Multisite Evaluation of a Point-of-Care Instrument for CD4+ T-Cell Enumeration Using Venous and Finger-Prick Blood: The PIMA CD4

Papa Alassane Diaw, PharmD, Géraldine Daneau, PhD,† Abdoul Aziz Coly, MSc,* Birahim Pierre Ndiaye, MD,* Djibril Wade, MSc,* Makhtar Camara, PhD,* Souleymane Mboup, PhD,* Luc Kestens, PhD,† and Tandakha Ndiaye Dieye, PhD**

Background: CD4+ T-cell enumeration (CD4 count) is used as a criterion to initiate antiretroviral therapy (ART) in HIV patients and to monitor treatment efficacy. However, simple, affordable, and reliable point-of-care (POC) instruments adapted to resource-limited settings are still lacking. The PIMA CD4 analyzer is a new POC instrument for CD4 counting that uses disposable cartridges and a battery-powered analyzer.

Methods: Whole blood samples were taken by venipuncture or by finger prick from 300 subjects, including HIV-infected patients and HIV (-) controls. CD4 counts were measured by PIMA (using venous or capillary blood) and by FACSCount (using venous blood) considered as the reference.

Results: Similar CD4 counts were obtained by PIMA and FACSCount using either HIV+ venous blood or HIV+ finger-prick blood samples. However, with a concordance coefficient of 0.88 and a Pearson correlation of 0.89, finger-prick blood performed not as good as venous blood (0.97 and 0.98, respectively). For a clinical decision to start ART at 200 CD4 cells per microliter, sensitivity of PIMA was 90%/91% and specificity 98%/96% for venous/finger-prick blood, respectively, and for a treatment threshold of 350 CD4 cells per microliter, the sensitivity was 98%/91% and the specificity was 79%/80% for venous/finger-prick blood, respectively. Repeatability (precision) on venous blood resulted in a coefficient of variation of 4%. Using finger-prick blood, the average instrument error frequency resulting in aborted analyses was 14%.

Conclusions: PIMA is a good POC instrument for screening adult HIV-infected patients in resource-limited settings for treatment eligibility. Its performance on finger-prick blood is not as good as on venous blood. Adequate training for correct use of finger-prick blood samples is mandatory.

Key Words: CD4+ T-cell count, HIV, finger prick, FACSCount, point-of-care, PIMA

(*J Acquir Immune Defic Syndr* 2011;58:103–111)

INTRODUCTION

The HIV pandemic affects approximately 33 million people worldwide, of which 67% are in sub-Saharan Africa. Although access to antiretroviral therapy (ART) has increased considerably during the last 5 years in settings with limited resources, laboratory monitoring of treatment is still a challenge in many regions.[1]

In the early years of the pandemic, CD4 cell counting was only used as a laboratory marker to assess the degree of immune deficiency.[2,3] Today, CD4 counting is mainly used to help clinicians to decide when to start ART and how to monitor treatment efficacy. Medical laboratories in resource-limited settings need affordable and robust CD4 counting instruments adapted to their needs. Apart from the cost of reagents and instrument purchases, indirect requirements such as water and power supply, cold chain assurance, laboratory space availability, maintenance, and human resources are important considerations for the choice of equipment. To meet these needs, alternative and new CD4 instruments are being developed to complement existing conventional but more demanding flow cytometry instruments.[4]

A new point-of-care instrument (PIMA CD4, Alere) to enumerate CD4+ T cells in whole blood from HIV-infected patients was released at the end of 2009. It is a small portable (2.54 kg) and very mobile device equipped with a rechargeable battery very suitable for remote and decentralized health care settings. It is based on digital imaging of fluorescent CD4 cells to count CD4+ T cells in a blood sample. The complete analysis takes approximately 20 minutes, and the absolute CD4+ T-cell count is automatically calculated and shown on a liquid crystal display (LCD); it can also be printed, if requested. This instrument can be operated in a tropical environment up to 40°C. All reagents and controls are included

Received for publication February 4, 2011; accepted September 1, 2011.

From the *Laboratoire de Bactériologie-Virologie, CHU Aristide le Dantec, Université Cheikh Anta Diop, Dakar, Sénégal; and †Department of Microbiology, Laboratory of Immunology, Institute of Tropical Medicine, Antwerp, Belgium.

Financial support was provided by the Belgian Directorate-Generale for Development Cooperation. PIMA Analyzers, disposable cartridges and lancets for finger-prick blood collection were kindly provided by Clondiag (Inverness Medical Innovations).

Presented at XVIII International AIDS Conference; July 18, 2010; Vienna, Austria and at the Uniting Streams Symposium; Utrecht, The Netherlands; October 6, 2010.

The authors Papa Alassane Diaw and Géraldine Daneau contributed equally.

The authors have no conflicts of interest to disclose.

Correspondence to: Dr. Papa Alassane Diaw, PharmD, Laboratoire de Bactériologie-Virologie, CHU Aristide le Dantec, BP 7325, Dakar, Sénégal (e-mail: padiaw21@orange.sn).

in a disposable cartridge that can be stored at room temperature (2–30°C) until use. Blood sampling can be performed by venipuncture or by a less invasive finger prick or heel prick.

Evaluation of this new instrument is still limited. Two studies, one in Zimbabwe[5] the other in Mozambique[6] were recently published. Both studies compared finger-prick blood samples obtained by laboratory technicians or nurses, with venous blood counted on a reference instrument. They concluded that PIMA was a very promising point-of-care instrument for voluntary counseling and testing clinics and for use at the primary health care level. Apart from these 2 published studies, 2 conference abstracts, comparing venous and finger-prick blood on PIMA in a reference center and in an HIV clinic in South Africa, were presented at international meetings.[7,8] They concluded that the instrument performed well but emphasized the importance of standardization of both operator training and consistency of finger-prick sampling to minimize variability of sampling procedure and/or use of capillary blood in PIMA. Obviously, more evaluations on clinical setting are required to validate the use of capillary blood on this new point-of-care instrument in different conditions.

