



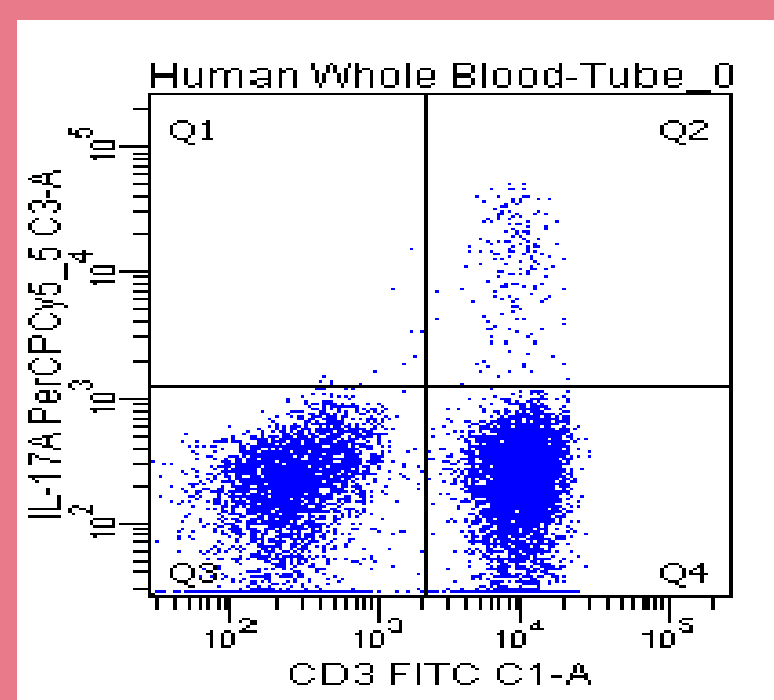
A NOVEL FLOW CYTOMETRY STIMULATION ASSAY USING CYTO-CHEX® BCT TUBES FOR USE IN CLINICAL TRIALS

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ABSTRACT

Rationale: The Cyto-Chex® BCT tubes from Streck Laboratories contain a priority fixative that stabilizes the blood at room temperature making flow cytometry analysis possible for up to one to two weeks after collection. This application has been advantageous for doctors and scientists in remote locations being able to transport specimens back to a testing lab that is not in close proximity to the draw site. These BCT tubes have also become very popular for use in clinical research trials for flow cytometry testing since humans are drawn from multiple sites and then the specimens are shipped to a central laboratory. One disadvantage of the tube is that since it contains a fixative, flow cytometry applications which require stimulation cannot be performed once the specimen is exposed to the fixative. In our laboratory we are working on ways to stimulate the specimen prior to placing in the Cyto-Chex® BCT tube. In one such method, we can measure the intracellular cytokine IL-17A expressed in T cells up to five days post-stimulation. This method can also be used to measure many phosphorylated proteins. These methods will greatly benefit the pharmaceutical and biotechnology industry in that whole blood specimens can be stimulated immediately after the draw, fixed and then shipped to a central testing facility.



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INTRODUCTION

Biomarker evaluation is important in clinical research trials. More and more researchers are using flow cytometry applications to measure these markers. Whole blood procedures are in most cases the best assay to employ because the kinetics of the test article can change when the plasma is removed by PBMC isolation. For surface marker evaluation, drawing whole blood into Cyto-Chex® BCT tubes has been advantageous since the tube contains a fixative to provide stability for most antibody markers. These tubes were developed for drawing patients in remote locations when testing of the specimen cannot be performed in a couple of days. The Cyto-Chex® BCT tube has become very popular for use in clinical research trials. However, since these tubes contain a fixative, the cells cannot be stimulated for evaluation of many intracellular proteins. The objective of this study was to develop two methods that could stimulate the whole blood prior to placing into the Cyto-Chex® BCT tube. The first method requires the whole blood to be drawn into a sodium heparin blood collection tube and then manually stimulated prior to transfer. The manual manipulation may be too complex for some draw sites and might introduce variability. The second method the whole blood is drawn into a FCStim™ tube developed by FCS Laboratory which can stimulate the cells immediately after the draw and then transfer the specimen to the Cyto-Chex® BCT tube. This method should provide more consistency for the marker evaluation. The experiments performed here are for proof of concept of these methods.

MATERIALS AND METHODS

Specimens - Human whole blood were drawn into sodium heparin or FCStim™ tubes. After stimulation of 4 hours the whole blood (5 mL) was transferred to Cyto-Chex® BCT tubes (Streck Laboratories) and stored at ambient room temperature until processing.

