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NOTES

Comparison of a New, Affordable Flow Cytometric Method and the Manual Magnetic Bead Technique for CD4 T-Lymphocyte Counting in a Northern Nigerian Setting

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We compared two techniques for CD4 T-lymphocyte counting: flow cytometry (Cyflow) and magnetic beads (Dynabead). Similar results with good correlation were obtained from the 40 adult blood samples counted ($P = 0.057$, $r = 0.93$). The Cyflow technique is more precise and cost-effective than the Dynabead method ($3 to $5 versus $12 to $22 per test, respectively), since as many as 200 samples can be measured per day.

Human immunodeficiency virus (HIV) infection is increasing at an alarming rate worldwide, and the burden is heaviest in sub-Saharan Africa (13). Obtaining accurate, reliable, and affordable CD4 T-lymphocyte counts is essential in determining disease stage and progression. It is also crucial in determining when to start or change antiretroviral (ARV) therapy and while monitoring ARV therapy (3).

Although flow cytometry is the "gold standard" for accurate and automated measurement of CD4 T lymphocytes, the technique is expensive and requires sophisticated equipment as well as trained personnel to perform it. In addition, the lack of ready access to technical support and quality assurance programs has limited the use of flow cytometry techniques in resource-constrained countries (3, 10). On the other hand, manual non-flow cytometric techniques such as the Dynabead and Coulter methods, which produce results comparable to those of flow cytometry, have their own limitations. They are labor-intensive, and only a few (10 to 15) CD4 T-lymphocyte counts can be performed by an analyst per day (3).

Previous reports (3, 4, 10) have shown that older generations of flow cytometers, such as those from Becton Dickinson, Coulter, and Ortho Systems, produce reliable and accurate results which are comparable and correlate with results obtained with non-flow cytometric techniques (e.g., Dynabead). In a study carried out by Crowe and colleagues (3) in 2003, it was found that the Dynal assay was easier to correlate with data from flow cytometry ($n = 54$, $r = 0.96$) than the manual Coulter assay was ($n = 27$, $r = 0.69$). Similar multicenter studies also showed a high correlation ($r = 8.9$ and $r > 0.9$) of flow cytometry (FACScount) with Dynabead CD4 T-lymphocyte counts (4, 10).

We recognize that the optimal design would compare techniques with similar principles and modes of operation (e.g., flow cytometry versus flow cytometry or non-flow cytometry versus noncytometry techniques); however, in our circumstances and setting as a developing country, we have chosen primarily to compare the two techniques (a simple, automated Cyflow method versus the manual Dynabead method) which are the present methods available to us for CD4 T-lymphocyte enumeration to determine which technique would be more cost-effective for CD4 T-lymphocyte estimation in order to meet the overwhelming demand for the test in our center.

This study was conducted at the AIDS Prevention Initiative Nigeria (APIN) laboratory, Jos University Teaching Hospital (JUTH), Jos, Nigeria, between January and March 2004. Blood samples (7 ml) were collected in EDTA Vacutainer containers from each of 40 adults consisting of healthy volunteers, pregnant women, and HIV-positive patients in order to obtain a wide range (low, medium, and high) of CD4 T-lymphocyte counts. All blood samples were analyzed in duplicate for CD4 T lymphocytes within 6 h of collection. This study was approved by the Ethical Committee at JUTH.

The Cyflow instrument (Partec, Munster, Germany) is a newer generation of flow cytometer which does not require optical alignment, and the protocol used for CD4 T-lymphocyte count in this instrument does not require washing or lysing of cells. It involves a one-step pipetting technique. The reagents and protocol for CD4 T-lymphocyte count were obtained from Partec. A total of 50 μl of well-mixed whole blood in EDTA was placed in the test tube provided, and 10 μl of CD4-PE EDU monoclonal antibody was added. The contents of the tube were mixed gently and incubated in the dark at room temperature for 15 min. Following incubation, 800 μl of dilution buffer was added to the tube to give a total volume of

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860 μl and a dilution factor of 17.2. The tube was mixed gently for 5 s to resuspend cells immediately before counting. Calibration of the Cyflow instrument was done with standard stained beads of known concentration to obtain the best peak and resolution for counting $CD4^+$ T cells.

Each blood sample was prepared as stated above in duplicate and measured directly with the Cyflow flow cytometer with FlowMax software (Partec). The Cyflow cytometer is equipped with only one excitation light source, of 532 nm (green solid-state laser). Data acquisition and analysis were performed in real time with a connected desktop computer.

The manual magnetic bead method (Dynabead) requires a three-step technique: depletion of monocytes in 125 μl of whole blood with 25 μl of CD14 antibody (Dynabead M-450 CD14), addition of 25 μl of CD4 antibody-labeled magnetic beads (Dynal M-450 CD4), and counting of acridine-stained CD4 nuclei in a Mallassez counting chamber. The CD4 nuclei counted were multiplied by a dilution factor of 2 to obtain CD4 T-lymphocyte concentration per microliter of whole blood. The reagents and protocol were provided by Dynal, Compiégne, France.

