Accurate and Reproducible Enumeration of Cd4 T Cell Counts and Hemoglobin Levels Using a Point of Care System: Comparison With Conventional Laboratory Based Testing Systems in a Clinical Reference Laboratory in Cameroon

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Article

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Abstract

Background

Measurements of CD4 T cells and hemoglobin (Hb) are used to determine the immunological state and information about disease progression for HIV-infected patients. We analyze the correlation in CD4 and Hb measurements using BD FACSPresto™ system compared with the BD validated FACSCanto™ II clinical software analyzer and Sysmex XN 1000 haematology analyzer. We evaluated the advantages of using the POC device in the era of scale up in access for HIV patient management in resource limited settings.

Method

The analytical performance of the BD FACSPresto compared with the FACSCanto II flow cytometer and the Sysmex XN 1000 haematology analyzer was evaluated testing 241 routine clinical specimens collected in EDTA tubes from patients attending the Immunology and Microbiology laboratory of Chantal BIYA International Reference Centre (Yaounde, Cameroon) between January and May 2016.

Results

The mean in absolute counts and percentage of CD4 T cells was 606 cells/mL and 25% respectfully via the FACSPresto and 574 cells/mL and 24% respectfully via the BD FACSCanto II. The mean concentration of Hb levels was 11.90 on the Sysmex XN 1000 and 11.45 via the BD FACSPresto. A high correlation ($R^2 = 0.95, P < 0.001$) of Hb level measurements was noted between the BD FACSPresto™ and Sysmex XN 1000 hematology analyzer. Overall, Bland-Altman plot of the differences between the two methods showed an excellent agreement for absolute and percentage CD4 counts and hemoglobin measurements between POC and conventional methods evaluated here. Furthermore, the study demonstrated the ease of use of the BD FACSPresto POC technology in remote areas.

Conclusion

The BD FACPresto is a suitable tool for CD4 enumeration in resource-limited settings, specifically providing a deployable, reliable POC testing option. The BD FACSPresto™ performed appropriately in comparison to the conventional reference standard technologies. The BD FACSPresto system provides accurate, reliable, precise CD4/%CD4/Hb results on venous blood sampling. The data showed good agreement between the BD FACSPresto, BD FACSCanto and Sysmex XN 1000 XN 1000 systems.

Introduction
In the context of HIV, CD4 T cells are infected with the virus and their number decreases [1]. Globally in 2018, more than 38 million people died because of HIV infection and key cells infected [2] are CD4 T cell lymphocytes. Phenotyping of lymphocytes using flow cytometry is extensively used [3, 4] for enumeration of the CD4 absolute cell counts to determine HIV-infection or AIDS disease status, for monitoring disease progression or co-infection, for patient staging, and for initiation of antiretroviral treatment (ART).

Anemia has been identified as an additional parameter to assess the HIV-disease progression and can be diagnosed by measuring the concentration of hemoglobin (Hb) in venous blood. [5]. Nutrient deficiencies, exposure to antiretroviral drugs such as zidovudine or concomitant conditions could be associated with anemia. Close monitoring of pregnant women with preexisting anemia and advanced HIV/AIDS is recommended [6, 7].

The burden of HIV infection is significant in low income countries where infrastructure is lacking or absent. In this context, many pharmaceutical companies proposed new instruments with innovative characteristics like point of care utility able to enumerate only CD4 absolute cell counts like the Abbott PIMA (previously Alere) or CD4 absolute counts and percentages combined with hemoglobin concentration like the FACSPresto from Becton Dickinson[8–10]. Many performance comparison studies have been done with the BD FACSCalibur as a validated reference flow cytometry instrument in different countries in Africa [11–14] or in Asia [15]. To date, there have been no studies published using the validated BD FACSCanto II clinical software to measure the T/B/NK cell populations and other parameters of research and clinical analysis. It is hypothesized that results for CD4% and Hb on the BD FACSPresto system are accurate and reproducible when samples are measured within 24 hours of phlebotomy. The study objective was to demonstrate that the performance of the BD FACSPresto system is comparable to a validated BD FACSCanto II clinical software system used for measuring absolute cell count and percentages of CD4 T cells stained with BD multitest reagents in BD Trucount tubes using venous blood specimens from patients and the hemoglobin concentration determined by the Sysmex XN 1000 hematology analyzer.