In the present study, we evaluated the performance of PIMA in Dakar, Senegal. We evaluated instrument precision and its performance on venous blood in a reference setting in Dakar, Senegal, and compared the results with those from the widely used flow cytometry technique (FACSCount, BD Biosciences). We also tested PIMA under field conditions using capillary blood that was collected by finger prick and directly analyzed in 3 different outpatient clinics by laboratory technicians, nurses, or physicians. This is the first time that physicians have been directly included in such an analysis. Because physicians' offices (or mobile clinics with reduced staff) are an intended setting for this device, it is important to consider PIMA's performance in this context.

METHODS

Study Population

Three hundred patients presenting for HIV follow-up or other laboratory examinations between December 2009 and February 2010 were enrolled in Dakar, Senegal. Patients presented for routine examinations in 4 different clinical sites where the staff was trained on the use of the PIMA instrument: 2 physicians at the Centre de Recherche Clinique (CR) and 1 laboratory technician and 1 nurse at the "Centre de Traitement Ambulatoire" in "Centre Hospitalier National de Fann," 1 physician at the "Institut d'Hygiène Sociale", and 1 laboratory technician at the "Centre Hospitalier Universitaire Aristide Le Dantec (CHU Le Dantec)". Venous blood was taken from all patients by venipuncture in K_3EDTA vacutainer tubes for $CD4^+$ T-cell enumeration by FACSCount (Becton Dickinson, Erembodegem, Belgium). From 200 patients, venous blood was analyzed by PIMA CD4 (Alere/Clondiag, Jena, Germany) as well. One hundred patients were HIV infected [HIV (+)], and 100 were HIV seronegative [HIV (-)]. Capillary blood from another group of 100 HIV (+) patients was obtained by finger prick and analyzed by PIMA. Capillary blood is generally obtained from a finger prick (adults) or a heel prick (eg, pediatric cases)" using a medical lancet which causes superficial bleeding from capillary veins.

Thirty extra HIV (+) patients were enrolled at CHU Le Dantec for repeatability testing on venous blood, measured by PIMA only.

This study was approved by the Senegalese Ethical Committee (Conseil National de Recherche en Santé), and all patients signed an informed consent document.

$CD4^+$ T-Cell Enumeration

$CD4^+$ T-cell counting by PIMA using venous blood was performed by introducing 25 μL of fresh whole blood (<8 hrs after venipuncture) into the capillary of a disposable PIMA cartridge. For $CD4^+$ T-cell counting in capillary blood, finger-prick blood was obtained from patients using dedicated lancets. Approximately, 2 drops of blood were directly loaded in the cartridge capillary. After adding the blood to the cartridge, it was immediately capped and inserted into the PIMA Analyzer within 5 minutes after capping, as instructed by the manufacturer.

A reference $CD4^+$ T-cell count was obtained using venous blood from the same patients. Blood samples were analyzed in the reference laboratory by FACSCount according to the manufacturer's instructions using dedicated commercial reagents. In short, 50 μL of whole blood was added to test tubes containing fluorescent anti-CD3 and either anti-CD4 or anti-CD8 antibodies and fluorescent beads. Samples were analyzed automatically by the built-in software. Analyses were always performed within 8 hours after venipuncture. Results are given as absolute number of $CD4^+$ T cells (cells/μL).

Instrument Error Reporting

If during sample analysis, the instrument encounters abnormalities that hinder normal counting of the cells (cell clumping, air bubbles etc), the instrument generates error codes and aborts the analysis (sample rejection, no result generated). The frequency of such errors was recorded at all the study sites.

Statistical Analysis

The CD4 results from PIMA were compared with those from FACSCount, the CD4 cell counting reference instrument, using MedCalc statistical software (version 10.0.2). The percentage of similarity of the PIMA data with the FACSCount data was calculated for each sample as $100 \times [(\text{PIMA} + \text{FACSCount})/2]/\text{FACSCount}$.[9] The percent similarity is expressed as average percent similarity ± relative standard deviation. Ideally, the average similarity should be 100% (measure of accuracy), and the relative standard deviation (measure of precision) as small as possible. Normal distribution of similarity plots was assessed with the d'Agostino-Pearson test. The Wilcoxon paired test, Kruskal–Wallis test, χ^2 test, and Spearman test for rank correlation (rho) were used where appropriate. The agreement between the paired test results from both instruments was assessed by the concordance correlation coefficient $\rho_c = \rho C_b$, which evaluates the degree to which pairs of

observations fall on the 45° line through the origin. This type of analysis also contains a measurement of precision (Pearson correlation ρ), and accuracy (bias correction factor C_b) which both can vary between zero and one.[10] The closer they get to 1, the better. Passing and Bablok regression analysis was applied to the data with no special assumptions regarding the distribution of the samples and the measurement outliers.[11] The Bland–Altman and Pollock analyses were performed to calculate the mean bias and limits of agreement (LOA) which are the 95% confidence intervals (±1.96 × SD) of the mean bias of all paired measurements.[12]

To determine the clinical significance of the measurement differences on the treatment decision, interrater agreement was used to calculate the kappa coefficient on the HIV (+) population.[13] To measure the sensitivity/specificity of the PIMA device to identify HIV+ patients eligible for treatment, the FACSCount CD4 results were used as gold standard values. The precision of the CD4 results from PIMA was calculated as the median percent coefficient of variation (CV) for a set of 30 duplicates (% CV = standard deviation/average × 100).

RESULTS

PIMA Performance on Venous Blood

We compared CD4+ T-cell counts from PIMA with those from FACSCount using venous blood (Table 1). The similarity plot of the PIMA results revealed 94.9% ± 10.0% similarity with the FACSCount results, indicating that PIMA underestimated the CD4 count relative to FACSCount. The concordance analysis revealed an overall concordance coefficient of 0.93 with a bias correction factor of 0.97 and a Pearson correlation of 0.96.