A reproducibility test was performed using a blood sample of known CD4 T-lymphocyte concentration in the low-borderline region (250 to 350 cells/μl) of clinical significance. The blood sample was tested as described in the above protocols for Dynabead and Cyflow for CD4 T-lymphocyte count, and 10 sets of separate and independent sample preparations were made for each method. The same analyst handled sample preparation and cell count for each method.

The mean of the duplicate CD4 T-lymphocytes counted for each of the 40 subjects by the two methods was calculated. The means of the total CD4 T-lymphocyte counts obtained with each technique were classified into three groups: (i) low CD4 T-lymphocyte count (<400 cells/μl; $n = 17$), (ii) medium CD4 T-lymphocyte count (400 to 700 cells/μl; $n = 11$), and high CD4 T-lymphocyte count (>700 cells/μl; $n = 12$). The total results and the subgroups were subjected to statistical analysis with the paired t test. Correlation coefficients were obtained for the total CD4 T-lymphocyte count for the two techniques. A P value of <0.05 was considered statistically significant. In addition, the coefficient of variation (CV%) was calculated for the separate sets of 10 CD4 T-lymphocyte counts obtained for each method in the reproducibility test. Number Cruncher Statistical System (NCSS) software and Microsoft Excel were used for data analysis.

The differences between the overall means ± standard deviations (SDs) of CD4 T-lymphocyte counts obtained by the Dynabead (475 ± 270 cells/μl) and Cyflow (508 ± 283 cells/μl) techniques were not statistically significant. Although the same phenomenon was observed for the differences between the means of the medium- and high-CD4-T-lymphocyte-count subgroups, those in the low subgroup showed a significant difference ($P = 0.048$). Cyflow CD4 T-lymphocyte counts in the low group were higher than the counts determined by Dynabeads.

There was a strong correlation ($r = 0.93$) between the total CD4 T-lymphocyte count obtained with the Dynabead method and that obtained by the Cyflow method (Table 1; Fig. 1). When the same analysis was performed for the subgroups, the values obtained were 0.83, 0.76, and 0.12, respectively, for the low, medium, and high subgroups. The strongest correlation was seen at the lowest level of CD4 T-lymphocyte counts. The 10 separate determinations of $CD4^+$-T-lymphocyte counts performed with the two techniques gave CV% of 7.8% for Dynabead and 1.9% for Cyflow (Table 2).

TABLE 1. Comparison of CD4 T-lymphocyte counts by manual (Dynabead) and flow cytometric (Cyflow) techniques[a]

CD4 T-lymphocyte count group	Count (mean ± SD)		P	r
	Dynabead	Cyflow		
Total ($n = 40$)	475 ± 270	508 ± 285	0.057*	0.93
Low, <400 cells/μl ($n = 17$)	211 ± 95	240 ± 68	0.048**	0.83
Medium, 400–700 cells/μl ($n = 11$)	521 ± 102	536 ± 85	0.575*	0.76
High, >700 cells/μl ($n = 12$)	807 ± 84	860 ± 160	0.285*	0.12

[a] Statistical comparison was performed by the paired t test. *, no significant difference; **, significant difference.

We have compared a new, highly simplified, low-cost, true volumetric flow cytometer (Cyflow) with a manual magnetic bead (Dynabead) method in a setting of a high HIV-AIDS prevalence rate and had three main findings.

Overall, the two techniques (Dynabead and Cyflow) produced comparable and well-correlated CD4 T-lymphocyte counts ($P = 0.057$, $r = 0.93$). These findings agree with earlier reports which involved the comparison of CD4 T-lymphocyte counts by Dynabead and other flow cytometry techniques (3, 4, 10).

The second important result was the finding that Cyflow produced more precise and reproducible CD4 T-lymphocyte counts than the manual method did. The analysis of the CD4 T-lymphocyte data obtained from 10 separate determinations performed on the same sample with the two methods showed CV% of 1.9% for Cyflow and 7.8% for Dynabead (Table 2). The performance of Cyflow in this study compared well with data generated with flow cytometers operating with single and multiple platforms (11, 12). The marked difference in CV% observed for Dynabead and Cyflow is not surprising. Manual microscopic techniques suffer limitations in accurately estimating cell counts in a sample with extremes of low and high cell populations compared with automated techniques (2). In the low-CD4-T-lymphocyte group, the Cyflow method gave results 10% higher on average than those of the Dynabead method. Does such a count make a difference? We contend that it does because the absolute CD4 T-lymphocyte count is used as a cutoff point to determine when to administer ARV drugs and also for monitoring immunologic response to ARV drug therapy. This is an exceptionally important consideration in our environment and other developing countries where ARV drugs are scarce.

