LBPE17

Evaluation of the point-of-Care Pima CD4 Assay and specimen collection methods at PMTCT and CTC sites in Dar-es-Salaam, Tanzania 2011

Luciana Kohatsu[1]*, Omotayo Bolu[1]*, Fausta Mosha[2], Mary Schmitz[1], Ruth Lemwayi[3], Nichole Arnett[1], Karen Chang[1], Patrick Rwehumbiza[1], Sehin Birhanu[1], Jennifer Sabatier[1], Michael Mwasekaga[1], John N. Nkengasong[1], Larry Westerman[1]

[1]Division of Global HIV/AIDS, U.S. Centers for Disease Control & Prevention (CDC); [2]National Health Laboratory Quality Assurance and Training Center (NHLQATC), Tanzania Ministry of Health and Social Welfare; [3] African Field Epidemiology Network
(*These two authors contributed equally to this study; Corresponding to: Dr. Larry Westerman, email: lew2@cdc.gov)

Introduction

The point-of-care (PoC) Pima CD4 Assay can reduce the time for CD4 staging, minimizing pre-treatment loss to follow-up of patients and increasing patient ART initiation within a reduced time period (Jani et al.[1], Zachariah et al.[2]). Determining the test reliability and appropriate collection method for the assay is fundamental prior to its implementation in HIV/AIDS Control Programs. This evaluation compares the performance of the Pima CD4 Assay using specimens collected from 1060 patients by three different methods and tested using the Pima assay at the site of its intended use in Dar-es-Salaam, Tanzania.

Materials and Methods

- Specimens from HIV-positive patients (Table 1 and Fig. 1) were collected in five sites with antenatal and HIV/AIDS Care and Treatment clinics (CTC) in Dar es Salaam, Tanzania.
- Collected specimens (Fig. 2) were tested with the Pima CD4 Assay by the healthcare worker at the site. Leftover venous specimens were transported and tested at the Tanzanian NHLQATC using the FACSCalibur and Pima instruments within 48 hours of collection.
- Operators were trained on analyzer operation by the manufacturer, and on fingerprick blood collection method by CDC technical personnel.
- Two types of controls were run daily:
  1) Pima Bead Cartridge (low and normal), provided by the manufacturer to control for analyzer function; and
  2) Intra-laboratory quality control, using commercially available CD4 Count Streck fixed blood (low and normal) for assay control.
- Pima CD4 results were analyzed for correlation (coefficient of determination R^2) and bias (Bland-Altman difference) vs. the FACSCalibur venous blood results and vs the specimen mean.
- The percentage of specimens with Pima CD4 results obtained, and those with invalid Pima CD4 tests were analyzed.

Table 1 Specimens from 1060 patients ages 8 – 65 years were collected upon obtaining their informed consent.

	Number of patients	Percentage of patients
On ARV	648	61%
CTC	751	71%
PMTCT	304	29%
Pregnant	162	15%
<18 years old	47	4%
18 – 39 years old	739	71%
39 – 65 years old	263	25%
Female	856	81%
Male	200	19%

Fig. 1 CD4 count distribution.

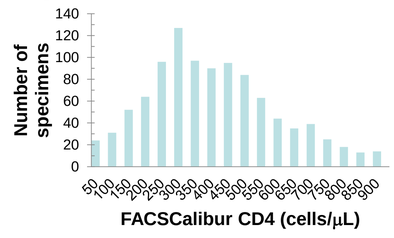


Fig. 2 Specimens were collected using three Specimen Collection Methods..

Fig.2a Standard venous blood: Pima-Venous.

Fig.2b Fingerprick blood directly applied to a Pima cartridge: Pima-Direct.

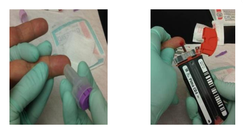
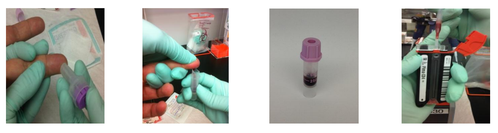


Fig.2c Fingerprick blood collected into a microtube: Pima-Microtube.



