Comparison between Fascalibur and Fascount Analyzers of CD4 Counts

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Abstract- The decision to commence antiretroviral therapy (ART) is clinical and made in consultation with the patient with the help of basic laboratory investigations. Clinical assessment includes staging of (Human Immunodeficiency Virus (HIV) diseases, determining other existing conditions, identifying other medications including traditional therapies, body weight, and assessment of patients' readiness for antiretroviral therapy. The laboratory play an important role in initiation and follow up of already enrolled clients. CD4 cells can be constantly estimated to obtain values before the initiation of therapy and even during chemotherapy. This can be achieved through the use of flow cytometry instruments for estimating CD4 cells in individuals, such as Fascalibur or Fascount ; which work under the same principle but occasionally produces different results. There is need to establish their accuracy, reliability and reproducibility. This study was aimed at investigating the accuracy and reliability between Fascount and Fascalibur in CD4 count enumeration.

Index Terms- Antiretroviral therapy, CD4 count, Fascalibur, Fascount, Guava

I. INTRODUCTION

The initiation of antiretroviral therapy depends on the stage of human immunodeficiency virus (HIV) infection which is determined through chemical tests *(Carlos et al, 2010*.). Once it is decided that an individual infected with the virus should start treatment, variety of factors should be considered when choosing the right combination of drugs for the individual, and the effectiveness of their treatment must be monitored so that if necessary treatment regimen can be switched (*Egger et al., 2002).*Starting treatment depends on the stage of HIV infection; most guidelines especially the World Health Organization (WHO) recommends that individuals should not start antiretroviral therapy until the patient is at the advanced stage of HIV infection (HIV/AID stage 3 and 4), because it is an important decision with long term consequence *(Mohamed et al,2011).*

In order to decide whether a HIV positive patient should start treatment, important clinical tests need to be carried out in the laboratory. These tests determine the stage of HIV infection and individual patient's willingness during treatment, which may depend on a variety of factors such as the patient medical history (Mohamed *et.al,* 2011).The first and the most important test is CD4. A CD4 test measures the number of T-help cells (in a cubic mm of blood) which is known as CD4 count. A non- infected HIV individual has between 500-1200 cells / mm^3 while in an infected individual, the CD4 count often declines gradually. (Bernard ,*et al.,*1998).HIV treatment is generally recommended when CD4 test shows fewer than 350 cells / mm^3. The World Health Organization 2010 guidelines recommend starting treatment for all patients with CD4 count of 350 cells / mm3 (Mohamed *et.al*, 2011). Although in most resource limited countries aim to fully observe WHO 2006 guidelines which starts treatment at less than 200 cell/mm3, apart from pregnant Mothers who start at CD4count of 350cells/mm3 and administer treatment if there are complications, such as if the patient has hepatitis B, or show signs of WHO clinical stage 3 and 4 which comes due the emergence opportunistic infections (such as PCP) and cancers*(Zolopa et.al, 2009).*

The CD4 count is performed using either the fascalibur which produces all CD4, CD8 and CD45 at the same time or a fascount which produces CD4 cells result and ratio (CD_3:CD_4) only.CD4 count tells the number of CD4 cells or T–cells in a sample of blood while CD4% tells how many CD4 cells one has, relative to other immune system in that sample. A-CD4%of 14%-28%, typically means CD4 count is in the range of 200 – 500cells/mm^3 (*Bartlett et.al, 2007).*

II. MATERIALS AND METHODS

Study Area

The study was conducted between June – August, 2012, in Kisumu District, Nyanza Province, Kenya, in the New Nyanza Provincial General Hospital Laboratory which is run by Ministry of Health, Kenya. Kisumu has a population 216,479 according to Geonames geographical data base.