Our third important finding is that, although the initial investment on purchasing the Cyflow instrument is about 22,000 euros, it is in the long term a cheaper, more cost-effective, and more practical method for use in tertiary or referral centers that handle large numbers of blood samples for CD4 T-lymphocyte estimation. As many as 200 samples can be analyzed per day compared to 10 to 15 samples with the Dynabead techniques (6). The cost per test for Cyflow is between $3.00 and $5.00, whereas that of Dynabead ranges from $12.00 to $22.00 and other flow cytometry techniques are as high as $30.00 to $100.00 per test (1, 6). Therefore, Cyflow is three- to fourfold more cost-effective. The Cyflow instrument, unlike the

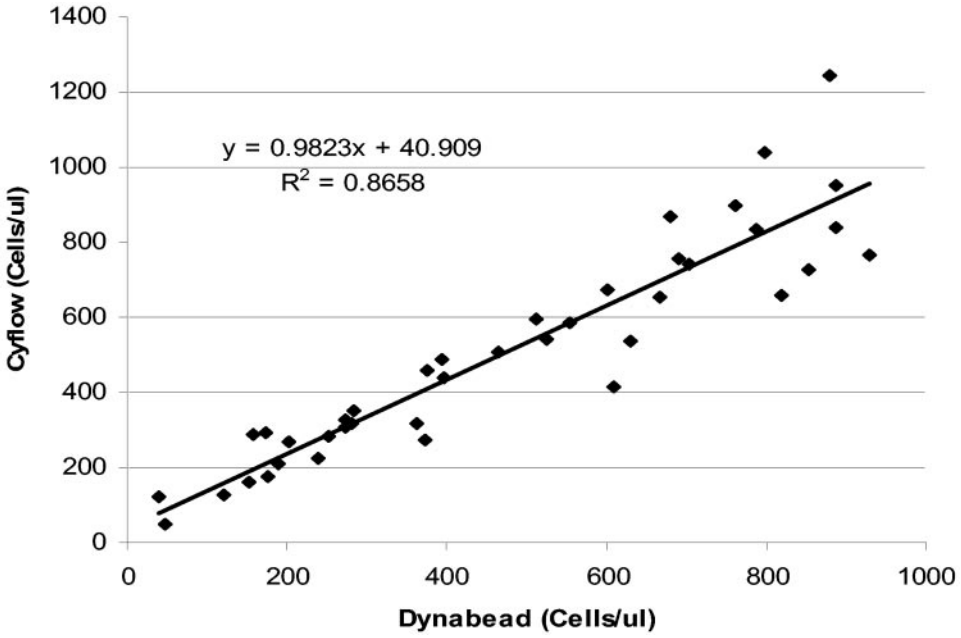

FIG. 1. Comparison of CD4 T-lymphocyte counts by Cyflow and Dynabead methods.

older generations of flow cytometers, uses one antibody step. The protocol does not require cell lysing, a procedure known to reduce CD4 T-lymphocyte count by 0 to 40% (5–7), and it has no need for frequent optical alignment by a well-trained operator.

Access to ARV therapy is affected by the cost of the drugs and laboratory monitoring tests. Recent protests aimed at reducing the cost of ARV drugs spearheaded by South Africa have forced some pharmaceutical companies to lower their drug prices by 90% (13). Similarly, the introduction of affordable monitoring tests such as CD4 T-lymphocyte counts and viral load assay will go a long way in helping to realize the proposal by the World Health Organization for the treatment of 3 million HIV-positive patients by the year 2005 (3, 8, 9).

In our center in Jos, north-central Nigeria, the reduction of the cost of CD4 T-lymphocyte counts from $22.00 to $5.00 by employing the Cyflow method facilitates our management of about 2,000 patients enrolled in our national ARV treatment project. There is no doubt that the manual CD4 T-lymphocyte enumeration techniques have their rightful place, especially in secondary and primary centers where only a few blood samples are tested per day. In these centers, the cost per test can further be reduced by the use of an ordinary light microscope instead of the more expensive and more sensitive UV fluorescence microscope.

In places where the HIV-AIDS burden is high and demand for CD4 T-lymphocyte enumeration is high, the Cyflow technique, which runs as many as 200 samples per day, is cost-effective and ideal.

TABLE 2. Precision and reproducibility of CD4 T-lymphocyte counts in a blood sample[a]

Sample no.	Count	
	Dynabead	Cyflow
1	252	344
2	292	341
3	254	351
4	278	347
5	264	354
6	258	356
7	300	356
8	296	344
9	280	359
10	316	360
Mean	279	351
SD	21.9	6.8
CV%	7.8	1.9

[a] Statistical comparison was done by a paired *t* test ($P < 0.05$).

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