

## Validation Plan

XXXXX Cryopreservation Bags

Prospective  X

Retrospective

Revalidation

### Purpose:

Process parameters and outcomes to be assessed in this study include:

Validate (COMPANY NAME) XXXXX bags as an alternative cryopreservation container to our current ZZZZZ Cryocyte

Assess the impact of consecutive freeze/thaw cycles for breakage.

Assess the viability of peripheral mononuclear cells with the XXXXX Bags.

Assess the sterility of the product.

Assess the nucleated cell recovery.

Introduce XXXXX bags as the primary cryopreservation container for the laboratory.

### Acceptable results:

For this study, the following minimal measurement parameters are required:

No breakage or leaks of the XXXXX bags during the freeze/ thaw cycles.

Viability of products greater than 75% post thaw for all products.

Sterile culture results from all products post thaw.

Nucleated cell recovery greater than 80%.

### Procedure:

1. Record all data on WFM.B.017 Equipment Validation worksheet.
2. Obtain buffy coat samples that are freshly collected. Perform a Trypan Blue Viability and cell count, to include MNC%. Bacterial and fungal cultures will be collected from the buffy coat bags after normal saline has been added to bring the volume up to approximately 25 mls.
3. Aliquot approximately 25mls of cells and 25mls of cyroperservative into the XXXXX bags. Heat seal entry port, and label, into the pocket of the bag. Bags 1-5 will be labeled with consecutive numbers 1 through 5, "strength" and total volume. Bags 6-10 will be labeled with consecutive numbers 6 through 10, "viability and sterility" and total volume.
4. Place XXXXX bags into pre-chilled cassettes and place in the -80-degree freezer over night.
5. The following morning, submerge in LN2 tank with approximately 7" of LN2. Refill each day as necessary to maintain approximately 7" level.
6. Approximately 48 hours after initial freeze, thaw 5 bags (strength) successively in a 37°C waterbath according to SOP #9312.
7. Inspect each thawed bag, especially on the seals and fill ports. Note any leaking bags, if there are any, discard. Document all results on validation worksheet.
8. Re-freeze the 5 bags for a total of 2 times (see step 4). These bags will be thawed along with the other 5 "viability and sterility" bags. After the second freeze; drop 1 bag approximately 4 feet from the floor, sideways and then upright. Note any points that may have started to leak.
9. Approximately 7 days after the initial freeze, thaw all bags according to SOP# \_\_\_\_\_. On Bags 6-10 (viability and sterility), obtain a sample for counts, bacterial and fungal cultures and viability from the bags.
10. Make a report or summary of the number of bags that leaked vs. intact bags as well as other observations. Include counts, percent recovery, viability (pre/post), and sterility results.

### SOP's and forms needed

SOP 9312, Thawing and Reinfusion of Cryopreserved Bone Marrow or Peripheral Blood Stem Cells

SOP 9314, Dye Exclusion Viability Testing

SOP 9507, Specimen Preparation for Bacterial and Fungal Cultures

SOP PM.A.002.01, Policy for Establishing Validation Criteria and Performing Validations

SOP TPM.J.001.01, Operation and Maintenance of Coulter A<sup>c</sup>•T diff Analyzer

### Expected results or function:

All XXXXX bags tested will withstand the freeze/ thaw procedure with no leaks or breakage.

Products will have > 80 recovery of nucleated cells along with >80% viability, post thaw.

Products will have negative cultures pre and post thaw.

The (COMPANY NAME) XXXXX bags will be used as an alternative cryopreservation container to our current ZZZZZ Cryocyte bags.

**Critical Control Points and Key Elements:**  
All processing of products and procurement of test samples must be done using aseptic technique.  
Samples must be properly mixed and diluted for performance of cell counts.  
Viability testing must be performed within 15 minutes of thawing of the product. Cells being tested for viability must be properly prepared and accurately counted.  
"Test" bags must be inspected thoroughly to check for leakage or cracks in the primary container after the thaw process

**Responsibilities:**

Study Author:	
Study Plan Approval:	
Data Review:	
Final Approval and Implementation:	

**Results:**

Inclusive dates of Study: From \_\_\_\_\_ To \_\_\_\_\_  
Work forms: See attached reports  
  
Raw Data: See attached reports.  
  
Statistical Analysis and/ or graphs: see attached reports  
Data Summary: See attached reports

**Summary Evaluation**

Overall description of study results:

This process is considered \_\_\_\_\_ Validated \_\_\_\_\_ Not validated

If not Validated give reason:

Required additional studies or monitoring after implementation: