

A Simple Method for CD4+ Lymphocyte Counting in Resource-Limited Countries

“CD4 Select Reagent”

Background

Human Immunodeficiency Virus (HIV) infection is increasing at an alarming rate worldwide⁽¹⁹⁾, especially in underdeveloped and developing countries. Progressive clinical and immunologic decline in HIV-infected persons is usually correlated with a falling CD4+ lymphocyte count⁽⁸⁾. Obtaining an accurate and reliable CD4+ lymphocyte count is essential in determining disease stage and progression, as well as, determining when to start or to change antiretroviral (ARV) therapy. In other word, it is used as part of therapeutic monitoring of patients with HIV^(7,12,13,20). Recently, ARV therapy is becoming more accessible to people living with HIV/AIDS in developing countries. A readily accessible laboratory test for enumeration of CD4+ cell count is very important tool for successful HIV/AIDS management program. At present, flow cytometry is the most sophisticated method and generally viewed as the best reference standard for accurate and automated measurement of CD4+ lymphocytes. However, the flow cytometric technology is too expensive and sophisticated to be used, especially, in the remote and limited-resources laboratories^(6, 16). The lack of CD4+ lymphocyte monitoring may render treatment less effective and could ultimately jeopardize the HIV/AIDS care program in developing countries. Alternative low-cost and reliable technologies for enumeration of CD4+ T lymphocytes are therefore needed to supplement or replace the standard flow cytometric method.

A monoclonal antibody (mAb) named “MT4”, specific to the CD4 molecule, was generated in our laboratory⁽¹⁴⁾. This mAb has a special feature compared with other reported CD4 mAbs. The MT4 mAb reacts only to CD4+ lymphocytes. On the other hand, it does not react

or very weakly react with CD4+ monocytes. Therefore, it can be indicated that MT4 mAb is useful as a tracer for determining CD4+ lymphocytes without interference by monocytes.

Recently, MT4 mAb is used to develop a simple method, called “CD4 SELECT”, for enumeration of CD4+ lymphocytes. It is simple, inexpensive, and accurate. It requires no flow cytometer other than an automatic hemato-analyzer (CBC machine), which is already available in most hospitals’ laboratories. The CD4 SELECT method is therefore an alternative for enumerating CD4+ T lymphocytes for assessing HIV/AIDS patients, particularly in limited-resource countries.

Characterizes of monoclonal antibody-MT4

A CD4 mAb, named MT4, was generated in our laboratory⁽⁸⁾. By staining WBC with MT4 mAb, CD4+ lymphocytes showed strongly positive reactivity (Fig. 1B; Donors #1 and #2).

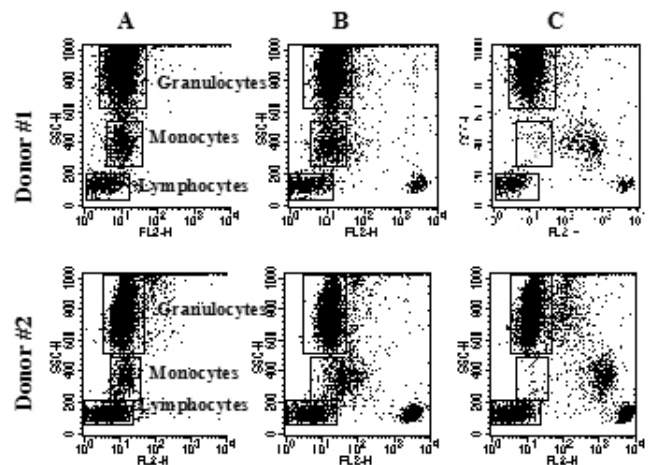


Figure 1. Immunofluorescence analysis of the reactivity of CD4 mAbs MT4 and Leu3a with peripheral blood leukocytes. Whole blood samples were stained with either irrelevant negative control mAb (A), CD4 mAb MT4 (B), or CD4 mAb Leu3a (C) purchased from Becton Dickinson and analyzed by lysed whole blood indirect immunofluorescence. Granularity (SSC) and PE fluorescence (FL-2) were plotted to show the binding of mAb to each leukocyte population. The fluorescence intensities of negative control mAb of all cell populations are marked by rectangles. Two subjects (donor #1 and donor #2) are shown as representative of four studied subjects.

