

## NEGATIVE DEPLETION CELL SORTING USING A QUADRUPOLE MAGNETIC CELL SORTER

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**INTRODUCTION:** Immunomagnetic cell sorting is a suitable technique for many therapeutic applications. Positive depletion using a Quadrupole Cell Sorter (QMS) has proved to effectively sort cells of interest in a short time with high throughput (Moore, et al. 2001, Nakamura, et al. 2001). Improvements made to the separation device have led to the application of the same apparatus to negative depletion.

Operating the QMS for negative depletion can be applied to depletion of T-cells for bone marrow transplant and to detection of cancer cells in circulating peripheral blood.

From the basic theory developed for QMS separation (Hoyos, et al., 2000) it is understood that parameters such as cell concentration, transport lamina thickness, and magnetization level, among others, govern cell separations. Therefore, it is necessary to evaluate each of those parameters to optimize separation to a desired cell purity level in the negative outlet.

We are showing partial results on negative depletion, and the effect of various parameters that govern this separation.

**OBJECTIVE:** The present study aims to evaluate and optimize the separation parameters governing negative depletion for future clinical application. The model cell system is composed of a human breast cancer cell line (MCF-7) and peripheral blood leucocytes (PBL).

**METHODS:** Peripheral blood buffy coats from apparently healthy donors were purchased from the American Red Cross, Central Ohio Region. Ficoll–Hypaque density gradient centrifugation (1.077 g/ml) was used to separate mononuclear cells from the buffy coat. No red cell lysis was carried out since we have found that leukocytes tend to clump after this treatment. Leukocytes were resuspended

in calcium- and magnesium-free PBS buffer containing 2mM EDTA and 0.5 % bovine serum albumin (BSA). In addition, monocytes and macrophages were removed by allowing them to adhere to plastic for two hours at 37 C in 5% CO<sub>2</sub>. Non-adherent cells were collected and resuspended in PBS buffer.

MCF-7 cells were grown to confluence on Eagle medium supplemented with 10% fetal bovine serum (FBS). MCF-7 cells were harvested using trypsin-EDTA for 5 minutes and then washed and resuspended in PBS buffer.

Cell number and size analysis was carried out using a Coulter Multisizer II system ((Beckmann-Coulter, Miami, FL). PBL cells were accounted in the range from 5.92 μm to 12.57 μm and MCF-7 cells were accounted from 12.57 μm to 40.1 μm. The great size difference allows us to obtain the ratio of MCF-7/PBL cells.

Cell mixture was prepared to a desired MCF-7 ratio by mixing calculated volumes of cell suspensions and adjusting to a desired concentration with PBS buffer. Final concentration of the mixture was determined before each set of experiments.

Leukocytes were labeled using a two-step labeling technique. Since we wanted to deplete our mixture of leukocytes, we chose anti-CD45-PE (Beckmann Coulter, Cat # IM2078) as primary antibody and Anti-PE microbeads (Miltenyi Biotech, Cat # 488-01) as a secondary antibody.

Cell separation was carried out using a QMS system with the latest version of separation column, MarkV1b.

Separation buffer was calcium- and magnesium-free PBS supplemented with 0.5 % of BSA and 2mM EDTA final concentration. Cells were pulsed using an injection valve with 500 μl capacity. Fig. 1 depicts the QMS column used for this separation.

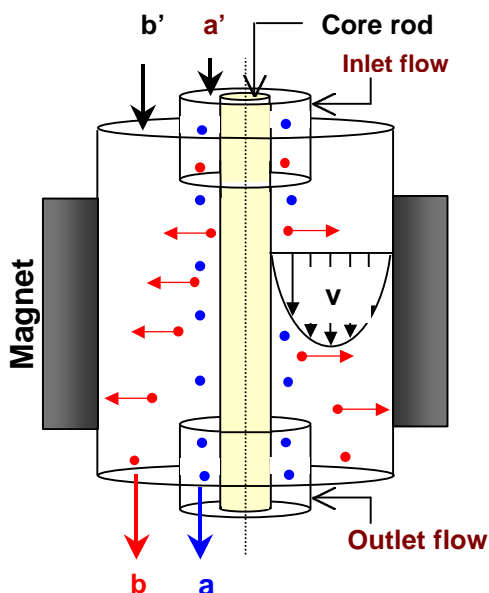


Fig. 1: QMS column showing flow distribution and cell path during separation.

