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EVALUATION OF THE SINGLE PLATFORM MUSE® AUTO CD4/CD4 % SYSTEM IN CAMEROON
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ABSTRACT

Background: **according to who revised guidelines for scaling up antiretroviral therapy (ART) in adults and children living in resource-limited settings, there is an urgent need for laboratory monitoring, including the numeration of CD4 T cells.** ***Objective:*** **the study compared the muse® auto CD4/CD4% System for CD4 t cell enumeration in absolute counts and in percentages, to the Guava® AutoD4/CD4% System.**
Design: **This was a prospective study using adults, adolescents, children and infant's samples.**
Setting: **The Centre International de Diagnostic Medical (CIDM), Yaounde, a research laboratory devoted to HIV screening and monitoring affiliated to the University of Yaounde I.**
Subjects: **K3-EDTA-blood samples from 111 patients (77 adults, 12 adolescents, 18 children and 4 infants) were collected and tested. All participants signed an informed consent form whereas the guardian and parent of children signed the assent form.**
Results: **the absolute CD4 t lymphocyte counts as well as the percentage CD4 lymphocyte of the Muse® AutoCD4/CD4% and GuavaAutoCD4/CD4% Systems, were highly correlated with an interclass correlation coefficient of 0.997 (95%CI: 0.996-0.998) and 0.991 (95% CI: 0.987-0.994) respectively. The Bland-Altman analysis limits of agreement were -5.79 cells/µl (95%CI: [-97.77; 86.19]) for the absolute CD4 T lymphocyte counts and -1.93 (95%CI: [-7.29; – 3.43]) for CD4 T lymphocyte percentage. The numbers of outliers were similar (6/111=5.41%) both for CD4 T lymphocyte counts and percentage. In addition, Cohen's Kappa ranged from 0.95 to 1 according to CD4 T lymphocyte counts thresholds (p<0.001), showing agreement between both methods.**

Conclusion: **this study demonstrates that the muse™ auto CD4/CD4% system constitutes a promising system for CD4 t cell counting comparable to existing reference methods, and should facilitate wider access to CD4 T cell enumeration for adults and children with HIV infection living in resource-limited countries.**

INTRODUCTION

According to the WHO revised guidelines for scaling up antiretroviral therapy (ART) in adults and children living in resource-limited settings [1], there is an urgent need for laboratory monitoring, based on CD4 T lymphocytes numeration for ART initiation and on HIV load to monitor treatment efficacy and early therapeutic failure [2-4].

More recently, the ambitious UNAIDS Fast-Track targets for 2020 urge countries to further accelerate their HIV responses in the coming years in line with the 90-90-90 targets [5]. This should lead to a significant reduction of HIV-related mortality and new infections. A thorough revision of the consolidated WHO guidelines on the use of ART was undertaken in 2015 [6].

The key recommendation was to initiate ART in everyone living with HIV regardless of CD4 T lymphocyte counts, especially since this biological marker may not be universally accessible [6]. However, CD4 T lymphocyte counts remain an important biological marker for ART monitoring for at least four major reasons: i) It is easily procured and used throughout Africa; ii) WHO thresholds for ART initiation as a priority remain based on CD4 T cell enumeration; iii) CD4 will still be used to diagnose immunological failure in the absence of viral load monitoring ; and finally iv) CD4 T lymphocyte count determination is necessary for initiation of prophylaxis for opportunistic infections. During the past decade, several low-cost new generation cytometers operating as single-platform CD4 enumeration have been manufactured and evaluated [7-21].

Against this background, the main aim of the present study was to evaluate the usefulness of the simplified Muse® Auto CD4/ CD4% system (EMD Millipore, CA, USA) for CD4 T lymphocyte numeration (in absolute count and in percentage) compared with a reference flow cytometry method. We focused our evaluation on bias and misclassification probabilities of different CD4 T cell thresholds that are important for ART initiation according to WHO guidelines [6; 22].

MATERIALS AND METHODS

Subjects: Blood samples from 111 patients [(median age:30 years (Interquartile range, 14-38years); range: one month-82 years; 47 (42.3%) males)], including 77 (69.4%) adults (> 19 years), 18 (16.2%) adolescents (10 to 19 years old), 12 (10.8%) children (>1 to 9 years) and 4 (3.6%) infants (< 1 year), were obtained by venipuncture in vacutainer tubes between March and April 2015. These vacutainer tubes contained tripotassium ethylenediamine tetraacetate (K3-EDTA; Becton Dickinson, Franklin Lakes, NJ, US). Participants were HIV-1-infected patients followed for routine biological monitoring at the Centre International de Diagnostic Médical (CIDM), Yaounde, a research laboratory devoted to HIV screening and monitoring affiliated to the University of Yaounde I.