Flow cytometry - Phenotype analyses were performed by Flow Cytometry. Cells were surface stained at ambient temperature for 15 - 20 min with CD3 and CD14, lysed with 1X BD FACS Lyse for 8 - 12 minutes at ambient temperature and washed once prior to intracellular staining. For intracellular analysis, cells were fixed, permeabilized and stained with IL-2, TNFα, IL-17A and IFNγ. After staining the cells were washed and acquired and analyzed using a BD FACSCanto™ flow cytometer equipped with BD FACSDiva™ 6.1.3 software.

RESULTS

Procedure 1: IL-17A Stability

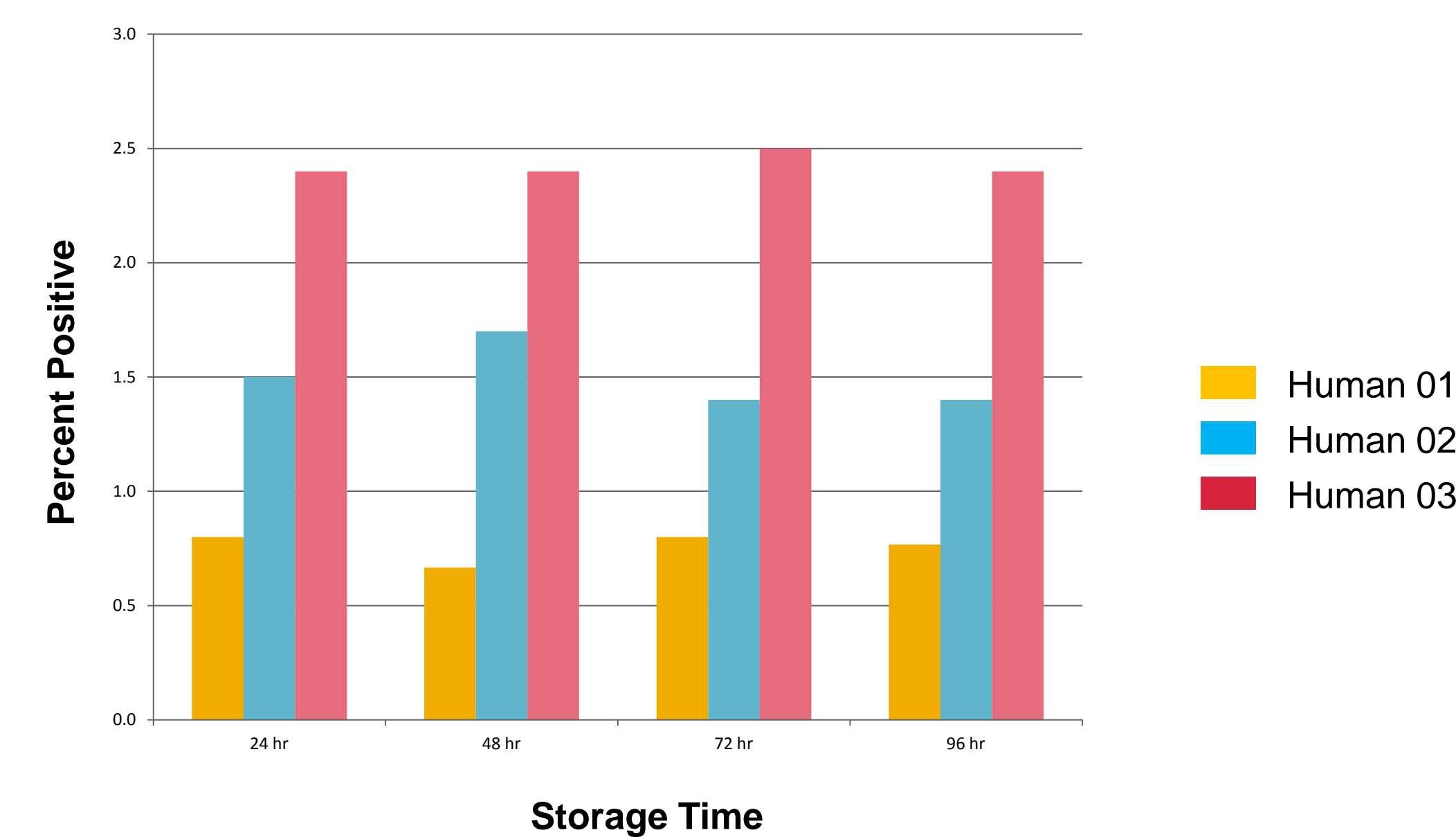
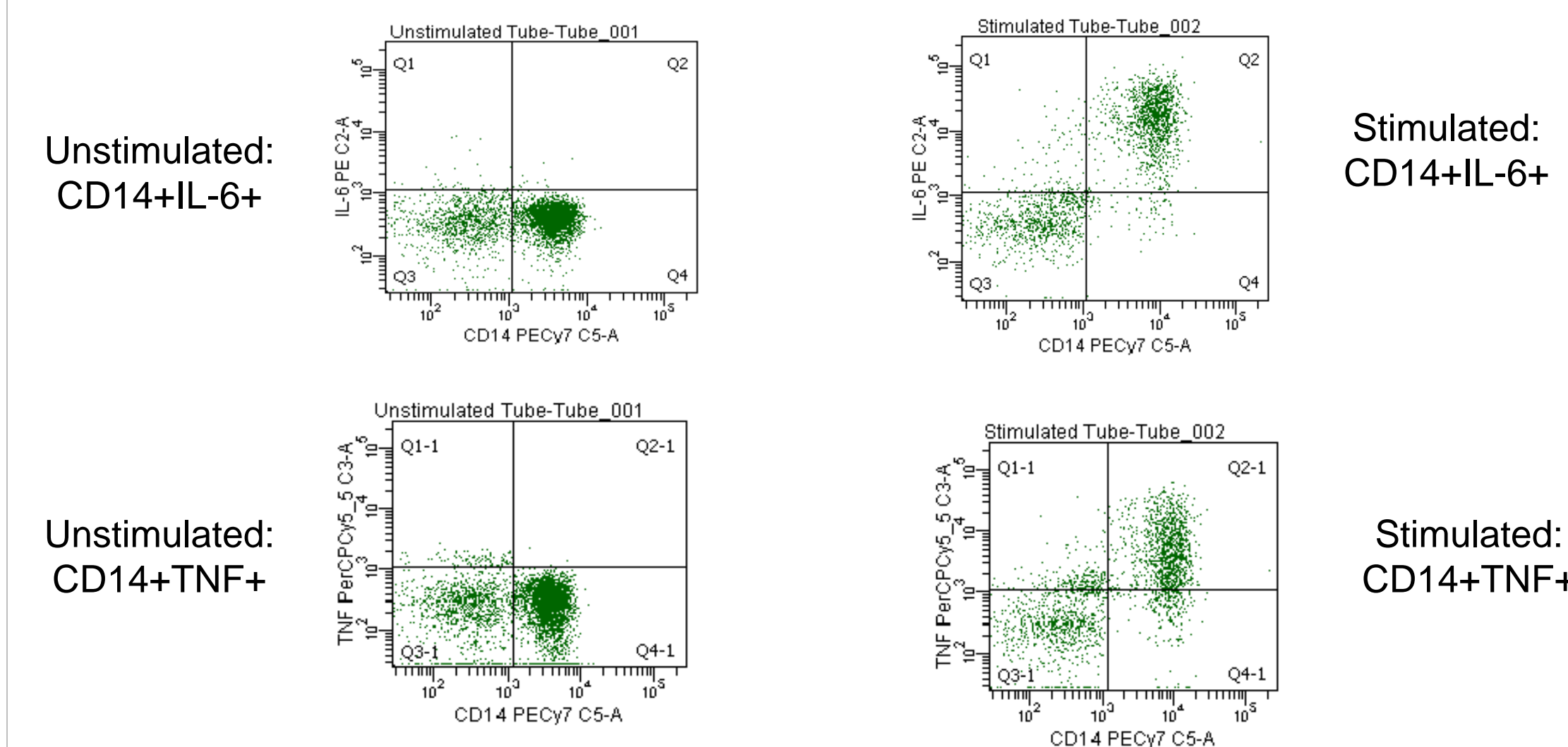


