Assessment of two POC technologies for CD4 count in Morocco

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Abstract

Background : In the era of “test and treat strategy”, CD4 testing remains an important tool for monitoring HIV-infected individuals. Since conventional methods of CD4 count measurement are costly and cumbersome, POC CD4 counting technique are more affordable and practical for countries with limited resources. Before introducing such methods in Morocco, we decided to assess their reliability. **Methods :** in this study 92 blood samples from HIV-infected patients, were tested by PIMA to generate absolute CD4 count and by FACSPresto to derive percentage and absolute CD4 count. Flow cytometry using FacsCalibur, was used as reference method for CD4 count comparison. Linear regression, Bland and Altman analysis were performed to assess correlation and agreement between POC methods and the reference method. In addition, sensibility and specificity, positive predictive value (PPV), negative predictive value (NPV) at 350 CD4 count threshold were also determined. Finally, because FACSPresto can also measure hemoglobin (Hb) concentration, 52 samples were used to compare FACSPresto against an automated hematology analyzer. **Results :** With PIMA technology, the coefficient of determination R^2 was 0.93. Regarding FACSPresto, R^2 was 0.93 and 0.96 for absolute CD4 count and percentage CD4 count, respectively. Bland and Altman analysis displayed a mean bias of -32.3 cells/ μ L with a limit of agreement (LOA): -181.3 to 116.8, for Pima. The mean bias for FACSPresto was -8.1 cells/ μ L with LOA: -158.2 to 142.0 for absolute CD4 count and 0.2 with LOA: -3.8 to 4.3, for CD4 percentage. Moreover, with a threshold of 350 CD4 count, sensibility, specificity, PPV, NPV, were 98%, 87%, 89%, 86%, and 88%, 96%, 93% and 92% for PIMA and FACSPresto, respectively. Finally, the hemoglobin measurement evaluation displayed an R^2 of 0.80 and a mean bias of -0.12 with a LOA between -1.75 and 1.51. **Conclusion :** When compared to the reference method, PIMA and FACSPresto have shown good performance, for CD4 counting. The introduction of such POC technology will speed up the uptake of patients in the continuum of HIV care, in our country.

Background

Since the advent of HAART therapy, HIV-infected patients have been treated according to CD4 count threshold. Initially the threshold was set to 200 CD4 count, as the question of when to start HAART therapy was not answered [1–3]. However, since 2009, studies demonstrated that treating patients till 200 cells/ μ L or less, was too late and could even jeopardize their life. On the other hand, when antiretroviral treatment is initiated at a higher threshold, significant reduction in risk of mortality and morbidity due to HIV/AIDS, was observed [4,5]. Therefore, this treatment threshold was raised to 350 in 2010 and then to 500 CD4 count, in 2013 [6,7]. Furthermore, in 2014, other studies revealed a benefit of early treatment for all patients, irrespective of their immunologic or virologic status [5,8]. In fact, it was reported that early treatment is not only beneficial for the HIV-infected individuals themselves, but it can also reduce the viral infectiousness and subsequently the ongoing HIV transmission [8]. This evidence prompted international guidelines to recommend early treatment of HIV-infected individuals. In this framework, in 2015, WHO advocated “Test and treat strategy”. In other words, once a person tested infected with HIV, they should be offered HAART therapy, immediately and regardless of their CD4 count [9]. In this respect, universal

access to the treatment has transformed the deadly HIV/AIDS to a chronic disease, in developed countries. In addition, they are also endeavoring to achieve the UNAIDS goal towards ending the epidemic, by 2030 [10–12]. In contrast with this context, the HIV/AIDS remains an important cause of death in resources limited countries, despite significant efforts that aimed at helping these countries access HAART therapy. Thanks to these efforts, 24.5 million patients accessed HAART treatment at the end of 2018 [13]. Nevertheless, 35% of 37.9 million among persons living with HIV/AIDS, are still not treated. Consequently, in these settings, access to HAART therapy still prioritized for patients most in need, by using CD4 count. Furthermore, CD4 count is still required to begin and stop opportunistic infections treatment, for patients that are diagnosed during advance stage of the infection. Therefore, CD4 count remains an essential tool for HIV management for resource limited countries that are struggling to implement “test and treat strategy” [14–16].

