

Cell Analysis System Based on Immunomagnetic Cell Selection and Alignment Followed by Immunofluorescent Analysis Using Compact Disk Technologies

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Background: Although the flow cytometer has become the standard in cell analysis, it has limitations. Recently, we introduced a new cell analysis method based on immunomagnetic selection and aligning of cells. No flow system is needed and cell analysis can be performed in whole blood.

Methods: Whole blood is incubated with fluorescent labels and immunomagnetic nanoparticles. The blood is injected into a capillary that is in a strong magnetic field. The immunomagnetic-labeled cells move upward and align themselves along ferromagnetic lines present on the upper surface of the capillary. An optical focus and tracking system analogous to that used in a conventional compact disk player focuses a 635-nm laser-diode on the magnetically aligned cells. The emitted fluorescence signals are projected on two photomultipliers. Allophycocyanin (APC)-labeled CD4 (CD4-APC) and Cyanin5.5 (Cy5.5)-labeled CD8 (CD8-Cy5.5) antibodies and Oxazine750, all red excited, are used as fluorescent labels.

Results: A differential white blood cell count performed in whole blood is obtained using the CD4-APC in combi-

nation with Oxazine750. The results are compared with the Technicon-H1 hematology analyzer. Correlation coefficients of 0.91 for neutrophilic granulocytes, 0.93 for lymphocytes, 0.93 for monocytes, and 0.96 for eosinophilic granulocytes were obtained. Immunofluorescence is demonstrated using CD4-APC and CD8-Cy5.5. The absolute counts obtained for CD4+ and CD8+ are compared with the Coulter Epics XL flow cytometer. Correlation coefficients of, respectively, 0.91 and 0.94 were obtained.

Conclusion: We conclude that our system is as capable as a standard flow cytometer or hematology analyzer for a reliable routine white blood cell analysis, including immunophenotyping, and can be used as an easy-to-handle disposable white blood cell test. *Cytometry* 43:31-37, 2001. © 2001 Wiley-Liss, Inc.

Key terms: cytometry; cell analysis; Cell Tracks; ferrofluorides; ferromagnetic nanoparticles; white blood cells

The most commonly used routine cell analysis method is flow cytometry (1). Although a flow cytometer is reliable, fast, and sensitive, the whole system requires handling by trained personnel in a clinical analysis laboratory. An important fundamental drawback is that the cells, once analyzed, are no longer available for repeated or further analysis, for example, microscopic examination of rare event cells. In addition, erythrocytes, present at a density of at least 1,000 times that of leukocytes, have to be removed prior to analysis, or alternatively, the blood sample has to be significantly diluted (2,3).

A number of alternative technologies have been developed that overcome one or more of these disadvantages but all introduce their own specific problems (4-6). We have recently developed a simple system in which cells are separated and aligned by immunomagnetic means.

Then, they are analyzed optically in a manner comparable to that in a flow cytometer (7). This Cell Tracks system allows routine cell analysis in whole blood. In addition, the magnetic forces keep the cells in a fixed position, making it possible to do repeated and further analysis on cells that already have been analyzed.

We compare the differential leukocyte count of a Technicon-H1 hematology analyzer (8) and the CD4 and CD8 count of a Coulter Epics XL flow cytometer with data obtained in our Cell Tracks system. We show that the

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