Methods and Applications

Assessment of POC CD4 Detecting Mode in District or County Labs — Jiangsu Province, China, 2021

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ABSTRACT

Objective: This study seeks to explore efficient and multiple-item detection modes in new-style HIV labs, as well as access the accuracy and reliability of CD4 cell count detected by point of care (POC) to analyze POC work feasibility in district or county labs.

Methods: POC devices adopted in grassroots-level labs and flow cytometers adopted in prefecture-level labs were used to analyze the same group of blood samples. The individual results were collected and compared for parametric tests in correlation and consistency.

Results: The Pearson correlation coefficients (r) between results detected by FACSPresto and those by FACSCalibur, FACSVia, FACSCantoII, and EPICSXL were 0.922, 0.938, 0.914, and 0.823, respectively; the average deviations were −25.64, 24.68, 3.05, and 70.97 cells/μL, respectively; the Pearson correlation coefficient (r) between results by Pima and FACSCalibur, FACSVia, FACSCantoII, and EPICSXL were 0.900, 0.950, 0.954, and 0.876, respectively; and the average deviations were −73.99, −40.78, −29.32, and −22.75 cells/μL, respectively.

Discussion: Strong positive correlations and good consistency were observed between the CD4 count tested by POC and flow cytometers. These findings provide theoretical support for new-style HIV labs and one-stop services, which can provide shorter testing duration and simpler testing processes, so that the most comprehensive testing results can be obtained in the shortest amount of time.

Accurate and reliable CD4 counts are the most specific indicator for monitoring the damage to the immune system of persons living with human immunodeficiency virus (PLWH); tracking them is thus crucial for human immunodeficiency virus (HIV) control and prevention. It is also a key indicator for identifying the stage of HIV infection, estimating complications, and evaluating the efficacy of antiviral therapy (*1*). According to the clinical practice guidelines, absolute CD4 count should be tested one to four times per year for PLWH based on patient context (*2*). However, the time and frequency of detection are limited as flow cytometry techniques, which require more time, are the primary means of follow-up detection for PLWH in most areas of China (*3*–*5*). In comparison, some recent studies have shown point of care (POC) technology to positively affect timely treatment and assessment of therapeutic efficacy for HIV (*6*–*7*).

In the past 5 years, the proportion of late diagnosis was about 20%–25% among newly infected HIV patients (baseline CD4 <200/μL), while the average time from discovery to treatment of PLWH in Jiangsu was about 1 month (data not published). Early treatment plays an important role in immune reconstruction and therapeutic efficacy for PLWH (*1*,*8*). Therefore, exploring a new and efficient, multiple-item way for early detection using the POC technology will ideally provide PLWH with timely antiviral treatment, better treatment efficacy, and improve the diagnostic capacity of district or county labs. However, the evaluation of consistency and correlation of results for the same sample with different instruments in different laboratories has been rarely reported, making it difficult to justify the deployment of this new detection process.

This research thus evaluated correlation and consistency between POC detector results and flow cytometry method test results. This was achieved by comparing CD4 counts of newly infected HIV patients at the first follow-up determined by POC laboratory technologies in district or county labs and flow cytometry in prefecture-level labs, respectively.

METHODS

Materials and Methods

In this study, blood samples were collected in

duplicate ethylene diamine tetraacetic acid (EDTA) tubes from newly HIV-diagnosed patients (PLWH) in 13 cities of Jiangsu Province from January 1 to December 31, 2021. One tube of each blood sample was retained in the district and/or county CDCs for immediate detection, and the other tube was transported to corresponding prefecture-level labs for sample processing and subsequent flow cytometry detection.

This study used two POC devices [BDFACSPresto (Becton, Dickinson and Company, BD Biosciences, San Jose, California, USA) and Pima Analyser (Abbott Rapid Diagnostics Jena Gmb, Jena, Germany)] and four flow cytometers [FACSCalibur, FACSVia, and FACSCanto Ⅱ (Becton, Dickinson and Company, BD Biosciences, San Jose, California, USA) as well as EPICSXL (Beckman Coulter, Inc. USA)] for CD4 counting. The POC devices were operated with reagents provided by their respective manufacturing companies, and the collection and analysis processes strictly followed the instructions provided. The flow cytometers used reagents registered with the National Medical Products Administration, and the instructions for device operation were strictly followed.