Because conventional methods used to measure CD4 count are costly, cumbersome and require highly skilled staff, point of care (POC) CD4 count technology, represents an excellent alternative to overcome such challenges.

Morocco is regarded as low HIV prevalence area, since this prevalence is less than 0.1%, and the current estimate of persons living with HIV/AIDS is around 22000 [17,18]. In early 90s the Moroccan ministry of health has developed and implemented a national response to curb the epidemic HIV/AIDS, within the country. The impact of this national response has been further strengthened since the advent of Global Fund in 2003, which has been helping scaling up HIV prevention treatment and care within the country.

Morocco has been followed WHO guidelines for initiating the antiretroviral treatment. Since 2015, “test and treat strategy” has been adopted by the Moroccan ministry of health. Despite the introduction of this strategy, CD4 testing is still required for the management of HIV-infected people, within Morocco. In fact, according to national guidelines, CD4 count is required for all newly HIV-diagnosed patients in order to decide starting and discontinuing the prophylaxis of opportunistic infections, for patient with late presentation. Besides, CD4 count is still measured for patients initiating HAART therapy, and once the HIV viral load is fully suppressed and CD4 count exceeds 350 cells/ μ l, the monitoring is based only on HIV viral load testing.

POC technologies are being deployed within the country, in order to facilitate the access to CD4 count. Because all HIV management centers are available in regional and some provincial hospitals, with laboratory facilities, it was decided to install this POC CD4 count technology within laboratories of these hospitals, in order to cover area where CD4 count is not yet available. In this framework, we decided to evaluate two POC CD4 count technologies, PIMA and FACSPresto, by comparing them to a reference method.

Methods

In this study, we have used 92 remnant samples collected from EDTA blood samples, regularly addressed for CD4 count monitoring, from HIV management center, for HIV-infected patients, in University Hospital

Center Ibn Rochd, between May 26 and September 4, 2015. Samples were routinely collected and tested in unlinked anonymous manner [19] to determine CD4 count. Ethical approval for this study was obtained from the Ethic committee of Biomedical Research, Medical School and Pharmacy, University Mohammed Vth, Rabat, Morocco.

The reference method

Throughout the entire evaluation, the reference method was a single platform flow cytometry with three-color reagent kit, performed on a standard clinical instrument. Samples were stained by mixing 50 µl of whole blood, with 10 µl of CD3FITC/CD4PE/CD45PercP (Beckton-Dickinson), in tubes containing beads (TrueCount, Beckton-Dickinson), and then incubated for 15 minutes. Samples were lysed and fixed during 15 minutes, by adding 450 µl of lysing solution (FACS lysing solution, Beckton-Dickinson). All the incubations were performed at room temperature. Percentage and absolute CD4 count were determined on flow cytometer (FacsCalibur, Beckton-Dickinson) by using CellQuest Pro software [20]. All steps were performed according to manufactures' instructions.

PIMA technique

PIMA CD4 technology consists of a portable device for CD4 count testing, using disposable cartridge that comprises dried reagents, made of anti-CD3 and anti-CD4 antibodies conjugated to dyes. The cartridge is opened and 25 µl of blood sample were added, then it is capped and loaded into the analyzer. After 20 minutes of incubation inside the analyzer, the absolute CD4 count is determined when all steps are successful, otherwise an error report is provided. The results are displayed on the screen device and printed automatically.

FACSPresto technique

FACSPresto CD4 system is a device that determines CD4 count, by using dried reagent preloaded in disposable cartridges. The reagents are made of anti-CD3, anti-CD4, anti-CD14 and anti-CD45RA antibodies conjugated to fluorescent dyes, as well as an integrated quality control (QC). A volume of 25 µL of blood sample is transferred to the cartridge which is capped and incubated at room temperature, during 18 minutes. The cartridge is loaded onto the FACSPresto analyzer for result reading. For each sample, the reading takes around 4 minutes before printing absolute and percentage CD4 count, as well as hemoglobin (Hb) concentration in g/dl. In case of reading problem, the analyzer generates an error report. Results are shown on the analyzer screen and printed automatically.

