



STEM CELL LABORATORY (STCL)



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DOCUMENT TITLE:

Manufacturing, Storage and Thawing of the Placebo Infusion (Sham Cord Blood Infusion) for the Phase II Randomized, Placebo Controlled Trial of Autologous Cord Blood Cells in Patients with Cerebral Palsy

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STCL-PROC-006
MANUFACTURING, STORAGE AND THAWING OF THE PLACEBO
INFUSION (SHAM CORD BLOOD INFUSION) FOR THE PHASE II
STUDY OF AUTOLOGOUS CORD BLOOD CELLS IN PATIENTS
WITH CEREBRAL PALSY

1 PURPOSE

- 1.1 To describe the process in which the placebo infusion product is prepared for patients enrolled on "Phase II Randomized, Placebo-Controlled trial for patients with cerebral palsy".

2 INTRODUCTION

- 2.1 The Pediatric Blood and Marrow Transplant Program is conducting a randomized, Phase II, placebo-controlled trial to test the efficacy of autologous cord blood infusion in infants and children with cerebral palsy.
- 2.2 Patients will be randomized to receive either washed cord blood cells or placebo.
- 2.3 One year later, each patient will cross over to receive the product they did not receive at their initial treatment.
- 2.4 This procedure describes the preparation of the placebo product. The type of product administered at each infusion will be blinded to the caretakers, medical staff, parents, and patient.
- 2.5 The randomization will be conducted by the CRSO, The EMMES Corporation and will be transmitted to the Stem Cell Laboratory staff on the day before the infusion. The STCL staff will be the only un-blinded staff at Duke participating in this study.

3 SCOPE AND RESPONSIBILITIES

- 3.1 The Medical Director, Laboratory Manager, and designated laboratory staff are responsible for ensuring that the requirements of this procedure are successfully met.

4 DEFINITIONS/ACROYMNS

- 4.1 CRSO Clinical Research Support Office
- 4.2 STCL Stem Cell Laboratory
- 4.3 mL milliliter
- 4.4 DMSO Dimethyl sulfoxide
- 4.5 LN2 Liquid Nitrogen
- 4.6 UCB Umbilical cord blood
- 4.7 CBU Cord Blood Unit

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- 4.8 NS Normal Saline
- 4.9 N/A Not Applicable
- 4.10 ISBT International Society Blood Transfusion
- 4.11 C Celsius

5 MATERIAL

5.1 Reagents

- 5.1.1 TC199
- 5.1.2 DMSO
- 5.1.3 0.9% Sodium Chloride Solution (Normal Saline)

5.2 Supplies

- 5.2.1 Pall Transfer/Freezing Bag Set
- 5.2.2 Sterile Disposable Syringes – 3, 10, 20, 30 and 60 mL
- 5.2.3 Sterile Needles – 16 and 19 gauge
- 5.2.4 Aerobic and Anaerobic Culture Bottles
- 5.2.5 Thermogenesis Overwrap bag
- 5.2.6 Labels – ISBT, Thermogenesis Demand 128, Demand 128
- 5.2.7 Cryogenic Nunc Vials
- 5.2.8 Sharpie Marker
- 5.2.9 Welding Wafers
- 5.2.10 300 mL Transfer Bags
- 5.2.11 150 mL Transfer Bags
- 5.2.12 Sampling Site Coupler
- 5.2.13 Alcohol Prep Pads or equivalent
- 5.2.14 ChloroPrep® SEPP or equivalent disinfectant
- 5.2.15 Disinfected Scissors
- 5.2.16 Amber Colored Bag Cover
- 5.2.17 Amber Tubing Sleeve Cover
- 5.2.18 Gloves
- 5.2.19 Sterile zip-lock bags or equivalent

6 EQUIPMENT

- 6.1 Thermogenesis Bioarchive LN2 Freezer
- 6.2 Bact/Alert 3D

- 6.3 LN2 Vapor Freezer
- 6.4 Heat Sealer
- 6.5 Thermogenesis Canister
- 6.6 Sterile Tubing Welder
- 6.7 Biological Safety Cabinet
- 6.8 Cryobag Overwrap Bag Sealer
- 6.9 Water Bath

7 SAFETY

- 7.1 Wear all appropriate personal protective equipment whenever handling potentially hazardous blood and body fluids to include, but not limited to, gloves, lab coat, goggles, etc.

8 PROCEDURE

8.1 Manufacturing

- 8.1.1 While working in a biological safety cabinet and using aseptic technique, inject 24.5 mL of TC199 into a MedSep transfer/freezing bag set cryopreservation bag.
- 8.1.2 Inject 0.5 mL of 50% DMSO solution to create a final concentration similar to thawed and washed UCB product.
- 8.1.3 Label the cryobag with an ISBT barcode label assigned to this placebo product, a Thermogenesis Demand 128 label and add a bag letter designation, ie. A, B, C, etc.
- 8.1.4 Assign the product the same expiration date that is assigned to the TC199 media.
- 8.1.5 Heat seal the cryobag between the 80 and 20 percent fractions.
- 8.1.6 Heat seal the cryobag tubing to create 3 segments.
- 8.1.7 Place the cryobag in an overwrap bag and seal according to standard practice as described in the *STCL-PROC-045 Cryopreservation and Storage of CBU* procedure.
- 8.1.8 Repeat steps 8.1.1 – 8.1.6 as appropriate for the amount of TC199 that is available to create a “lot/batch” of placebo product.
- 8.1.9 In a syringe, pull up ~2 mL of residual TC199 and ~1 mL of residual DMSO for sterility cultures.
- 8.1.10 Using aseptic technique, inoculate ~1.5 mL into each bottle type (aerobic and anaerobic).
- 8.1.11 Load culture bottles into BacT/Alert 3D system per *STCL-EQUIP-011 Sterility Culture Using the Bact-Alert Microbiology System*.

