Evaluation of Transfix-treated blood on Partec Cyflow as a potential test material in an external quality assessment (EQA) program for CD4 T-cell enumeration

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Introduction
CD4 T-cell enumeration remains an important prognostic marker in the management of HIV disease and is currently regarded as the most direct assessment of a patient’s overall immune status and risk for opportunistic infections. Accurate CD4 T-cell results are thus essential in the HIV treatment monitoring cascade. External quality assessment (EQA) programs provide an objective means of assessing reliability of clinical measurements. The utility of whole blood stabilizing products as test reagents in EQA programs has previously been demonstrated in a number of currently available international EQA schemes. Since then, many of these products have become available. In the present study, we evaluated the performance of Transfix-treated blood on Partec Cyflow as a potential blood-stabilizing product for CD4 T-cell enumeration in a national EQA program.

Methods and Materials
Blood from HIV-infected individuals (n=25) was treated with Transfix at a ratio of 5:1 and incubated at room temperature and at 37°C. Stabilized samples were analysed on Partec Cyflow for days 0, 3, 6, 17 and 20. Percentage CD4 and Absolute cell counts from stabilized blood samples were compared to those of untreated blood.

Results
At room temperature, average CD4+ T-cell counts for day 0, 3,6,17 and 20 gave correlation coefficients of R²=0.604, 0.920.0.858, 0.906 and 0.909 respectively when compared to counts from day 0 untreated blood. RF was 0.897 for day 3 treated blood stored at 37°C. The best correlation coefficient for the percent CD4+ T-cell counts were obtained at day 17 at RF=0.842. The average percent differences were 0.76%, -0.8%, -2%, -1.8% and -1.5% for days 0,3,6,17 and 20 respectively.

Conclusions
Performance characteristics for Transfix-treated blood imply that the blood remains viable for CD4 T-cell immuno-phenotyping using Partec Cyflow for up to 20 days at room temperature and 3 days at 37°C. Whole blood stabilization with Transfix allows testing of aged samples in a low volume, inexpensive format with minimal reagent use, and may therefore be used in EQA programs in resource-poor settings.

References

Figures
Figure 1. Partec Cyflow counter
Figure 2. Transfix blood stabilizer

Chart 1. Linear regression analysis for CD4 Absolute cell counts at room temperature (figures A-E) and at 37°C (figure F). Values from treated Transfix-stabilized blood at day 0, 3, 6, 17 and 20 were compared to values from fresh, untreated blood. Figure G shows the correlation coefficient for percent CD4 between day 17 values and fresh whole blood

Chart 2. Bland Altman analysis for CD4 T-cell counts using Transfix-treated blood for days 0 and 20 at room temperature when compared to fresh whole blood