Inter-laboratory Comparison of the TransFix® /EDTA Vacuum Blood Collection Tube (TVT) with the 5 mL Cyto-Chex® BCT

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Introduction

The "TBNK panel" in flow cytometry (FC) immunophenotyping has become a staple of most clinical FC laboratories worldwide. The panel is used to identify and enumerate the lymphocyte subsets within patient blood samples and is typically used for immune monitoring individuals with HIV. In addition, the panel is used to evaluate unexpected changes in cell numbers, indicative of certain immunological diseases, and as a method of demonstrating the pharmacodynamic effects of drugs such as rituximab [2].

The "gold standard" collection tube is the EDTA Vacutainer (BD Vacutainer), but requires that FC analysis be performed 6 hours, or a maximum of 48 hours from venipuncture to prevent cellular degradation from affecting the immunophenotypic profile [1, 3]. Blood samples >48 hours old exhibit indistinguishable cell subsets and inaccurate absolute cell counts leading to erroneous clinical results. In these situations fresh samples need to be collected, incurring extra costs, inconvenience to the patients involved and delayed analysis. Indeed in locations that do not have the infrastructure to test such specimens, samples must be transported for clinical evaluation and subsequently destroyed if this deadline cannot be met. Furthermore, for multisite clinical trials the preservation of patient samples is of utmost importance in order to achieve reliable results when varying transit times are unavoidable [2].

For these reasons, manufacturers Caltag Medsystems (Buckingham, UK) and Streck (Omaha, NE, USA) have developed the TransFix Vacuum Blood Collection Tube (TVT) and BCT, respectively to preserve the integrity of such critical specimens.

Objectives

This study aimed to assess the performance of the TransFix Vacuum Blood Collection Tube (TVT) and BCT devices across three clinical FC sites for their shared intended purpose of stabilizing lymphocytes for immunophenotyping via flow cytometry for up to 14 days.

TVTs and BCTs exhibit good clinical accuracy

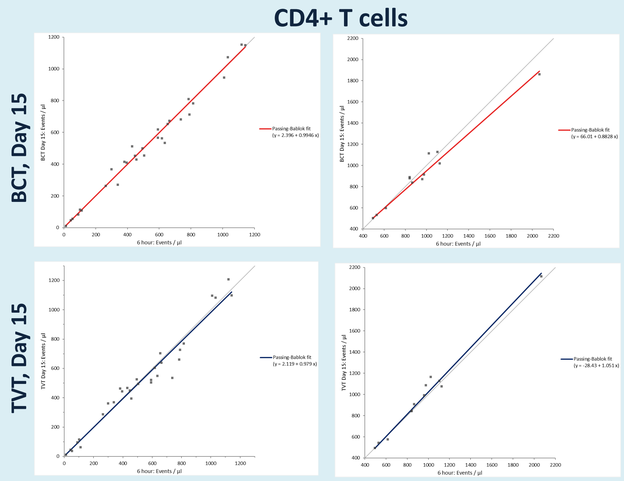

Figure 1. Regression analyses showing the correlation between CD4+ T cell absolute counts recovered from HIV+ or healthy (HC) samples stored in the test device (BCT or TVT) for 15 days versus the Vacutainer result (<6 hours post venipuncture).

Graphs show that both devices are clinically accurate for the CD4 T-cell marker as trend lines closely mirror the control results from both cohorts.

Methods

- Sites (NY, USA and UK) recruited 30 HIV+ subjects (CD4 T cell absolute values: <50 to >500) and 12 HC subjects. From each subject, three blood samples were collected: a 4mL Vacutainer, a 5mL BCT and a 3mL TVT. Samples were analyzed for a TBNK panel (BD Multitest IMK kit) on a BD FACS Canto II (FCM) at baseline (<6 hours), on Day 11 and on Day 15 (TVTs and BCTs only).
- To assess clinical accuracy, recovery of cells (lymphocyte absolute counts) was recorded per device (BCT and TVT) over the 15 day testing period. The Passing-Bablok regression analysis was performed to assess the relationship between the measurement from each time point and the control (BD Vacutainer) result. Slope point estimates and correlation coefficient point estimates (r value) were compared between the TVT and BCT in relation to the control (Vacutainer) result.
- 2D dot plots were visually assessed to determine whether lymphocyte sub populations could still be easily identified and to evaluate cell morphology.
- Using 'fcs' files provided (HIV+ n =25 and HC n=8), the averaged mean fluorescence intensities (MFIs) of lymphocyte markers were calculated using Infinicyt. MFIs were then presented as box and whisker plots and statistically evaluated by assessing p-values generated by a paired two-tailed t-test.