The study was approved by ethics committees of the University of Yaounde I. Administrative authorizations were also obtained from the University of Yaounde I, The Chantal Biya International Research Center for HIV/AIDS, The University Teaching Hospital of Yaounde and The Hopital de District de la Cité Verte of Yaounde. All participants signed an informed consent form whereas the guardian and parent of children signed the assent form.

Clinical specimens and processing: Two aliquots were kept at an ambient temperature of 25oC. All blood samples were anonymously labelled, unlinked to identifiers, and no mention of antiretroviral treatment was made. Each aliquot was first subjected to CD4 T cell count by Muse™ Auto CD4/CD4% system within 1 hour at the CIDM, and the second aliquot was used in

parallel within 1 hour for measurement by Guava® AutoCD4/CD4% Assay System (EMD Millipore, Hayward, CA, USA), chosen as flow cytometry reference analyzer. CD4 T cell measurements: Parallel CD4 T lymphocyte measurements on both instruments, the Muse® Auto CD4/CD4% system and Guava® AutoCD4/ CD4% system were available for a total of 111 tested blood samples.

The Guava® AutoCD4/CD4% Assay System, a previously validated reference method for CD4 counting [19], was used as predicate single platform flow cytometer for CD4 T lymphocyte counts, in absolute and percentage values according to the instructions provided by the manufacturer. The Guava® AutoCD4/CD4% Assay System is manufactured and marketed by the life science business of EMD Millipore, Hayward, CA, USA.

The system consists of a cell analysis instrument called Personnal Cell Analyzer (PCA), comprising a 532-nm green diode laser with a forward scatter detector and 2 fluorescence detectors of yellow (580 nm) and red fluorescence (675 nm), a laptop computer with the Guava® AutoCD4/CD4% software and reagents. The system works on principles of flow cytometry with some modifications.

It uses microcapillary as a flow cell unlike the conventional flow cytometry. The whole blood sample is incubated with a cocktail of antibodies that recognize all lymphocytes and CD4+ cells allowing for the distinction of CD4+ T cells. The CD4+ T cell number is then estimated as the cells simultaneously expressing lymphocytes and CD4 markers. The volumetric control system allows a precise count of cell numbers and measurement of fluid volume and is regulated by a variable-speed fluid (stepper motor syringe) pump that does not require sheath fluid. CD4 T cell measurements with

The Guava® AutoCD4/CD4% Assay system were done according to the manusfacturer's instructions, using 10 µl of EDTA-blood. The Muse® Auto CD4/CD4% system, currently CE-IVD marked, consists of a compact, portable, and easy-to-use cell analyzer, software, and optimized reagents. The Muse® Auto CD4/CD4% Assay includes three modules: Muse® Auto CD4/ CD4%, System Check, and Complete System Clean. The kit is intended to identify and quantify both absolute CD4 T-cell counts and CD4% values in whole blood samples. The CD4% values are the absolute counts of the CD4 T-helper cells expressed as a percentage of total lymphocytes in EDTA whole blood samples from adult and pediatric donors.

The Muse® Auto CD4/CD4% Kit consists of the Muse® Auto CD4/ CD4% antibody cocktail, a proprietary mixture of antihuman lymphocyte antibodies and a monoclonal anti-human CD4 antibody and a lysing solution. The anti-human lymphocyte antibodies detect all human lymphocytes. The CD4 antibody identifies human CD4-T cells. The Muse® AutoCD4/CD4% system is based on touch screen analysis and requires users to mainly input sample name and sample related details. The system performs automated acquisition and gating to provide test results on CD4 T cell count, CD4% of lymphocytes and total lymphocyte counts. The Muse® Cell Analyzer is also based on principles of microcapillary cytometry and provides absolute counts without the use of external beads and generated low biohazardous waste.

Briefly, 10 µl of whole blood samples were added to 10 µl of Muse® Auto CD4/CD4% antibody cocktail, vortexed and incubated for 15 minutes at room temperature in the dark. After incubation, 380 µl of Muse® 1X lysing solution was added to the samples, vortexed and incubated at room temperature in the dark for 15 minutes. The samples were then acquired and analyzed using the Muse® cell analyzer and the Muse Auto CD4/CD4% software module within 4 hours of preparation. In addition, external quality control of the flow cytometry platform was performed on a regular basis.

Statistical analysis:

The following definitions for adults, adolescents, children and infants were used according to the 2015-revised WHO recommendations [6]: an adult is a person older than 19 years, an adolescent is a person 10–19 years old inclusive, a child is a person younger than 10 years old, and an infant is a child younger than one year of age.