Study Population

This was an experimental study that specifically focused on patients who are HIV positive and were under care and treatment whose CD4 samples were collected and analyzed in both Fascalibur and fascount to establish accuracy, precision and reproducibility of each instrument then analyzed in the third instrument, Guava EasyCD4 as a Gold standard. The sample size was determined using Fisher's (1998) formula for sample size determination; n = z2Pq/d2 Where n = Desired sample size population <10,000; Z = standard normal deviate – set at 1.96 at 95% confidence level. P = proportion of the subjects having the correct CD4 results. For this Study P will be estimate at 0.19, q = 1 –p. Therefore the desired sample size was calculated as follows: $= \frac{1.96^2 \times 0.19 \times 0.71}{0.05^2} = 199$

It follows that 199 blood samples were collected for the study and CD4 estimation.

Inclusion and Exclusion Criteria of the Study Subjects

The study was only carried out on patients who attended the laboratory for the CD4 count both in ART programme and those with new HIV infections and give signed consent to enroll in the study.

Blood Sample Collection and Determination of CD4 cell counts

Blood Samples collected both in the laboratory and from peripheral sites were analyzed for CD4 count in the CD4 section using BD fascalibur (BD sciences). BD fascalibur is a four – color dual laser, bench top flow cytometry system that provides both cell analysis and sorting and uses approximately 50µl of blood stained with 4 colour antibodies having FITC, APC,PerCEP and APC and Lysed with 450mls of lysing solution diluted in distilled water and estimated according to SOPS. New clients with CD4 of less than 350 were introduced to ART programmed according to WHO guidelines 2010.

All the results which were between 300-350 cell/mm3 were re-analysed on the BD fascount, a single platform based estimated instrument which also uses 50ul in a CD3/CD4 reagent and fixed with a fixative which was added after one hour of incubation and then the sample was fed manually into the BD fascount and analysed.

The two results from different instruments but of the same sample were then analysed on the third confirmatory instrument guava (Guava technologies) which showed the level of variation, accuracy and precise of the two instruments (fascalibur and fascount)

III. DATA ANALYSIS

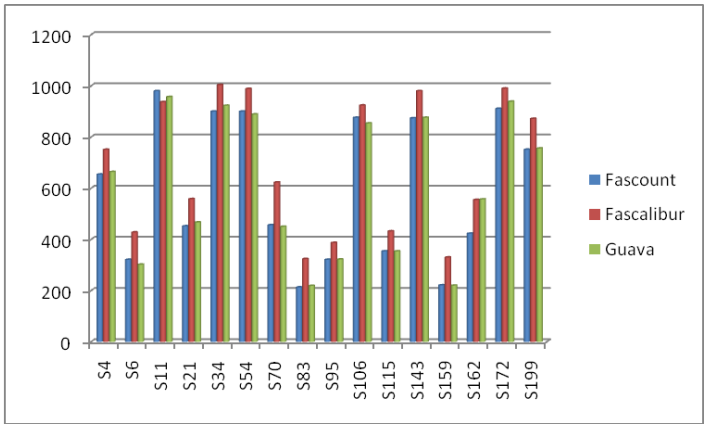
The data was collected using standard check sheet, fascalibur instrument, fascount and Guava instruments.CD4 samples were analyzed, recorded in a structured check sheet, then used to generate pie charts to give comparative results. Quality control involved analyzing respective control samples in both instruments and performing calibration in fascalibur using calibrite beads every day. Reagents were stored under recommended temperatures and no expired material or reagent was used in the study. Guava instrument was taken as the gold standard, the performance of fascalibur and fascount instrument in CD4 estimation was compared to generate summary statistics. Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS version 18) software; generated data was then presented and interpreted using charts.

IV. RESULTS

Out of 199 samples analyzed for CD4 count in both Fascalibur and Fascount, 16 samples had varying results with over 50cells\mm^3.

Fascalibur instrument consistently produced results which tended to be higher than fascount. When the 199 samples were analyzed using Guava as the Gold standard, most of the results produced were closely comparable as opposed to Fascalibur. Fascalibur and Fascount showed very varied results especially on samples with higher CD4 count of above 500cells/mm3 and samples with 450-200cells/mm^3.There was no major variation in results of samples with less than 100cells/mm^3

Table1.Comparison of CD4 measurements using Fascount, Fascalibur and Guava



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