For both POC CD4 count methods, the number of samples per hour was estimated. Furthermore, sensitivity, specificity, PPV, VNP, were calculated for a CD4 count threshold of 350, for PIMA and FACSPresto. Finally, Hb concentration measured by FACSPresto, was compared to Hb determined by an automated hematology analyzer (Coulter Ac.T diff, Beckman Coulter) on 52 samples.

Quality control

For the reference method, maintenance is performed on daily basis for the cytometer FACS Calibur cytometer. In addition, maintenance of each 3 months consisted of verifying laser alignment as well as the status of the machine is performed by specialist engineer. Finally, the instrument is calibrated in each run, with beads (Calibrite Beads, Beckton-Dickinson).

Regarding the POC PIMA, there are two quality control cartridges, one with low CD4 count and another with high CD4 count, which are tested in each run.

For FACSPresto, two types of QC, one for CD4 count and another for Hb measurement, are printed automatically, each time the device is switched on.

In addition, a commercial stabilized blood (Immunotrol Cells, Beckman Coulter), was used as an internal quality control throughout the study and tested in each run, by the reference method as well as by PIMA and FACSPresto techniques. The technologists were trained on using the PIMA and FACSPresto techniques as well as on the reverse pipetting. All tests were performed during 24 hours after venipuncture, by the same technologist.

Statistical analysis

The mean of CD4 count, the SD and the % of CV were calculated when necessary. We have also performed a linear regression analysis to determine the regression equation and the graphic to assess the strength between these POC techniques and the reference method. The coefficient of determination (R^2) was also provided. Furthermore, the agreement between these technologies and the reference method was studied by Bland-Altman analysis. In this case, the mean bias as well as the 95% limits of agreement (LOA) i.e. mean bias $\pm$ 1.96 SD, were measured. Finally, for Hb measurement linear regression and Bland and Altman were performed as well to evaluate the results obtained by FACSPresto, using an automated hematology analyzer as a reference technique.

Result

Quality control

In the present study, PIMA low control and high control gave a mean $\pm$ SD and %CV of 153.27 $\pm$ 4.76 and 3.10, 1015.36 $\pm$ 8.79 and 0.87; respectively. The internal quality control QC of FACSPresto was OK during all the runs, throughout the study.

During this study, the CD4 count of a commercial stabilized blood (with CD4 count target 598 $\pm$ 165) tested by the reference method and by PIMA and FACSPresto techniques, was determined (Table 1). Regarding absolute CD4 count, the reference method gave a mean $\pm$ SD of 650.89 $\pm$ 60.91 and a %CV of 9.3. PIMA and FACSPresto had a mean $\pm$ SD and %CV of 686.78 $\pm$ 53.22, 7.75 and 633.56 $\pm$ 52.57, 8.30; respectively. As far as the CD4 percentage concerned, the reference method and FACSPresto gave a mean $\pm$ SD and a %CV of 47.73 $\pm$ 2.87, 5.75; 45.18 $\pm$ 2.32, 5.13; respectively (data not shown).

Table 1: Stabilized blood analyzed by the three methods

	Reference method	PIMA	FACSPresto
Mean	650.89	686.78	633.56
SD	60.91	53.22	52.57
%CV	9.36	7.75	8.30

Performance of PIMA and FACSPresto

During this study, we have tested 92 samples with the reference method and by PIMA and FACSPresto technologies. There were six samples that PIMA instrumentation was unable to read and five no reads by FACSPresto. Table 2 depicts the statistics related to studied samples by all three methods.

Tables 2: Characteristics of samples studied by the reference method, PIMA and FACSPresto techniques

	Reference Method		FACSPresto		PIMA
CD4 count	Absolute count	%	Absolute count	%	Absolute count
Number	92	92	87	87	86
Range	(14-1501)	(1-44)	(27-1481)	(1-45)	(34-1019)
Mean±SD	466.81±301.26	21.44±10.89	479.63±280.04	22.55±10.56	446.33±236.01
95% CI for the mean	404.43-529.20	19.19-23.70	419.95-539.32	20.30-24.80	395.64-497.01