Results

A. Pima-Venous and Pima Microtube show better correlation and less bias than the Pima Direct collection method.

Fig. 3 Correlation by Linear Regression of CD4 counts obtained with the Pima assay vs FACSCalibur CD4 values for CD4 results <500 cells/mL.

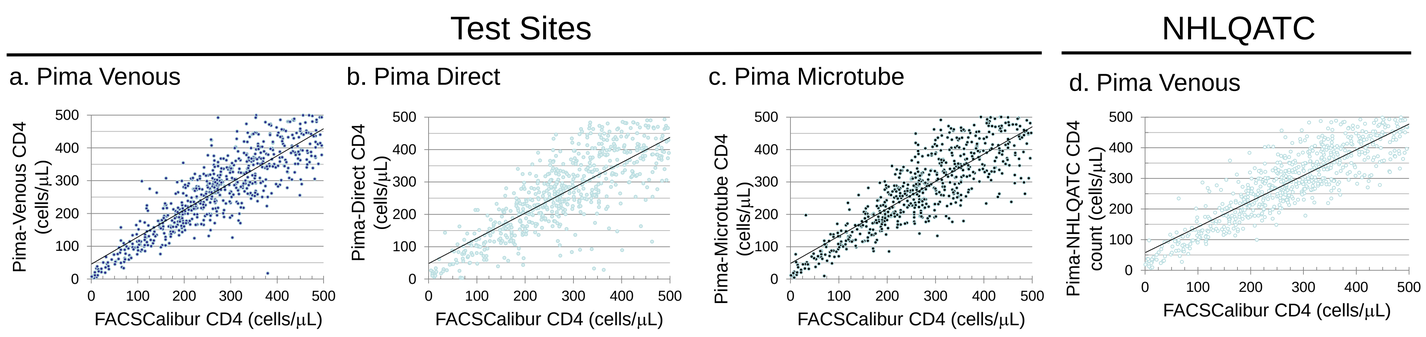


Fig. 4 Bland-Altman analysis comparing the CD4 Count results vs average CD4 results.

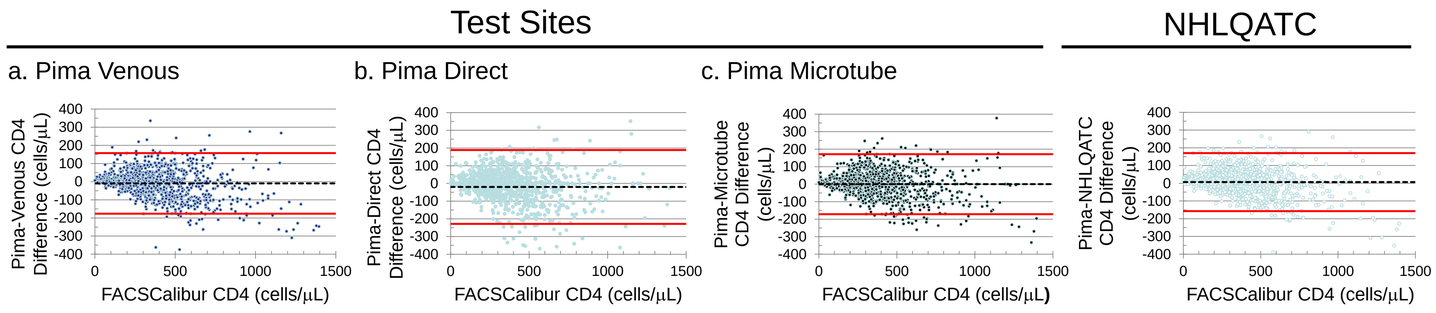


Table 2 Statistical analysis of CD4 count results over the entire range, <500, <350, and <200 cells/mL including R^2 (coefficient of determination) by linear regression and bias by Bland-Altman analysis.

