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RELIABLE AND ACCURATE CD4 T CELL COUNT AND CD4 PERCENT OF THE NEW PORTABLE FLOW CYTOMETER CYFLOW MINIPOC

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Background

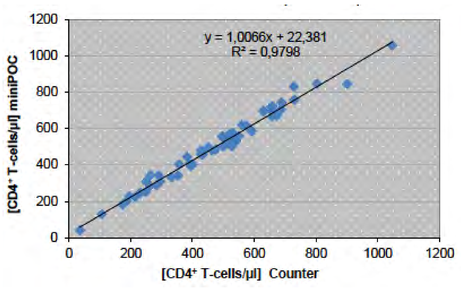
An accurate, reliable, and affordable CD4 T cells count is essential in several parts of the world. Flow cytometry is the "gold standard" for CD4 T cell count, but this technique is expensive and requires sophisticated equipment and trained personnel. In addition, the lack of ready access to technical support and quality assurance programs limits the use of flow cytometry techniques in resource-constrained countries. Instruments are now available that can solve these problems. We have tested the new portable flow cytometer for CD4+ T cell count percentage, named CyFlow MiniPOC (Partec), and analyzed its accuracy, carry-over contamination, repeatability and sensitivity.

Methods

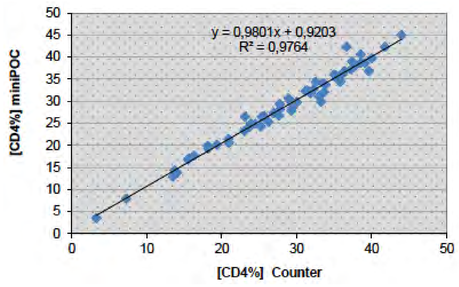
Venous blood from 59 adult HIV-1+ patients (age: 25-58 years; sex: 43 males) was collected in EDTA blood, stained with the Partec MiniPOC CD4% count kit - dry kit, and analysed within a maximum of 2 hours.
CD4 T cell count and percentage were determined by the CyFlow MiniPOC instrument, equipped with a 30 mW, 532 nm laser and three optical parameters for detection of side scatter, orange and red fluorescence. To evaluate the MiniPOC accuracy, CD4 T cell count and percentages were measured in parallel by a reference system, the CyFlow Counter (Partec) and by an indipendent reference system (routinely used in the clinic setting at the NOCSAE Hospital, Baggiovara), the dual platform system based upon Cytomic FC 500 (Cytostat tetrachrome kit for mAbs) and Coulter HMX (for absolute cell count). All measures were performed in triplicate.

Results 1: Accuracy

A

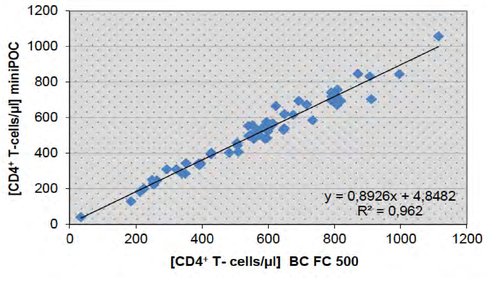


B

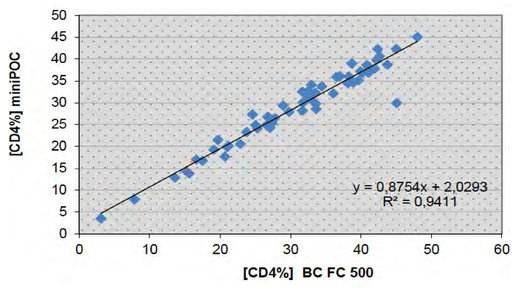


Linear regression analysis of CD4 absolute count (A) and percentage (B) shows that the correlation of CyFlow MiniPOC (y-axis) against the CyFlow® Counter (x-axis; reference system) values is well.