The method validator software, version 1.1.9.0 (Philippe Marquis, France); IBM-SPSS Version 21 (Armonk, NY: IBM Corp.), STATA Version 14 (College Station, TX: StataCorp LP), and SAS 9.3 (SAS Institute, Cary NC) were used for statistical analysis. Intraclass correlation coefficients were computed between Muse® AutoCD4/CD4% System and Guava®AutoCD4/CD4% System in absolute count and percentage.

The agreement between Guava® AutoCD4/CD4% System and Muse® AutoCD4/CD4% system was depicted by difference plots as proposed by Bland and Altman [23-24]. The Bland-Altman analysis examines, in a discriminative fashion, whether the methods agree sufficiently well to be used interchangeably. The average of values obtained by the two methods is displayed on the x axis and plotted against the difference between the two methods shown on the y axis. The average difference between the two methods, referred to as bias, was marked on the graph by a horizontal line, and the limits of agreement with a 95% confidence interval (CI) were also depicted. Since CD4 (percent and counts) deviated from normality, median with interquartile range was reported followed by the nonparametric related sample Wilcoxon signed rank test for comparison.

To assess the clinical impact of using the Muse® Auto CD4/CD4% system instead of the Guava® AutoCD4/CD4% Assay System in this setting, the sensitivity and the specificity of the Muse® Auto CD4/CD4% system was calculated to identify patients who had with the Guava® AutoCD4/CD4% Assay System a CD4 T cell count below 200 cells/µl, the threshold of immune-restauration under ART and the threshold for therapeutic initiation according to the 2006-revised WHO recommendations [25], 350 cells/µl, the threshold for ART initiation for adults and children aged more than 5 years according to the 2010-revised WHO guidelines [26] and the threshold for ART initiation as a priority for adults, adolescents and children aged more than 5 years according to the 2015-revised WHO guidelines [6], or 750 cells/µl and %CD4+ ≤25%, the absolute and percent CD4 T cell count according to the 2010-revised WHO thresholds for ART initiation in children aged between 24 and 59 months [27] and the thresholds for ART initiation as a priority for children aged more than 2 years and less than 5 years according to the 2015-revised WHO guidelines [6]. For clinical significance of the measurement differences on treatment decision, the Cohen's k coefficient was calculated on the study population [28].

RESULTS

Precision of CD4 T cell measurements by the Muse™ Auto CD4/CD4% system: The Bland-Altman analysis measured the limit of agreement between Muse® and Guava® (Figure 1 and Figure 2).

Figure 1

Bland-Altman plot between Muse® and Guava® in CD4 count

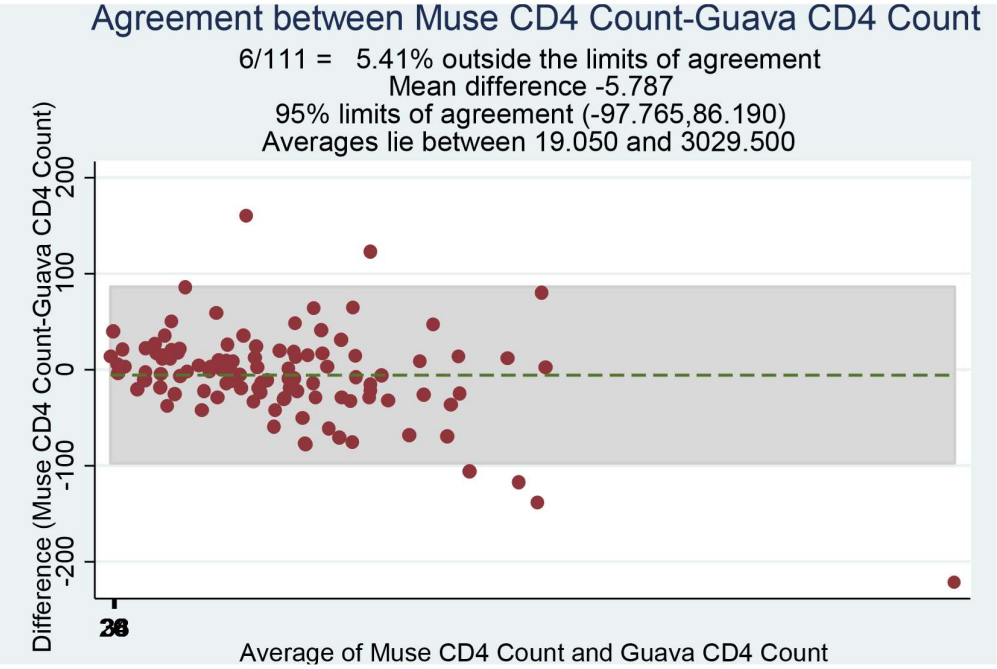
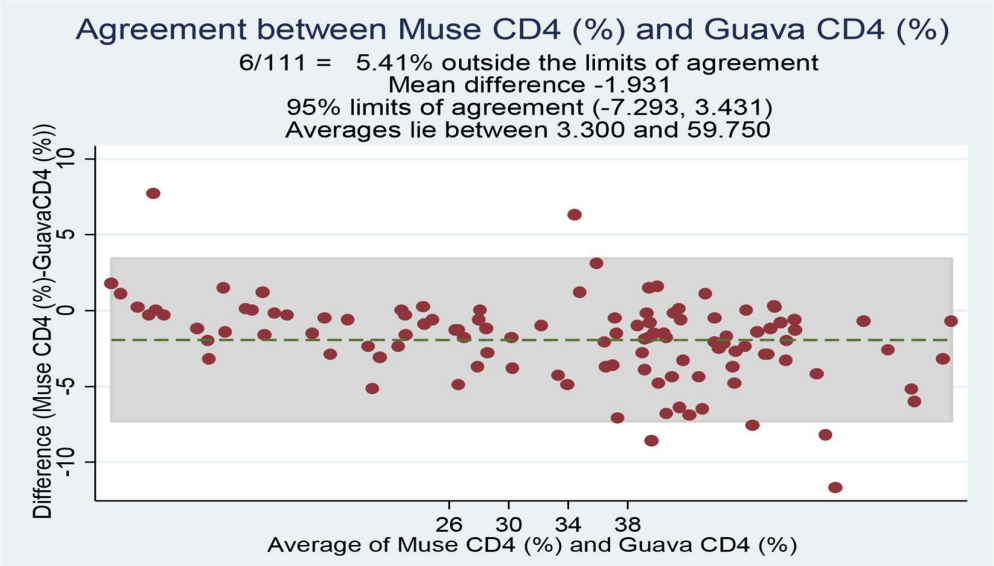


