# SHIPPING OF NON-CLINICAL SPECIMENS: THE IDEAL CONDITION FOR CONSISTENCY OF SAMPLE HANDLING THROUGHOUT THE DRUG DEVELOPMENT PROCESS

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## ABSTRACT

**Rationale:** A wide variety of specimen types, including whole peripheral blood, peripheral blood mononuclear cells (PBMC) and various tissues can be analyzed by flow cytometry during the drug development process. Cell integrity, morphology and antigenic site activity is often questionable when shipping samples due to unfavorable environmental conditions and possible delivery delays. However, shipping of samples has many benefits such as an increase in choice of laboratories, the improved consistency through using the same laboratory for non-clinical as well as clinical analysis and maximizing the expertise of laboratories that specialize in specific type of testing, such as flow cytometry. **Experimental Procedures:** In our study we tested non-human primate (NHP) whole blood specimens shipped overnight in Cyto-Chex® BCT tubes and compared the results to the more traditional anticoagulants such as Na Heparin and EDTA. Whole blood immunophenotyping of CD3, CD4, CD8, CD159a, and CD20 on NHP blood contained in Na Heparin, EDTA, Cyto-Chex® BCT tubes was evaluated using flow cytometry analysis on a BD FACSCanto™ instrument equipped with BD FACSDiva™ software. Rodent tissue specimens were tested shipped in 100% FBS and the resulting cell suspensions were evaluated for cell count and viability on a Guava PCA instrument equipped with Viacount® software. **Results: :** Our studies showed that NHP whole blood specimens can keep their integrity for 24-48 hours using EDTA tubes held at 2-8ºC, and 72 hours using Na Heparin tubes held at 2-8ºC and up to one week at 2-8°C storage using Cyto-Chex® BCT tubes. For the tissue specimens, viability and cell count is directly impacted by shipping conditions. Shipping the specimens on frozen gel packs, as opposed to refrigerated gel packs, caused a decrease in viability range from 82.2% – 91.1% to 50.7% - 72.2%. **Conclusion:** Despite the risks of shipping, monitoring of shipping conditions and recent advances that help maintain specimen integrity for longer periods have made shipment of non-clinical samples more reliable and allow for greater flexibility in the choice of flow cytometry laboratory.

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## INTRODUCTION

Flow Cytometry is considered a high complexity testing and is becoming more and more important in during the drug development process. The need for a laboratory with highly trained individuals is essential. Pharmaceutical and biotech companies should have the flexibility to choose the most qualified laboratory to perform their testing. There has been a fear that choosing the most qualified laboratory could not be done because specimens could not be shipped due to instability of the markers of interest. However, if the stability of a marker is in question, would it not be better to determine this in preclinical testing where stability testing can be determined within the scope of the validation for GLP studies?

Flow cytometry specimens can be shipped in the preclinical stage because all timepoints contain a control group which can be used to normalize environmental effects. There are also reliable shipping services available to deliver specimens to the test site within 24 hours or less. In addition, as drug development moves into the clinical phase, the clinical specimens will be shipped from multiple draw sites to a central facility so for consistency shipping of preclinical samples is recommended. Finally, having specimens analyzed in a GLP lab with highly qualified individuals adds value to the preclinical study.

These experiments were conducted to show that many surface, activation or intracellular markers can be stable for several days once drawn. Investigators should have no concerns when it comes to shipping.

## MATERIALS AND METHODS

**Specimens** - NHP whole blood specimens were obtained in sodium heparin, EDTA, and Cyto-Chex BCT blood collection tubes.

Rodent bone marrow specimens were attained in 100% FBS solution and processed into a single cell suspension. The Guava® PCA™ equipped with Viacount software was used for bone marrow cell counts and to evaluate viability.

**Flow cytometry** - Phenotype analyses were performed by Flow cytometry. Cells were stained at ambient temperature for 15 - 20 min with CD3, CD4, CD8, CD159a,CD20, CD69 and CD25. For intracellular analysis, cells were stimulated, fixed, permeabilized and stained with IL-2, TNFα and IFNγ. After staining the cells were washed and subjected to flow cytometry analysis at 24 hrs, 48 hrs, 72 hrs, 96 hrs and 7 days performed by a BD FACSCanto™ equipped with BD FACSDiva™ 6.1.3 software.