Passing and Bablok regression on all 199 samples including HIV+ and HIV- patients, revealed a linear relation with a slope of 0.87 (95% CI: +0.84 to +0.90) and an intercept of 16.67 (95% CI: +0.84 to +31.65). The mean absolute bias between PIMA and FACSCount CD4 results measured by Bland–Altman was −78 cells per microliter (relative relative bias was −10.1%) with LOA from −327 to +171 cells per microliter over the entire range of CD4 results.

The performance of PIMA was remarkably better in HIV (+) patients (Percent similarity was 96 ± 11.7%, Pearson correlation was 0.98, bias correction factor was 0.99, and overall concordance was 0.97) than in HIV-negative patients (percent similarity was 93.9 ± 7.3%, Pearson correlation was 0.89, bias correction factor was 0.91, and overall concordance was 0.81) (Table 1). Of note, control patients had a higher median CD4+ T-cell count than HIV-infected patients ($P < 0.001$). Passing and Bablok regression on all 100 samples from HIV+ revealed a linear relation with a slope of 0.87 (95% CI: 0.84 to 0.93) and an intercept of +14 (95% CI: +6 to +27) (Fig. 1A). Passing and Bablok regression on all 99 samples from HIV-negative patients revealed a linear relation with a slope of 0.87 (95% CI: 0.81 to 0.94) and an intercept of +7 (95% CI: −54 to +65) Fig. 2A.

The mean absolute bias between PIMA and FACSCount was −32 cells per microliter (relative bias was −6.6%) with LOA from −146 to +84 cells per microliter for samples from HIV+ patients (Fig. 1B) and a mean absolute bias of −125 cells per microliter (relative bias was −14%) with LOA from −434 to +184 cells per microliter for samples from HIV-negative patients (Fig. 2B).

We also analyzed the CD4 data applying clinically relevant CD4+ T-cell ranges in HIV (+) patients (Table 2). It is clear from that table that the absolute bias increased with higher CD4+ T-cell counts, whereas the similarity decreased with increasing CD4+ T-cell counts (Spearman rho −0.229; $P = 0.023$). The concordance between the PIMA and FACSCount results is much smaller when only small ranges of CD4 counts are analyzed (eg, 200–350 and 350–500 cells/μL) instead of the entire range of CD4 counts from all HIV+ samples (Tables 1, 2).

The precision of absolute CD4 cell counting by PIMA was assessed by testing the repeatability on 30 venous HIV (+) samples. Each sample was measured in duplicate, using different cartridges inserted in 2 PIMA Analyzers in parallel. The overall CV was 4.0% (95% CI: 2.7 to 5.5) for a median

TABLE 1. Comparison of CD4+ T Lymphocyte Counts Measured by PIMA and FACSCount in Blood Samples Obtained by Venipuncture

	All	HIV (−)	HIV (+)
N	199	99	100
Female sex, (%)	56.3	50.5	62.0
Age (yrs), median (IQR)	36 (28–42)	32 (26–40)	38 (32–46)
CD4 cells/μL (FACSCount), median (IQR)	591 (324–923)	912 (721–1112)	330 (213–498)
CD4 cells/μL (PIMA), median (IQR)	543 (286–811)	798 (623–978)	304 (223–453)
% Similarity, median (95% CI)	94.9 (94 to 96.3)	93.9 (92 to 96)	96 (94 to 98)
Relative SD, (%)	10	7.3	11.7
Concordance ρ_c, median (95% CI)	0.93 (0.91 to 0.94)	0.81 (0.74 to 0.86)	0.97 (0.96 to 0.98)
Pearson ρ (correlation)	0.96	0.89	0.98
Bias correction factor C_b	0.97	0.91	0.99
Absolute (cells/μL) bias and LOA, mean (± 1.96 SD)	−78 (−327, +171)	−125 (−434, +184)	−32 (−146, +84)
Relative bias and LOA, mean % (± 1.96 SD)	−10 (−46, +26)	−14 (−45, +18)	−6.6 (−46, +33)

Venous samples from control patients [HIV (−)] or HIV-infected patients [HIV (+)] were analyzed together (all) and separately.

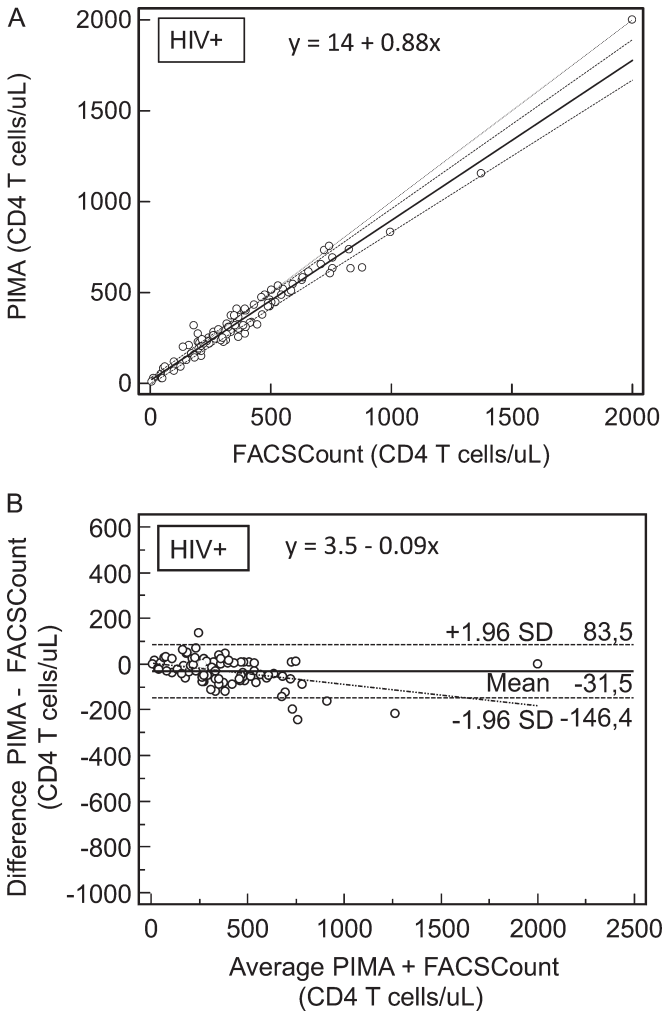

FIGURE 1. Comparison of CD4+ T-lymphocyte counts measured by FACSCount and PIMA in blood samples obtained by venipuncture from 100 HIV+ patients. A, Passing and Bablok regression. The solid line represents linear regression between both methods, with 95% CI shown as dashed lines, and the line of perfect similarity is shown for reference (dotted line). B, Bland–Altman graph for all 100 patients. Horizontal lines indicate the mean of the bias (solid line) and the limits of agreement (dashed lines) representing ±1.96 SD (95% CI) of the mean bias. Regression is plotted (dashed-dotted line) and the equation indicated.

