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E.O. Idigbe, PhD, Director General, R.A. Audu, PhD, Senior Research Fellow, C.T. Oparaugo, FIMLS, MSc, Principal Medical Laboratory Scientist, D. Onwujekwe, BSc, MBBS, Senior Research Fellow, C.C. Onubogu, PhD, Senior Research Fellow, J. Adedoyin, MSc, Research Fellow II, A.G. Mafe, FIMLS, Chief Technologist, R. Okoye, FIMLS, MSc, Chief Technologist, J.I. Onyewuche, BSc, AIMLS, Principal Medical Laboratory Scientist, E.O. Funso-Adebayo MSc, Junior Research Fellow and E. Meshack, MSc, Junior Research Fellow, Nigerian Institute of Medical Research, P.M.B 2013, Yaba, Lagos, Nigeria

Request for reprints to: Dr. R.A. Audu, Nigerian Institute of Medical Research, P.M.B 2013, Yaba, Lagos, Nigeria

## COMPARISON OF DYNABEADS AND CAPCELLIA METHODS WITH FACSCOUNT FOR THE ESTIMATION OF CD4 T-LYMPHOCYTE LEVELS IN HIV/AIDS PATIENTS IN LAGOS, NIGERIA

E.O. IDIGBE, R.A. AUDU, C.T. OPARAUGO, D. ONWUJEKWE, C.C. ONUBOGU, J. ADEDOYIN, A.G. MAFE, R. OKOYE, J.I. ONYEWUCHE, E.O. FUNSO-ADEBAYO and E. MESHACK

### ABSTRACT

**Objective:** To compare the efficacy of the Capcellia assay and the Dynabeads technique against the FACScount technique in the estimation of CD4 T-lymphocytes within a Nigerian setting.

**Design:** Prospective study.

**Setting:** Urban area in Nigeria.

**Subjects:** Ninety seven subjects (51 HIV seronegative and 46 HIV seropositive adults) seen in the blood banks and two out patient clinics in Lagos within the study period.

**Main Outcome Measures:** Dynabeads technique with a higher correlation coefficient is a feasible alternative to the FACScount method.

**Results:** There was an overall correlation coefficient of  $r = 0.75$  for CD4 cell counts as determined by the Dynabeads technique in comparison with the FACScount method. Also, an overall correlation coefficient of  $r = 0.17$  for CD4 cell counts as determined by the Capcellia technique against the FACScount method.

**Conclusion:** Dynabeads technique is simple to carry out and cheaper in terms of demand for human expertise and infrastructural requirements than the FACScounts. Therefore, it was recommended for use in the laboratory for monitoring of ARV therapy in Nigeria and any other resource poor country.

### INTRODUCTION

One of the most important laboratory parameters in monitoring HIV disease progression is the CD4 cell counts (1-3). Absolute CD4 T-lymphocyte count has also been used as a surrogate marker for classification and staging of Acquired Immune Deficiency Syndrome (AIDS). The CD4 T-lymphocyte count is also used as an important test in the monitoring of response by infected patients to clinical management with antiretroviral drugs (4).

Flowcytometry is the standard method for performing CD4 T-cell counts by immunophenotyping but it is expensive, requires highly trained personnel to operate (5-8) elaborate quality control procedures to ensure accuracy and precision of counts (9,10). With these requirements, the flowcytometer is seldom used for routine tests in the developed countries and virtually not available in developing countries. Other simpler and cheaper alternative automated techniques for the determination of CD4 and CD8 cell counts have also

been developed. One of such techniques is the FACScout method which correlates very well with the flowcytometry technique (11,12). In addition, the FACScout method has the advantage of estimating absolute counts from unlysed whole blood and does not need a haematological analyser. Despite these, the cost of the FACScout technique is still outside the reach of most developing countries. The increasing burden of HIV infections in most developing countries of the world demands new techniques for counting CD4 T-lymphocytes at substantially reduced cost (6).

In the effort to have methods that are accessible and affordable by developing countries a few ELISA and manual techniques have been developed. These are much cheaper than the flowcytometry and FACScout techniques and less demanding with regards to requirement for human expertise. Some of the techniques are now available in the market, however, it is important that these techniques are properly evaluated before their use in any setting. The need for routine CD4, CD8 cell enumeration in Nigeria has increased enormously and thus there is the need to adopt an affordable and reliable technique for use in the country.