Overall, 903 blood samples were analyzed by FACSPresto — 374 duplicates of which were analyzed using FACSCalibur, 329 by FACSVia, 171 by FACSCantoII, and 29 by EPICSXL, respectively. A total of 955 blood samples were analyzed using Pima, of which 569 duplicates were detected using FACSCalibur, 240 by FACSVia, 130 by FACSCantoII, and 16 by EPICSXL. All the final data were submitted to provincial CDCs.

Statistical Analyses

Microsoft Excel 2016 (Microsoft Corporation, CA, USA), SPSS 26.0 (IBM, NY, USA), and GraphPad Prism 9.3 (GraphPad Software, CA, USA) were used to calculate the descriptive statistics and correlation measures between the 2 measurements taken by POC devices and flow cytometers. Measurement data (absolute CD4 count) was reported as median values. The differences between the two techniques were determined through the Wilcoxon signed-rank test. Pearson correlation was used for each pair of the results generated by different devices. All statistical tests were two-sided, and those with $P<0.05$ were considered statistically significant. To determine the accuracy of each device, Bland–Altman analysis (deviation analysis) was done, and the Bland–Altman plots were produced to visualize bias and limits of agreement [LOA = mean bias ± 1.96 standard deviation (SD) of the differences in the results obtained].

RESULTS

Performance Comparison

Strong positive correlations were observed between results by FACSPresto and 3 flow cytometers (FACSCalibur, FACSVia, and FACSCanto Ⅱ) with Pearson correlation coefficients of (r) 0.922, 0.938, and 0.914. The Pearson correlation coefficient (r) between EPICSXL and FACSPresto was 0.823. The Pearson correlation coefficients (r) were all >0.8, showing a significant linear correlation ($P<0.001$), as shown in Figure 1.

Positive correlations were also observed between Pima and 3 flow cytometers (FACSCalibur, FACSVia, and FACSCantoII). The Pearson correlation coefficients (r) were 0.900, 0.950 and 0.954, respectively. The Pearson correlation coefficient (r) between EPICSXL and Pima was 0.876. The Pearson correlation coefficients (r) were all >0.8, showing a significant linear correlation ($P<0.001$), as shown in Figure 2.

Comparison of CD4 Count Results

The non-parametric test results showed that the CD4 cell count results for PLWH using FACSPresto and FACSCantoII tests were not statistically different (Table 1). However, the median FACSPresto test results were significantly lower than FACSCalibur results whilst higher than the FASCVia and EPICSXL test results ($P<0.05$).

There was no statistically significant difference between the CD4 cell count test results using Pima and results from tests using EPICSXL on blood samples collected from PLWH. However, the median value of CD4 cell count using Pima was lower than the other three flow cytometers ($P<0.05$). Details are in Table 2.

Consistency Analysis

Bland–Altman plots of the CD4 count results detected by FACSPresto and the 4 flow cytometers yielded mean relative deviations of –25.64, 24.68, 3.05, and 70.97 cells/μL, respectively (Figure 3). Among PLWH, approximately 94.65%, 95.14%, 94.15%, and 93.10% of participants in each group were within the mean ± 1.96 SD of the relative deviation, respectively.

The Bland–Altman plots of the CD4 count results

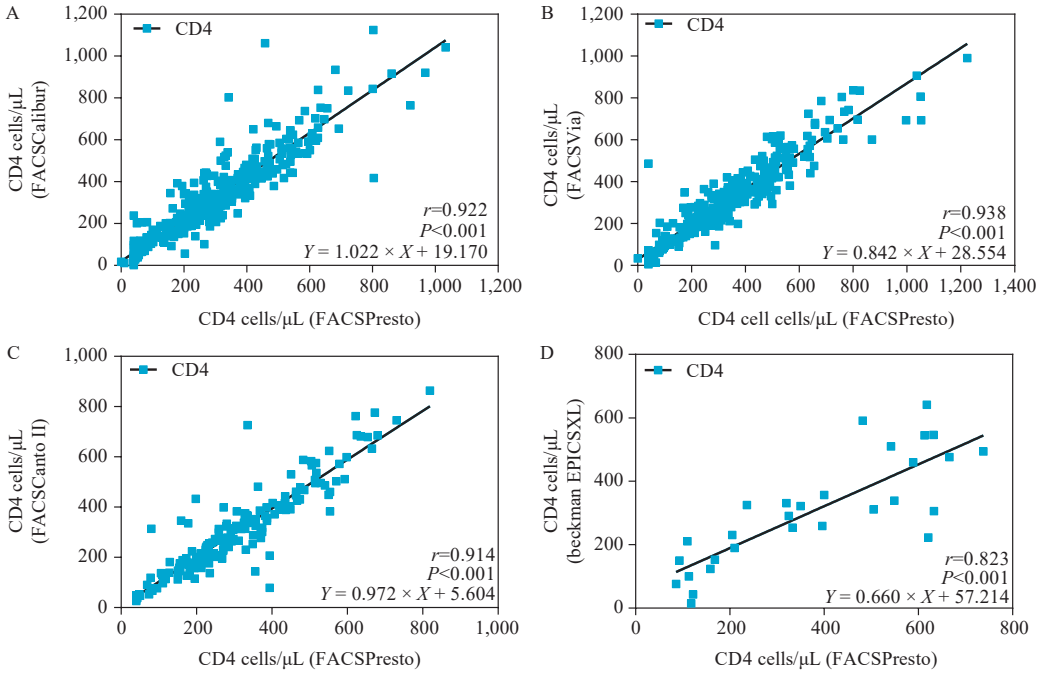

FIGURE 1. Correlation between the CD4 counts with FACSPresto and those with four types of flow cytometers. (A) Correlation between absolute CD4 cell counts (AbsCD4) in venous blood using FACSPresto and that using FACSCalibur. (B) Correlation between AbsCD4 in venous blood using FACSPresto and that using FACSVia. (C) Correlation between AbsCD4 in venous blood using the FACSPresto and that using FACSCanto II. (D) Correlation between AbsCD4 in venous blood using FACSPresto and that using EPICSXL.
Abbreviation: AbsCD4=absolute CD4 cell counts.