CD4 count of 325 cells per microliter. For specific CD4 cell count ranges, the CV was 9.0% for <200 cells per microliter (n = 9), 4.0% for 200–500 cells per microliter (n = 16), and 3.2% for >500 cells per microliter (n = 5).

PIMA Performance on Finger-Prick Blood

For 95 HIV+ patients, we compared CD4+ T-cell counts in finger-prick blood using the PIMA with CD4 counts in venous blood on the FACSCount (Table 3). The similarity between both methods was 97.2 ± 18.1% although the distribution was not normal. The concordance analysis gave a Pearson correlation of 0.89 and a bias correction coefficient of 0.98 and an overall concordance correlation coefficient of 0.88. Passing and Bablok regression gave a linear relationship with a slope of 0.93 (95% CI: 0.86 to 1.01) and an intercept of 2.20 (95% CI: −19.04 to +21.02) (Fig. 3A). The mean absolute bias measured by Bland–Altman was −39 cells per microliter (relative mean bias was −8.7%) with LOA from −258 to +179 cells per microliter (Fig. 3B).

We looked for a potential effect of different operators (eg, influencing sampling quality) on the results by comparing performance between different study sites (Table 3). The patients were different but median CD4+ T cell counts were

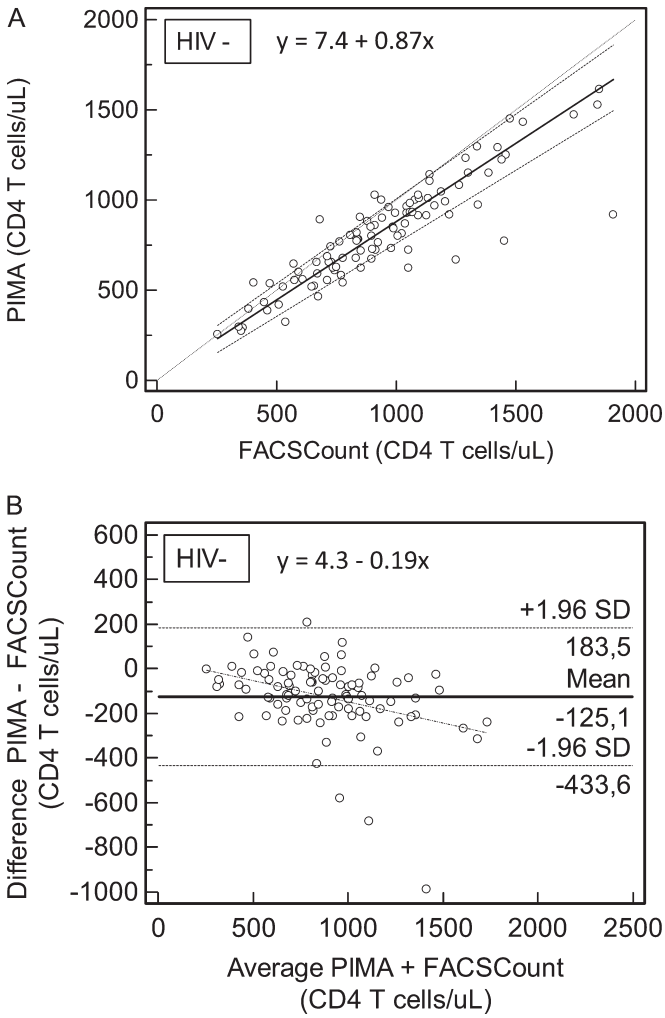

FIGURE 2. Comparison of CD4+ T-lymphocyte counts measured by FACSCount and PIMA in blood samples obtained by venipuncture from 99 HIV-negative patients. A, Passing and Bablok regression. The solid line represents linear regression between both methods, with 95% CI shown as dashed lines, and the line of perfect similarity is shown for reference (dotted line). B, Bland–Altman graph for all 99 patients. Horizontal lines indicate the mean of the bias (solid line) and the limits of agreement (dashed lines) representing ±1.96 SD (95% CI) of the mean bias. Regression is plotted (dashed-dotted line) and the equation indicated.

TABLE 2. Comparison of CD4+ T Lymphocyte Counts Measured by PIMA and FACSCount in Blood Samples Obtained by Venipuncture, According to Clinically Relevant CD4 Ranges

	HIV (+)			
	<200 CD4/μL	200–350 CD4/μL	350–500 CD4/μL	>500 CD4/μL
N	20	32	24	24
Female sex (%)	60	66	67	54
Age (yrs), median (IQR)	36 (31–43)	40 (35–49)	37 (31–41)	42 (31–52)
CD4 cells/μL (FACSCount), median (IQR)	132 (53–175)	263 (220–303)	397 (366–464)	683.5 (564–793)
CD4 cells/μL (PIMA), median (IQR)	124 (60–181)	250 (227–269)	376 (322–425)	610 (516–710)
% Similarity, median % (95% CI)	102 (90 to 120)	98 (93 to 100)	95 (93 to 98)	95 (93 to 97)
Relative SD (%)	20.4	6,7	6,5	4.1
Concordance ρ_c, median (95% CI)	0.82 (0.63 to 0.92)	0.67 (0.43 to 0.82)	0.49 (0.21 to 0.69)	0.95 (0.89 to 0.97)
Pearson ρ (correlation)	0.85	0.71	0.64	0.98
Bias correction factor C_b	0.96	0.94	0.77	0.97
Absolute (cells/μL) bias and LOA, mean (± 1.96 SD)	9.4 (−76, +94)	−15 (−82, +51)	−42 (−139, +56)	−77 (−217, +63)
Relative (%) bias and LOA, mean % (±1.96 SD)	3.5 (−75, +68)	−6.1 (−33, +21)	−11 (−38, +16)	−11 (−28, +6)

Results from HIV (+) patients were subdivided in 4 CD4+ T-cell count strata, as determined by FACSCount.

