

# True Point-of-Care CD4 and CD4% Testing with Highest Accuracy and Precision for Adult and Pediatric HIV/AIDS Patients

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**Abstract:** The absolute number of CD4+ T-lymphocytes is the most widely used parameter for the immune status monitoring of HIV-infected individuals and AIDS patients undergoing antiretroviral therapy. CD4 count analysis is commonly organized in larger centralized laboratories. Until now, this has resulted in a limited availability of this life-saving diagnostic procedure for patients living in remote areas or not being capable to travel to laboratories in larger cities. With a new technological approach, a portable and mobile useable instrument was developed and designed for true point-of-care ("close to the patient") HIV/AIDS immune status monitoring, offering both, CD4 absolute and CD4 percentages among lymphocytes (CD4%) results from one quick sample run with a maximum capacity of up to 250 patient tests per day. Being the first point-of-care solution offering besides CD4 absolute also CD4% results, pediatric patients can now be safely included in the required diagnostic services. The CyFlow® miniPOC can be easily employed without any requirements of a standard laboratory infrastructure. The device has a fixed optical, align-free set-up. Dry lyophilized CD4/CD4% monoclonal antibody test kits eliminate cold chain and cold storage. A disposable syringe is filled with the buffer solution and attached to the device following mixing with cells and antibodies. A full reading of the sample usually takes not more than 40-60sec. Performance tests show a reproducibility of less than 5%. Comparison studies, e.g. from the field in Zimbabwe, show that the CyFlow® miniPOC yields results in high correlation and agreement with other well established CD4 counting flow cytometers (e.g. CyFlow® Counter and CyFlow® SL\_3, Partec GmbH, Germany). The full technical possible range of CD4 absolute counts and CD4 percentages is covered with high precision, accuracy and reproducibility, until today only known from significantly more expensive and much larger, complex flow cytometers. The stand-alone instrument has a weight of less than 5 kg and can thus be easily moved close to the patients. The CyFlow® miniPOC runs on regular power supplies, laptop type Li-Ion batteries and car battery or optionally on solar panels. It is therefore ideally suited as true point-of-care (POC) solution for primary health centers and PMTCT sites in all settings, contributing to further significant decentralization and scaling-up of the obligatory required diagnostic services to HIV/AIDS patients.

**Introduction:** The demand for easy-to-use, accurate and affordable devices performing precise determinations of CD4+ T-lymphocytes absolute counts for adult patients and of CD4 percentages among total lymphocytes (CD4%) required for pediatric patients within HIV monitoring and AIDS patient follow-up is rapidly increasing. Many countries, especially in Sub-Saharan Africa, Asia and Latin America, have started to decentralize and to significantly scale up treatment programmes for HIV/AIDS patients, requiring a wide expansion of activities and services into remote regions, rural areas and primary health stations. In most cases, it is nearly impossible or utmost time- and effort-demanding to introduce a standard laboratory infrastructure for helping the patients in such settings. Furthermore, the patients are geographically widespread in small villages up-country. Centrally organized blood samples logistics often fail. The travel of patients to the nearest center usually is not feasible and those patients not getting the diagnostic results instantly for targeted treatment tend not to return to the clinics. Therefore, entirely new point-of-care (POC) solutions are obligatory needed. The requirements to such an instrument are manifold:

- Compactness of instrument and suitable for transportation (hand-carry) and battery operation / solar panel operation
- Single-platform true absolute counting (no reference particles needed)
- Easy-to-use operation and automatic result reading / printing
- Reliable and efficient reagent protocol for CD4 absolute count and CD4% determination (full diagnostic range)
- Fast sample analysis (less than a minute)
- High correlation and agreement with conventional reference laboratory methods

Additional factors of highest relevance are the robustness of the hardware and the reduction of service/maintenance to a neglectable minimum as required for such instruments in field use. The purpose of this study is to validate the performance of a new POC device (CyFlow® miniPOC) for the determination of CD4 absolute counts and CD4% for use close to the patients, including evaluation of correlation and agreement compared to accepted and well established reference methods for CD4/CD4% analysis.