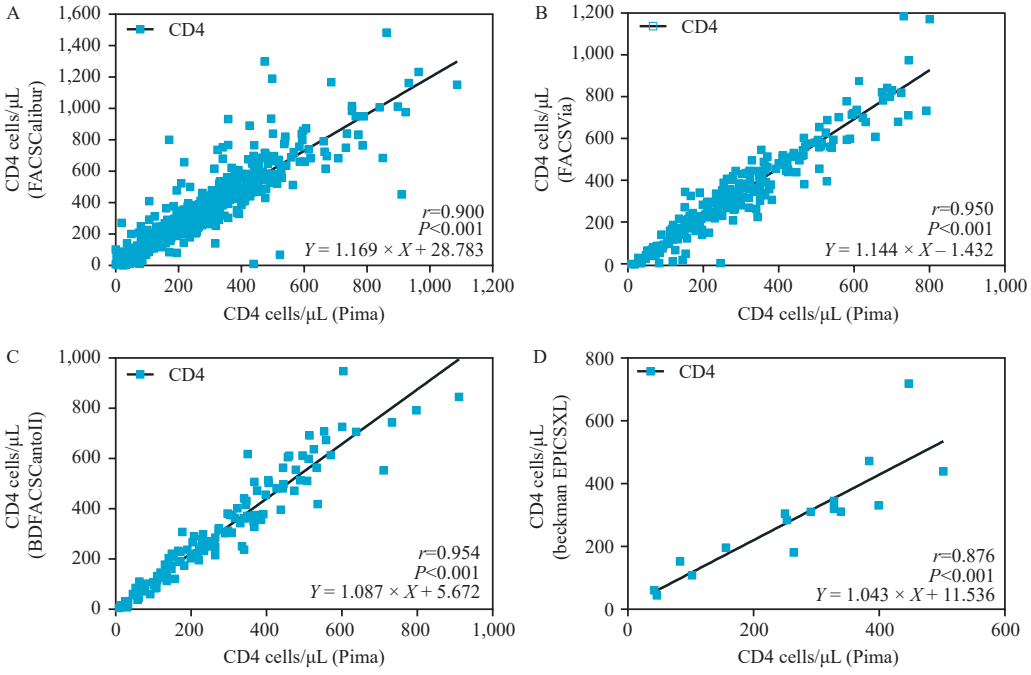

FIGURE 2. Correlation between the CD4 counts with Pima and those with four types of flow cytometers. (A) Correlation between AbsCD4 in venous blood using Pima and that using FACSCalibur. (B) Correlation between AbsCD4 in venous blood using Pima and that using FACSVia. (C) Correlation between AbsCD4 in venous blood using Pima and that using FACSCanto II. (D) Correlation between AbsCD4 in venous blood using Pima and that using EPICSXL.
Abbreviation: AbsCD4=absolute CD4 cell counts.

TABLE 1. Comparison of CD4 count results detected by FACSPresto and other flow machines of the same samples (HIV/AIDS patients newly diagnosed with HIV) in Jiangsu 2021.

Comparison group	Flow cytometer	N	Median (cells/μL)	*Z*	*P*
Group 1	FACSCalibur	374	296	−6.393	<0.001*
	FACSPresto	374	277		
Group 2	FACSVia	329	294	−6.902	<0.001*
	FACSPresto	329	314		
Group 3	FACSCanto Ⅱ	171	273	−1.765	0.078
	FACSPresto	171	279		
Group 4	EPICSXL	29	306	−2.844	0.004*
	FACSPresto	29	350		

**P* value <0.05 is considered significant.

TABLE 2. Comparison of CD4 count results detected by Pima and other flow machines of the same samples (HIV/AIDS patients newly diagnosed with HIV) in Jiangsu 2021.

Comparison group	Flow cytometer	N	Median (cells/μL)	*Z*	*P*
Group 1	FACSCalibur	569	299	−17.249	<0.001*
	Pima	569	243		
Group 2	FACSVia	240	307	−8.821	<0.001*
	Pima	240	265		
Group 3	FACSCanto Ⅱ	130	251	−5.239	<0.001*
	Pima	130	243		
Group 4	EPICSXL	16	308	−1.034	0.301
	Pima	16	278		

**P* value <0.05 is considered significant.

detected by Pima and the four flow cytometers yielded mean relative deviations of –3.99, –40.78, –29.32, and –22.75 cells/μL, respectively (Figure 4). Among PLWH, approximately 95.96%, 95.00%, 95.38%, and 93.75% of participants in each group were within the mean ± 1.96 SD of the relative deviation, respectively.

DISCUSSION

Since flow cytometry is complicated to operate and expensive, it has a higher demand on minimum personnel and laboratory requirements (*9*). As a result, only prefecture-level labs can satisfy the laboratory test demands, which leads to a longer waiting time for results from large batches of test samples and contributes to potential risks in sample transportation. The POC CD4 testing machine is a portable device that is easy to carry, simple to operate onsite, and adaptable to various environments — which helps realize a shorter detection time for a single sample. It can ultimately help PLWH get their CD4 count test results faster and facilitate their timely initiation into or adjustment to antiviral treatment regimens (*9–10*). In some countries, studies have shown that adopting POC CD4 detection instruments can meaningfully improve the timeliness of initiating antiviral therapies (*6,11–12*). Timeliness plays a vital role in the care and therapeutic evaluation of HIV patients; this is especially witnessed in mobile HIV testing services (HTS) delivery, which patients are often more willing to use (*11*). Therefore, using POC CD4 cell detectors, based on their accuracy and reliability, for PLWH in districts or counties without high-standard laboratories is a novel strategy in HIV prevention and control work that will help promote detection accessibility.

