Performance of Aged, TransFix-Treated Blood in the Guava EasyCD4 and EasyCD8 Assays

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ABSTRACT

Background: Counting CD4 and CD8 T cell counts has become essential in research on blood from healthy individuals and transplant patients, such as those with HIV. Most procedures for CD4 and CD8 T cell enumeration require the blood to be collected and assayed in a timely manner. This can be problematic in settings where samples can only be transported long distances or samples need to be transported for a few days due to a lack of instrumentation or operator availability. It has been reported that TransFix can stabilize blood samples for up to 72 hrs old for accurate results. This can often be a problem in clinical settings where samples can be used for a period of 0–30 days. In this study, we demonstrate the performance of TransFix-treated blood in the EasyCD4 and EasyCD8 assays as shown in the Figure above, are simple two-color assays that use antibody staining of 10 µL of blood, lysing of stained samples followed by analysis of samples on the Guava PCA. The %CVs of triplicates were calculated for the TransFix-treated and fresh blood samples and plotted as shown in this study.

RESULTS: With TransFix-treated HIV-seronegative donors, the CD4 and CD8 T cell counts were 99.3 ± 5.1 and 98.6 ± 7.0, respectively, supporting that good precision could be obtained on the TransFix-treated blood samples.

CONCLUSIONS: The %CVs of triplicates were calculated for the TransFix-treated and fresh blood samples and plotted as shown in this study.

INTRODUCTION

CD4 and CD8 T cell enumeration has become an important component of basic and clinical research and clinical monitoring of several infectious and inflammatory diseases, such as HIV. Most procedures for CD4 and CD8 T cell enumeration require blood samples to be counted on the day of draw. It is important to be able to accurately count T cells in blood samples even after a period of days when CD4 counting technology is not immediately accessible and samples need to be transported long distances or samples need to be transported for a few days due to a lack of instrumentation or operator availability. The %CVs of triplicates were calculated for the TransFix-treated and fresh blood samples and plotted as shown in this study.

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MATERIALS AND METHODS

Materials

The EasyCD4 and EasyCD8 assays were performed according to manufacturer’s instructions. 1% of samples were quality controlled prior to each run. The %CVs of triplicates were calculated for the TransFix-treated and fresh blood samples and plotted as shown in this study.

Methods

The %CVs of triplicates were calculated for the TransFix-treated and fresh blood samples and plotted as shown in this study.

Figure 1. Accuracy of CD4 Counts from TransFix-treated Aged Blood Samples

Figure 2. Accuracy of CD4/CD8 Ratio for TransFix-treated Samples

Figure 3. Precision Data for EasyCD4 Assay on TransFix-treated Blood Samples over a Period of 30 Days

Figure 4. EasyCD8 Staining Patterns of Blood Samples TransFixed and Assayed at Different Times

Figure 5. EasyCD8 Staining Patterns of Blood Samples TransFixed and Assayed at Different Times

Figure 6. Performance of TransFix-treated Blood in the Guava EasyCD4 and EasyCD8 Assays

Figure 7. Accuracy of CD4/CD8 Ratio for TransFix-treated Samples

CONCLUSIONS

- Use of Transfix as a stabilizer is a ±10% ratio when added to whole blood shows good compatibility for use with the EasyCD4 and EasyCD8 assays to obtain accurate and precise CD4 and CD8 counts.
- The staining pattern from Transfix treated samples over 15 days is almost identical to the staining pattern at day 0. At 30 days the staining pattern is starting to show signs of deterioration, however accurate counts could still be obtained.
- Accurate CD4 and CD8 T cell counts were obtained from Transfix treated samples over a period of 0–15 days (within 10% of untreated control) when compared to fresh untreated blood samples.
- The 30 day Transfix-treated samples show an increased negative bias compared to samples treated for 15 days or less, however in most cases counts were within 20% of counts from untreated samples from Day 0 to Day 15.
- Accurate CD4/CD8 ratios could be obtained for the entire period of 30 days.

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