The absolute bias represents the mean of all paired measurement differences (PIMA–FACSCount) in CD4 cells/μL. The relative bias represents the mean of all paired relative differences: (PIMA-FACSCount) × 100/average (%). The LOA are the 95% (1.96 SD) confidence intervals around the absolute or relative bias.

The % Similarity is the agreement between the PIMA and the FACSCount CD4 results.

IQR, interquartile range (25%–75%).

similar between sites ($P > 0.05$). The mean similarities between both methods were not significantly different between the sites. However, in one site, C.R, where the % similarity tended to be lower then the other sites, the absolute bias between PIMA and FACSCount results analyzed by Bland-Altman was significantly higher than in Centre de Traitement Ambulatoire ($P = 0.018$). After stratification of the CD4 data according to clinically relevant CD4+ T-cell count ranges (Table 4), we found that the % similarity decreased significantly with increasing CD4+ T-cell counts (Spearman correlation rho = −0.239; $P = 0.020$).

From Tables 1, 3, it is clear that the concordance correlations between PIMA and FACSCount results were lower with finger-prick blood than with venous blood on PIMA. Finger-prick blood resulted in lower CD4 counts on PIMA than venous blood on FACSCount except for low CD4 counts (<200 cells/μL) (Table 4).

Clinical Agreement

We calculated the agreement between both methods using 2 clinically important cut-off values for CD4+ T cells that are currently used for initiating treatment: 200 and 350 CD4+ T cells per microliter.[3] We considered CD4+ T-cells counts within a 10% bilateral error around the cut-off to be similar, for example, for a cut-off of 200, values from 190 to 210 cells per microliter were considered similar.

Of 100 HIV (+) patients sampled by venipuncture, 18 of 20 subjects had a concordant result of <200 CD4+ T cells by FACSCount and by PIMA and would receive treatment, regardless of the instrument used; 78 of 80 patients had a concordant CD4 count >200. Only 4 patients would have been misclassified by relying on PIMA. Two patients would receive treatment based on the PIMA results (false positives, CD4 counts by PIMA/FACSCount of 149/211 and 179/214), and 2 other patients would not receive treatment based on the

TABLE 3. Comparison of CD4+ T Lymphocyte Counts Measured by PIMA and FACSCount in Blood Samples Obtained by Finger Prick

	All	IHS	CR	CTA
N	95	21	37	36
CD4 cells/μL (FACSCount), median (IQR)	364 (212–550)	490 (342–699)	310 (209.5–552.75)	343 (202–475)
CD4 cells/μL (PIMA), median (IQR)	313 (209–482)	434 (263–619)	280 (170.5–420.75)	372.5 (196–482)
% Similarity, median (95% CI)	97.2 (95 to 99)	98.6 (92 to 104)	94.6 (87.1 to 98.9)	99.0 (95 to 106)
Relative SD (%)	18	11	27	9.5
Concordance ρ_c, median (95% CI)	0.88 (0.82 to 0.91)	0.85 (0.68 to 0.93)	0.87 (0.77 to 0.93)	0.90 (0.81 to 0.95)
Pearson's ρ (correlation)	0.89	0.88	0.90	0.90
Bias correction factor C_b	0.98	0.96	0.96	1.00
Absolute (cells/μL) bias and LOA, mean (± 1.96 SD)	−39 (−258, +179)	−59.1 (−291, +173)	−64 (−296, +169)	−4.2 (−186, +177)
Relative (%) bias and LOA, mean % (± 1.96 SD)	−8.7 (−65, +48)	−10.4 (−61, +40)	−15.0 (−86, +56)	−1.8 (−42, +39)

Finger prick results were analyzed for all HIV (+) patients and subdivided based on analysis site (IHS, CR, CTA).

CR, Centre de Recherche Clinique de Fann; CTA, Centre de Traitement Ambulatoire; IHS, Institut d'Hygiène Sociale.