Materials And Methods

This study was conducted at CIRCB, the CD4 reference laboratory in Yaounde, Cameroon. The instrument comparison of the POC device, BD FACSPresto against conventional laboratory technologies BD FACSCanto and Sysmex XN 1000 hematology analyzers using venous blood specimens. Blood samples were obtained from patients undergoing routine CD4 monitoring at the reference laboratory. All specimens were processed within 24 hours of collection. EDTA venous blood samples were analyzed on the BD FACSPresto, BD FACSCanto and Sysmex XN 1000 hematology analyzer. Results obtained from BD FACSPresto device were intended for research use only and were not used for clinical patient management. All procedures were conducted under good clinical laboratory practices and good clinical practice guidelines to ensure quality of laboratory testing, safety and confidentiality of subject's participation in the study and quality of results.
Samples

Venous blood (4mL) were collected in K3-EDTA tubes and inverted several times to ensure proper mixing. Samples were excluded from analysis because of instrument problems or not processed as per protocol. Exclusion criteria included the following: poor sample quality, process controls were not tested prior to sample testing, lymphosum failure, samples processed outside the time window for sample staining and/or acquisition, acquisition did not satisfy the minimum number required of lymph events, beads, and time and reagent storage issues.

Analysis on BD FACSPresto

Venous blood specimens collected were analyzed on the BD FACSPresto machine. For analysis on the FACSPresto, a drop of blood from a Pasteur pipette was loaded onto the FACSPresto cartridge and capped and incubated at room temperature for 18 minutes; following incubation the cartridge was loaded onto the analyzer. On each day, prior to testing, the BD FACSPresto instrument was turned on, the instrument QC test automatically run, and results printed. CD4 and Hb external quality controls were run on the corresponding instruments before testing patient samples. Specimens with valid results were analyzed. Results were considered invalid if testing did not comply with the protocol procedures (inclusion or exclusion criteria, testing outside the recommended time window or if system errors suppressed results).

Analysis on FACSCanto II

This BD flow cytometer is a validated in vitro diagnostic instrument, housed at the Reference laboratory of CIRCB. The analysis on the FACSCanto was performed with a BD FACSCANTO validated clinical software for CD4 count. BD CS&T beads and BD FACS 7-color Setup Beads were used. In brief, 20µl of BD Multitest fluorescent conjugated monoclonal antibodies, and 50µl of whole blood were added to the TruCOUNT tube and vortexed for 5 seconds. The Multitest consists of CD3-FITC/CD8-PE/ CD45-PerCP/CD4 APC reagent. The mixture was incubated for 15 min at room temperature in the dark before adding 450µl of FACS™ lysing solution containing 15% formaldehyde and 50% diethylene glycol (BD Biosciences, San Jose, CA) and incubating for an additional 15 minutes in the dark prior to acquisition on the validated FACSCanto Clinical Software with automated gating and analysis. The operator had received appropriate training on the reverse pipetting technique and on the performance of the assay by manufacturer’s guidelines prior to initiation of the evaluation. Internal quality control was monitored routinely and the laboratory staff participate in external quality assurance program with the Cameroon National Quality Assurance program for CD4 enumeration in collaboration with QASI® (Quality Assessment and Standardization of Indicators) in Canada and the UKNEQAS (United Kingdom National External Quality Assurance service for CD4 testing.

The Sysmex XN 1000 hematology analyzer
To determine the percentage of hemoglobin, the Sysmex XN 1000 automated Hematology analyzer was used according to manufacturer instructions. The rate of hemoglobin was measured directly from the venous blood tube. Three levels of the Hb controls (low, normal, and high levels) were used. Sysmex control tests were used for quality control assurance.

Statistical methods

CD4 + T cell counts obtained from the FACSPresto device were compared to counts collected on the FACSCanto. The amount of Hb obtained from FACSPresto device was compared to levels measured on the Sysmex XN 1000 Analyzer at CIRCB. Descriptive statistics were used for the data. Differences in parameters between the two groups were determined by Wilcoxon signed rank test and paired t-test. Passing-Bablok regression was used for the method correlation and correlation coefficients were determined. To determine the bias between the platforms, Bland-Altman analysis was done. The bias was defined as the mean difference between two methods. The limits of agreement were calculated as the mean ± 1.96 Standard Deviation (SD) of the differences of the results obtained. Confidence intervals for the bias and the limits of agreement were calculated. Pollock analysis was done to calculate the relative bias and the limits of agreement, which were defined as the mean ± 1.96SD of the relative mean bias of paired measurements. The data was plotted with the y-axis representing the % difference relative to the absolute value (x-axis) of the comparator test. The percentage similarity between a sample pair was determined and defined as the average between the methods divided by the comparator method multiplied by 100. The analysis was done for comparing CD4% values on the FACSPresto and the BD FACSCanto.

Ethical Review

The Cameroon National Ethics Committee approved the protocol prior to implementation with the number 2016/08/754/L/CNERH/SP. Written informed consent was required for residual samples from routine services CD4 testing. Residual blood from routine testing was used for the analysis. No personally identifiable information was made available to the researchers.

