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 patients 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

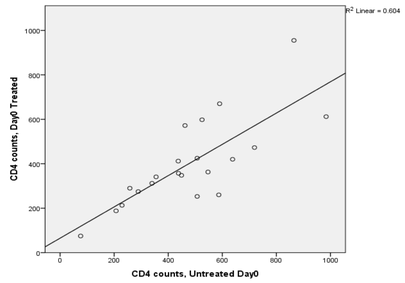
Blood from HIV-infected individuals (n=25) was treated with Transfix at a ratio of 5:1 and incubated at room temperature and at 37°C. Stabilized samples were analysed on Partec Cyflow for days 0, 3,6,17 and 20. Percentage CD4 and Absolute cell counts from stabilized blood samples were compared to those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

Figure 1. Partec Cyflow counter

Figure 2. Transfix blood stabilizer

Results

At room temperature, average CD4+ T-cell counts for day 0,3,6,17 and 20 gave correlation coefficients of R^2=0.604, 0.920,0.858, 0.906 and 0.909 respectively when compared to counts from day 0 untreated blood. R^2 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 R^2=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.



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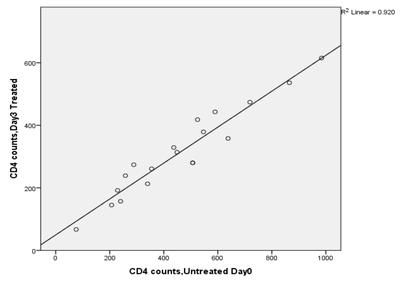

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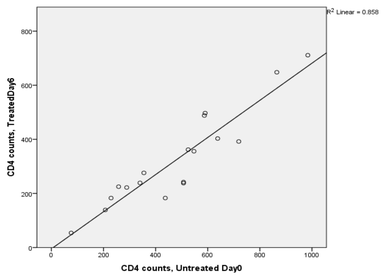

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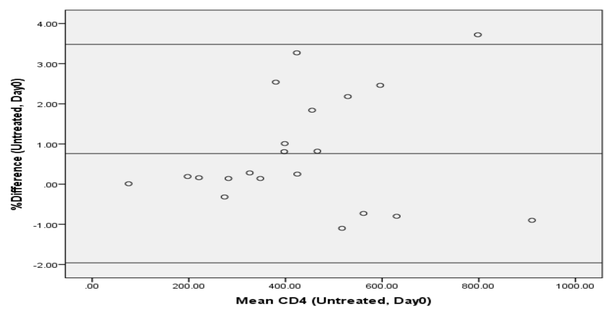

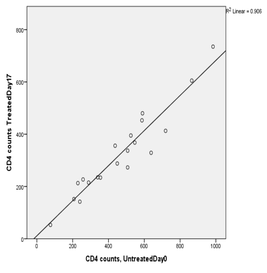

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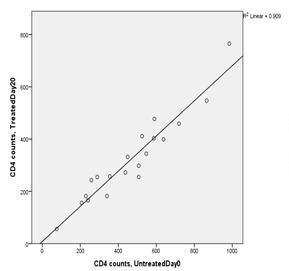

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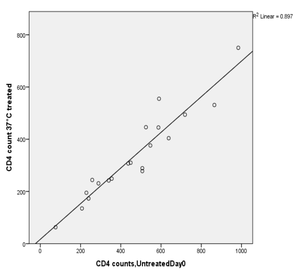

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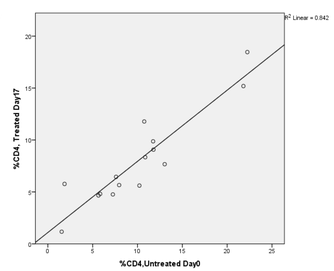

G

Chart 1. Linear regression analysis for CD4 Absolute cell counts at room temperature (figures A-E) and at 37C (figure F). Values from treated Transfix-stabilized blood at day0, 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

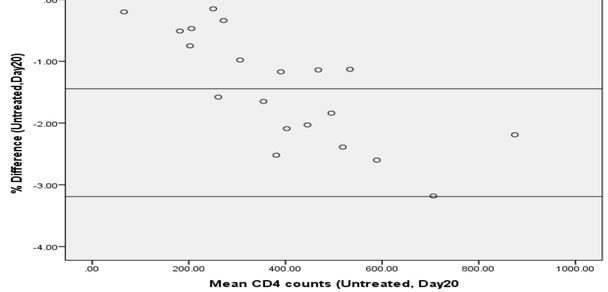

Chart 2. Bland Altman analysis for CD4 T-cell counts using Transfix-treated blood for days 0 and 20 at room temperature when compared to fresh whole blood

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 37C. 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.

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References

1. TransFix: a sample stabilising fluid for use in cellular haematology and immunology. Barnett D., Granger V, Pockley A. G., Saxton J. M., Storie L., Whitby L., and Reilly J. T.. 1998. Cytometry 38:88.
2. Affordable CD4+ T cell counts by flow cytometry. II. The use of fixed whole blood in resource-poor settings. Jani V, Janossy G, Iqbal A, Mhalu FS, Lyamuya EF, Biberfeld G, Glencross DK, Scott L, Reilly JT, Granger V, Barnett D. J Immunol Methods. 2001 Nov 1;257(1-2):145-54.
3. Evaluation of leukocyte stabilisation in TransFix-treated blood samples by flow cytometry and transmission electron microscopy. Canonico B, Zamai L, Burattini S, Granger V, Mannello F, Gobbi P, Felici C, Falcieri E, Reilly JT, Barnett D, Papa S. J Immunol Methods. 2004 Dec;295(1-2):67-78