This pilot study evaluated the efficacy of the Dynabead technique (Manual) and the Capcellia kit (ELISA technique) in the estimation of CD4 cells. These techniques were compared against the FACScout technique as the standard.

## MATERIALS AND METHODS

*Study centres:* The study was carried out at the Nigerian Institute of Medical Research (NIMR) Yaba, Lagos in collaboration with the Department of Haematology, University of Lagos Teaching Hospital, Idi-Araba, Lagos. The study was carried out between September and November 2001.

*Study population:* The study population comprised 97 adults of both sexes aged 18 years and above. This age cut-off was necessary because children aged 17 years and below were not included in the Nigerian ARV programme as at then. The population was drawn from patients who presented at the outpatient clinics and blood bank of the study centres within the period of study. The population comprised of 46 HIV seropositive patients who had earlier been diagnosed at these centres and were at different stages

of infection. The other 51 patients comprised of HIV seronegative patients who had presented at these clinics with mild pathological conditions and from apparently healthy blood donors.

Before commencement of the study, approval of the institutional review board was obtained. The subjects were enrolled after a detailed explanation of the purpose of the study and their informed written consent was obtained. Approximately 10ml of venous blood were aseptically obtained from each of the subjects between 10.00 hours and 12.00 hours on the day's samples were taken. Approximately 4ml of the blood sample were immediately dispensed into sterile ethylenediamine tetra-acetic acid (EDTA) tubes for CD4 cell determination. The other 6ml of blood was dispensed into plain serum bottles for other serological tests, which included HIV screening and confirmatory tests. All the blood samples were immediately transported to the laboratory in Nigerian Institute of Medical Research (NIMR). The blood samples were assayed for both HIV antibodies and CD4 counts within 24 hours of collection.

The bio-data and other clinical information of each of the patients were obtained using a questionnaire adopted for the study.

*Serological methods:* The HIV serostatus of each of the subjects was rescreened at NIMR laboratory. This was necessary to ensure that the serostatus of each of the subjects as earlier established at the clinics was correct. The blood samples were screened for HIV antibodies using the Cappillus HIV-1 / HIV-2 test kit (Trinity Biotech Plc.). The positive results were confirmed using Genie II HIV-1 / HIV-2 EIA kit (Bio-Rad).

The level of CD4 T-lymphocytes in each of the samples was determined using three parallel methods namely the FACScout, the Capcellia and the Dynabead techniques.

*FACScout method:* The FACScout system which is a Becton Dickson's automated instrument is designed for the enumeration of absolute CD4, CD8 T-lymphocytes in unlysed whole blood sample. When the whole blood was added to the reagents, fluorochrome-labeled antibodies in the reagent bind specifically to lymphocyte surface antigens. After a fixative solution is added to the reagent tubes, the samples were run on the instrument. Here, the cells

come in contact with the laser light which causes the fluorochrome-labeled cells to fluoresce. This fluorescent light provides the information necessary for the instrument to count the cells.

**Capcellia method:** This is an enzyme immunoassay based technique for the estimation of CD4 and CD8 cells in blood samples. The method requires the use of manual plate washer and microplate reader as well as magnetic frame. In this study a Dynatech Manual washer (Guernsey Channel Islands) and a Biotech Instrument Microplate reader were used. Sanofi Diagnostic Pasteur, France produced the Capcellia CD4/CD8 kits. The assay is based on time specific capture of the CD4 or CD8 lymphocytes with paramagnetic microparticles coated with capture antibodies. The test was carried out according to manufacturers procedure.

**Dynabeads method:** This is a manual technique that needs essentially a light or fluorescent microscope, Dyna Mechanical rotator and the Dynabeads assay kits (DynaL A/S, Oslo, Norway). It is an immunomagnetic cell isolation method which uses Dynabeads magnetic particles, coated with antibodies to CD4 antigen, to capture and isolate CD4 T-lymphocytes from whole blood. The isolated cells are lysed and the nuclei are counted by light microscopy after staining with turks solution.