Results

For this study for method comparison, 241 specimens were enrolled. Table 1 shows the characteristics of study participants. Table 2 shows the comparison of hemoglobin testing between BD FACSPresto and Sysmex 1000. There were valid results for 233 specimens for BD FACSPresto and 230 for BDS FACSCanto II. 11 samples were excluded from analysis because of instrument problems or not processed as per protocol described above.
Table 1
Characteristics of study participants

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects (%)</td>
<td>61 (25)</td>
<td>180 (75)</td>
<td>241 (100)</td>
</tr>
<tr>
<td>Median age</td>
<td>36</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>Median CD4 cells/µL (BD FACSCanto™)</td>
<td>346</td>
<td>605</td>
<td>447</td>
</tr>
<tr>
<td>CD4 &lt; 100 cells/µL</td>
<td>8 (13,11)</td>
<td>13 (6,70)</td>
<td>21 (8,24)</td>
</tr>
<tr>
<td>100 &lt; CD4 &lt; 350 cells/µL</td>
<td>22 (36,07)</td>
<td>50 (25,77)</td>
<td>72 (28,24)</td>
</tr>
<tr>
<td>350 &lt; CD4 &lt; 500 cells/µL</td>
<td>11 (18,03)</td>
<td>34 (17,53)</td>
<td>45 (17,65)</td>
</tr>
<tr>
<td>CD4 &gt; 500 cells/µL</td>
<td>20 (32,79)</td>
<td>90 (46,39)</td>
<td>110 (43,14)</td>
</tr>
</tbody>
</table>

Table 2
FACSPresto comparing with Sysmex XN 1000 XT on the Hemoglobin concentration testing

<table>
<thead>
<tr>
<th></th>
<th>BD FACSPresto venous vs Sysmex XN 1000 XT (venous)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>119</td>
</tr>
<tr>
<td>Hgb dl/%L BD FACSPresto (median)</td>
<td>12 (6,5–16)</td>
</tr>
<tr>
<td>Hgd dl/µL Sysmex XN 1000</td>
<td>12 (6,6–16,1)</td>
</tr>
<tr>
<td>Coefficient of determination R2</td>
<td>0,94</td>
</tr>
<tr>
<td>Absolute mean bias (dl/µL) (LOA)</td>
<td>-0,419 (-0,578, -0,261)</td>
</tr>
<tr>
<td>Percentage of similarity</td>
<td>99,1 (2%)</td>
</tr>
</tbody>
</table>

The Deming regression results for the CD4 absolute count, CD4 percentage and the rate of hemoglobin gave R2 = 0.98, R2 = 0.94 and R2 = 0.96 (Fig. 2). Figure 3 illustrates the strong linear correlation between CD4 enumeration machines and hemoglobin instruments.

Performance comparisons were done between the BD FACSPresto system and Sysmex XN 1000 automatic hematology analyzer for the measurement of Hb concentrations. For Hb concentrations in venous blood samples, we observed significant correlations p < 0.001 between the values generated by the BD FACSPresto system and the reference standard, where slope values were 0.91 and R2 value of 0.98, for all participants, In addition an approximately ≥ 95.8% of participants were within the mean ± 1.96 SD of the relative deviation.

Discussion And Conclusions
The first study done in Cameroon on the BD FACSPresto performance was published comparing BD FACSPresto versus PIMA, BD FACSCount and BD FACSCalibur [12]. The CIRCB is a large reference laboratory working under the ISO 15189–2012. Before using new equipment and its implementation throughout the country, a comparison of results generated by a new instrument with the existing gold standard must be performed. The BD FACSPresto is a POC instrument capable of doing CD4 absolute and percentage counts as well as hemoglobin levels. The BD FACSCanto II is the existing instrument at CIRCB for T/B/NK measurements [16] for research with a validated clinical software for CD4 enumeration for HIV infected patients throughout Cameroon. Installing a POC instrument in remote areas with consistent performance characteristics is very important. Analyzing internal laboratory data, and according to WHO and NIH issued guidelines recommending initiation of the ART regardless of the CD4 cell counts with a shift to use viral load for HIV+ subjects diagnostic and monitoring [17], CD4 enumeration still important in the management of HIV infected patient in rural areas of developing countries because not all patients have access to viral load testing. In addition, there is low adoption of viral load as a routine central laboratory test for patients staging prioritization with ART availability is limited [18]; therefore, enumeration of the CD4 cells counts still has a role when viral load is not easily available. CD4 results contribute to the interpretation of HIV genotyping testing (personal data). However, HIV infected subjects have higher risks of co-morbidity and are more vulnerable to opportunistic infections [19]. The present study is the first published paper to demonstrate that BD FACSPresto provides excellent results when compared with a validated BD FACSCanto II clinical software, showing very good agreement between both instruments. This study supports that CD4 T cells measurements were not influenced by the counting method used. The BD FACSPresto results were reproducible when operated by trained operators (but not laboratory technical experts) such as nurses after minimum training (personal data). It is easy to use attesting the fact that BD FACSPresto can accurately identify those patients with ART failure irrespective of technical laboratory expertise.