Figure 2

Bland-Altman plot between Muse® and Guava® in percent (%)



The limits of agreement were -5.79 cells/µl (95%CI: -97.77 - 86.19); and -1.93 (95%CI: -7.29 – 3.43) in absolute count and percentage. In addition, the percentages of data points outside the limit of agreement (outliers) were 5.41% (6/111); and 5.41% (6/111) respectively in absolute count and percentage. Thus, Bland-Altman analysis on the relative differences between the absolute and percentage CD4 T cell counts obtained with Muse® Auto CD4/CD4% system and Guava® AutoCD4/ CD4% Assay System showed a concordance between both methods. Accuracy of CD4 T cell measurements by the Muse™ Auto CD4/CD4% system: The accuracy of CD4 T cell measurements by the Muse® Auto CD4/ CD4% system was carried out against the results obtained by the Guava® AutoCD4/CD4% Assay System chosen as reference for CD4 T cell counting (Table 1).

Table 1

CD4 T cell counting in absolute count and percentage in 111 HIV-1-infected patients living in Cameroon, including 77 adults, 18 adolescents, 12 children and 4 infants, by the single-platform Muse® Auto CD4/CD4% system, and by the Guava AutoCD4/CD4% Assay System.

Category	Total	Children and infants (<5years, 16(14.4%))	Adults, Adolexcents, and Children (>=5 years, 95(85.6%))
Absolute CD4 T cells (cells/ml)			
Muse® Auto CD4/CD4% (Median (IQR[a]))	551.20(266.60-829.80)	973.05(643.30-1384.43)	513.50(246.40-769.00)
Guava® AutoCD4/CD4% (Median (IQR))	564.30(254.40-852.00)	919.51(653.73-1385.23)	527.10(228.90-754.80)
Percent CD4 T cells (%CD4)			
Muse® Auto CD4/CD4% (Median (IQR))	37.20(22.80-43.20)	36.30(26.68-39.78)	37.50(19.80-43.60)
Guava® AutoCD4/ CD4%™ (Median (IQR))	39.20(23.80-45.60)	37.10(29.65-41.18)	39.40(22.90-46.60)

[a] IQR: Interquartile range

Median (IQR, Interquartile range) numbers of CD4 T cells/µl expressed in absolute number was 551.20(266.60-829.80) cells/µl (range, 25.7-2918.6) by Muse® Auto CD4/CD4% system, and 564.30(254.40-852.00) cells/µl (range, 9.10-3140) by Guava® AutoCD4/CD4% system(P=0.22) (Table 1).