C



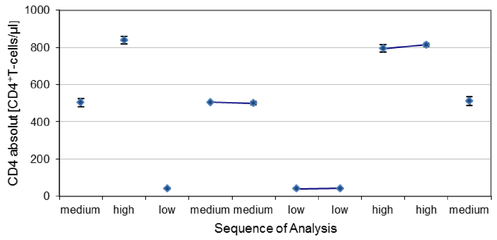
D



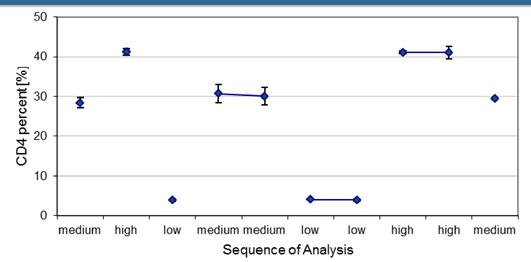
Linear regression analysis of CD4 absolute count (C) and percentage (D) shows that the correlation of CyFlow MiniPOC (y-axis) against the BC FC 500 (x-axis; independent reference system) values is well.

Results 2: Carry-Over Contamination

A



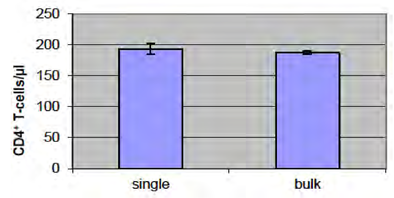
B



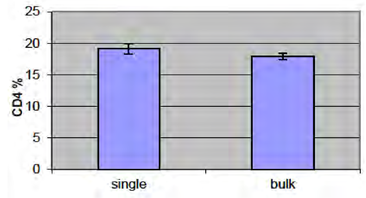
CD4 absolute count (A) and percentage (B) of 3 samples were analyzed in this sequence (Medium – high – low – medium – medium – low – low – high – high – medium) in triplicate. There are no significant differences among all samples with low, medium or high CD4 count and percentage, regardless of their position in the sequence of analysis on the CyFlow® miniPOC.

Results 3: Repeatability

A



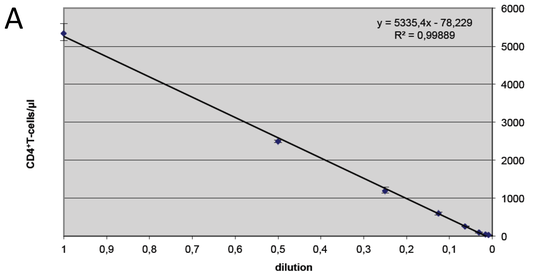
B



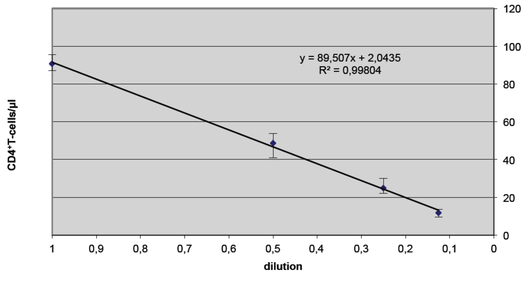
CD4 absolute count (A) and percentage (B) of one sample were analyzed 10 times in single or in bulk. The intra-assay precision of CyFlow® miniPOC is below +/- 10% of deviation.

Results 4: Analytical Sentivity

A



B



Titration analysis of CD4 absolute count of purified lymphocytes (A) and of whole blood (B). Eight dilutions of the same sample were prepared and measured in triplicate. The analytical sensitivity of CyFlow® miniPOC is linear within the investigated range of 0 - 5000 CD4+ T-cells/µl.

Conclusions

The cost-effective and portable instrument MiniPOC produces reliable and accurate results that are fully comparable with highly expensive dual platform systems. Indeed, data perfectly correlate with those obtained with Cytomic FC 500/Coulter HMX. Finally, using CyFlow MiniPoc permits to perform in a fast and easy way a very high number of CD4 T cells counts per day.