Figure 1. IL-17A Stability of Human Whole Blood Specimens. Procedure 1: Whole blood was drawn into a sodium heparin blood collection tube and then manually stimulated in conjunction with a transport inhibitor for 4 hours. Whole blood was then transferred to a Cyto-Chex® BCT tube. Tubes were stored at ambient room temperature until processing. The average percent for each specimen was 0.8%, 1.5% and 2.4%, respectively with a %CV of 8.3%, 9.4% and 2.1%, respectively.

Procedure 2: IL-6 and TNF Staining on Monocytes



Procedure 2: IFNγ and IL-2 Staining on Lymphocytes

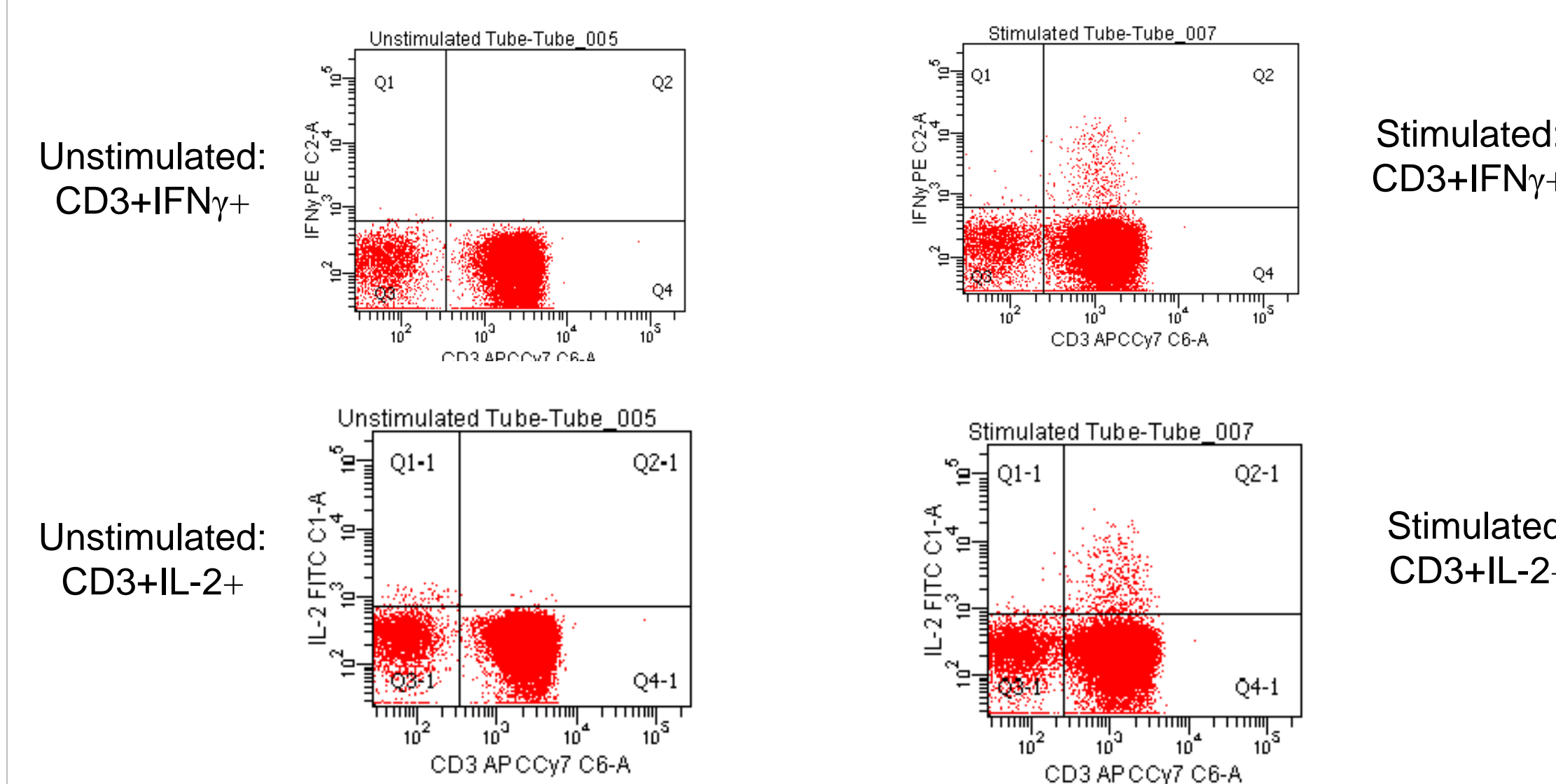


Figure 2. IL-6, TNF, IFNγ and IL-2 staining of human whole blood. Procedure 2: Whole blood was drawn into FCStim™ Tubes, inverted 10 times and placed into CO₂ incubator for 4 hours. After incubation, the contents in the FCStim™ tube is transferred to a Cyto-Chex® BCT tube and stored at ambient temperature before processing.

RESULTS (cont.)