Both were highly correlated with an intraclass correlation coefficient of 0.997(95%CI: 0.996-0.999). The relation between Muse® Auto CD4/CD4%system and Guava®AutoCD4/CD4% System did not differ from linearity (P=0.6) (Fig Analysis of CD4 T cell count measurement expressed in percentage showed, similarly to CD4 T cell count expressed in absolute numbers, a high correlation and a close agreement between both CD4 T cell counting methods (Table 1). Median (IQR) CD4 T cell count in percentage was 37.20(22.80-43.20) %CD4+ (range, 4.2-59.4) by Muse® Auto CD4/CD4% system, and 39.20(23.80-45.60) %CD4+ (range, 2.30-60.80) by Guava® AutoCD4/CD4% system (P=0.26).

Results of CD4 T cell count in percentage by Muse® Auto CD4/ CD4% system and Guava® AutoCD4/CD4% system shows high correlation with an intraclass correlation of 0.991 (95% CI: 0.987-0.994). CD4 T cell values (in absolute count and percentage) from 111 HIV-1-infected patients living in Cameroon, by Muse® AutoCD4/CD4% system, and by the Guava® AutoCD4/CD4% Assay System, at various CD4 T cell count ranges showed comparable results (Table 2).

Table 2

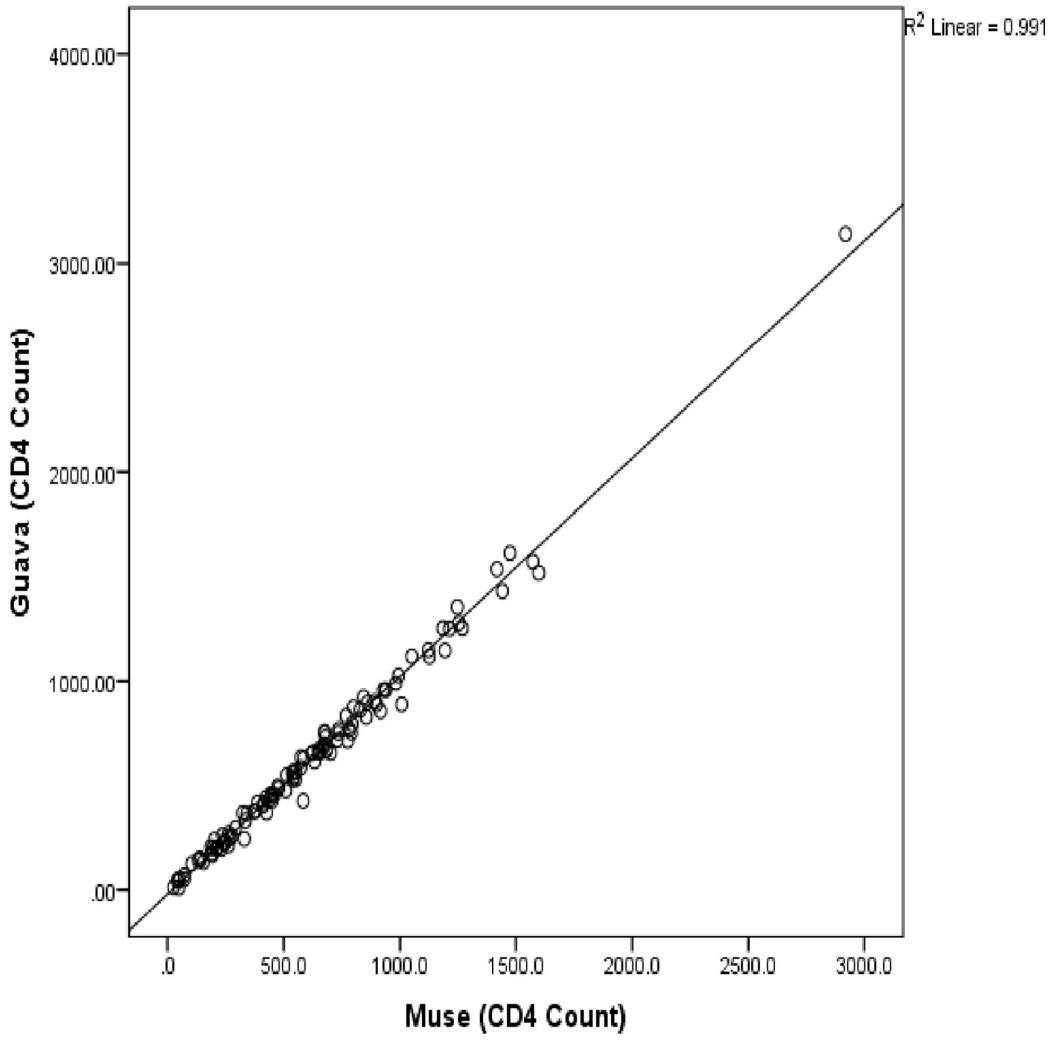
CD4 T cell counting in absolute count and percentage in 111 HIV-1-infected patients living in Cameroon, by the single-platform Muse™ Auto CD4/CD4% system, and by the Guava® AutoCD4/CD4% System, at various CD4 T cell count ranges according to the Guava® AutoCD4/CD4% Assay System results.