## RESULTS

### Surface Marker Stability

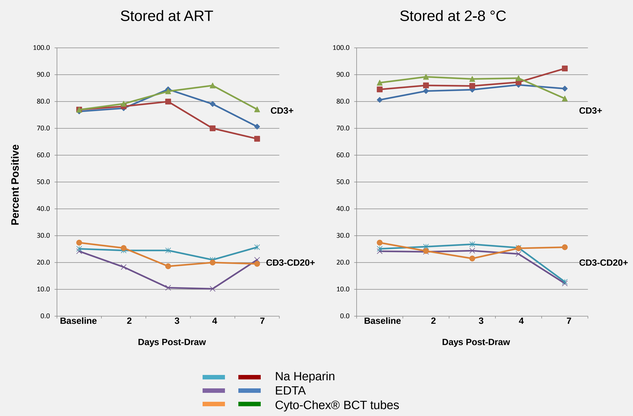


**Figure 1**. CD3, CD3+CD4+, CD3+CD8+, CD3-CD20+ and CD3-CD159a+ staining in NHP whole blood comparing Na Heparin. EDTA and Cyto-Chex® BCT tubes at RT and 2-8 °C. More variability is seen at ART versus 2-8 °C. There is a decline in the CD3-CD20+ population when using EDTA and Sodium Heparin as an anticoagulant versus when using Cyto-Chex® BCT tubes the population is more stable at 7 days.

### Surface and Activation Marker Stability

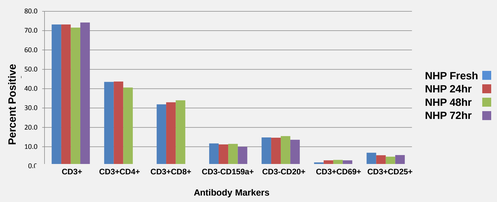


**Figure 2**. Staining in NHP whole blood using Na Heparin as the anticoagulant, fresh, 24 hour, 48 hour and 72 hour with 2-8°C storage.

### Intracellular Marker Stability

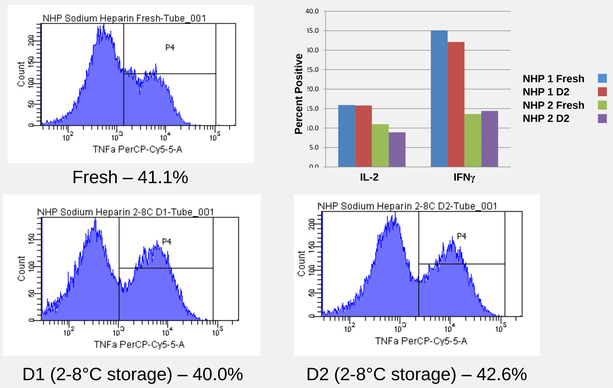


**Figure 3**. Intracellular marker staining of IL-2, IFNγ, and TNFα in NHP whole blood using Na Heparin as the anticoagulant at 2-8°C storage.

## RESULTS (cont.)

### Tissue Shipping Stability

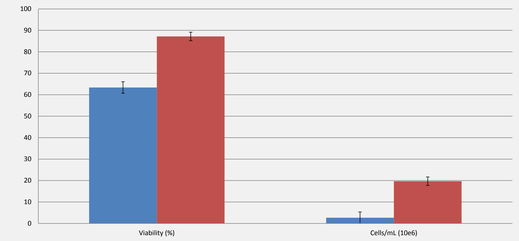


**Figure 4**. Shipment of rat bone marrow specimens. **Blue**: Describes the average percentage viable and number of cells/mL of three specimen samples shipped on frozen gel packs. **Red**: Describes the average percent viable and number of cells/mL of six specimen samples shipped on refrigerated packs.

## CONCLUSIONS

- NHP whole blood specimens can keep surface marker, activation marker and intracellular marker integrity for extended periods depending on anticoagulant and storage conditions.
- In rodent tissue specimens, cell viability and cell count can be directly impacted by shipping conditions with maintaining the specimens on refrigerated gel packs being the most optimal.
- Flow cytometry can be a valuable tool in drug development. As best practice, it is recommended that specimens be shipped so there is consistency of specimen handling throughout the drug development process and so they are analyzed by highly qualified and trained individuals.

## REFERENCES

Plate,M.M. et al. *Viral Immunol*. **22**, 329-32 (2009).

Reddy,M. et al. *Validation of Cell-Based Assays in the GLP Setting: A Practical Guide.* [John Wiley & Sons, Ltd, Chichester, West Sussex, England] (2008).

Warrino,D.E et al. *J. Immunol. Methods* **305**,107-19 (2005).

Weinberg,A. et al. *Clin. Diagn. Lab Immunol* **5**, 804-7 (1998).

## ACKNOWLEDGEMENTS

We kindly thank the University of Washington Primate Center and Bioreclamation for the NHP whole blood specimens and Southern Research Institute for the rat bone marrow specimens. Also, we thank Julie Hill (Full Spectrum Cytometry) for sponsoring this abstract.