Cyto-Chex® BCT allows for accurate T-cell counts by flow cytometry 14 days post sample collection

Introduction

Accurate counts of lymphocyte subsets are used by clinicians to help assess general immune health with CD4 counts in HIV infected persons being of predominate importance (1). These subsets are distinguished by flow cytometry, which uses fluorescent antibodies to detect the type and relative amount of antigens on each cell's surface. The flow cytometry "TBNK" assay measures the absolute counts and percentages of T, B, and Natural Killer (NK) lymphocyte subsets by determining the total CD3+ (T-cells), CD3+/CD4+ (Helper T-cells), CD3+/CD8+ (Cytotoxic T-cells), CD3-/CD19+ (B-cells), and CD3-/CD16/56+ (NK cells) in whole blood. Several IVD cleared assays, such as BD Multitest, are commercially available.

Recent world events have led to new importance in monitoring immune cells in that lymphocyte numbers, especially decreased T-cells, are associated with poor outcomes in COVID-19 disease (2-5). COVID-19 has also stressed the clinical lab with an increased amount of patient samples causing a reduction in the available time and/or labor necessary to prepare and run complicated samples in the required time frame. Moreover, increased biosafety measures currently required to safely process COVID-19 whole blood for Flow Cytometry analysis could prove detrimental to the timely processing of patient samples (6). Thus, it would prove beneficial to be able to batch and run samples when favorable to the time and staffing constraints of the clinical laboratory while not taking up valuable cooler space. Cyto-Chex® BCT is an FDA approved blood collection tube intended to stabilize CD3, CD4, CD8, CD16/CD56, CD19 and CD45 on lymphocytes in whole blood for up to 14 days at room temperature post blood draw, thereby demonstrating its efficacious role during this pandemic.

A previous publication measured the aforementioned CD markers stability in Cyto-Chex BCT with the BD Biosciences FACSCalibur and the Beckman Coulter EPICS XL, the most common clinical instruments at the time of publication (7). However, flow cytometers have become more advanced and new clinical software packages have been cleared for use with the TBNK panel of CD markers. We chose to reevaluate the function of Cyto-Chex BCT

on the FACSCanto II using the clinical software package with the knowledge that the FACSCanto II is the predominate instrument in the clinical lab and in the hope of ensuring that clinicians can have confidence in the accuracy of their results during these uncertain times.

As expected, it was demonstrated that Cyto-Chex BCT maintains CD3, CD4, CD8, CD19, and CD16/56 lymphocyte absolute counts and percentages as measured on the BD FACSCanto II for 14 days post draw to an equivalent amount as a day 0 K_2EDTA blood collection tube.

Methods:

Blood from eight self-reported healthy donors were drawn into one K_2EDTA BD Vacutainer and one Cyto-Chex BCT each. Samples were processed with the BD Multitest IMK kit as instructed in the manufacturers IFU within four hours after draw. Briefly, 50µL of reverse pipetted whole blood was stained with 20 µl of BD Multitest CD3/CD8/CD45/CD4 cocktail or 20 µl of the BD Multitest CD3/CD16+CD56/CD45/CD19 cocktail in BD Trucount tubes, lysed in 450µL of BD FACS lysing solution and run on a BD FACSCanto II under the IVD clinical software package.

Three separate preparations of each cocktail and whole blood were made, and each preparation was run in triplicate within 6 hours post draw for a total of nine runs per donor per blood collection tube. The whole blood filled Cyto-Chex BCT tubes were stored at room temperature (18 °C to 22 °C) for 14 days post draw and then processed and run as before. The whole blood filled K_2EDTA blood collection tube cannot be stored and run at room temperature for that length of time as the cells die and fragment (7).

The BD FACSCANTO II was monitored and calibrated daily by running the BD CS&T beads and the BD FACS 7-color Setup Beads as per manufacturer's directions.

