 enrichment from U266-spiked in peripheral blood. (A) U266 cells were enriched from 2.5% (spike-in concentration) to 64.5% purity, compared to 26.2% when using a different separation system. U266 cells were pre-stained with CD298 APC. (B) MARS magnetic separation recovered 69% of the spiked-in U266 Cells.

To demonstrate a 2 step workflow recommended for samples with very low endogenous plasma cells, CD138+ cells were purified from bone marrow samples with 0.15% plasma cell count (Fig. 4).

After using a workflow with MARS Cell Processing Chip (CPC) acoustic wash and MARS magnetic separation, CD138+ U266 bone marrow target cell purity was evaluated with flow cytometry using CD298 APC staining. As a reference, a control spiked bone marrow sample was processed only with direct magnetic separation, following the workflow illustrated in Figure 1 that we used for CD138+ cell isolation from peripheral blood.

The analysis revealed that CD138+ cell enrichment from U266-spiked in bone marrow reached 59% purity, while the purity in the sample prepared without the initial acoustic sample preparation reached 17% (Fig. 5).

DISCUSSION

Purified CD138+ cells are used in both research and preclinical studies. MARS® system provides an automated solution to rapidly isolate CD138+ plasma cells with high purity and recovery from both peripheral blood and bone marrow samples.

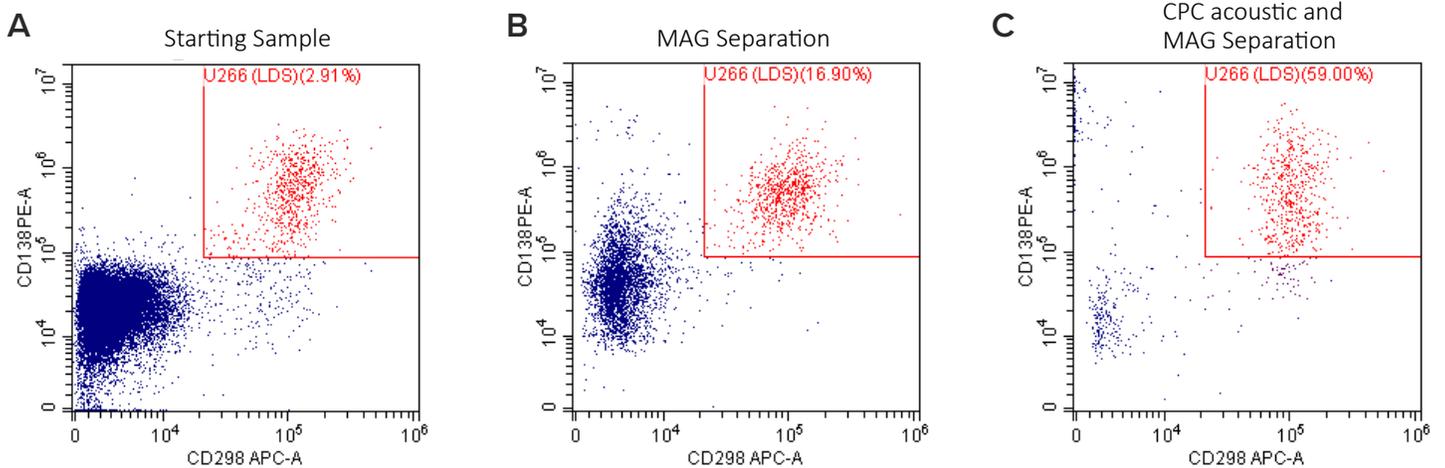


FIGURE 5. CD138 enrichment from U266-spiked in bone marrow reached 59% purity using a workflow involved MARS Cell Processing Chip (CPC) acoustic wash followed by magnetic separation. CD138 expressing U266 target cell purity was determined in cell population, by using flow cytometry: (A) CD138+ purity in the sample before process, (B) CD138+ purity in the sample processed only with direct magnetic separation (MAG), a workflow as illustrated in Figure 1, and (C) CD138+ purity in the sample processed with acoustic module (CPC) wash prior to magnetic (MAG) separation, a workflow illustrated in Figure 4.

CONCLUSIONS

Depending on the desired endogenous cell level in the purified sample, Applied Cells MARS CS solutions offer alternative workflows for high purity and high-efficiency purification with no harm to cell viability and functionality.

Additionally, MARS® fluidic line can be sterilized and replaced. The whole workflow can be performed sterily to generate enriched CD138+ cells for downstream applications.

REFERENCES

1. Shaji Kumar, M.D, Teresa Kimlinger, B.A, and William Morice, M.D, Ph.D., Immunophenotyping in multiple myeloma and related plasma cell disorders, *Best Pract Res Clin Haematol.* 2010 Sep; 23(3): 433–451.



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