**Material and Methods:**

**Instrumentation:** The CyFlow® miniPOC works as a single-platform absolute counting (miniature) flow cytometer. The device is fully covered by a transportation case with handle. In transportation mode, the device is completely sealed for splash water and dust protection. In active mode (line voltage or battery) the built-in sheath and waste containers are both level controlled (non contact) for safe operation. The fluidic system works with a syringe pump for disposable plastic syringes (one for each test, supplied with the reagent kits) and transports the sample at a (software-controlled) volume rate (µl/s range). The accuracy and precision of the disposable syringes is checked with a precounted fluorescent bead suspension (Partec CountCheck Beads green, Partec GmbH, Germany). The optical system includes a miniature diode pumped solid state laser in a fixed optical photodetector setup. The built-in computer controls the data acquisition and sample run. The user mode is a completely automated routine with result display in order to avoid false data analysis. The expert mode, based on full histogram / scatterplot display, allows the experienced user to display and reanalyze data files (see figures at right). The system can be operated by touchscreen or external keyboard and mouse.

**Blood samples and reagents:** One hundred twenty five (125) blood samples were collected from the Beatrice Road Infectious Diseases Hospital (BRIDH, Harare, Zimbabwe) into EDTA tubes. The blood samples were processed within 6hrs of collection. Each blood sample was processed twofold for the CyFlow® miniPOC and the reference instrument. When samples were not processed within 6hrs, the tubes were stored at 2° to 8°C according to the recommendation of the manufacturer. Original Partec reagents have been used according to the manufacturer's protocols (CyFlow® CD4 easy count kit and CyFlow® CD4% easy count kit, available in both, liquid and lyophilized form). Liquid reagent kits were used for the CyFlow® SL\_3 and dry reagent kits were used for the CyFlow® miniPOC. Subsets of lymphocytes and leukocytes are identified according to the recognition of surface antigens present especially on CD4+ T lymphocytes. Subpopulations of leukocytes show different expression levels and thus leading to different (weaker) signals in the computer plots (see "Expert mode display" in the picture on the right side).

**Analysis:** The study is divided into four parts with twofold analysis on CyFlow® miniPOC and the reference instruments CyFlow® SL\_3 : 1) CD4 sample analysis for the complete set of patient samples (n=125) covering the full range of CD4+ T cell concentrations (low to high, between 0 and 800 CD4+ cells/µl) and 2) comparisons of CD4 easy count kits in liquid and in lyophilized form (n=77 as extracted data set from the entire data set) and 3) CD4% (0% to 27%, n=27). For part 1) to 3) the correlation method and the Bland-Altman plots are shown. 4) Finally, Count Check Beads green of known concentration for accuracy and precision QC runs are analyzed (10 repetitions). The coefficient of variation (CV) is determined for the determination of precision.

**Results and Discussion:** The new approach presented in this study is the CyFlow® miniPOC, a true point-of-care instrument for robust and reliable CD4 and CD4% determinations, covering adult and pediatric patients. The performance of this miniaturized portable flow cytometer has been validated against larger flow cytometers established as reference methods (e.g. in Zimbabwe, Nigeria, etc.). The results are presented in four parts according to the analysis layout mentioned above. The degree of correlation (correlation coefficient  $r^2$ , fig 1a, 2, 3) is extremely high over the entire measurement range, yielding 0.99 for CD4, CD4 dry/liquid and CD4%, respectively. The Bland-Altman statistics (fig 1b) show a high agreement between the two instruments and methods, yielding a bias of -6.6 CD4+µl (95% CI, -43.8 to 30.6) for the complete data set (n=125). The accuracy of the quality control runs (precounted beads, fig 4) is better than 99% (mean difference from target value < 1%, n=10) for both instruments and the precision is well below the 5% level (3.2% for the CyFlow® miniPOC and 3.7% for the CyFlow® SL\_3). The results therefore underline an excellent level of correlation and agreement to reference methods as well as highest accuracy and reproducibility. In summary, the CyFlow® miniPOC performs equally to conventional, larger, more complex and more costly flow cytometry instruments.

**Conclusion:** This study demonstrates that the miniaturized flow cytometer CyFlow® miniPOC is a highly suitable solution for close-to-patient diagnostic services offering reliable HIV/AIDS immune status monitoring in patient support programmes operating in remote areas far away from central laboratories. The use of newest ultracompact state-of-the-art components (e.g. fluidic, electronics, lasers, detectors, computer boards) allow to have a portable instrument with less than 5 kg but with full functionality. The CD4 absolute and CD4% results have the identical quality as of conventional large flow cytometers.