Category	< 200 cells/ml 16(14.4%)	200 – 350 cells/ml 16(14.4%)	> 350 cells/ml 79(71.2%)
Absolute CD4 T cells (cells/ml)			
Muse® Auto CD4/CD4% (Median (IQR[a]))	118.25(49.63-182.00)	246.85(213.90-273.35)	682.00(538.90-937.20)
Guava AutoCD4/CD4% (Median (IQR))	129.20(48.15-161.25)	234.65(206.65-259.73)	712.20(527.20-954.50)
Percent CD4 T cells (%CD4)			
Muse®Auto CD4/CD4% (Median (IQR))	9.25(5.85-17.88)	26.00(14.40-42.43)	38.80(31.20-43.90)
Guava® AutoCD4/CD4% (Median (IQR))	9.90(5.25-22.48)	26.45(14.63-43.55)	41.20(32.70-46.90)

Further, the relation between Muse®Auto CD4/CD4% system and Guava® AutoCD4/CD4% Assay System did not differ from linearity (P=0.55) (Figure 4).

Figure 4

relation (CD4 count) between muse® autoCD4/CD4% System and guava® autoCD4/CD4% System



Sensitivity and specificity to identify clinically relevant thresholds by the Muse™ Auto CD4/CD4% system: The sensitivity and specificity of CD4 T cell counting in absolute number on the Muse® Auto CD4/CD4% system to identify relevant thresholds of CD4 T cell count according to the 2015-revised recommandations are depicted in Table 3. The capability of the Muse® Auto CD4/CD4% system to identify patients having less than (or more than) 200 CD4 T cells/µl was evaluated on the 111 available CD4 T cell count measurements.

Taking into account a 10% bilateral range (i.e., counts between 190 and 210 CD4 T cells/µl were considered similar), the concordance between the Muse® Auto CD4/CD4% system and Guava® AutoCD4/CD4% System was high (k=0.95; P<0.001). The decision differed for only two study blood samples.

The Muse® AutoCD4/CD4% system had a sensitivity of 94.7% and a specificity of 98.6% to identify individuals with CD4 T cell counts below 200 cells/µl when compared with the Guava® AutoCD4/CD4% System results. The sensitivity and specificity of CD4 T cell counting in absolute number on the Muse® Auto CD4/CD4% system to identify patients having less than (or more than) 350 CD4 T cells/µl was also evaluated on the 111 available CD4 T cell count measurements. Considering a 10% bilateral range (i.e., counts between 332 and 367 CD4 T cells/ µl), the concordance between the Muse® Auto CD4/ CD4% system and Guava® AutoCD4/CD4%Assay System was high ((k=0.98; P<0.001). The decision differed for only one study blood sample. The Muse™ Auto CD4/CD4% system had a sensitivity of 99.8% and a specificity of 99.3% to identify individuals with CD4 T cell counts below 350 cells/µl when compared with the Guava® AutoCD4/CD4% Assay System results.

Similar sensitivity (100%), specificity (100%) and k index (k=1) were found for the capacity of the Muse® Auto CD4/CD4% system to identify children having less than (or more than) 750 CD4 T cells/µl, considering a 10% bilateral range (i.e., counts between 675 and 825 CD4 T cells/µl), as well as children having less than (or more than) %CD4+ ≤25%, considering a 10% bilateral range (i.e., counts between 22.5 and 27.0 %CD4+).

Table 3

Sensitivity and specificity of CD4 T cell counting by the Muse® Auto CD4/CD4% system to identify patients having less than (or more than) 200 CD4 T cells/µl, 350 CD4 T cells/µl, 750 CD4 T cells/µl and 25 %CD4+.

		Sensitivity (%)	Specificity (%)	Cohen's k coefficient[$]
Thresholds	200 CD4 T cells/µl*,[a]	94.7	98.6	0.95
	350 CD4 T cells/µl**,[a]	99.8	99.3	0.98
	750 CD4 T cells/µl***,[b]	100.0	100.0	1.00
	25 %CD4+***,[b]	100.0	100.0	1.00

A IQR: Interquartile range* 200 CD4 T cells/µl: Threshold of immunerestauration under antiretroviral treatment and the threshold for therapeutic initiation according to the 2006-revised WHO recommendations; ** 350 CD4 T cells/µl.