This research included 13 prefecture-level labs and 90 district or county labs. The findings indicated a good consistency and relevance between POC devices and flow cytometry test results with correlation coefficients >0.8. Moreover, the consistency was also close to the results reported in other research (*11,13–14*). This study did find variations in the CD4 cell counts test results for the same PLWH samples from different laboratories using POC devices and flow

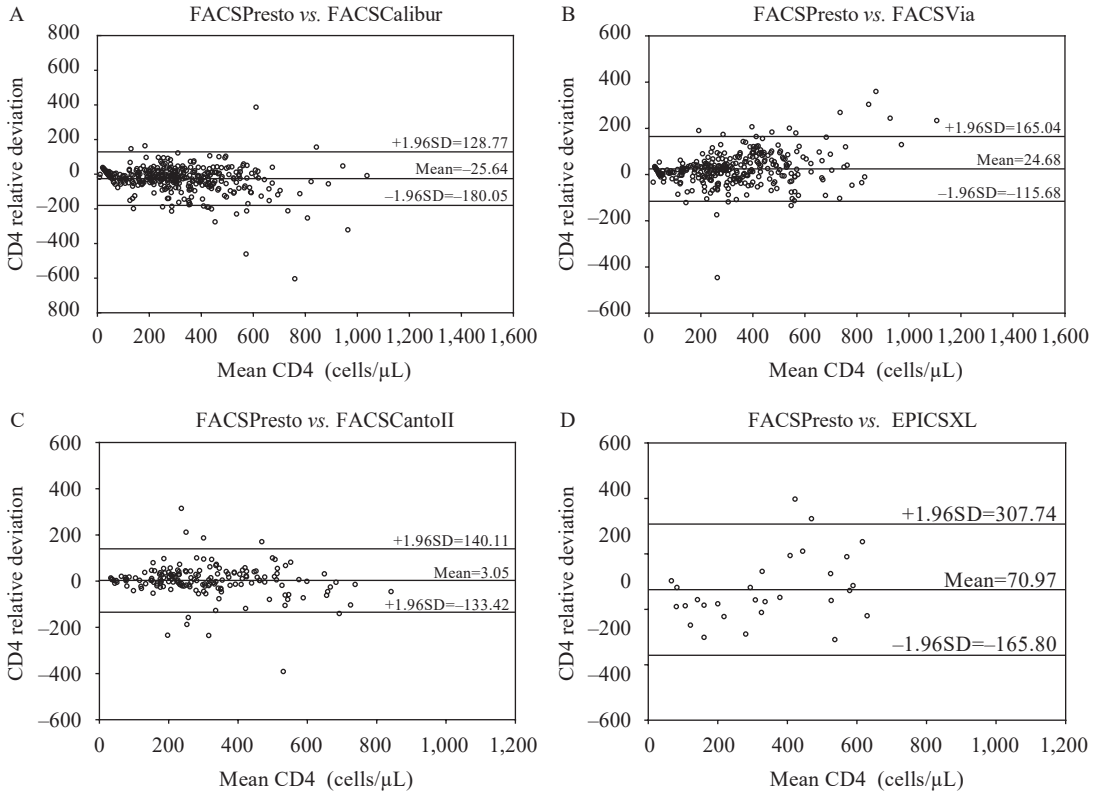

FIGURE 3. Bland-Altman analyses of relative deviations (FACSPresto to the four flow cytometers). (A) Deviation of AbsCD4 in venous blood using FACSPresto to that using FACSCalibur. (B) Deviation of AbsCD4 in venous blood using FACSPresto to that using FACSVia. (C) Deviation of AbsCD4 in venous blood using FACSPresto to that using FACSCanto Ⅱ. (D) Deviation of AbsCD4 in venous blood using FACSPresto to that using EPICSXL.
Note: Mean CD4: Mean of CD4 counts detected by each comparison group. CD4 relative deviation: CD4 counts detected by FACSPresto minus the results detected by flow cytometers.
Abbreviation: AbsCD4=absolute CD4 cell counts.