Correlation and agreement assessment between PIMA, FACSPresto and the reference method are represented in figure 1. The comparison of CD4 count measurement between PIMA and the reference method, displays a determination coefficient $R^2=0.93$ and a regression equation $y= 0,84x + 46.24$. Regarding FACSPresto technology, the comparison gives an $R^2=0.93$ and regression equation $y = 0,91x+ 34.07$, for absolute CD4 count. Concerning the percentage of CD4 count, the regression equation is $y=0.99x+0.48$ and $R^2= 0.96$. Furthermore, the agreement between each technique and the reference method, studied by Bland and Altman analysis, shows a mean bias of -32.3 cells/ μ L, with LOA ranging from -181.3 to 116.8, for PIMA technology. When the testing is performed by FACSPresto method,

the mean bias is -8.1cells/ μ L, with LOA varying from -150.8 to 141.0. The mean bias obtained by FACSPresto is lower than that obtained by PIMA (p=0.0384, data not shown). The mean bias is -0.2 with a LOA between -3.8 and 4.3, for the CD4 count percentage generated by FACSPresto. We have also measured the sensitivity, specificity, PPV, NPV, using a threshold of 350 CD4 count (Table 3). PIMA displays a sensitivity, specificity, PPV and NPV of 91.17% (0.75-0.98), 92.31% (0.81-0.98), 88.57 % (0.72-0.96) and 94.11% (0.84-0.98), respectively. FACSPresto shows a sensitivity, specificity, PPV and NPV of 88.23% (0.72-0.96), 96.23% (0.86-0.99), 93.75% (0.87-0.99) and 92.73% (0.81-0.98), respectively.

Table 3: sensibility, specificity, PPV, and NPV by PIMA and FACSPresto

	SS(95%CI)		PPV(95%CI)	NPV(95%CI)
	SP(95%CI)			
PIMA	91.17% (0.75-0.98)	92.31% (0.81-0.98)	88.57 % (0.72-0.96)	94.11% (0.84-0.98)
FACSPresto	88.23%(0.72-0.96)	96.23% (0.86-0.99)	93.75% (0.87-0.99)	92.73% (0.81-0.98)

Since FACSPresto can also generate the hemoglobin concentration, we have compared the results of 52 samples tested by FACSPresto to those measured by an automated hematology analyzer. Results (figure 1) show an R²= 0.80 and a regression equation, y=0.92x+1.00. The mean bias was -0.12 with a LOA between -1.75 and 1.51.

Finally, we have assessed the throughput of both CD4 count techniques (data not shown). Regarding PIMA, the number of samples that can be tested per hour is three samples; whereas FACSPresto can perform seven tests an hour.

Discussion

In the era of the UNAIDS 90 90 90 target aimed at eliminating HIV/AIDS by 2030, cost and complexity of technology still represent the most prohibitive challenges that hamper scaling up HIV tests used to monitor HIV-infected people, in developing countries. This brings about delay or inability to access HIV care. In this context, POC CD4 counting technology, known to be cheaper and user-friendly, represents an excellent tool to speed up the linkage of HIV-infected individuals to cascade care and therefore help optimize the management of HIV-infected persons, for these settings. However, the assessment of such techniques is a major prerequisite before their introduction in HIV management. In fact, their performance should be evaluated to avoid tests that generate unreliable results, which may put patients to unnecessary risk of morbidity and mortality, associated with HIV/AIDS [21]. Additionally, the evaluation is

crucial to inform and guide decision-making, regarding the appropriate choice of the reliable and affordable techniques [22].

In this study, we have evaluated two POC CD4 count techniques, PIMA and FACSPresto.

PIMA has displayed good performance when compared to the reference method. In fact, we have found a mean of bias of -32.3 cells/ μ l. This result concords with other studies that found a mean bias ranging from -32 to -22 cells/ μ l [23,24]. Actually, PIMA is a well established technology that has been used for many years, in developing countries, particularly in sub-Saharan Africa, for the measurement of CD4 count, to monitor HIV-infected people [25,26].

As far as FACSPresto method concerned, this technology performs well when compared to the reference method, since the mean bias is -8.1 cells/ μ l and 0.2%, for absolute count and CD4 percentage, respectively. These findings are in line with previous studies that report similar results [27,28].