- 8.1.11.1 If the sterility culture should turn positive, the entire “lot/batch” of placebo product will be discarded.
 - 8.1.11.2 Record all lot numbers of reagents, supplies and equipment used on the appropriate lot number forms.
- 8.2 Cryopreservation
 - 8.2.1 Cryopreserve the bag(s) in a designated Thermogenesis Bioarchive freezer per standard procedure *STCL-PROC-045 Cryopreservation and Storage of CBU*.
- 8.3 Thawing
 - 8.3.1 Remove the designated cryobag from the designated Bioarchive freezer.
NOTE: The placebo product will appear yellow in color when removed from the freezer, as the temperature of the product rises, the color will revert back to a pink/red color.
 - 8.3.2 Working in the vapor phase on the LN2 tank, remove product from metal canister.
 - 8.3.3 Confirm with a second technologist or designee the ISBT barcode on the product. Document the label confirmation by both individuals.
 - 8.3.4 Remove plastic overwrap, if present, and visually inspect the cryobag for damage.
 - 8.3.5 Remove segments and place in a labeled nunc vial (include recipient name, medical record number, “CP Study”, date and ISBT barcode assigned at the time of the infusion).
 - 8.3.5.1 These segments will be retained for potential microarray testing and APO-E testing.
 - 8.3.6 Store nunc in the current/designated nunc vial box and record storage location on thawing worksheet *STCL-PROC-043 Thawing and Infusion Worksheet*.
 - 8.3.7 Place the cryobag inside a sterilized zip-lock bag; remove the air and then seal the bag.
 - 8.3.8 Thaw product in a 37° C water bath until the product reaches a liquid consistency. This generally takes ~5 minutes.
 - 8.3.9 Dry the outside of the zip-lock bag containing the cryobag and transfer to the biological safety cabinet.
 - 8.3.10 Remove the cryobag from the zip-lock bag.
 - 8.3.11 Clean the outside port covers with ChloroPrep® SEPP.
 - 8.3.12 Cut both port covers with sterile or disinfected scissors.
 - 8.3.13 Clean cut surfaces with ChloroPrep® SEPP applicators followed by alcohol prep.

- 8.3.14 Allow the cut surfaces to air dry.
- 8.3.15 Insert a sampling site coupler into the dry and disinfected ports (one at a time).
- 8.3.16 Using a needle and a syringe, aseptically transfer the thawed placebo product into a labeled 300 mL transfer bag.
- 8.3.17 Sterile weld a 150 mL transfer pack containing ~100 mL of sterile normal saline to the 300 mL transfer bag; the normal saline will be used to rinse the product from the bag post infusion.
- 8.3.18 Remove a 2 mL sample from the thawed product to test for sterility as per standard operating *procedure STCL-EQUIP-011 Sterility Cultures Using the Bact/Alert Microbiology System*.
- 8.3.19 Label the final product with the appropriate ISBT and Demand 128 labels.

NOTE: DO NOT identify the product type the study recipient will be receiving since the study is double blinded.

- 8.3.20 Obtain an amber product bag cover and cut a small opening into the bottom of the bag.
 - 8.3.21 To further “blind” the recipient and clinical infusion team, place the labeled 300 mL product bag into the amber product bag cover and seal the amber bag cover, leaving the attached 100 mL normal saline bag outside the amber bag cover.
 - 8.3.22 Attach a tie tag with the appropriate recipient/donor demographic information to the product through the cut out hole in the amber bag cover.
 - 8.3.23 Label the outside of the amber bag cover with the appropriate ISBT and Demand 128 labels.
- NOTE: DO NOT** identify the product type that the study recipient will be receiving since the study is double-blinded and no one outside the laboratory is supposed to know the product type.
- 8.3.24 Complete all appropriate paperwork to accompany the product to the infusion site.
 - 8.3.25 Include one amber tubing sleeve cover with the product to be infused for use by the clinical infusion team.
 - 8.3.26 Record all lot numbers of reagents, supplies and equipment used on the appropriate lot number forms.
 - 8.3.27 Place product in designated transport container, validated to maintain ambient temperature, and deliver to clinical unit for infusion.

9 RELATED FORMS/DOCUMENTS

9.1 STCL-PROC-045 Cryopreservation and Storage of CBU

STCL-PROC-006 Manufacturing, Storage and Thawing of the Placebo Infusion (Sham Cord Blood Infusion) for the Phase II Study of Autologous Cord Blood Cells in Patients with Cerebral Palsy.
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9.2 *STCL-PROC-043 Thawing and Infusion Worksheet*

9.3 *STCL-EQUIP-011 Sterility Culture Using the Bact-Alert Microbiology System*

10 REFERENCES

- 10.1 American Association of Blood Banks. Standards for Hematopoietic Progenitor Cell and Cellular Product. Current edition.
- 10.2 Foundation for the Accreditation of Hematopoietic Cell Therapy (FACT) and Netcord. International Standards for Cord Blood Collection, Processing, Testing, Banking, Selection and Release Current edition.

11 REVISION HISTORY

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