cytometers. However, this observation is consistent with the findings of other studies (*3*,*13*–*15*) and may be related to differences in detection principles, laboratory conditions, and/or operators. This suggests that the same institutions with the same machines should be used for follow-up tests on the same patients to provide better stability in evaluating changes in CD4 by avoiding differences caused by devices and personnel, if possible. The correlation results of the 2 POC devices (FACSPresto and Pima) and 3 flow cytometers (FACScalibur, FACSVia, and FACSCanto Ⅱ) exceeded 0.9. This showed a good correlation between them: consistent with the findings of other researches (*3*,*13*–*15*). However, the correlation coefficient in this study was slightly lower. This may be due to the fact that previous studies compared test results in the same laboratory using different machines, which reduced the error from influencing factors such as personnel and laboratory conditions. The correlation coefficient between the 2 POC detectors and EPICSXL was >0.8, which is lower than the other 3 models. This may be related to the small sample size, the testing machine's long service time, or the instrument's aging. The high consistency of the test results between the 2 POC detectors and the 4 flow cytometers was similar to the results of other researches (*3*,*13*–*15*) in terms of deviation mean value and degree (especially the average deviation of the laboratory using FACSPresto in comparison to the one using FACSCanto II, which were within 5 cells/μL of each other).

PLWH with $CD4^+$ T cell count less than normal values are the key population targeted in many practical public health interventions. Thus, this study made another analysis for low-CD4 populations (CD4 <200/μL). The nonparametric test results showed that CD4 counts detected by POC and flow cytometers were similar in results (Supplementary Tables S1 and S2, available in https://weekly.chinacdc.cn/). Furthermore, both FACSPresto and Pima showed a good correlation with the other flow cytometers (FACScalibur, FACSVia, and FACSCanto Ⅱ)