The BD FACSPresto analyzer has the advantage of having the complete incubation process outside the machine and it can test approximately 60–80 samples daily. The analyzer is an easy to use instrument, providing daily internal quality controls before starting the assay. The analyzer provides absolute, percentage of CD4 + cells and hemoglobin levels [15] useful for monitoring the paediatric population and anaemic condition in pregnancy. Placing this instrument in rural areas will reduce the loss of follow up of patients and will support management of their infections. This POC technology is ideal for cross-training lab staff and representing a breakthrough solution to the most basic operational challenges of flow cytometry guaranteeing the independence of non-expert staff.

Given the introduction of POCs in rural areas for viral load and early infant diagnosis, their validation could be a valuable tool for laboratories facing the accreditation process [20, 21]. The BD FACSPresto could improve the standardization of results which remains a critical point to make clinical diagnoses comparable between different laboratories. Based on internal experience, FACSPresto and FACS CANTO II differ in size/weight, complexity and battery life. The ease of use, the small size/lightness of weight of the BD FACSPresto as well as the time to process a sample, can contribute significantly to improving the turnaround time (TAT) in results to patient. TAT is influenced by the less complex software used with the
BD FACSPresto combined with its deployability, closer to patients being tested. This reduces the need for specimens or patients to be transported to a centralized testing facility for accurate and reliable test results (both diagnostic and monitoring of known infections.

Moreover, another important advantage of FACSPresto is the improvement of safety for the operator. The use of capped cartridges technology on FACSPresto minimizes the exposure to open blood tubes containing infectious material.

BD FACSPresto has showed, in this study, highly accurate results for the routine analysis of lymphocyte CD4 T cells when compared with the widely used conventional flowcytometer FACSCanto II.

In conclusion, the method comparison demonstrated equivalent performance between the BD FACSPresto system and the standard-of-care, the BD FACSCanto II system, the Sysmex 100 XN analyzer for enumeration of the CD4 T cells and Hb in human blood anti-coagulated with EDTA from HIV infected very necessary in limited resources countries.

**Abbreviations**

ART: Antiretroviral therapy

BD: Becton Dickinson

CIRCB: Chantal BIYA International Reference Centre for Research on HIV/AIDS

EDTA: Ethylene diamine tetraacetic acid

Hb: Hemoglobin

HIV: Human Immunodeficiency Virus

MOH: Ministry of Health

NIH: National Institute of Health

POC: Point Of Care

QASI: Quality Assessment and Standardization of Indicators)

TAT: Turn Around Time

TBNK measurements: T lymphocytes, B lymphocytes and Natural Killer lymphocyte measurements

UKNEQAS: United Kingdom National External Quality Assurance service for CD4 testing

WHO: World Health Organization
Declarations

Ethics approval and consent to participate

The study was approved and reviewed by the Cameroon National Ethics Committee under the number 2016/08/754/L/CNERH/SP and was conducted with the consent of the subjects and following the Declaration of Helsinki.

Consent for publication

All the authors consent for publication

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Contributions

BS: contributed in study design, literature search, and manuscript preparation. VC, JT, and AG: helped to conceptualize, write the original draft, and review and edit the manuscript. FMG: review and edit the manuscript. SMS, SM: contributed in study design, literature search, and manuscript preparation. This manuscript was handled by: AN. All authors read and approved the final manuscript.

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References


Figures
Figure 1

Clinical evaluation of the BD FACSPresto system flowchart. Evaluation of the performance of the BD FACSPresto system using venous blood specimens from subjects attending a routine clinic visit.
Figure 2

Deming regression analysis, Deming regression plots for CD4 cell counts, %CD4 and Hb in venous blood. BDFACSPresto vs BD FACSCanto or Sysmex systems. Deming regression results are depicted for venous blood (1A, 1B, and 1C). The CD4 count and %CD4 cells results are shown from weighted Deming regression in 1B and 1C; for Hb, the unweighted Deming regression in 1A. The y-axis displays the predicate method for CD4 cell counts, or %CD4 cells, and the x-axis corresponds to the BD FACSPresto system.
Figure 3

Hemoglobin, CD4 absolute counts, and %CD4 bias in venous blood. Bland-Altman plots illustrate the biases for venous samples with limits of agreement. Biases hemoglobin in 2A, for % CD4 cell counts are shown 2B, for CD4 absolute count in 2C. The x-axis displays the average (Hb, CD4 counts, or %CD4 cells) and the y-axis is the difference (Hb, CD4 counts, or %CD4 cells) the solid line represents the mean bias, the dashed line represents mean bias ±1.96SD.