Inlet ports **a'** and **b'** receive cell suspension to be separated and carrier buffer respectively. Immunomagnetically labeled cells migrate toward outlet **b** whereas non-labeled cells elute at outlet **a**. All experiments were performed on a closed system, using a syringe pump to inject buffer and a syringe pump for suction from the outlets. Effluents were analyzed using a Coulter Multisizer II (Beckmann Coulter, Miami, FL), relating size distributions, ratio, purity and recovery percentages of both cell types in each effluent.

**RESULTS:** To accumulate representative results, each experiment was performed three times on different days so as to minimize donor variability. Total cell number on each fraction was calculated by knowing the total volume in calibrated tubes.

There were control samples for this type of separation; labeled leukocytes were the positive control and non-labeled MCF-7 cells as the negative control. In the case of the positive control, 8 % of injected cells went to outlet **a**, 45.4 % went to outlet **b**, and the remaining cells presumably hit the column wall. For negative control (MCF-7), 97 % of the injected cells went to outlet **a**, and 1 % were found in outlet **b**.

Results are shown in such a way that they represent the fraction of the originally injected cells that appear in either outlet **a** or **b**. Then,  $Fe_{tg}$  means fraction of target cells in enriched fraction (outlet **a**)  $Fd_{tg}$  means fraction of non-target cells in depleted fraction (outlet **b**). The same definition applies for non-target cells, which are designated by the subscript **nt**.

Summarized results can be found in Tables 1 and 2.

Table 1. Effect of transport lamina thickness on QMS performance. Cell concentration  $\gg 1 \times 10^7$  cells/ml. Amount of primary and secondary antibody is constant at  $5 \mu\text{l}$  per  $10^6$  cells.

Transport Lamina ( $\mu\text{m}$ )	Feed MCF-7 (%)	$Fe_{tg}$ %	$Fd_{tg}$ %	$Fe_{nt}$ %	$Fd_{nt}$ %
32	1.27	17.50	57.72	2.09	23.45
42	5.78	23.96	59.91	8.38	42.57

Table 2. Effect of cell concentration. Primary and secondary antibody maintained to  $20 \mu\text{l}$  per  $10^6$  cells. Transport lamina thickness constant at  $42 \mu\text{m}$

Cell conc. ( $10^6/\text{ml}$ )	Feed MCF-7 (%)	$Fe_{tg}$ %	$Fd_{tg}$ %	$Fe_{nt}$ %	$Fd_{nt}$ %
5.06	4.69	44.23	42.78	8.75	47.19
3.38	3.19	25.37	54.28	13.15	39.92

**DISCUSSION AND CONCLUSIONS:** A saturation study previously carried out (data not shown) indicates that  $5 \mu\text{l}/10^6$  cells of antibody is insufficient for saturation. In fact,  $20 \mu\text{l}/10^6$  cells is required.

The QMS system is still under development and it is necessary to improve the design of the column as well as to include in the current mathematical model some phenomena we have observed with other apparatus also developed in our laboratory. From results obtained when running the control samples, we have noticed that when there is a single cell population inside the column, the effluent compositions are very close to the theoretical predictions. However, when two different cell populations are present, the theory does not closely match the effluents.

This effect has been observed during Cell Tracking Velocimetry (CTV) experiments and it is apparently related to cell concentration (McCloskey, K., et al 2001). The higher the cell concentration, the greater this effect seems to be. We are attributing those results to a drafting effect occurring inside the column. This drafting effect occurs due to the large number of immunomagnetically labeled cells susceptible to the magnetic field. Consequently, we have reduced the number of cells injected into the column. We expected to obtain better results by this measure; however, we did not find any improvement in the results. This can be attributable to the low cell concentration in each effluent. It is necessary to try methods such as filtration to obtain a measurable cell concentration with low variation.

Our results suggest that there may be cell interaction (PBL-MCF-7) inside the column and

that this plays an important role in cell separation. It is necessary to run experiments using different cell lines, since MCF-7 cells are known to form clumps, making quantitation rather difficult. It is important to mention that it is necessary to perform more experiments in order to completely characterize the instrument for different cell systems and begin clinical applications that will show its relevance in the field.

**REFERENCES:** Moore, Lee R., et al. (2001) *J. Magnetism Magnetic Materials* **44**:115-130. Nakamura, M., et al. (2001) *Biotechnol Bioeng* **17**(6):1145-1155. Hoyos, et al. (2000) *J Chromatography A* 903:99-116.

**ACKNOWLEDGEMENTS:**

This work was supported by the National Cancer Institute (R33 CA81662-01 to JJC, RO1 CA62349 to M.Z., and CA16058-25 to OSU) the National Science Foundation (BES-9731059 to J.J.C).