TVTs and BCTs exhibit a decrease in cell marker expression

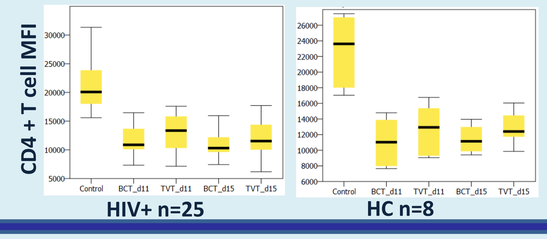

Figure 2. Box and whisker plots show that both devices exhibit a decrease in cell marker MFI, for the target cell (in this case CD4+ T cells) over the 15 day stability period, compared to the control.

TVTs exhibit background CD3 MFI results for B and NK cells that are equivalent to the control.

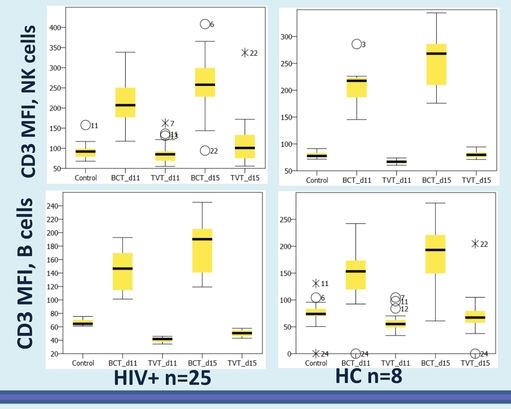

Figure 3. Box and whisker plots show that there was an increase in CD3 MFI auto-fluorescence for cells identified to be CD3- (NK cells and B cells) from samples stabilized within BCTs over the 15 day stability period, compared to the control. For TVT samples CD3 MFI for the same subsets, were significantly equivalent to the control.

Lymphocyte FC gating characteristics are well maintained in TVTs

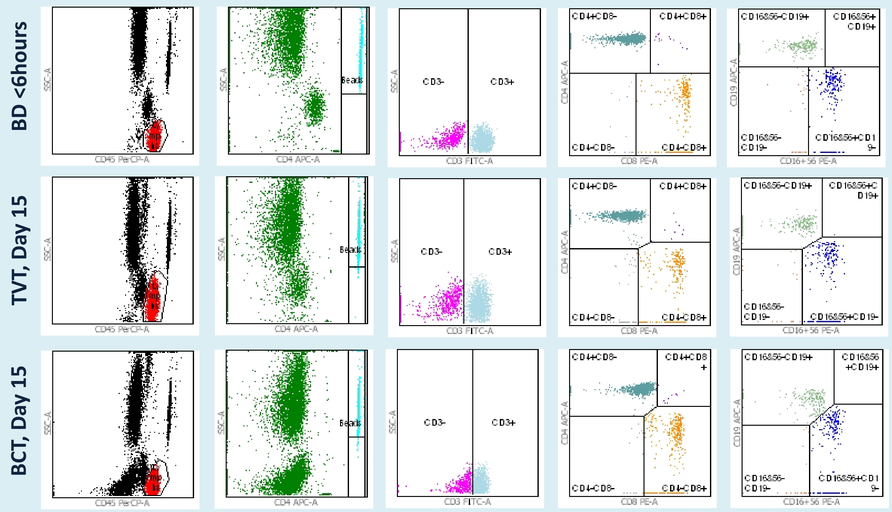

Figure 4. FC gating for a HIV+ subject - representative of the cell morphology observed for this cohort. Figure shows the visualization of the lymphocytes tested via cell markers CD3, CD4, CD8, CD19, CD16+56, CD45 for the control (BD <6 hours, Day 0), TVT on Day 15 and BCT on Day 15, respectively.
There was poorer separation of CD3+ (light blue) and CD3- (pink) lymphocytes and increased cell debris (CD45low, SSC low – black events) observed for BCT samples versus TVT samples. Lymphocytes from TVT samples exhibited higher side scatter (SSC) properties than the equivalent cells in the BD Vacutainer and BCT tubes.

Conclusions

- The TVT and BCT exhibited good clinical agreement with the "gold standard" BD Vacutainer result with regard to lymphocyte absolute count recoveries over the 15 day testing period.

- There were improved performance characteristics, in relation to the cell marker auto-fluorescence for lymphocytes recognized as CD3- and cell debris, from samples that were stabilized by the TVT over the BCT.

References

1. Laboratory Guidelines for enumerating CD4 T Lymphocytes in the context of HIV/AIDS, World Health Organization, 2007.
2. Flow cytometry: a flexible tool for biomarker research, R.M Barnard, Future Science, 2012.
3. BD Multitest IMK Kit, Product Insert, 2016.