Procedure 2: Stability

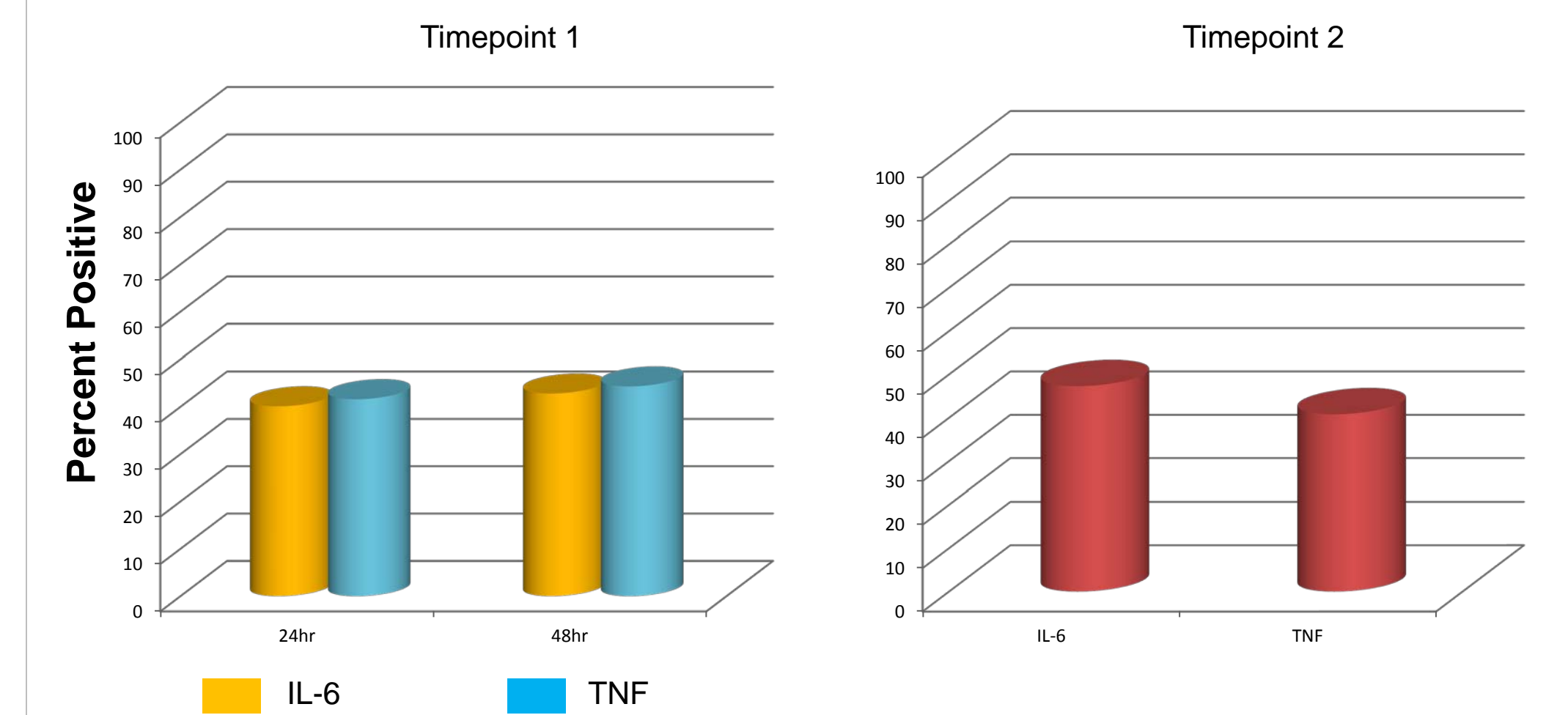


Figure 3. Timepoint 1: Stability of IL-6 and TNF tested 24hr and 48hr after draw into a FCStim™ tube and transferred to a Cyto-Chex® BCT tube. Timepoint 2: A repeat draw testing day to day variability of IL-6 and TNF using the FCStim™ tube and Cyto-Chex® BCT tube. The stability of the markers is consistent between days and similar between timepoints.

CONCLUSIONS

- These proof of concept experiments showed two methods of whole blood stimulation that could be used for clinical trial specimens.
- Procedure 1 employs a method of manual manipulation of the whole blood specimen which is then transferred to a Cyto-Chex® BCT tube. This process may not be feasible for some draw sites because of the complexity and training involved.
- Procedure 2 employs a method where human whole blood is drawn into a special FCStim™ tube developed by FCS Laboratory. The tube is placed in a CO₂ incubator and after an incubation the whole blood is transferred to a Cyto-Chex® BCT tube. The stabilized specimen can then be shipped to the central lab. This procedure could be very feasible for the draw sites because there would be minimal training involved.

REFERENCES

- Warrino, D., & McCarthy. Cyto-Chex BCT Stabilizes Light Scatter and Cell Morphology. Streck Laboratories, Application Note, Issue 2.
- Belouski, S., Wilkinson, J., Thomas, J., Keith, K., Wang, S., Suggs, S. & Ferbas, J. 2010. Utility of lyophilized PMA and ionomycin to stimulate lymphocytes in whole blood for immunological assays. Clinical Cytometry B, vo. 78B, pages 59-64.

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