WHO threshold for antiretroviral treatment initiation in adults and children aged more than 5 years according to the 2010-revised WHO guidelines and threshold for antiretroviral treatment initiation as a priority for adults, adolescents (10-19 years old) and children aged more than 5 years according to the 2015-revised WHO guidelines (WHO, 2015);*** 750 CD4 T cells/µl and 25 %CD4+:

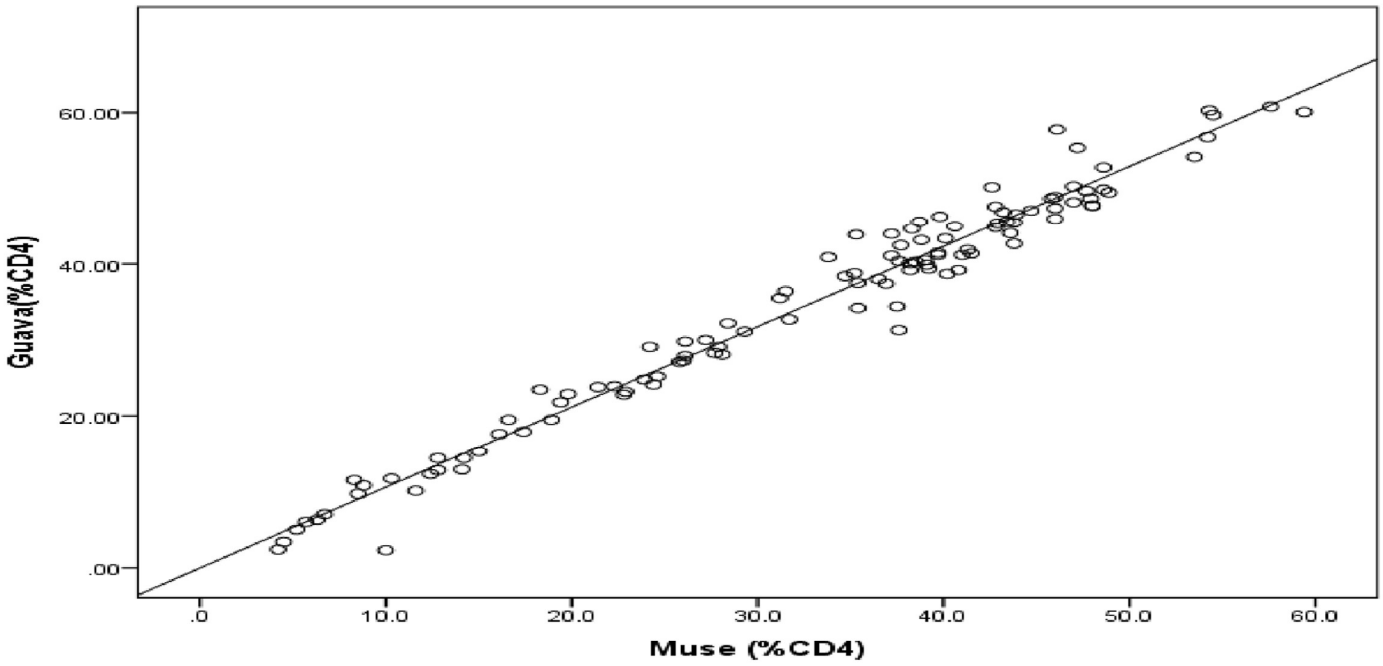
Absolute and percent CD4 T cell count 2010-revised WHO thresholds for antiretroviral treatment initiation in children aged between 24 and 59 months (WHO, 2010a) and thresholds for antiretroviral treatment initiation as a priority for children aged more than 2 years and less than 5 years according to the 2015-revised WHO guidelines (WHO, 2015)a.

The sensitivity and specificity of CD4 T cell counting by the Muse® Auto CD4/CD4% system to identify patients having less than (or more than) 200 CD4 T cells/µl and 350 CD4 T cells/µl, were calculated on the 111 available CD4 T cell count measurements from infants, children, adolescents and adults; b.

The sensitivity and specificity of CD4 T cell counting by the Muse® Auto CD4/CD4% system to identify patients having less than (or more than) 750 CD4 T cells/µl and 25 %CD4+, were calculated on the 20 available CD4 T cell count measurements from children aged more than 2 years and less than 5 years;$.