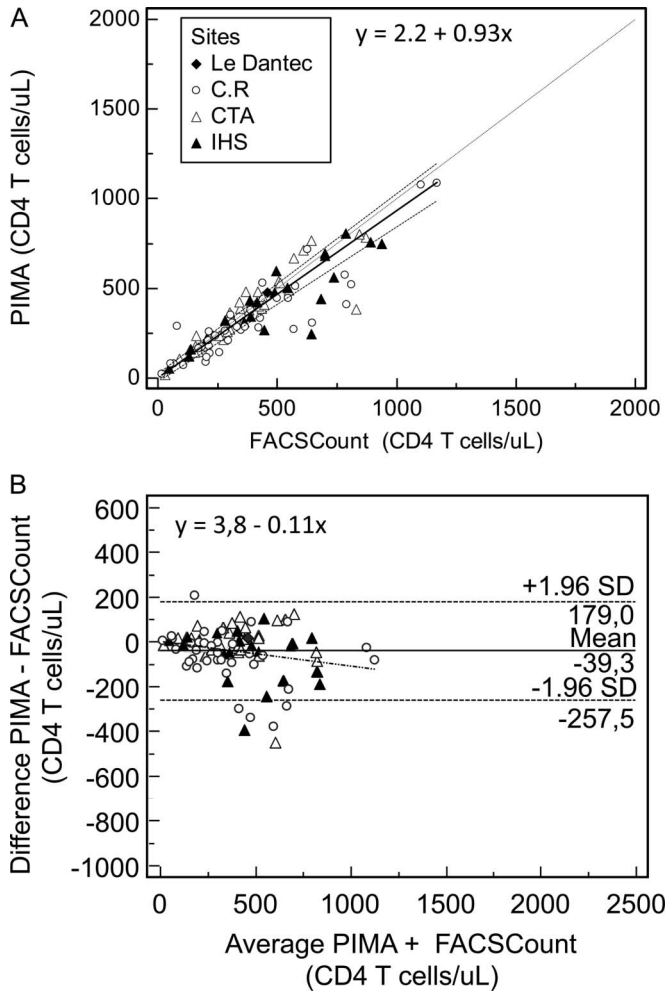

FIGURE 3. Comparison of CD4⁺ T lymphocyte in finger prick blood on PIMA with those in venous blood by FACSCount in 95 HIV+ blood samples. A, Passing and Bablok regression on all 95 results with the site of analysis indicated [Le Dantec hospital, Centre de Recherche Clinique de Fann (C.R.), Centre de Traitement Ambulatoire, Institut d'Hygiène Sociale]. The solid line represents linear regression between both methods, with 95% CI shown as dashed lines; the line of perfect similarity is shown for reference (dotted line). B, Bland–Altman graph for all 95 patients. Horizontal lines indicate the mean of the bias (solid line) and the limits of agreement (dashed lines) representing ± 1.96 SD (95% CI) of the mean bias. Regression is plotted (dashed-dotted line) with 95% CI (curved solid lines), and the equation is indicated.

PIMA results (false negatives, CD4 counts by PIMA/FACSCount of 211/160 and 317/182). The kappa coefficient was 87.5%. Considering FACSCount as the reference instrument, the sensitivity for identifying HIV patients eligible for ART was 90% and the specificity was 98%.

Of 95 patients sampled by finger prick, 20 of 22 had a concordant result of <200 CD4 cells by FACSCount and by PIMA and would receive treatment, regardless of the instrument used; 70 of 73 patients had a concordant CD4 count >200 CD4 T cells. Only 5 patients would have been misclassified by relying on PIMA. Three patients would receive treatment based on the PIMA results (false positives, CD4 counts by PIMA/FACSCount of 175/214, 139/218, and 143/258), and 2 other patients would not receive treatment based on the PIMA results (false negatives, CD4 counts by PIMA/FACSCount of 289/80 and 235/161). The kappa coefficient was 85.4%. Sensitivity for identifying HIV patients eligible for ART was 91%, with 97% specificity. Based on a prevalence of low CD4 cell counts of 21.5% in our overall HIV (+) population ("disease prevalence"), the positive predictive value was 88% and the negative predictive value was 97%.

Using 350 CD4⁺ T cells as the threshold for ART treatment initiation, the kappa coefficient was 77.8% for venous samples and 70.6% for finger-prick blood. Sensitivity and specificity were, respectively, 98% and 79% for venous samples, and 91% and 80% for finger-prick blood.

External Quality Assessment

Two samples from the African Regional External Quality Assessment (EQA) (AFREQUAS) were analyzed by both PIMA and FACSCount. The first sample had a low CD4⁺ T-cell count (141 cells/μL by FACSCount; 148 cells/μL by PIMA). The second sample had a higher CD4⁺ T-cell count (704 cells/μL by FACSCount, 579 cells/μL by PIMA).

Instrument Error Reporting

As depicted in Table 5, error codes leading to automatic abortion of the analysis occurred more frequently on PIMA using finger-prick blood (14% of all samples with a maximum of 23% of the samples in one study site) than after using venous blood (<5%) ($P = 0.0003$). When the first analysis did not give a result, a second measurement was performed. If the second analysis was also aborted, the error was considered repetitive, and the sample was excluded from the comparison analysis. No significant difference was seen between the different sites, even though frequencies were quite different. When comparing error rates for venipuncture samples, the rate using PIMA was 4.8%, whereas FACSCount had a lower failure rate of 0.5% ($P = 0.0039$).

DISCUSSION

The results from the comparisons between PIMA and FACSCount indicate that PIMA gives similar results but has a tendency to underestimate the CD4 counts at higher CD4 counts (>500 cells/μL). Individual cell recognition by PIMA software is based on the cell shape and size and $CD3^+CD4^+$ fluorescence. This may explain the bias toward underestimation at high CD4⁺ T-cell counts, as more cells tend to stick together and would therefore be excluded from analysis by the instrument because of incompatible size. This phenomenon could also be important for some EQA samples using a fixation medium that changes cell morphology, but according to the manufacturer, fixed blood used in several EQA are compatible with the instrument. This was partly confirmed in our study by the AFREQUAS controls, which were correctly analyzed for low CD4 counts.

The underestimation of the absolute CD4 counts was relatively smaller at lower CD4 ranges and disappeared for

TABLE 4. Comparison of CD4+ T Lymphocyte Counts Measured by PIMA and FACSCount in Blood Samples Obtained by Finger Prick, According to Clinically Relevant CD4 Ranges

	<200 CD4/μL	200–350 CD4/μL	350–500 CD4/μL	>500 CD4/μL
N	19	26	22	28
CD4 cells/μL (FACSCount), median (IQR)	135 (70–160)	278 (216–305)	423 (386–444)	691 (573–822)
CD4 cells/μL (PIMA), median (IQR)	146 (79–171)	249 (209–307)	405 (336–475)	568.5 (468–754)
% Similarity, median (95% CI)	106 (96 to 109)	96.4 (90.6 to 101.9)	98 (94 to 102)	95 (88 to 100)
Relative SD (%)	29	11	8.9	13
Concordance ρ_c, median (95% CI)	0.62 (0.28 to 0.83)	0.66 (0.45 to 0.80)	0.41 (0.12 to 0.64)	0.55 (0.29 to 0.73)
Pearson ρ (correlation)	0.66	0.78	0.53	0.66
Bias correction factor C_b	0.95	0.85	0.77	0.83
Absolute (cells/μL) bias and LOA, mean (±1.96 SD)	15 (−89, +118)	−20 (−126, +87)	−16 (−160, +127)	−112 (−429, +204)
Relative (%) bias and LOA, mean % (±1.96 SD)	8 (−59, +75)	−12 (−61, +38)	−5.6 (−42, +31)	−20 (−79, +39)

Finger prick results were analyzed for CD4+ T-cell count range, as measured by FACSCount.
IQR, interquartile range (25%–75%). Detailed explanation, see legend of Table 1.