QC Run No.	QC target value (beads/ml)	CyFlow® SL_3	Difference to Lot	CyFlow® miniPOC	Difference to Lot
1	24360	23640	-2.96%	23851	-2.09%
2	24360	25170	3.33%	23872	-2.00%
3	24360	24645	1.17%	25522	4.77%
4	24360	25105	3.06%	24311	-0.20%
5	24360	25055	2.85%	24791	1.77%
6	24360	24645	1.17%	24546	0.76%
7	24360	25725	5.60%	24462	0.42%
8	24360	22540	-7.47%	22546	-7.45%
9	24360	24695	1.38%	23992	-1.51%
10	24360	24015	-1.42%	23836	-2.15%
	Mean	24524/ml		24173/ml	
	Mean - Lot Concentration	164/ml	0,67%	-187/ml	-0,77%
	Standard Deviation	914/ml		777/ml	
	CV	3,73%		3,21%	

Fig. 4: Low concentration QC runs with precounted beads, showing results from ten repeated measurements on two instruments, the CyFlow® miniPOC and the reference method CyFlow® SL\_3 with very high accuracy >99% and high precision (CV <4%)



CyFlow® miniPOC in transport: full portability / mobility



CyFlow® miniPOC in operation: stand-alone solution for HIV monitoring

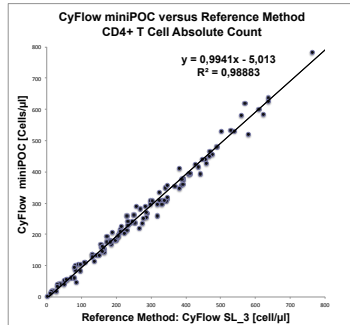
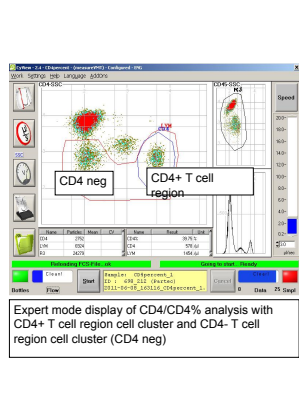
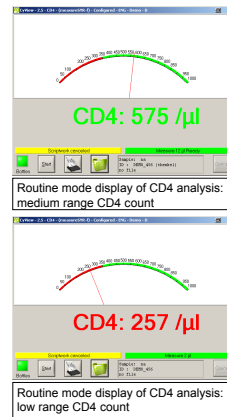


Fig. 1a: CD4+ T cell concentration (CD4 count) comparison on two instruments (n=125), CyFlow® miniPOC and the reference

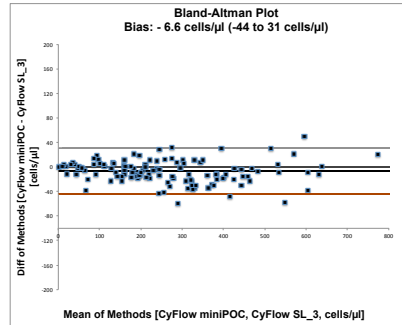


Fig. 1b: Bland-Altman Plot of CD4+ T cell concentration (CD4 count) comparison on two instruments, CyFlow® miniPOC and the reference

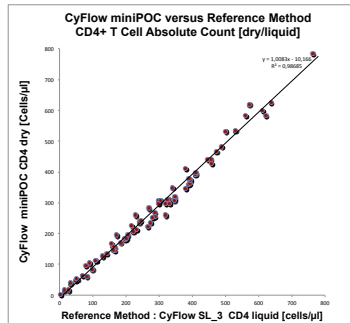


Fig. 2: CD4+ T cell count comparison on two instruments, CyFlow® miniPOC and the reference method CyFlow® SL\_3 with dry and liquid reagents (n=77 out of 125 samples)

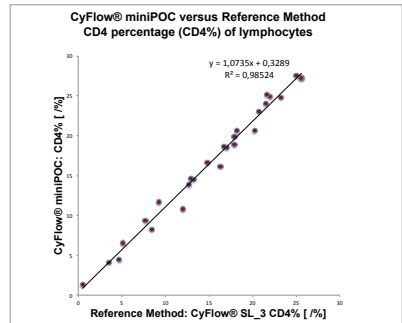


Fig. 3: CD4+ T cell percentage (CD4%) among total lymphocytes on two instruments (n=27), CyFlow® miniPOC and the reference method CyFlow® SL\_3