The sensitivity, specificity, PPV and NPV of both POC techniques were also evaluated against the reference method at a threshold of 350 CD4 count. The results display a sensitivity and specificity around 90% and PPV and NPV around 90%. Our data are similar with those reported in others studies [24,29] and witness the reliability of CD4 count measurement by PIMA and FACSPresto technologies, for monitoring HIV-infected individuals.

It is worthwhile noting that the device FACSPresto can provide also the CD4 count percentage, which is important for monitoring HIV-infected children who are less than 5 years [30]. In addition, it has also the advantage to measure the level of hemoglobin, which is essential for a timely management for HIV-infected individuals with hemoglobin lower level, during hematological abnormalities [31]. Such technologies that can perform simultaneous tests with the same reagents and the same instrument are important for developing countries, since they could help providing a rapid testing and optimizing the available resources.

In this study, we have assessed the throughput of both POC CD4 count techniques. Regarding PIMA, the number of samples that can be tested per hour is three samples; whereas FACSPresto can perform up to seven tests an hour. Therefore, if we can assume that, the working time per day is 6 hours; PIMA can analyze up to 18 samples a day, while FACSPresto can measure up to 42 samples. This difference in the daily throughput is due to the fact that the incubation (around 18 minutes) takes place within the machine, for PIMA. Consequently, the time between consecutive samples is always 20 minutes. On the contrary, for FACSPresto, the incubation of samples occurred outside the analyzer (18 minutes), and each sample reading takes around 5 minutes.

The rate of no read errors by both methods is 5% for PIMA and 6% for FACSPresto. All samples that lead to this reading failure have less 100 CD4 count, except for one. These reading problems need more investigation to figure out the cause, as well as to define the CD4 count that may lead to such results.

The POC techniques are all-important for developing countries, particularly in settings that lack laboratory infrastructure. In this regard, POC CD4 count methods have been used on finger prick blood, in basic healthcare center, in order to speed up the continuum of care of HIV-infected persons. However, the performance of such technology on finger prick seems to be lower than that of venous blood. In this regard, trainings on using these technologies as well as the way of collecting capillary blood are essential for reliable results [32].

The decentralization process of HIV management in Morocco is based on the creation of HIV/AIDS management activity in hospitals as well as the deployment of the HIV tests including diagnosis tests, viral load and CD4 count testing, in the hospital laboratory. The main goal of such decentralization is to provide an immediate linkage to care and therefore strengthen a timely management of HIV-infected people, which is primordial for the UNAIDS three 90s goal.

Limitations: FACSPresto has the advantage to generate also the percentage CD4 count which is important for children; nevertheless, in our study, this technique was evaluated only on adults' samples.

Conclusion

In this study, we have demonstrated that both technologies PIMA and FACSPresto can be used to generate reliable CD4 count among HIV-infected patients. These methods can enhance the linkage to care for HIV-infected persons, in developing countries.

List Of Abbreviations

PIMA, FACSPresto, CD4 count, HIV monitoring, Morocco

Declarations

Ethics approval and consent to participate

Ethic committee of Biomedical Research, Medical School and Pharmacy, University Mohammed Vth, Rabat, Morocco. Committees's reference: 196/2015

Consent for publication

Not applicable

Availability of data and materials

The data generated or analyzed during the current study are available from the corresponding author on reasonable request

Competing interests

The authors declare that they have no competing interests

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Author's contribution

Research conception and design were provided by HO and EE. Research was performed by HA, EH, HO and EE. Data was analyzed and results interpreted by EE and HO. Manuscript was prepared by EE, HO. It was critically revised by RB and KME. Final manuscript was approved by HA, HE, RB, KME, EE, and HO

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Figures

Figure 1

Linear regression and Bland and Altman analysis for comparison between PIMA, FACSPresto techniques and the reference method. Linear regression (A) and Bland and Altman analysis (B) for absolute CD4 generated by PIMA. Linear regression (C) and Bland and Altman analysis (D) for absolute CD4 and % CD4 (E and F) determined by FACSPresto. Linear regression (G) and Bland and Altman analysis (H) for

FACSPresto for hemoglobin level. The reference method for CD4 count is the tritest run on FacsCalibur and for hemoglobin is Coulter Ac.T diff.