CD4 cell counts of < 200 CD4+ T cells per microliter. This means that when PIMA is used as a screening tool to identify patients eligible for ART with cut-offs of 200 or 350 CD4+ T cells per microliter, results are sufficiently accurate to avoid significant misclassification of patients. The instrument showed a remarkably good agreement with the reference instrument for values <200 CD4+ T cells per microliter, both for venous and finger-prick blood. This is important for reliable screening of patients at the point-of-care in resource-limited countries as the <200 CD4 cell cut-off is still frequently used in these setting to initiate treatment. Taking into account the new 2010 WHO guidelines recommending a cut-off of 350 instead of 200 CD4+ T cells per microliter to initiate treatment,[3] the "clinical" agreement between both instruments was still acceptable. Indeed, PIMA's sensitivity for identifying patients eligible for treatment was still 98% for venous samples and 91% for finger-prick blood, despite lower specificities of 79% and 80%, respectively. This would be acceptable from the patient's perspective, as several patients would receive treatment slightly earlier than scheduled (at 250–300 CD4+ T cells per microliter instead of at <200). From the treatment program's perspective, however, this would increase the total cost of treatment as more people would be treated than strictly required. At high CD4+ T-cell counts, which are more relevant for monitoring immune reconstitution during treatment, measurement precision using venous blood was consistently good. Unfortunately, finger-prick blood seemed to be less precise at high CD4 cell ranges.

Two field evaluations of PIMA in Zimbabwe and in Mozambique using finger-prick blood have been published recently.[5,6] Our results on finger-prick blood are similar those of the Mozambique study that found a slightly larger bias of −52.8 cells per microliter and comparable limits of agreement (−250.9 to +145.2) than our study. The Zimbabwe study, however, found a smaller bias (+8 cells/μL) and narrower limits of agreement (−174 to 189 cells/μL) than our and the Mozambique finger-prick blood study. This may be the consequence of differences between the study populations. Indeed, the study population in Zimbabwe had a significantly lower average CD4+ T-cell count (226 cells/μL) than our (364 cells/μL) and the Mozambique (376 cells/μL) study population, which is associated with a better performance in our study as well.

Our study showed that PIMA's performance was better on venous blood than on finger-prick blood. This was also the observation of 2 studies presented at recent conferences by Glencross et al.[7,8] For venous blood in a laboratory environment (phase I), they reported an average bias of −17 cells per microliter with narrow LOA (89 to 55 cells/μL) in a study population with an average CD4 count of 337 cells per microliter. For

TABLE 5. Frequency of Instrument Errors During Sample Analysis (No CD4 Result Provided)

		Finger Prick					Venous
		All	IHS	CR	CTA	Le Dantec	All
Total patients	n	100	23	37	39	1	200
Total measurements	n	116	30	40	45	1	210
Errors due to PIMA	n (%)	16 (13.8)	7 (23.3)	3 (7.5)	6 (13.3)	0 (0.0)	10 (4.8)
Repetitive error	n (%)	4 (4.0)	2 (8.7)	0 (0.0)	2 (5.1)	0 (0.0)	0 (0.0)
Errors due to FACSCount	n (%)	1 (1.0)	0 (0.0)	0 (0.0)	1 (2.6)	0 (0.0)	1 (0.5)

Total number of instrument errors reported during reading of finger prick blood samples per site [IHS, CR, CTA, and Hôpital Le Dantec (Le Dantec)]. Total number of instrument errors reported during analyses of venous blood samples are shown separately.
IHS, Institut d'Hygiène Sociale; CR, Centre de Recherche Clinique de Fann; CTA, Centre de Traitement Ambulatoire.

venous blood evaluated in a clinical environment (phase III), they reported a bias of −21 cells per microliter with LOA (−164 to 121 cells/μL) in a study population with an average of 373 CD4 cells per microliter, which is similar to our observation in venous blood from HIV+ patients. On finger-prick blood, however, they found a bias of −38 cells per microliter, with considerably larger LOA (−388 to 311 cells/μL), which was also our finding. The discrepancy between venous and finger-prick blood CD4 counts could be due to technical issues during finger-prick sampling (eg, squeezing). It is very unlikely that the higher variability of the results with finger-prick blood is related to the different CD4 counts in venous and finger-prick blood in vivo. Indeed, a recently published study compared CD4 counts in finger prick with those in venous blood and found the results were in close agreement.[14]

Compared with other alternative but flow cytometry-based CD4 instruments currently used in the field, the PIMA results show less agreement with reference instruments than some alternative flow cytometric instruments like the Cyflow, but the results are similar to those from instruments like the Guava.[15–19]

The strengths of this instrument are its small size, its affordability, its ease of use at the point-of-care, and the small blood volume required per analysis. It is considerable less expensive than flow cytometry-based instruments. The fact that the cartridges contain all required reagents, ready-to-use, without the need for delicate pipetting (finger-prick blood) is an asset as less training of staff is required. Blood sampling by finger prick or heel prick would offer several other advantages, for pediatric cases in particular, because it is less invasive and requires a very small blood sample per test. Unfortunately, the instrument does not measure CD4 percentages yet which is preferred over absolute counts in pediatric HIV cases. Furthermore, finger prick blood results of PIMA agree much less with the reference results of the FACSCount than venous blood, and for reasons that are not clear, lead to a higher frequency or instrument error codes leading to automatic analysis abortion. Such errors can occur for instance as a consequence of blood coagulation during incubation, with subsequent impeded sample circulation in the cassette. Another source of error is the occurrence of air bubbles in the blood sample, which will lead to rejection of the cassette by the instrument and the generation of error codes. Therefore, once blood has been inserted in the cartridges, it must be processed immediately by the instrument. This means that finger-prick blood sampling requires the immediate availability of an instrument.