Results:

Tube A of the BD Multitest IMK kit stains for CD3, CD4, CD8, and CD45 and the BD FACSCanto clinical software automatically gates the CD3+/CD4+ and the CD3+/CD8+ lymphocytes. Representative dot plots from donor three are shown as examples of the visual appearance of the populations when drawn into K_2EDTA or Cyto-Chex BCT (Figure 1). There are no visual differences in the K_2EDTA vs. Cyto-Chex BCT population plot on Day 0. There is a slight decrease in brightness of both the CD4+ and CD8+ population plots on Day 14 due to the stabilizing reagents effect on the antibody epitopes, however this decrease does not prevent staining or affect the auto gating feature. Moreover, the populations remain distinct.

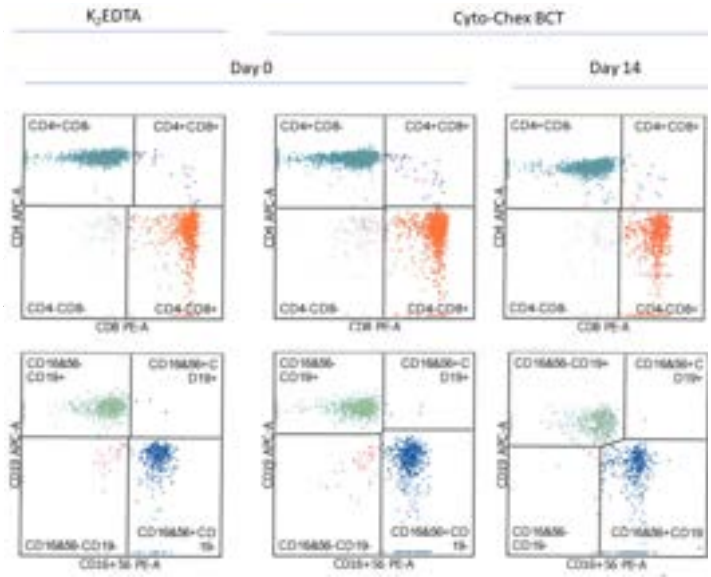

Figure 1. Example plots taken from the automated report of the TBNK assay are shown from donor 3. All donors had equal results. The blood was drawn into a K_2EDTA and Cyto-Chex BCT run on the day of draw and 14 days later.

The TBNK assay has intrinsic variability from preparation to preparation and run-to-run as reported in the assay's IFU. Therefore, it is not acceptable to use data from one preparation/ one run to determine statistically significant differences between tube types and timepoints as one tube's data point may be at the low end and the other tube's data point at the high end so that a difference would be falsely claimed. We prepared each donor sample in triplicate and ran each sample three times. This gives us nine data points per donor per tube type and we can therefore make clear comparisons between the blood collection tubes only without the assay itself contributing to any differences seen.

The data collected from the reports generated by the automated gating of the FACSCanto II clinical software are summarized in Figures 2 and 3. No statistically significant differences in counts or percentages are seen in blood drawn into Cyto-Chex BCT Day 0 and Day 14 as compared to K_2EDTA at Day 0.

