Novel Three-Color Reagent for Measurement of CD4 and CD8 Positive Lymphocytes by Flow Cytometry

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ABSTRACT

In HIV infection, the absolute number of CD4 positive lymphocytes is an important indicator for classification of the state of disease, prognosis, treatment decision and monitoring of therapy. Flow cytometric analysis is the current standard method for CD4+ lymphocyte measurement. This flow cytometric method generates accuracy and reliable results but the reagent used is very expensive. In this study, a novel three-color flow cytometric reagent for enumeration of CD4 and CD8 positive lymphocytes was developed. The developed reagent consists of FITC labeled CD3, PE labeled CD4 or CD8 mAb and 7-aminoactinomycin D (7-AAD) solution. 7-AAD is used to intercalate into DNA of white blood cells and allows white blood cells, but not red blood cells, to be detected with FL3 detector of flow cytometer. Fluorochrome labeled CD3, CD4 or CD8 mAb allows determination of CD4 and CD8 positive cells with FL1 and FL2 detector. Comparing to standard flow cytometric reagent, a very good correlation between the developed reagent and the reference reagent for both CD4 and CD8 positive lymphocyte counts was obtained. As the cost of the developed reagent is much lower than the commercially-available reagent, the developed reagent should be appropriate for using in low-income countries.

Key Words: Flow cytometry, Immunophenotyping, Three-color reagent, CD4 positive lymphocytes, CD8 positive lymphocytes, HIV

INTRODUCTION

Human immunodeficiency virus (HIV) is a retrovirus that infects cells which possess the CD4 receptor (Giorgi et al., 1987; De Wolf et al., 1988). This infection causes the depletion of CD4 positive lymphocytes which is a major clinical finding in progressive infection (Giorgi et al., 1987; Fahey et al., 1990). In HIV infection, the absolute number of CD4 positive lymphocytes is an important indicators for prognosis, classification of the state of disease, treatment decision and monitoring of therapy (Fahey et al., 1990; Volberding et al., 1990; Centers for Disease Control, 1992).