The choice of an instrument like the PIMA would be primarily determined by the type of setting and by the available resources. The entire process from incubation to reading and printing of results takes place inside the PIMA analyzer, and monopolizes the instrument. Therefore, the sample throughput is relatively low, with for instance 1 sample every 20 minutes or even longer, including preparation time. This restriction could be lifted by installing 2 or more instruments per site and running multiple analyses in parallel. Another restriction is that once cassettes are loaded with blood, they should be analyzed immediately by the instrument. This means that patients can only give finger-prick blood when an instrument is available. The use of anticoagulated venous blood overcomes this restriction, but the instrument is intended to be used with capillary blood. Instruments like the PIMA would be of interest to health care workers who are looking for a very simple and affordable instrument to test a limited number of samples per day at the point-of-care. Patients can wait until the results are ready to discuss treatment, if required. Furthermore and not unimportant, patients do not need to return to the clinic just to be informed about the results of the test, decreasing the risk of being lost to follow-up. This hope was confirmed in a preliminary implementation in Mozambique.[20]

In conclusion, this instrument has demonstrated a good performance under field conditions. This could be to the benefit of patients in resource-limited settings as it can help clinicians screen patients for treatment eligibility at the point-of-care. However, the performance of the instrument on finger-prick blood is not as good as on venous blood and could be a matter of concern.

ACKNOWLEDGMENTS

We thank physicians Dr. I. Ndiaye, Dr. S. Diop, Dr. M. C. Dia, Dr. I. Traoré, Dr. N.F. Ngom, and the paramedical staff from Fann Hospital, Institut d'Hygiène Sociale, who contributed to patient enrollment and CD4$^+$ T-cell analysis.

REFERENCES

1. UNAIDS. *Report on the Global AIDS Epidemic*. Geneva, Switzerland: UNAIDS; 2008.
2. Lynen L, Thai S, De MP, et al. The added value of a CD4 count to identify patients eligible for highly active antiretroviral therapy among HIV-positive adults in Cambodia. *J Acquir Immune Defic Syndr*. 2006; 42:322–324.
3. World Health Organization. *Antiretroviral Therapy for HIV Infection in Adults and Adolescents: Recommendations for a Public Health Approach*. WHO. 2010.
4. Mandy F, Janossy G, Bergeron M, et al. Affordable CD4 T-cell enumeration for resource-limited regions: a status report for 2008. *Cytometry B Clin Cytom*. 2008;74(suppl 1):S27–S39.
5. Mtapuri-Zinyowera S, Chideme M, Mangwanya D, et al. Evaluation of the PIMA point-of-care CD4 analyzer in VCT clinics in Zimbabwe. *J Acquir Immune Defic Syndr*. 2010;55:1–7.
6. Jani IV, Sitoe NE, Chongo PL, et al. Accurate CD4 T-cell enumeration and antiretroviral drug toxicity monitoring in primary healthcare clinics using point-of-care testing. *AIDS*. 2011;25:807–812.
7. Glencross DK, Coetzee L, Lawrie D, et al. Encouraging point-of-care PIMA CD4 testing performance in a laboratory setting: Johannesburg, South Africa. Presented at: 17th Conference on Retroviruses and Opportunistic Infections; San Francisco, CA; 2010.
8. Glencross DK, Coetzee L, Lawrie D, et al. Performance evaluation of PIMA CD4 point-of-care (POC) analyzer, Johannesburg, South Africa. Presented at: XVIII International AIDS Conference; Vienna, Austria; 2010.
9. Scott LE, Galpin JS, Glencross DK. Multiple method comparison: statistical model using percentage similarity. *Cytometry B Clin Cytom*. 2003;54:46–53.
10. Lin LI. A concordance correlation coefficient to evaluate reproducibility. *Biometrics*. 1989;45:255–268.
11. Passing H, Bablok. A new biometrical procedure for testing the equality of measurements from two different analytical methods. Application of linear regression procedures for method comparison studies in clinical chemistry, Part I. *J Clin Chem Clin Biochem*. 1983;21:709–720.

12. Bland JM, Altman DG. Measuring agreement in method comparison studies. *Stat Methods Med Res*. 1999;8:135–160.
13. Cohen J. A coefficient of agreement for nominal scales. *Educ Psychol Meas*. 1960;20:37–46.
14. Sitoe N, Luecke E, Tembe N, et al. Absolute and percent CD4+ T-cell enumeration by flow cytometry using capillary blood. *J Immunol Methods*. 2011;372:1–6.
15. Cecelia AJ, Balakrishnan P, Mohanakrishnan, et al. Effective evaluation of novel low-cost CD4 monitoring assays. *J Immunol Methods*. 2006; 316:158–162.
16. Dieye TN, Vereecken C, Diallo AA, et al. Absolute CD4 T-cell counting in resource-poor settings: direct volumetric measurements versus bead-based clinical flow cytometry instruments. *J Acquir Immune Defic Syndr*. 2005; 39:32–37.
17. Lynen L, Teav S, Vereecken C, et al. Validation of primary CD4 gating as an affordable strategy for absolute CD4 counting in Cambodia. *J Acquir Immune Defic Syndr*. 2006;43:179–185.
18. Pattanapanyasat K, Phuang-Ngern Y, Lerdwana S, et al. Evaluation of a single-platform microcapillary flow cytometer for enumeration of absolute CD4+ T-lymphocyte counts in HIV-1 infected Thai patients. *Cytometry B Clin Cytom*. 2007;72:387–396.
19. Spacek LA, Shihab HM, Lutwama F, et al. Evaluation of a low-cost method, the Guava EasyCD4 assay, to enumerate CD4-positive lymphocyte counts in HIV-infected patients in the United States and Uganda. *J Acquir Immune Defic Syndr*. 2006;41:607–610.
20. Janik I, Sitoe N, Alfai E, et al. Point-of-care CD4 improves patient retention and time-to-initiation of ART in Mozambique. Presented at: XVIII International AIDS Conference; Vienna, Austria; 2010.