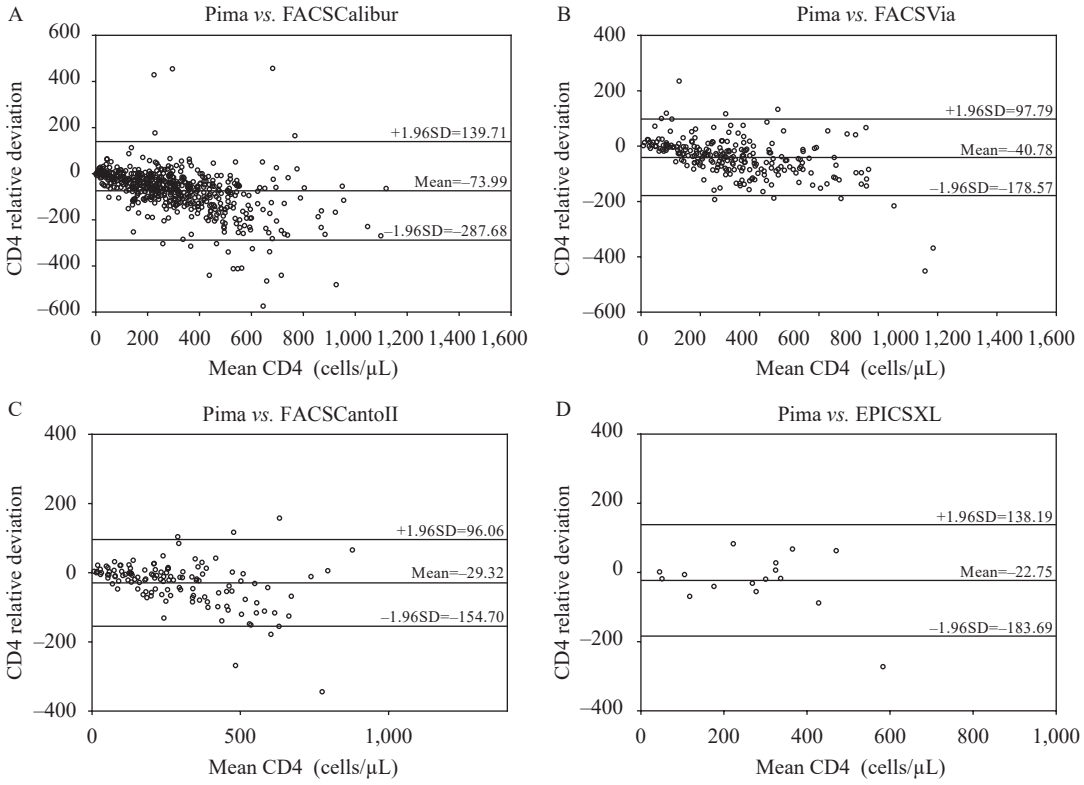

FIGURE 4. Bland-Altman analyses of relative deviation (Pima to the four flow cytometers). (A) Deviation of AbsCD4 in venous blood using Pima to that using FACSCalibur. (B) Deviation of AbsCD4 in venous blood using Pima to that using FACSVia. (C) Deviation of AbsCD4 in venous blood using Pima to that using FACSCanto II. (D) Deviation of AbsCD4 in venous blood using Pima to that using EPICSXL.
Note: Mean CD4=Mean of CD4 counts detected by each comparison group. CD4 relative deviation=CD4 counts detected by Pima minus the results detected by flow cytometers.
Abbreviation: AbsCD4=absolute CD4 cell counts.

(P<0.001). Compared with other samples, the Bland-Altman results showed a lower degree of dispersion tendency. Therefore, this study believes that the above devices have good stability and accuracy in detecting CD4 counts in populations with low CD4 cells (<500 cells/μL).

This study was subject to some limitations. Due to variations in device models, case origin and distribution, and nuances in detection schemes between municipal laboratories, the number of tested samples between the two POC devices and flow cytometry instruments varied greatly between study groups. These variations were especially salient for those labs using EPICSXL, whose sample sizes were too small and thus limited the evaluation of the results. However, the overall results obtained from the POC equipment used in district or county labs were reliable and comparable to those obtained from large-scale flow cytometry.

Changes in CD4 count can effectively reflect the status and degree of a viral infection. Therefore obtaining CD4 count test results early is essential to prompt treatment initiation and the early achievement of immune reconstitution effect in PLWH (*1*,*8*). Our findings show that POC CD4 machines in district or county labs could provide relatively accurate and reliable test results to facilitate treatment initiation. In addition, this assessment provides strong support for exploring modern, new-style AIDS laboratories, which could simplify CD4 count testing, shorten the waiting time for results, and enable one-stop-shop service delivery.

Conflicts of interest: No conflicts of interest.

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. Comparison of CD4 count of HIV/AIDS patients newly diagnosed between FACSPresto and four types of flow cytometry in Jiangsu 2021.

Comparison group	Flow cytometer	N	Median (cells/μL)	*Z*	*P*
Group 1	FACSCalibur	307	252	−5.052	<0.001*
	FACSPresto	307	240		
Group 2	FACSVia	259	250	−5.861	<0.001*
	FACSPresto	259	266		
Group 3	FACSCanto Ⅱ	143	239	−2.781	0.005*
	FACSPresto	143	2,245		
Group 4	EPICS XL	17	211	−1.349	0.177
	FACSPresto	17	205		

**P* value <0.05 is considered significant.
Abbreviation: HIV=human immunodeficiency virus; AIDS=acquired immune deficiency syndrome.

SUPPLEMENTARY TABLE S2. Comparison of CD4 count of HIV/AIDS patients newly diagnosed between Pima and four types of flow cytometry in Jiangsu 2021.

Comparison group	Flow cytometer	N	Median (cells/μL)	*Z*	*P*
Group 1	FACSCalibur	438	248	−14.240	<0.001*
	Pima	438	195		
Group 2	FACSVia	191	265	−7.111	<0.001*
	Pima	191	227		
Group 3	FACSCanto Ⅱ	104	220	−3.885	<0.001*
	Pima	104	203		
Group 4	EPICSXL	14	294	−1.099	0.272
	Pima	14	258		

**P* value <0.05 is considered significant.
Abbreviation: HIV=human immunodeficiency virus; AIDS=acquired immune deficiency syndrome.