**Analysis of data:** All the data generated in the study were collated and analysed statistically using the EPI-INFO version 6.0 software. A linear regression was performed to determine time correlation

coefficients ( $r$ ) for absolute CD4-T-lymphocyte counts obtained with the Dynabeads and Capcellia methods versus corresponding values obtained by the FACScout method. The CD4 counts of the Dynabeads and Capcellia methods were plotted against the FACScout to establish any constant or distinct difference in the two sets of counts.

## RESULTS

Comparisons were made between the CD4<sup>+</sup> T-lymphocyte counts obtained for the Dynabeads and Capcellia with those from the FACScout (Table 1). The overall correlation coefficient obtained by CD4<sup>+</sup> T-lymphocyte counts obtained by Dynabeads method versus FACScout were higher ( $r = 0.75$ ). The  $r$ -value obtained for Capcellia was quite low (0.17). There were four samples that had CD4<sup>+</sup> T-cell counts less than 50 cells/ $\mu$ l which was recorded by the FACScout machine because the software program used for this study could perform analysis and record counts of samples within T-lymphocyte subset counts below 50 and above 2000 cells/ $\mu$ l.

Figure 1 shows the line of regression between Dynabeads CD4 count and FACScout where most of the values lay close to the regression line showing that there was a good agreement between Dynabeads and FACScout methods. Figure 2 shows the line of regression between Capcellia CD4<sup>+</sup> counts and FACScout. Most of the values are scattered away from time line of regression showing that there was a poor correlation between the Capcellia and FACScout methods.

Table 1

*Correlation coefficients of CD4 T-lymphocyte subset counts obtained by Dynabeads and Capcellia CD4 methods when compared with FACScout values*

	FACScout	Dynabeads		Capcellia	
	(n)	r	(n)	r	(n)
All Individuals	97	0.75	97	0.17	81
HIV Seronegative	51	0.67	51	-0.05	45
HIV Seropositive	46	0.49	46	0.061	36
CD4 0-199 cells/ $\mu$ l	10	0.06	17	0.02	17
CD4 200-499 cells/ $\mu$ l	23	0.21	18	-0.24	18
CD4 $\geq$ 500 cells/ $\mu$ l	13	0.63	11	0.15	10

Figure 1

Regression between CD4 counts obtained by FACScout compared with Dynabeads method