The MT4 mAb does not react significantly with monocytes (Fig. 1B; Donor #1 and #2). Granulocyte population in all tested subjects was negative (Fig. 1B; Donors #1 and #2). In contrast, CD4 mAb clone Leu3a (Becton Dickinson) reacted to both CD4+ lymphocytes and monocytes but not to granulocytes in all tested subjects (Fig.1C; Donors #1 and #2). These results indicated that MT4 mAb is a unique CD4 mAb that recognizes CD4 protein on CD4+ lymphocytes, but reacts weakly or not at all to CD4 on monocytes.

“CD4 SELECT”: A Simple manual resetting method for determination of CD4+ lymphocytes

As MT4 mAb strongly reacts with CD4+ lymphocytes but does not react significantly with monocytes, this mAb is advantageous for development of any method for enumeration of CD4+ lymphocytes. Using MT4 mAb, we developed a reagent named “CD4 SELECT”. Purified MT4 mAb was coated on ferrous beads. The MT4-coated beads were then incubated with whole blood, and the CD4+ lymphocytes were depleted by a magnetic field. The CD4 depleted blood were then count by an automatic hemato-analyzer and the %CD4+ lymphocytes was obtained by an established formula.

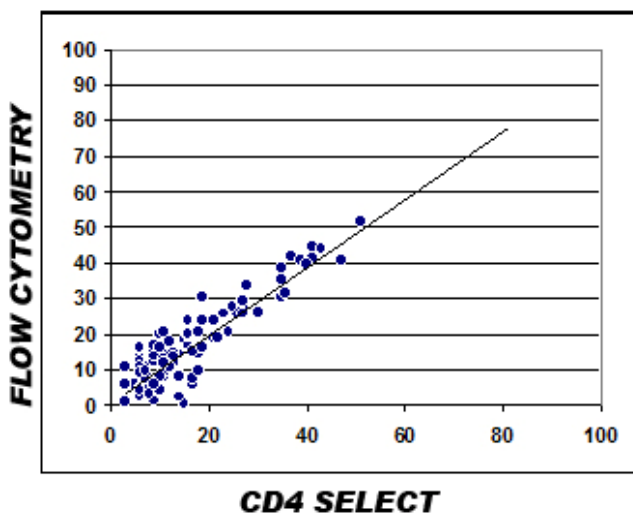


Figure 2. Comparison of %CD4+ lymphocytes by flow cytometry and the CD4 select method (n=100)

As shown in Figure 2, high correlation between CD4 select and standard flow cytometry was obtained (correlation coefficient (r) = 0.920). The Bland-Altman analysis for comparing % CD4+ values generated by the CD4 select and flow cytometry methods was shown in figure 3.

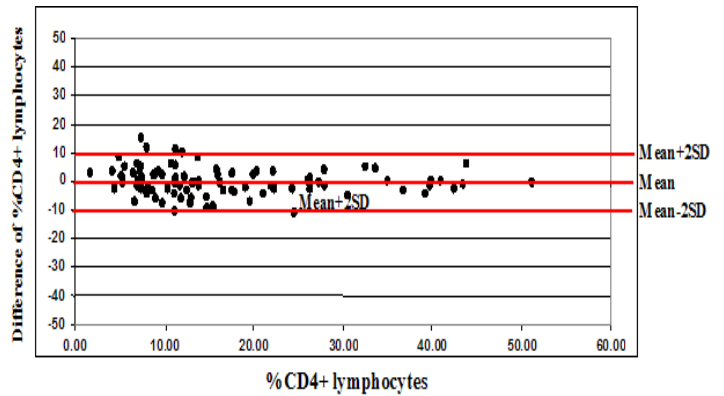


Figure 3. Bland-Altman plot of the differences versus the means of % CD4+ lymphocyte counts by flow cytometry and the CD4 select method.

Conclusion

The standard flow cytometric method is, in general, too expensive and sophisticated to be used in limited-resource countries ^(5, 10). Alternative low-cost and reliable technologies for enumeration of CD4+ T lymphocytes are definitely needed to replace flow cytometry. We have developed a new reagent, called **CD4 SELECT**, for enumeration of CD4+ lymphocytes. It is inexpensive, easy to perform, reliable in identifying those individuals with CD4+ lymphocyte counts less than 200 cells/ μ l, and has good correlation to flow cytometry. Moreover, the same CD4+ lymphocyte cutoff number suggested for flow cytometric method by CDC can also be applied for the **CD4 SELECT** method. By this method, only instruments that are needed to perform the assay is a hemato-analyzer. Then, **CD4 SELECT** will be useful for any laboratories where have limited equipment and small budgets.