A 10% bilateral range (i.e., counts between 190 and 210 CD4 T cells/µl for the threshold at 200 CD4 T cells/µl; counts between 332 and 367 CD4 T cells/µl for the threshold at 350 CD4 T cells/µl; counts between 712 and 787 CD4 T cells/µl for the threshold at 750 CD4 T cells/µl; and counts 23.7 and 26.2 %CD4+ for the threshold at 25 %CD4+) was considered similar

Figure 3
relation (%CD4) between muse® autoCD4/CD4% System and guava® autoCD4/CD4% System



DISCUSSION

Our findings clearly demonstrate that the Muse® Auto CD4/CD4% system flow cytometer results correlate with those obtained with the Guava AutoCD4/CD4% System used as the reference method. As demonstrated by the Passing-Bablok regression analysis, the correlation was maintained at different CD4 range values in absolute number and in percentage.

The Muse® Auto CD4/CD4% system accurately assessed the clinically relevant range in absolute number (thresholds of 200 cells/µl, 350 cells/ µl and 750 cells/µl) or in percentage (25 %CD4+). The procedure lasted only 30 minutes against 45 minutes for the Guava® AutoCD4/CD4% Assay. Thus, our findings highlight the fact that the Muse® Auto CD4/ CD4% system is a reliable, reproducible and robust alternative flow cytometer for CD4 T cell counting in absolute number and in percentage and should facilitate wider access to CD4 T cell enumeration for adults and children living with HIV in developing countries.

WHO strongly recommends evaluation of newly introduced affordable CD4 T cell measurement technologies by laboratories of resource-limited setting, and independently of manufacturers [22]. Therefore, the present independent evaluation of the Muse® Auto CD4/CD4% system by our reference laboratory involved in CD4 T cell counting analyzers [19-20], fulfilled the WHO recommendations for evaluation of CD4 T cell assays in resource-poor settings [22].

The Guava® AutoCD4/CD4% Assay System used as the reference system in this study, has been previously evaluated in comparison to standard flow cytometry [21]. A previous assay on the Guava PCA system, which provides CD4 T cell counts, the Guava® EasyCD4™ Assay has been evaluated in comparison to BD FACSCount for CD4+ T cell enumeration in HIV-infected persons in large urban settings in Uganda, Thailand, India,,

in rural Burkina Faso and in the United States [29-34]. Our results are similar to a previous evaluation of the Muse® Auto CD4/CD4% system against the reference BD Multitest assay (Becton Dickinson, San Jose, California) carried out on a limited number of samples [35]. It should be noted that the unique, patented micro-capillary flow cell technology of the Muse® Auto CD4/CD4% system eliminates the requirement for complicated sheath flow fluids and enables absolute cell counts without the need for reference beads, making the system extremely compact, easy to maintain and simple to use.

The scaling up of public ART programs globally has led to an increased demand for CD4 T cell count tests [5], especially to assess treatment eligibility. Given the fact that ART may not be universally provided, WHO defined priorities for ART initiation to all adults and adolescents with severe or advanced HIV clinical disease (WHO clinical stage 3 or 4) and individuals with CD4 count ≤350 cells/mm3, as well as all children from aged 3 to <10 years old with severe or advanced HIV clinical disease (WHO clinical stage 3 or 4) and individuals with CD4% <25% (if <5 years old) or CD4 count ≤350 cells/mm3 (if ≥5 years old) [6].

The accuracy of the determination of CD4 T lymphocytes is of paramount importance when caring for adults or children infected with HIV or suffering from AIDS particularly in developing world settings where viral load determination is not widely available [19-20]. Further, the speed of implementation of CD4 T lymphocyte count facilities has been unrivalled in recent years in resource-limited countries, and has met challenges with technology selection, laboratory infrastructure development, human resource training, cost-effectiveness, instrument maintenance, and ensuring testing access and quality [8; 10; 13; 3638].

These CD4 dedicated flow cytometers were introduced on the market to ensure decentralization of the HIV-monitoring services [8; 18]. Indeed, pointof-care CD4 T cells counting technologies reduce the time and increase patient retention along the testing and treatment cascade compared to conventional laboratory-based testing [39], which are therefore considered as useful tools to perform CD4 T cell counting for expedite result delivery.

In conclusion, the Muse™ Auto CD4/CD4% system constitutes a promising system for CD4 T cell counting comparable to existing reference methods, and should facilitate wider access to CD4 T cell enumeration for adults and children with HIV infection living in resource-limited countries.

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CONFLICT OF INTERESTS.

The authors declare that they have no conflict of interests.

AUTHORS' CONTRIBUTIONS.

FXMK, LB, MAS conceived and designed the research; GCK, JMN, AN were involved in patients recruitment and follow up; FXMK, FTM, JA, HGK performed the experiments; GNT performed statistical analyses; FXMK, GNT, GCK, LB, MAS analyzed the results and drafted the manuscript. All authors approved the final draft of the manuscript.

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