**DOCUMENT NUMBER:** STCL-PROC-022 JA3

**DOCUMENT TITLE:**
Thawing of Frozen Products for HPCA Testing

**DOCUMENT NOTES:**

**Document Information**

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STCL-PROC-022 JA3
THAWING OF FROZEN PRODUCTS FOR HPCA TESTING

1 PURPOSE
1.1 To describe the procedure by which a frozen cellular product from a contiguous segment, sample vial, or freezer bag can be thawed and tested.

2 INTRODUCTION
2.1 In the event that a culture plate yields no growth or is contaminated, a frozen aliquot, if available, can be used to retest the sample. Autologous cord blood units, being considered for infusion to a recipient, are sometimes tested as part of the workup process. Cryopreserved allogeneic cord blood unit usually have contiguous segments attached to the freezer bag which can be used for testing without compromising the integrity of the entire unit. Sample vials from cellular products being cryopreserved can be used for testing if a contiguous segment is not available. Common tests include, but are not limited to, human identity testing by DNA sequencing or HLA typing, ABO typing, confirmatory HLA typing (CT), hemoglobinopathy screening, nucleated cell counts, CD34 counts, cell viability, and enumeration of hematopoietic colony forming units.

3 SCOPE AND RESPONSIBILITIES
3.1 This procedure is performed by designated staff in the HPCA area of the Stem Cell Laboratory. Cells, post thaw, may be redistributed to other areas of the STCL, or to other performing laboratories outside the STCL, if additional testing is required. The medical director, laboratory manager, and designated STCL staff are responsible for ensuring that the requirements of this procedure are successfully met.

4 DEFINITIONS/ACRONYMS
4.1 UCB Umbilical Cord Blood
4.2 HPCA Hematopoietic Progenitor Cell Assay
4.3 HLA Human Leukocyte Antigen
4.4 DNA Deoxyribonucleic Acid
4.5 CT Confirmatory Typing / Testing
4.6 mls milliliters
4.7 DA Dextran Albumin
4.8 BSC Biological Safety Cabinet
4.9 STCL Stem Cell Laboratory
5 MATERIALS
5.1 Vial or segment from cord blood or other cellular product
5.2 Forceps
5.3 Scissors
5.4 Dextran-Albumin Solution
5.5 Stock Albumin 25% (bottle with 12.5gm/50ml or 0.25gm/ml)
5.6 Dextran 40 – 10% Gentran 40 in 0.9% Sodium Chloride Solution 500ml
5.7 3 cc sterile syringe with 17-20 gauge sterile needle
5.8 10 ml syringe
5.9 15 ml conicals
5.10 Alcohol pads
5.11 STERILE 12 x 17 test tubes
5.12 Trypan Blue
5.13 Dry Ice in designated container
5.14 Cell Pack diluent
5.15 STERILE Volumetric Pipette

6 EQUIPMENT
6.1 Biological Safety Cabinet (BSC)
6.2 Sysmex XS-1000i automated hematology analyzer or equivalent
6.3 Water bath (37 degrees Celsius)

7 SAFETY
7.1 Wear personal protective equipment (PPE) when handling any potentially
hazardous blood or body fluid to include, but not limited to, gloves, lab coat,
goggles, etc.

8 PROCEDURE
8.1 Prepare Dextran-Albumin solution per standard protocol
8.1.1 Under sterile conditions, add 2 mls (0.5g) of Human Serum Albumin to
8 mls of Dextran 40 into a 15 ml sterile conical tube. Final volume is
10 mls and the final concentration of albumin is 5%. An Expiration
date of 24 hours from date of preparation is placed on conical.
8.1.2 Keep the dextran-albumin (DA) solution cold by placing it in a
monitored refrigerator at 1-8 degrees Celsius, while not in use.
8.1.3 Remove 1 - 4 mls of DA solution and place in a sterile 15 ml conical tube. The amount of DA added will depend on the number of tests that have been requested on the sample.

8.2 The specimen will be delivered to the HPCA area by laboratory staff.

8.2.1 If the test sample is from a contiguous segment, prepare a 3 ml syringe by aspirating 0.5 ml of DA solution using a 17-20 gauge needle.

8.2.1.1 Warm segment by rubbing it between gloved hands then wipe it with alcohol prep pad to ensure sterility. Allow alcohol to dry.

8.2.1.2 While working in a biological safety cabinet (BSC) to maintain aseptic technique, gently puncture one side of the segment with the needle; reposition the needle, if necessary, in order to aspirate the contents from the segment.

**NOTE:** USE EXTREME CAUTION when performing this step and use ONE-handed SCOOP technique when recapping the needle to ensure that needle stick does not result.

8.2.1.3 Mix contents to remove all cellular material from segment. Document the volume of the sample mixture on the proper form.

8.2.2 If the test sample comes from a vial, proceed as follows:

8.2.2.1 Partially thaw the frozen vial in a 37 degree water bath while leaving some ice crystals in suspension. (Do NOT thaw completely).

8.2.2.2 Working in the BSC, wipe the outside of the vial with alcohol prep pads.

8.2.2.3 Add 0.5 mls of cold DA, one drop at a time, to the partially thawed cells in the vial using a sterile 2 ml volumetric pipet.

8.2.2.4 Mix the contents of the vial using a pipet to ensure adequate mixing.

8.3 Transfer the contents of the syringe or vial, containing the resuspended cells, into a 15 ml sterile conical tube containing the remainder of the DA solution. Mix the suspension thoroughly.

8.4 Remove ~200 µl of the cell suspension for cell count and viability. Record values on the appropriate laboratory form. (NOTE: In an effort to conserve sample, the cell suspension can be diluted with cell pack or equivalent diluent).

8.5 Plate 4 x 10⁵ cells/ml in duplicate according to HPCA procedure or at densities otherwise specified.

8.6 Deliver remaining sample to other laboratory areas as needed for further testing.
9 RELATED DOCUMENTS/FORMS

9.1 STCL-PROC-022 FRM 1 STCL Clinical HPCA Worksheet
9.2 STCL-SOP-052 FRM 1 Progenitor Assay Form

10 REFERENCES

10.1 N/A

11 REVISION HISTORY

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## STCL-PROC-022 JA3 Thawing of Frozen Products for HPCA Testing

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