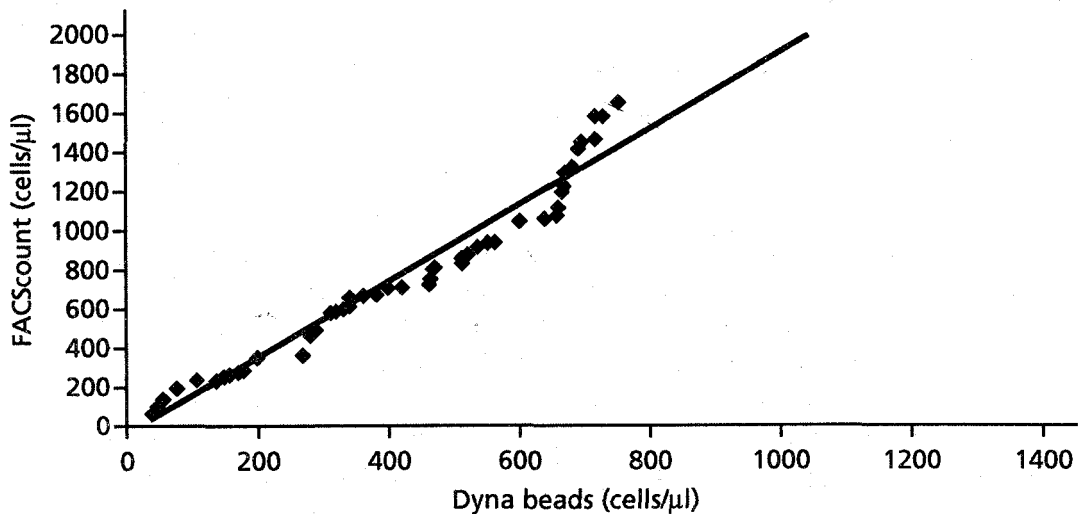
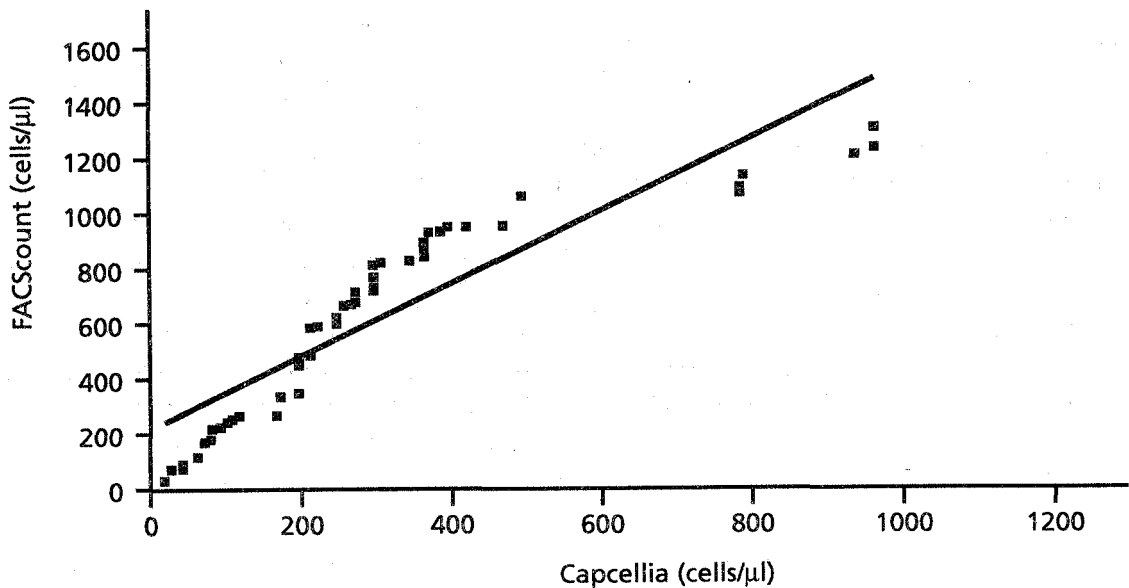


Figure 2

Regression between CD4 counts obtained by FACScout compared with Capcellia method



The FACScout, Dynabeads and Capcellia assays were evaluated for their ability to correctly segregate absolute CD4<sup>+</sup> T-lymphocyte values at the 200 level. Table 2 shows that the Dynabeads method was comparable to time FACScout in segregating patients. The sensitivity was 83% and the specificity was 80%, with a predictive value for CD4<sup>+</sup> T-lymphocyte count  $\geq 200\mu\text{l}$  of 95%. While the comparison of Capcellia and FACScout was not as good as the Dynabeads in segregating

patients (Table 3). The sensitivity was 77% and the specificity was 56% with a predictive value for CD4<sup>+</sup> T-lymphocyte count  $\geq 200$  of 90%. Since there was a better-correlation between the Dynabeads and the FACScout with a higher sensitivity for the Dynabeads, hence a correction factor was determined by linear regression method to be  $Y = 1.29(x) + 171.82$ , where 'x' is the CD4 count as obtained by Dynabeads and 'Y' is the corrected count.

Table 2

Segregation of individuals with use of CD4 T-lymphocytes: FACScout versus Dynabeads

FACScout		Dynabeads		Total
		+(n) CD4/ $\mu$ l <200	-(n) CD4/ $\mu$ l $\geq$ 200	
CD4/ $\mu$ l <200	+(n)	15	3	18
CD4/ $\mu$ l >200	-(n)	16	63	79
Total		31	66	97

Data are number of individuals.  $P < 0.001$  (Chi-Square test). Sensitivity = 83%; Specificity = 80%; Predictive value of CD4/ $\mu$ l  $\geq$ 200 = 95%.