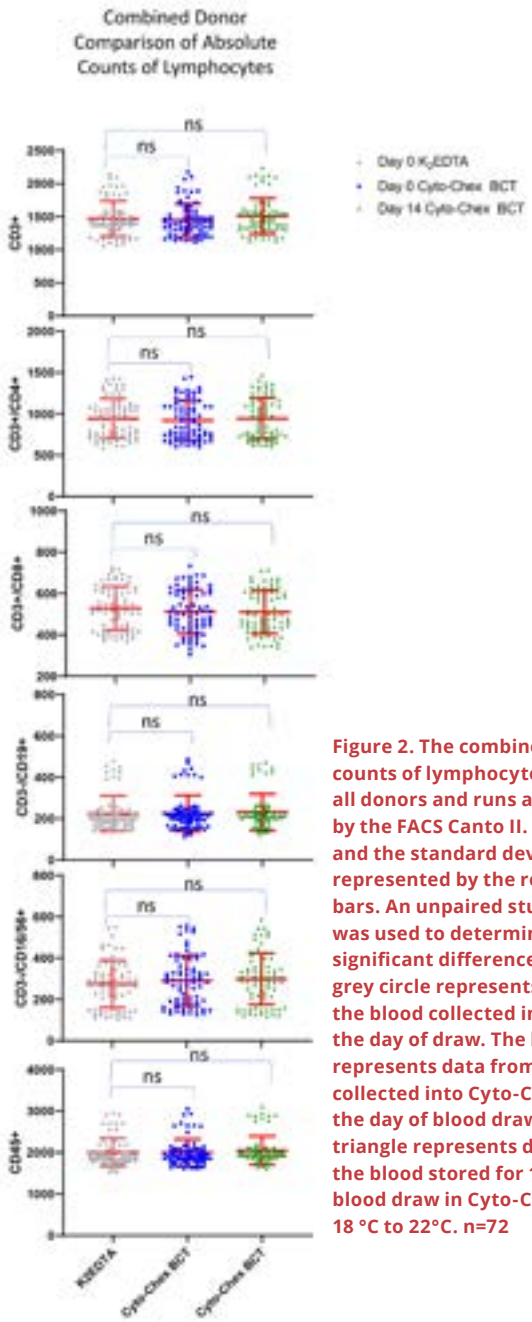

Figure 2. The combined absolute counts of lymphocyte subsets from all donors and runs as measured by the FACS Canto II. The mean and the standard deviation is represented by the red lines and bars. An unpaired student's T test was used to determine statistically significant differences, $p<0.05$. The grey circle represents data from the blood collected into K_2EDTA on the day of draw. The blue square represents data from the blood collected into Cyto-Chex BCT on the day of blood draw. The green triangle represents data from the blood stored for 14 days after blood draw in Cyto-Chex BCT at 18 °C to 22°C. n=72

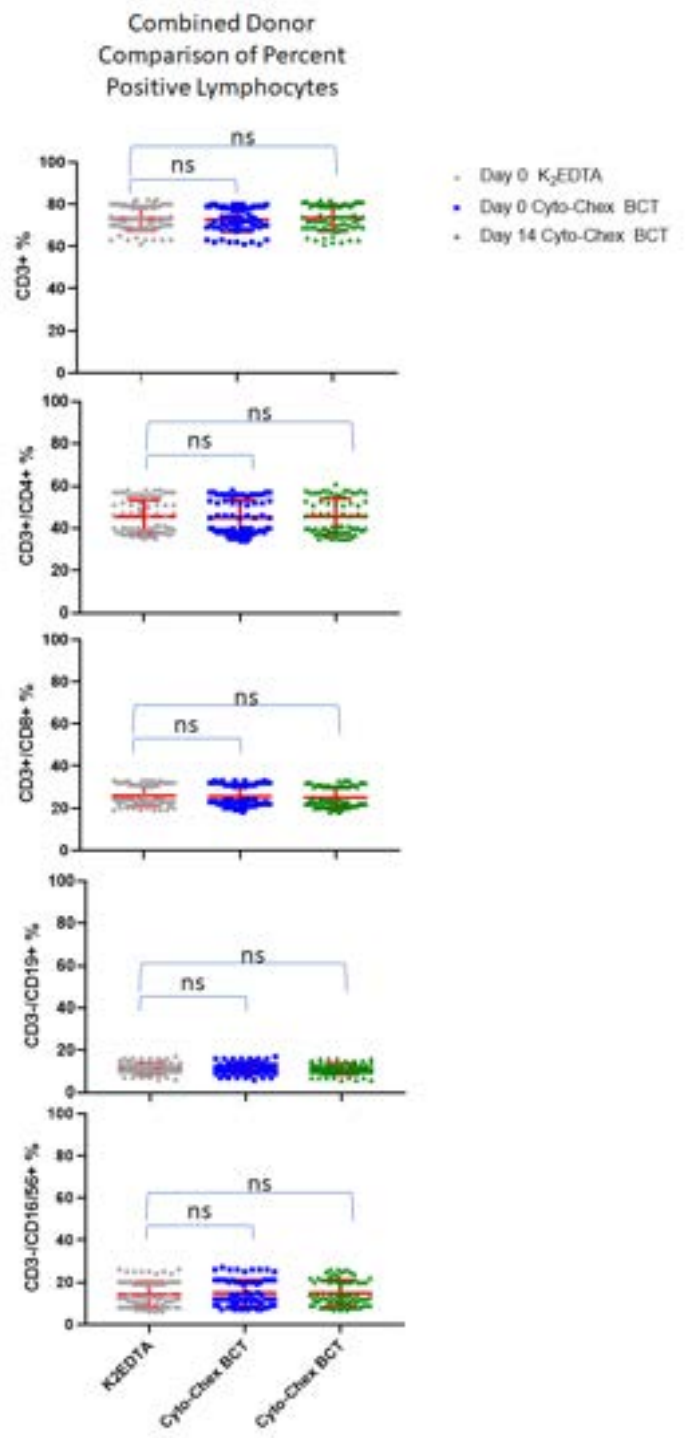

Figure 3. The percent positive lymphocyte subsets from all runs and donors as measured by the FACS Canto II. The mean and the standard deviation is represented by the red lines and bars. An unpaired student's T test was used to determine statistically significant differences, p>0.05. The grey circle represents data from the blood collected into K_2EDTA on the day of draw. The blue square represents data from the blood collected into Cyto-Chex BCT on the day of blood draw. The green triangle represents data from the blood stored for 14 days after of blood draw in Cyto-Chex BCT at 18 °C to 22 °C. n=72

Humans have inherent variability in their numbers of leukocytes and percentages of lymphocytes and this biological variability could hide differences in individual donors. Therefore, each donor was also compared individually.

In figure 4, the average of each donors' absolute counts of CD3+, CD3+/CD4+, CD3+/CD8+, CD3-/CD16/56+, CD19+, and CD45+ were compared. All donor to donor variability is within the normal population distribution and tube to tube variability is within the normal variability of the assay.

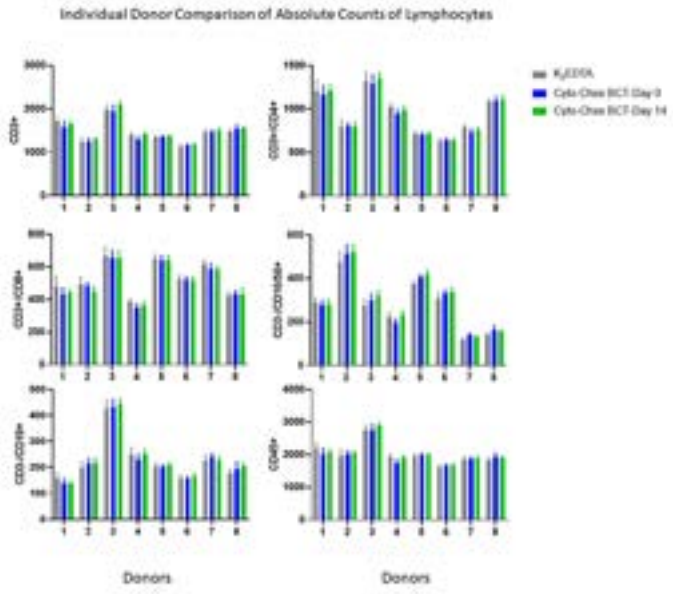

Figure 4. The absolute counts of lymphocyte subsets from each individual donor's runs as measured by the FACS Canto II. The grey column shows the data from the blood collected into K_2EDTA on the day of draw. The blue column shows the data from the blood collected into Cyto-Chex BCT on the day of blood draw. The green column shows the data from the blood stored for 14 days after of blood draw in Cyto-Chex BCT at 18 °C to 22 °C. n=9

In figure 5, the average of each donors' percent positive of CD3+, CD3+/CD4+, CD3+/CD8+, CD3-/CD16/56+, and CD19+ were compared. All donor to donor variability is within the normal population distribution and tube to tube variability is within the normal variability of the assay.

As a final comparison to show that there are no differences in cell numbers and percent recoveries, the data from the K_2EDTA blood draw was graphed against the data from Day 0 and the Day 14 Cyto-Chex BCT blood draw and a linear regression analysis was run (Figures 6 and 7). All slopes are near 1.0 showing equivalence to K_2EDTA at time of draw (Table 1 and 2).

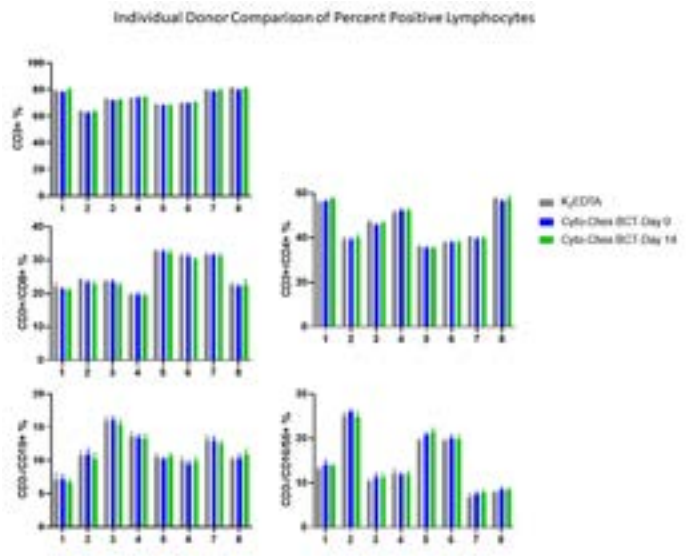

Figure 5. The percent positive of lymphocyte subsets from each individual donor's runs as measured by the FACS Canto II. The grey column shows the data from the blood collected into K_2EDTA on the day of draw. The blue column shows the data from the blood collected into Cyto-Chex BCT on the day of blood draw. The green column shows the data from the blood stored for 14 days after of blood draw in Cyto-Chex BCT at 18 °C to 22 °C. n=9

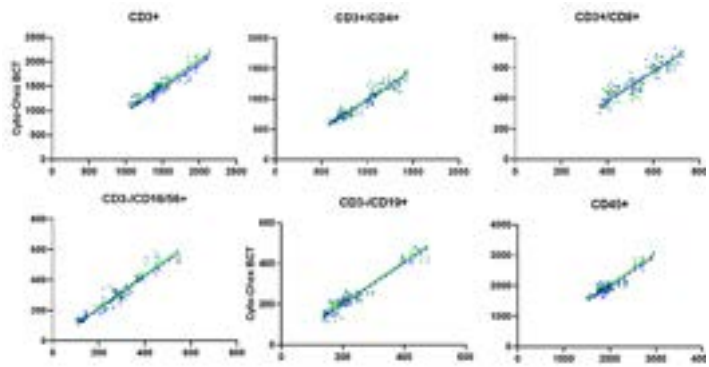

Figure 6. Regression analysis for the absolute counts of lymphocyte subsets from all runs and donors from Cyto-Chex BCT Day 0 (blue lines) and Cyto-Chex BCT Day 14 (green lines) versus the K_2EDTA result at Day 0. n=72

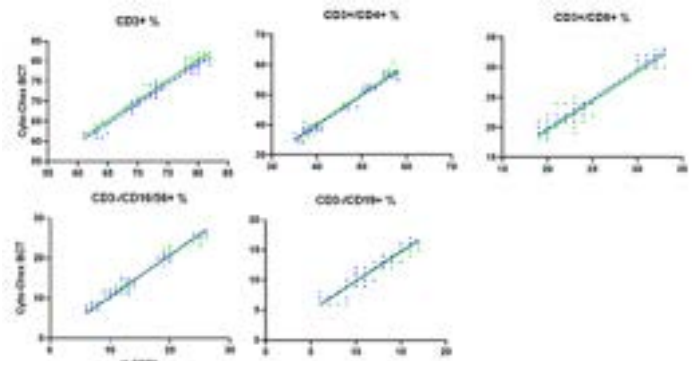

Figure 7. Regression analysis for the percentage of lymphocyte subsets from all runs and donors from Cyto-Chex BCT Day 0 (blue lines) and Cyto-Chex BCT Day 14 (green lines) versus the K_2EDTA result at Day 0. n=72

CD Marker	Day	Slope	Std Error	95% CI for Slope	R
CD3	0	0.982	0.007	0.9673 to 0.9958	0.9383
	14	1.023	0.008	1.008 to 1.038	0.9355
CD4	0	0.971	0.009	0.9533 to 0.9876	0.9542
	14	0.998	0.010	0.9785 to 1.018	0.9453
CD8	0	0.966	0.009	0.9485 to 0.9843	0.9227
	14	0.961	0.010	0.9404 to 0.9820	0.8926
CD16/56	0	1.051	0.014	1.023 to 1.078	0.9583
	14	1.084	0.014	1.056 to 1.112	0.9600
CD19	0	1.001	0.012	0.9766 to 1.026	0.9579
	14	1.026	0.013	1.000 to 1.051	0.9561
CD45	0	0.992	0.008	0.9769 to 1.007	0.9198
	14	1.020	0.008	1.004 to 1.035	0.9253

Table 1. Summary of regression analysis for the absolute counts of lymphocyte subsets.

CD Marker	Day	Slope	Std Error	95% CI for Slope	R
CD3	0	0.992	0.002	0.9881 to 0.9952	0.9812
	14	1.003	0.002	0.9999 to 1.007	0.9845
CD4	0	0.993	0.003	0.9863 to 1.000	0.9862
	14	1.005	0.004	0.9971 to 1.012	0.9844
CD8	0	0.988	0.004	0.9799 to 0.9966	0.9821
	14	0.973	0.006	0.9610 to 0.9839	0.9657
CD16/56	0	1.044	0.008	1.027 to 1.060	0.9853
	14	1.036	0.009	1.018 to 1.054	0.9823
CD19	0	0.989	0.009	0.9717 to 1.007	0.9448
	14	0.977	0.010	0.9564 to 0.9969	0.9222

Table 2. Summary of regression analysis for the percentage of lymphocyte subsets.

Conclusions

We have shown, with a modern flow cytometer and clinical software, that Cyto-Chex BCT stabilizes white blood cells and selected CD markers (TBNK panel) such that accurate recoveries are ensured for 14 days post blood draw. This stabilization allows for whole blood samples to be stored without any processing or cold chain management and still guarantees an accurate patient result.

Cyto-Chex BCT is an IVD blood collection tube that has been used for over 15 years to safely store and ship normal and HIV+ whole blood samples all over the world (7). It has been an invaluable resource to clinics in rural areas who may not have easy access to flow cytometers and large clinical trials in which samples must be shipped to a central location (8-10). It also has been shown to inactivate viruses while maintaining white blood cell integrity thus allowing for greater laboratory safety while processing for flow cytometry analysis (11). During the COVID-19 fight, all possible tools should be utilized to assist our overtaxed health system. Cyto-Chex BCT is a known and proven technology that can be of benefit in the current pandemic for the processing of whole blood samples for use with the flow cytometry TBNK assay.

References

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