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

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Abstract

Background—An inter-laboratory study was performed to compare the performance of the 3mL TransFix®/EDTA Vacuum Blood Collection Tube (TVT), with the 5mL Cyto-Chex® BCT tube (BCT). Both devices are intended for collection and storage of whole blood specimens for immunophenotyping of leukocytes by flow cytometry for up to 14-days.

Methods—One site in the USA and two in the UK tested samples from 10 HIV positive patients and four healthy subjects for a total of 42 samples. From each subject, three blood samples were collected: a BD 4 mL K₃ EDTA Vacutainer (Vacutainer), a TVT, and a BCT. At all sites, samples were analyzed on a BD FACS Canto II flow cytometer for a full lymphocyte subset count within 6 hours of collection (all devices) and on Day 11 and Day 15 (TVT and BCT only). Data obtained from the Vacutainer were used as the control data set with which TVT and BCT data were statistically compared.

Results and Conclusions—Statistical concordance was demonstrated for both devices in relation to cell absolute count recovery. For cell marker signal, both devices exhibited a significant decrease in mean fluorescence intensity (MFI) for the detection of lymphocyte subsets and their target markers. There was a marked increase in auto-fluorescence observed for BCT stabilized lymphocytes whereas values for the TVTs were comparable to the control. There were eight instances of statistical equivalence between the level of antibody auto-fluorescence observed in the control tube and the TVT across both patient cohorts, versus two for the BCT.

Keywords

Immunophenotyping; HIV; Hematology

Introduction

The “TBNK panel” or full lymphocyte subset count in flow cytometry (FC) immunophenotyping is a routine investigation performed by most clinical FC laboratories worldwide. The panel is used to identify and enumerate T cells including CD4 and CD8 T cell subsets, B cells (CD19+ cells) and NK cells (CD16/56+ cells) within patient blood samples and is typically used for monitoring individuals with HIV. CD4+ T cell enumeration, along with plasma HIV RNA (viral load) are the predominant biomarkers for assessment of patient compliance and disease progression [1, 4, 5, 11]. 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, 9].

The recommended collection tube for this assay is the EDTA Vacutainer (Vacutainer), but requires that FC acquisition be performed within 48 hours of venipuncture collection to prevent cellular degradation from significantly affecting the immunophenotypic profile and is ideally performed within 6 hours [1, 3, 8]. Blood samples older than 48 hours can exhibit indistinguishable cell subsets and inaccurate absolute cell counts leading to erroneous clinical results. In these situations, fresh samples may 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 [4, 7]. Furthermore, for multisite clinical trials the preservation of patient samples is of utmost importance in order to achieve reliable results when it is considered that varying transit times are unavoidable [2, 9].

For these reasons, manufacturers Caltag Medsystems (Buckingham, UK) and Streck (Omaha, NE, USA) have developed the TVT and BCT, respectively to preserve the integrity of such critical specimens [6, 9, 10]. This study aimed to assess the performance of these devices across three clinical FC sites for their shared intended purpose.

Materials and Methods

Study Design

One site in the USA (Buffalo, NY) and two sites in the UK (Brighton and Sheffield) were asked to recruit four healthy subjects (individuals with no known disease process and 21 years of age), and 10 HIV positive subjects (individuals which represented a range of CD4 T cell absolute values from <50 to >500). This corresponded to 12 healthy subjects, and 30 HIV positive subjects across the study.

Blood was collected from consenting donors according to the guidelines and recommendations of each respective institution’s IRB / ethical committee.

From each subject, three peripheral whole blood samples were collected at the same time: one into a 4mL Vacutainer, one into a 5mL BCT and one into a 3mL TVT. The sample collected into the Vacutainer was analyzed for a full lymphocyte subset count/TBNK panel

(BD Multitest IMK kit: CD3 FITC, CD4 APC, CD8 PE, CD19 APC, CD16/56 PE and CD45 PerCP, BD, San Jose, CA, USA), in duplicate, on a BD FACS Canto II flow cytometer (FCM) at baseline, defined as within 6 hours of collection only (refer to Table 1). Samples taken into the TVT and BCT tubes were similarly analyzed for a full lymphocyte count in duplicate within 6 hours of collection, on Day 11, and on Day 15 (Table 1). The 6-hour Vacutainer time was chosen to be consistent with the manufacturer's preferred time point for the TBNK panel. Day 15 was chosen to qualify the product's stability for at least two weeks (14 +1 day) and Day 11 was selected as an interim time point.

Sample Preparation, Data Acquisition and Analysis

Staining was performed according to the manufacturer's instructions for the BD Multitest IMK kit (BD, San Jose, CA, USA). At all sites, acquisition was performed on a 3 laser BD FACSCanto II and the data were analyzed using the accompanying BD FACSCanto Clinical Software. Before patient sample acquisition, all instrument parameter and compensation settings were established daily using BD 7 color setup beads which harmonizes the median fluorescence intensity. Each laboratory was responsible for the clinical analysis. BD FACSCanto II reports were initially reviewed by the technologist. If the regions established by the software algorithm required additional adjustments an electronic copy of the initial report was saved before the technologist made any region adjustment which were also saved and reviewed by the laboratory director. The 2D dot plots generated from the clinically validated FC reports were visually assessed by at least one author to determine whether lymphocyte sub populations could still be satisfactorily resolved on the CD45 versus side scatter plot and that separation of T cell subsets, B cells and NK cells were resolved. On completion, the clinically validated FC reports were given to the study sponsor – Caltag Medsystems with which a study database was populated with the clinically approved data. All absolute cell count data presented in this study was generated from the first of these reports, per subject.

In addition, sites provided the sponsor with the raw acquisition 'fcs' files to enable an independent operator to generate MFI data, per cell marker, using the FC analysis program: Infinicyt (Cytognos, Salamanca, Spain). Using all the available raw 'fcs' files provided by sites (HIV n=25 and HC n=8), the averaged (from duplicate tests) MFIs of lymphocyte cell markers were calculated and presented as box and whisker plots. N.B. Duplicate files from five HIV patients and four healthy donors were missing from one sites' electronic archiving system.

Statistics

The Passing-Bablok regression analysis was performed to assess the relationship between the subset absolute count measurement from each time point and the control (Vacutainer) result. This method was chosen for this data set as it allows multiple subject results, for a particular cell marker, to be collated to demonstrate statistical concordance.

For statistical concordance to be proven, the target criteria for the Passing-Bablok regression were slope point estimates from 0.90 – 1.10, and correlation coefficient point estimates (r value) of 0.90 when comparing both the TVT and BCT to the control (Vacutainer) result.

The slope point estimate range and r value criteria are statistical standards rather than measures of clinical acceptability. Therefore, the concordance evaluation was made between the TVT and the BCT in the context of their performance against the reference Vacutainer tubes.

Cell marker MFIs were displayed as box and whisker plots. These data were statistically evaluated by assessing p-values generated by a paired two-tailed t-test, between the control Vacutainer lymphocyte marker MFI versus the TVT and BCT lymphocyte marker MFIs at time points Day 11 and Day 15.

Results

Concordance – Absolute Cell Counts

In order to assess the stability of cells collected into each of the blood collection tubes (BCT and TVT), the absolute counts of each lymphocyte subtype were measured over the 15 day testing period and compared to the day 0 standard Vacutainer. For this study, only the first available replicate result for each tube was used in order to mimic a routine clinical testing situation.

HIV Positive Subjects

Figures 1a, 1c, 1e (BD vs BCT) and 1b, 1d, 1f (BD vs TVT) demonstrate that both devices are suitably equivalent to the baseline Vacutainer result for the CD4 T cell marker. Trend lines closely mirror the control result at all time points.

The results for HIV subjects (n=30) in Table 2 show that both the BCT and TVT vs BD (Vacutainer) tube target criteria are met in all cases except for the slope point estimate for TVT tubes at 6 hours for CD8 (slope = 1.12). As the 6-hour (Day 0) time point is the baseline, the slightly increased slope does not point to an issue with stabilization up to 14 days. The specification criterion for the correlation coefficient (r value) point estimate is achieved in all cases.

Table 3 shows that the 30 HIV patients used for this study have wide ranging absolute cell counts for the lymphocyte subsets tested. CD4+ T cells range from 13 cells/ μ l to 1142 cells/ μ l, which meet the criterion set out in the study design (CD4 T cell absolute values from <50 to >500).

Healthy Subjects

Figures 2a, 2c, 2e (BD vs BCT) demonstrate that the observed CD4+ T cell absolute counts are lower than expected for healthy donor samples collected in BCTs. This is particularly true for the higher CD4 counts. Figures 2b, 2d, 2f (BD vs TVT) demonstrate that the observed CD4 T cell absolute counts are similar to the control Vacutainer results for healthy donor samples collected in TVTs as trend lines closely mirror the Vacutainer result at all time points.

The results for the healthy controls (n=12) in Table 4 show that slope point estimates are out of specification for the BCT tubes in seven cases (often in the 0.8 range), and are out of

specification for the TVT tubes in one case (CD45 at 11 days, at 1.14). The correlation coefficient point estimate specification is achieved for both tubes in all cases except for TVTs at Day 11 for CD16/56, at 0.89.

Table 5 shows that the 12 healthy subjects used for this study have wide ranging absolute cell counts for the lymphocyte subsets tested. CD4+ T cells range from 493 cells/ μ l to 2068 cells/ μ l.

Table 6 shows that for all subjects there are nine slope point estimate results that do not meet the criterion set – seven out of 36 for the BCT tube and two out of 36 for the TVT. There is one failure for the HIV cohort from the TVTs for the CD8 marker at Day 0, but as this is at this time point it does not point towards an issue of the device's stability characteristics.

Cell Scatter and Staining Characteristics – Flow Cytometry Dot Plots

2D FC dot plots are used by flow cytometrists to identify certain cell populations based on light scatter characteristics and cell marker positivity (antibody staining). Gating strategies are employed by FC software programs which automatically place fields around cell subsets so that they can be categorized and enumerated. However, the technologist and the lab director will need to review these to ensure the clinical outcome is correct. If cell subsets are poorly separated from one another on a dot plot then it becomes harder to categorize them and ultimately count them.

HIV Positive Subjects

Figures 3a – 3c show the flow cytometry gating for a HIV subject in the study, and are representative of the cell scatter and staining characteristics observed for this cohort. Figures 3a, 3b and 3c show the visualization of the lymphocyte subsets tested via cell markers CD3, CD4, CD8, CD19, CD16+56, CD45 for the Vacutainer control (BD <6 hours, Day 0), TVT on Day 15 and BCT on Day 15, respectively.

Healthy Subjects

Figures 4a – 4c show the flow cytometry gating for a healthy subject in the study, and are representative of the cell scatter and staining characteristics observed for this cohort. Figures 4a, 4b and 4c show the visualization of the lymphocyte subsets tested via cell markers CD3, CD4, CD8, CD19, CD16+56, CD45 for the Vacutainer control (BD <6 hours, Day 0), TVT on Day 15 and BCT on Day 15, respectively.

A visual review of all the 2D dot plots from both healthy donors and HIV patient consistently revealed a poorer separation of CD3+ (light blue) and CD3- (pink) lymphocytes and an increase in cell debris (CD45 low, SSC low – black events) observed for BCT samples versus TVT samples. We also noted that lymphocytes from TVT samples consistently exhibit higher side scatter (SSC) properties at both the 11 and 15 day time point than the equivalent cells in the Vacutainer and BCT tubes.

Cell Marker Signal – Mean Fluorescence Intensity

As well as absolute counts, MFI is an important parameter within the field of immunophenotyping. This parameter will demonstrate the strength of the fluorescence signal associated to a particular cell marker indicating a degree of positivity for certain cell populations. MFI is also a good parameter for assessing the device stability (in this case for both TVT and BCT tube types) because if antigens degrade over time the MFI will decrease. Also, in certain cell types which do not naturally express the marker increased fluorescence can occur due to non-specific binding (i.e. increased auto-fluorescence). If the MFI for a particular cell marker observed within the Vacutainer (Day 0) is similar to that of the BCT or TVT (Day 11 or Day 15), then it indicates that the device provides good cell marker stability.

HIV Positive Subjects

Box and whisker graphs in figures 5a – 5f show the relationship between the mean control (Vacutainer, Day 0) MFI signal for cell subsets and their target markers tested within HIV subjects (n=25) versus the equivalent BCT and TVT MFI results for time points Day 11 and Day 15. The graphs demonstrate that for all lymphocyte subsets, there is a significant reduction ($p<0.05$) in MFI for their target marker (e.g. CD3 MFI for T cells) within BCTs and TVTs. Therefore, both devices performed equivalently.

Box and whisker graphs in figures 5g – 5j show the relationship between the background fluorescence of the negatively staining populations for each cell subset within HIV subjects (n=25) versus the equivalent BCT and TVT MFI results for time points Day 11 and Day 15. These assessments of auto-fluorescence particularly look at: CD3 MFI within NK cells, CD3 MFI within B cells, CD4 MFI within Cytotoxic T cells, and CD8 MFI within T helper cells. Conditions with an asterisk adjacent to them indicate that the result is statistically significant from the control i.e., asterisk = $p < 0.05$.

For the HIV cohort, these data demonstrate that TVT samples exhibit the statistical equivalent ($p>0.05$) level of auto-fluorescence as the Vacutainer control tube on four occasions: CD3 MFI within NK cells on Day 11, CD3 MFI within B cells on Day 15 and CD4 MFI within Cytotoxic T cells for both time points. BCT samples all exhibit statistically higher ($p<0.05$) levels of auto-fluorescence compared to the control.

Healthy Subjects

Box and whisker graphs in figures 6a – 6f show the relationship between the mean control (Vacutainer, Day 0) MFI signal for cell subsets and their target markers tested within healthy subjects (n=8) versus the equivalent BCT and TVT MFI results for time points Day 11 and Day 15. The graphs demonstrate that for all lymphocyte subsets, there is a significant reduction ($p<0.05$) in MFI for their target marker within BCTs and TVTs. Therefore, both devices performed equivalently.

Box and whisker graphs in figures 6g – 6j show the relationship between the background fluorescence of the negatively staining populations for each cell subset within healthy subjects (n=8) versus the equivalent BCT and TVT MFI results for time points Day 11 and

Day 15. Conditions with an asterisk adjacent to them indicate that the result is statistically significant from the control i.e., asterisk = $p < 0.05$.

For the healthy cohort, these data demonstrate that TVT samples exhibit the statistical equivalent ($p > 0.05$) level of auto-fluorescence as the control tube on four occasions: CD3 MFI within NK cells on Day 15, CD4 MFI within Cytotoxic T cells for both time points and CD8 MFI within T helper cells on Day 15. BCT samples exhibit the statistical equivalent ($p > 0.05$) level of auto-fluorescence as the control tube on only two occasions: CD4 MFI within Cytotoxic T cells for both time points. However, similarly to the HIV cohort, BCT samples exhibit a marked increase in levels of auto-fluorescence compared to the control.

Discussion

While there have been comparative studies between the TransFix solution (Caltag Medsystems, UK) and BCT / Streck Cell Preservative products conducted prior to this one [12, 13, 15], the authors believe that this study is the first assessment of the performance of the 3mL TVT device with the 5mL BCT device with regard to their shared intended use of stabilizing leukocytes for 14 days where statistically relevant donor numbers have been evaluated. It was also designed to demonstrate that the devices performed consistently for donor samples of differing disease status, hence the inclusion of healthy and HIV+ samples.

The results demonstrated that both the BCT and the TVT were concordant, in terms of lymphocyte subset absolute count recovery over 15 days, when compared to the control Vacutainer result. Regression analysis results showed that there was only one case where the correlation coefficient (r value) failed to meet the 0.90 target (TVT vs BD = 0.89 for CD16/56 on Day 11) for both devices. These results highlight that both devices can be used in a capacity where a centralized analysis laboratory for immunophenotyping is required for clinical development purposes. For the TVT, its utility has already been described in this fashion in publications such as Poirier et al. [9] and Sommer et al. [14].

However, the data indicated that there were nine instances where the slope point estimate target of 0.90 – 1.10 was not achieved. There were seven out of specification slope point estimates (five out of these were in the 0.8 range) for the BCT, all for healthy control samples, whereas, for the TVTs, there were two slope point estimates that were out of specification for the HIV and healthy control samples at 1.12 and 1.14, respectively. This equated to an approximate overestimation of a high absolute count when the slope point estimate exceeded 1.10 and an underestimation of a high absolute count when the slope point estimate was less than 0.90.

In general, the TVT tubes compared more favorably to the EDTA Vacutainer 6 hour reference than the BCT device. Given the number of observations in each comparison ($n=30$ HIV, $n=12$ healthy controls), the resulting slope point estimates at or near identity indicated good quantitative agreement between the TVT and the Vacutainer reference. This analysis emphasized how TVT tubes were closer to the recognized reference (Vacutainer) than the BCT, in this study.

For the purposes of CD4+ T cell enumeration for monitoring patients with HIV, the results showed that both the TVT and BCT devices were diagnostically accurate throughout the 15 day storage period. These findings have important implications for resource-limited locations that rely upon centralized laboratories to enumerate CD4+ T cells via flow cytometry from samples that are shipped from further afield. Examples of these technological and financial problems related to CD4+ T cell enumeration have been discussed in such papers as Cheng et al. [16] and MacLennan et al. [17] who address the cost and complexity implications of flow cytometry and evaluate more cost effective options. However they both acknowledge flow cytometry to be the gold standard for CD4+ T cell enumeration due to the accuracy of the technology (particularly with regard to CD4 counts of <200 cells/ μ l) and discuss the importance of preservation of samples. With this in mind, these results demonstrate the possibility of enhanced accessibility of samples to flow cytometric testing, the most accurate method for CD4+ T cell enumeration and mean CD4 percentages, using these devices.

The data for the 2D dot plots showed that there was poorer separation of CD3+ from CD3- lymphocytes and increased cell debris observed for BCT stabilized samples versus TVT samples. However, lymphocytes from TVT samples exhibited higher side scatter (SSC) properties than the equivalent cells in the Vacutainer and BCT tubes. These characteristics were true for both TVT and BCT tubes at all time points.

With regard to the cell marker signal results, there were no marked differences between the two devices when it came to decreased MFI for cell markers: CD3, CD4, CD8, CD16+56, CD19 and CD45 for their target cells. However, there was a marked increase in auto-fluorescence observed for lymphocyte subsets that were stabilized within the BCT versus the control. These increases were significantly higher than the control Vacutainer values ($p < 0.05$), except for two instances (CD4 MFI within Cytotoxic T cells for both time points). Whereas the equivalent MFI values for the TVTs were comparable and were not significantly different on eight occasions (refer to figures 5g – 5i, 6g, 6i, 6j). In real terms, this suggested that it would be more problematic to categorize BCT stabilized lymphocytes than TVT stabilized cells. This was also observed in Figures 3a – 3c and 4a – 4c. These findings are consistent with Ng et al. [15] who concluded that the TransFix fixative was preferable at preserving marker expression versus the BCT.

Conclusions

In summary, both the TVT and BCT exhibited good agreement with the baseline Vacutainer result with regard to lymphocyte subset absolute counts over the 15 day testing period. Both devices are clinically safe for their intended use of stabilizing lymphocytes. We establish both will preserve CD3, CD4, CD8, CD16/56, CD19 and CD45 antigens on the surface of lymphocytes. While we would anticipate that other leukocyte antigens would be equally preserved with TVT and BCT tubes, it is recommended investigators independently establish stabilities in their own system for other antigens.

However, there were marked increases in auto-fluorescence and cell debris from samples that were stabilized by the BCT in comparison to the TVT and Vacutainer results. These

findings suggest that for certain cell markers, it will be easier for an operator to identify specific cell subsets when using a TVT due to the decreased auto-fluorescence (compare CD3 staining for TVT versus BCT in figures 3 and 4) and reduced amount of debris.

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Figures 1a – 1f. Regression analyses for BCT (red trend line) and TVT (blue trend line) CD4+ T cell absolute counts versus the equivalent Vacutainer (BD) result, at each time point, for the HIV cohort, n=30.

Figures 1a, 1c, 1e (BD vs BCT) and 1b, 1d, 1f (BD vs TVT) demonstrate that both devices were suitably equivalent to the baseline Vacutainer result for the CD4 T cell marker. Trend lines closely mirror the control result at all time points.

Figures 2a – 2f: Regression analyses for BCT (red trend line) and TVT (blue trend line) CD4+ T cell absolute counts versus the equivalent Vacutainer (BD) result, at each time point, for the healthy donor cohort, n=12.

Figures 2a, 2c, 2e (BD vs BCT) demonstrate that the observed CD4+ T cell absolute counts are lower than expected for healthy donor samples collected in BCTs. This is particularly true for the higher CD4 counts. Figures 2b, 2d, 2f (BD vs TVT) demonstrate that the observed CD4 T cell absolute counts are similar to the control Vacutainer results for healthy donor samples collected in TVTs as trend lines closely mirror the Vacutainer result at all time points.

3a – 3c. Flow cytometry gating for a HIV subject in the study.

Figures 3a – 3c: Flow cytometry dot plots from a clinically approved FC report for a HIV positive subject.

Figures 3a – 3c show the flow cytometry gating for a HIV subject in the study, and are representative of the cell scatter and staining characteristics observed for this cohort. Figures 3a, 3b and 3c show the visualization of the lymphocyte subsets tested via cell markers CD3, CD4, CD8, CD19, CD16+56, CD45 for the Vacutainer control (BD <6 hours, Day 0), TVT on Day 15 and BCT on Day 15, respectively.

4a – 4c. Flow cytometry gating for a healthy subject in the study.

Figures 4a – 4c: Flow cytometry dot plots from a clinically approved FC report for a healthy subject.

Figures 4a – 4c show the flow cytometry gating for a healthy subject in the study, and are representative of the cell scatter and staining characteristics observed for this cohort. Figures 4a, 4b and 4c show the visualization of the lymphocyte subsets tested via cell markers CD3, CD4, CD8, CD19, CD16+56, CD45 for the Vacutainer control (BD <6 hours, Day 0), TVT on Day 15 and BCT on Day 15, respectively.

5a – 5j

Figures 5a – 5f: Box and whisker graphs of the mean control (4mL Vacutainer, Day 0) MFI signal for cell subsets and their target markers (i.e. a: T cell CD3 FITC, b: T helper cell CD4 APC, c: Cytotoxic T cell CD8 PE, d: NK cell CD16/56 PE, e: B cell CD19 APC and f: lymphocyte CD45 PerCP) tested within HIV subjects (n=25) versus the equivalent BCT and TVT MFI results for time points Day 11 and Day 15. Box and whisker plots that have an asterisk adjacent to them indicate that the condition result is statistically significant from the control i.e. asterisk = $p < 0.05$.

The graphs demonstrate that for all lymphocyte subsets, there is a significant reduction ($p < 0.05$) in MFI for their target marker (e.g. CD3 MFI for T cells) within BCTs and TVTs. Therefore, both devices performed equivalently.

Figures 5g – 5j: Box and whisker graphs of the background fluorescence (Control) of the negatively staining populations for each cell subset within HIV subjects (n=25) versus the equivalent BCT and TVT MFI results for time points Day 11 and Day 15. I.e. g: NK cell CD3 FITC, h: B cell CD3 FITC, i: Cytotoxic T cell CD4 APC, j: T helper cell CD8 PE within HIV subjects (n=25) versus the equivalent BCT and TVT MFI results for time points Day 11 and Day 15. Box and whisker plots that have an asterisk adjacent to them indicate that the condition result is statistically significant from the control i.e. asterisk = $p < 0.05$. These assessments of auto-fluorescence particularly look at: CD3 MFI within NK cells, CD3 MFI within B cells, CD4 MFI within Cytotoxic T cells, and CD8 MFI within T helper cells. For the HIV cohort, these data demonstrate that TVT samples exhibit the statistical equivalent ($p > 0.05$) level of auto-fluorescence as the Vacutainer control tube on four occasions: CD3 MFI within NK cells on Day 11, CD3 MFI within B cells on Day 15 and CD4 MFI within Cytotoxic T cells for both time points. BCT samples all exhibit statistically higher ($p < 0.05$) levels of auto-fluorescence compared to the control.

6a – 6j

Figures 6a – 6f: Box and whisker graphs of the mean control (4mL Vacutainer, Day 0) MFI signal for cell subsets and their target markers (i.e. a: T cell CD3 FITC, b: T helper cell CD4 APC, c: Cytotoxic T cell CD8 PE, d: NK cell CD16/56 PE, e: B cell CD19 APC and f: lymphocyte CD45 PerCP) tested within healthy subjects (n=8) versus the equivalent BCT and TVT MFI results for time points Day 11 and Day 15. Box and whisker plots that have an asterisk adjacent to them indicate that the condition result is statistically significant from the control i.e. asterisk = $p < 0.05$.

The graphs demonstrate that for all lymphocyte subsets, there is a significant reduction ($p < 0.05$) in MFI for their target marker within BCTs and TVTs. Therefore, both devices performed equivalently.

Figures 6g – 6j: Box and whisker graphs of the background fluorescence (Control) of the negatively staining populations for each cell subset within healthy subjects (n=8) versus the equivalent BCT and TVT MFI results for time points Day 11 and Day 15. I.e. g: NK cell CD3 FITC, h: B cell CD3 FITC, i: Cytotoxic T cell CD4 APC, j: T helper cell CD8 PE within healthy subjects (n=8) versus the equivalent BCT and TVT MFI results for time points Day 11 and Day 15. Box and whisker plots that have an asterisk adjacent to them indicate that the condition result is statistically significant from the control i.e. asterisk = $p < 0.05$. For the healthy cohort, these data demonstrate that TVT samples exhibit the statistical equivalent ($p > 0.05$) level of auto-fluorescence as the control tube on four occasions: CD3 MFI within NK cells on Day 15, CD4 MFI within Cytotoxic T cells for both time points and CD8 MFI within T helper cells on Day 15. BCT samples exhibit the statistical equivalent ($p > 0.05$) level of auto-fluorescence as the control tube on only two occasions: CD4 MFI within Cytotoxic T cells for both time points. However, similarly to the HIV cohort, BCT samples exhibit a marked increase in levels of auto-fluorescence compared to the control.

Table 1:

Testing regime for samples taken into each collection tube from each healthy and HIV subject

	BD Vacutainer	TVT	BCT
Tested <6hrs from collection (Day 0)	FCMx2	FCMx2	FCMx2
Tested on Day 11		FCMx2	FCMx2
Tested on Day 15		FCMx2	FCMx2

FCMx2= flow cytometer testing performed in duplicate.

Table 2:

Summary of regression analysis for HIV positive subjects as compared to control (n=30) (absolute counts in cells/ μ l)

Marker	Tube	Time Interval	Passing-Bablok Regression			
			Intercept	Slope	95% CI for Slope	R
CD3	BCT	6 hour	-3.4	0.99	0.9478 to 1.039	1.00
	TVT	6 hour	-18.1	1.05	0.9773 to 1.142	0.99
	BCT	11 day	28.9	1.01	0.9071 to 1.074	0.99
	TVT	11 day	-31.7	1.05	0.9493 to 1.207	0.98
	BCT	15 day	52.2	0.95	0.8882 to 1.034	0.99
	TVT	15 day	-13.8	1.04	0.9425 to 1.154	0.97
CD4	BCT	6 hour	5.3	0.96	0.9325 to 1.013	1.00
	TVT	6 hour	0.6	1.02	0.9636 to 1.089	0.99
	BCT	11 day	1.9	0.96	0.9076 to 1.024	0.99
	TVT	11 day	11.5	0.97	0.9159 to 1.027	0.99
	BCT	15 day	2.4	0.99	0.9222 to 1.027	0.99
	TVT	15 day	2.1	0.98	0.8558 to 1.067	0.98
CD8	BCT	6 hour	-12.2	1.02	0.9467 to 1.074	1.00
	TVT	6 hour	-60.5	1.12	1.0481 to 1.196	1.00
	BCT	11 day	-0.3	1.04	0.9398 to 1.142	0.99
	TVT	11 day	-26.3	1.08	0.9823 to 1.191	0.98
	BCT	15 day	47.9	0.97	0.8793 to 1.055	0.99
	TVT	15 day	4.1	1.03	0.8790 to 1.179	0.97
CD16/56	BCT	6 hour	3.8	1.08	0.9451 to 1.189	0.98
	TVT	6 hour	1.5	1.05	0.9278 to 1.172	0.96
	BCT	11 day	4.1	1.10	0.9681 to 1.205	0.97
	TVT	11 day	10.3	0.96	0.7929 to 1.118	0.95
	BCT	15 day	20.3	0.96	0.8554 to 1.090	0.96
	TVT	15 day	15.7	0.95	0.8079 to 1.139	0.96
CD19	BCT	6 hour	0.4	1.01	0.9735 to 1.072	1.00
	TVT	6 hour	3.2	1.02	0.9361 to 1.078	0.99
	BCT	11 day	-5.8	1.05	0.9779 to 1.109	0.99
	TVT	11 day	-4.8	1.07	0.9437 to 1.149	0.98
	BCT	15 day	-2.2	0.96	0.8950 to 1.031	0.99
	TVT	15 day	-5.8	1.05	0.9489 to 1.123	0.99
CD45	BCT	6 hour	13.6	1.00	0.9501 to 1.049	1.00
	TVT	6 hour	-10.5	1.07	0.9978 to 1.153	0.99
	BCT	11 day	19.3	1.02	0.9435 to 1.101	0.99
	TVT	11 day	-32.5	1.05	0.9718 to 1.167	0.98
	BCT	15 day	9.4	1.00	0.9384 to 1.076	0.99

			Passing-Bablok Regression			
Marker	Tube	Time Interval	Intercept	Slope	95% CI for Slope	R
	TVT	15 day	5.3	1.05	0.9232 to 1.137	0.97

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Table 3:

Dynamic range summary for HIV positive samples collected into the Vacutainer (n=30) (absolute counts in cells/ μ l)

Marker	Tube	Time Interval	Mean	Min	Max
CD3	BD	6 hour	1415.9	226.3	3741.1
CD4	BD	6 hour	520.3	12.5	1142.4
CD8	BD	6 hour	878.0	217.4	3017.8
CD16/56	BD	6 hour	227.6	33.2	656.6
CD19	BD	6 hour	199.7	0.2	520.7
CD45	BD	6 hour	1853.7	298.6	4556.0

Table 4:

Summary of regression analysis for healthy subjects as compared to control (n=12) (absolute counts in cells/ μ l)

Marker	Tube	Time Interval	Passing-Bablok Regression			
			Intercept	Slope	95% CI for Slope	R
CD3	BCT	6 hour	75.9	0.92	0.7386 to 1.048	0.99
	TVT	6 hour	-14.3	1.03	0.9415 to 1.226	0.99
	BCT	11 day	189.6	0.83	0.6452 to 0.965	0.99
	TVT	11 day	-178.6	1.10	1.0392 to 1.445	0.99
	BCT	15 day	38.9	0.95	0.7709 to 1.107	0.99
	TVT	15 day	-69.5	1.05	0.9630 to 1.221	0.99
CD4	BCT	6 hour	33.4	0.94	0.8592 to 1.131	1.00
	TVT	6 hour	19.0	0.97	0.8749 to 1.209	0.99
	BCT	11 day	123.9	0.82	0.7653 to 0.925	0.99
	TVT	11 day	-76.7	1.04	0.9407 to 1.212	0.99
	BCT	15 day	66.0	0.88	0.8084 to 1.180	0.99
	TVT	15 day	-28.4	1.05	0.9592 to 1.303	0.99
CD8	BCT	6 hour	66.0	0.86	0.6987 to 0.976	0.99
	TVT	6 hour	20.5	0.99	0.5831 to 1.176	0.97
	BCT	11 day	35.3	0.91	0.7903 to 1.021	0.99
	TVT	11 day	32.8	0.90	0.4914 to 1.015	0.97
	BCT	15 day	37.2	0.98	0.8106 to 1.090	0.98
	TVT	15 day	48.1	0.90	0.4299 to 1.032	0.98
CD16/56	BCT	6 hour	23.4	0.95	0.5042 to 1.117	0.93
	TVT	6 hour	-23.0	1.10	0.6485 to 1.403	0.92
	BCT	11 day	-24.4	1.23	0.6715 to 1.528	0.90
	TVT	11 day	6.0	0.96	0.6636 to 1.311	0.89
	BCT	15 day	-30.2	1.19	0.8838 to 1.363	0.91
	TVT	15 day	-11.7	1.00	0.7030 to 1.377	0.90
CD19	BCT	6 hour	-4.1	1.06	0.8942 to 1.233	0.99
	TVT	6 hour	14.4	1.00	0.5285 to 1.238	0.98
	BCT	11 day	-1.6	0.97	0.8060 to 1.451	0.95
	TVT	11 day	2.7	0.99	0.7014 to 1.248	0.98
	BCT	15 day	-0.3	0.94	0.7719 to 1.258	0.97
	TVT	15 day	-5.0	1.08	0.8658 to 1.369	0.99
CD45	BCT	6 hour	131.5	0.92	0.5996 to 1.018	0.98
	TVT	6 hour	-78.1	1.05	0.8239 to 1.354	0.98
	BCT	11 day	295.4	0.82	0.5367 to 1.248	0.96
	TVT	11 day	-322.8	1.14	0.9225 to 1.427	0.97
	BCT	15 day	137.9	0.91	0.7036 to 1.095	0.97

			Passing-Bablok Regression			
Marker	Tube	Time Interval	Intercept	Slope	95% CI for Slope	R
	TVT	15 day	-42.4	1.04	0.7490 to 1.293	0.98

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Table 5:

Dynamic range summary for healthy samples collected into the Vacutainer (n=12) (absolute counts in cells/ μ l)

Marker	Tube	Time Interval	Mean	Min	Max
CD3	BD	6 hour	1493.1	772.2	2819.4
CD4	BD	6 hour	953.6	493.8	2068.0
CD8	BD	6 hour	522.1	220.5	990.6
CD16/56	BD	6 hour	279.3	113.7	497.3
CD19	BD	6 hour	211.3	122.0	428.0
CD45	BD	6 hour	2009.0	1453.2	3452.4

Table 6:

Summary of point estimate slope results that were out of specification

Subset	Time Interval	Slopes beyond 0.9 and 1.1	
		BCT vs BD	TVT vs BD
CD3	Day 11	0.83 (HC)	
CD4	Day 11	0.82 (HC)	
CD4	Day 15	0.88 (HC)	
CD8	Day 0	0.86 (HC)	1.12 (HIV)
CD16/56	Day 11	1.23 (HC)	
CD16/56	Day 15	1.19 (HC)	
CD45	Day 11	0.82 (HC)	1.14 (HC)

HC = result for healthy subjects and HIV = result for HIV positive cohort.