

Automated Four Colour CD4/CD8 Analysis of Leukocytes by Scanning Fluorescence Microscopy Using Quantumdots

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Background: Flow cytometer and Laser scanning cytometer are usually used for the multi-color phenotyping of leukocytes. The Scanning Fluorescence Microscope (SFM), the new technique for automated motorized microscopes, could also be able to measure fluorescent labelled slides [1, 2]. The T-Helper / T-Cytotoxic (CD4+/CD8+) lymphocyte cell count and ratio are important features of the immune system for immune diagnostics. With the spread of HIV there is emerging demand for automatic methods to determine the CD4/CD8 cell ratio.

Aims: Development of quadruple fluorescent labelling method for leukocytes and application of SFM for their automated analysis and counting on peripheral blood specimen. Study the bleaching of some currently developed dyes.

Materials and methods: EDTA anticoagulated blood samples were stained by the whole blood method by CD4 PE-Alexa-610 (Caltag) and CD8 FITC (BD-Biosciences) antibodies. Parallel staining was made by CD4 FITC (BD-Biosciences) and CD8 biotin/Streptavidin Qdot-605 or Qdot-655 (Quantum Dot Corp.). For the correct T-cell analysis CD3 APC and nuclear counterstaining by Hoechst 33342 were additionally used. An aliquot was measured by flow cytometer (FACScalibur, BD-Biosciences). The remaining suspension was transferred to glass slides and different mounting media were used for saving the fluorescence. In the SFM the specimens were scanned and digitised using four fluorescence filter sets: for Hoechst 33342 at G 365 nm, FT 395 nm, LP 420 nm, for APC BP 575-625 nm, FT 645 nm, BP 660-710, for Qdot605/Qdot655 and PE-Alexa-610 at BP 546/12 nm, FT 560 nm, BP 575-640 nm and for FITC at BP 450-490 nm, FT 510 nm, BP 515-565 nm (all filters from Carl Zeiss, Axioplan-2 MOT). Automated cell detection (based on Hoechst 33342 fluorescence), CD3, CD4 and CD8 detection were performed, CD4/CD8 ratio was calculated.

Results: Fluorescence signals were well separable on both systems. Qdot605 Qdot655 and PE-Alexa-610 were well measurable with by 488 nm laser excitation and FL2/FL3 emission (FCM) or with BP 546/12nm, FT 560 excitation and BP 575-640 nm emission (SFM).

Significant correlation between the SFM and FCM CD4/CD8 ratio results could be observed ($p < 0,05$). In the SFM technique bleaching of dyes were affected by the mounting. In contrast of the organic fluorescent dyes the inorganic Quantumdot staining was very stable in PBS, but bleached shortly after mounting with antioxidant and free radical scavenger mounting media. This finding shows the problematic of staining combination of organic and quantumdot dyes.

Conclusions: Slide based multiple fluorescent labelling system and automated SFM are applicable tools for the CD4/CD8 ratio determination in peripheral blood samples.

1: V.S. Varga; J. Bocsi, F. Sipos, G. Csendes, Z. Tulassay, and B Molnar, "Scanning fluorescent microscopy is an alternative for quantitative fluorescent cell analysis,"

Cytometry, 60A(1), 53-62 (2004).

2: J. Bocsi; V.S. Varga, B. Molnar, F. Sipos, Z. Tulassay and A. Tarnok, "Scanning fluorescent microscopy analysis is applicable for absolute and relative cell frequency determinations", *Cytometry*, 61A(1), 1-8 (2004).

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