Table 3

Segregation of individuals with use of CD4 T lymphocytes: FACScout versus Capcellia CD4 methods

FACScout		Capcellia		Total
		+(n) CD4/ $\mu$ l <200	(n) CD4/ $\mu$ l $\geq$ 200	
CD4/ $\mu$ l <200	+(n)	13	4	17
CD4/ $\mu$ l $\geq$ 200	-(n)	28	36	64
Total		41	40	81

Data are number of individuals.  $P < 0.001$  (Chi-Square test). Sensitivity = 77%; Specificity = 56%; Predictive value of CD4/ $\mu$ l  $\geq$ 200 = 90%.

## DISCUSSION

There is an increase in the demand for CD4<sup>+</sup> T-lymphocyte determinations in clinical management of HIV infected individuals in Nigeria. This is so because the Nigerian government has embarked on a large programme to provide ARV to ten thousand HIV-1 infected patients. The purpose of the assay is routine and each ARV centre is expected to run 10-14 samples daily. Due to the high demand for CD4<sup>+</sup> T-lymphocyte determination, and being a resource limited country this study was carried out to evaluate and establish a suitable method for CD4<sup>+</sup> cell count estimation in clinical samples in Nigeria. The FACScout is more expensive than Dynabeads and Capcellia techniques with regards to reagents, human expertise and infrastructural requirements (13).

In Nigeria, CD4<sup>+</sup> T-lymphocyte enumeration by FACScout is done only in the HIV reference

laboratory. Hence, the other two alternative methods, Dynabeads and Capcellia, which are cheaper, were evaluated. The performance of time Dynabeads as compared with the FACScout correlates well ( $r = 0.75$ ). This correlation is not as strong as that obtained in other studies ( $r = 0.9$ ) (14) but this could be because a light instead of a fluorescent microscope was used as it is the most commonly affordable type in a resource constrained country such as ours. Absolute CD4<sup>+</sup> T-lymphocyte values <200 is one criteria for AIDS classification. With the use of a value of <200 cells/ $\mu$ l as a cut-off, the Dynabeads method was again comparable to the FACScout with a sensitivity and specificity of 83% and 80% respectively. The Dynabeads and FACScout have been reported by other workers to correlate well within flowcytometer, which is the gold standard (12,15). The Dynabeads is a manual counting technique, which is labourious, and to some extent subjective especially when the nuclei

are fragmented. Counting could sometimes be difficult when there is crowding and overlapping of some nuclei but this could be overcome by diluting such samples before incubation with anti CD4 coated beads. However, a correction factor was determined for Nigerian patients to relate the results obtained by Dynabeads to that of the FACScount. The Dynabeads method permits processing of a few samples at a time which must be analysed within 24 hours of collection hence can be used for only a few patients a day.

The Capcellia CD4/CD8 ELISA is a one-step immunoenzymatic assay used for counting CD4<sup>+</sup> and CD8<sup>+</sup> T-lymphocytes in human blood. It can measure CD4<sup>+</sup> T-lymphocytes from within the range of 15-1250 cells/ $\mu$ l. This method can run 19 samples within 2 hours which should be suitable for laboratories with a limited number of personnel. Though this method is simple to perform, the plotting of graph and extrapolation of the results is very subjective and requires some level of skill. The correlation between this method and the FACScount for CD4 was low. This was further confirmed by the lower sensitivity and specificity (77% and 56% respectively) obtained for correctly establishing CD4 counts <200 cells/ $\mu$ l. This low performance of this immunoenzymatic assay is similar to findings of other workers on another enzyme linked immunosorbent assay-TRAx CD4 ELISA (14,16). The reason for low performance in the TRAx CD4 ELISA assay was attributed to higher CD4<sup>+</sup> counts obtained by the assay as it is not specific for the T-lymphocytes. But in this case, the Capcellia assay is designed to be specific for CD4<sup>+</sup>/CD8<sup>+</sup> T-lymphocytes yet it has a lower sensitivity and specificity for capturing these cells.

In conclusion, the Dynabeads method has a better correlation with the FACScount and had a higher sensitivity and specificity than the Capcellia assay. Though, only few samples are processed at a time by the Dynabeads method and the visual counting is quite subjective, it will be more suitable for use in Nigeria and other resource poor countries for measuring CD4<sup>+</sup> T-lymphocytes.

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