CD4 Count	Statistical Measure	Pima-Venous	Pima-Direct	Pima-MicroTube	Pima-NHLQATC
All	**R^2**	0.89	0.82	0.88	0.89
	Bias	-10	-20	0	7
	S.D.	83	104	86	82
<500	**R^2**	0.74	0.64	0.71	0.78
	Bias	-5	-17	2	11
	S.D.	69	80	73	63
<350	**R^2**	0.70	0.55	0.65	0.70
	Bias	-2	-13	2	14
	S.D.	53	67	59	54
<200	**R^2**	0.66	0.48	0.66	0.64
	Bias	0	-10	3	14
	S.D.	38	46	38	40

B. Greater number of CD4 results were obtained from Pima-Venous specimens, followed by the Pima-Microtube specimens. Pima-Direct specimens resulted on highest number of specimens without a final CD4 result.

Table 3 1060 specimens per collection method were obtained. A total of 5128 Pima tests were conducted. CD4 results could not be obtained from a number of specimens tested. Tests were repeated at least once before stop testing the specimen. Some specimens were tested more than once to obtain a result.

	Pima-Venous	Pima-Direct	Pima-Microtube	Pima-NHLQATC	FACSCalibur
# of specimens with CD4 results	1050/1060	955/1060	1012/1060	1058/1060	1055/1060
% of specimens without CD4 results	0.9%	9.9%	4.5%	0.2%	0.5%

C. Low under-treatment and over-treatment frequencies were observed when comparing the CD4 values obtained with the Pima CD4 assay vs FACSCalibur.

Table 4 Analysis of CD4 count results for all specimens tested on the Pima compared to the FACSCalibur analyzer above and below the 350 cells/ µL CD4 threshold. Reported FACSCalibur Coefficient of Variations (%) are between 7.5 to 9.9 (Jani et al.[3], Hultin et al.[4])**.**

		FACSCalibur CD4	
		> 350 cells/mL	**< 350 cells/mL**
Pima CD4	**> 350 cells/mL**	1995 (92.7%*)	116 (6.2%**) under-treatment
	< 350 cells/mL	157 (7.3%*) over-treatment	1769 (93.8%**)

* % = (# of Pima specimens / # of FACSCount specimens with CD4 values >350 cells/mL) x 100
** % = (# of Pima specimens / # of FACSCount specimens with CD4 values <350 cells/mL) x 100

Conclusions

Pima CD4 Assay demonstrated acceptable correlation with the FACSCalibur. Greater variation and bias were registered with Pima-Direct. Specimen collection and loading impact assay performance and require appropriate training. The estimated low percentage for ART over- and under-treatment using Pima technology support the value of this analyzer, especially for sites with challenging access to standard CD4 testing. Pima CD4 implementation should include backup plans for specimens without results obtained, and a Quality Assurance strategy. This evaluation provided guidance for Pima CD4 testing implementation in Tanzania, and may be valuable to other countries.

References

1. Jani IV, Sitoe NE, Alfai ER, et al. Lancet **2011** 378(9802):1572-9.
2. Zachariah R, Reid SD, Chaillet P, et al. Trop. Med. and Intl. Health **2011**; 16(1): 37-41
3. Jani IV, Sitoe NE, Chongo PL, et al. AIDS **2011** 25:807–812
4. Hultin LE, Chow M, Jamieson BD., et al. Clin. Cytom. Part B 2010, (788) 194-200

Acknowledgements

We thank the Tanzanian MoHSW, and personnel from participating district hospitals, Health Centers, and the NHLQATC laboratory. Especial thanks to Dotto Kalovya, who coordinated the CD4 testing at the NHLQATC. Pima analyzers were donated to the Republic of Tanzania by the Clinton Foundation. Pima Operation training to supporting MoH personnel was provided by ALERE. This research has been supported by the President's Emergency Plan for AIDS Relief (PEPFAR) through the Centers for Disease Control and Prevention.