Clinical and Translational Support Laboratory

Viable Cell Cryopreservation

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Department: Processing Laboratory

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Date	06 JAN 2017	6 Jan 2017	Jan 6, 2017

1. OBJECTIVE

This Standard Operating Procedure (SOP) defines the procedures used in the Clinical and Translational Support Laboratory (CTSL) to ensure that cryopreservation of cells for future viable cell applications are processed in a uniform manner. This procedure provides the basis for documenting compliance with accepted practices, such as Good Clinical Practices (GCP), and serves as a basis for application to protocols.

2. SCOPE

2.1. The SOP applies to CTSL and Clinical Research Center (CRC) personnel conducting cryoprotected cell processing and aliquoting for Indiana Clinical and Translational Sciences Institute (CTSI) CTSL. It is intended to provide the basic procedure but defers to protocol defined processing when provided.

2.2. Samples for this procedure may have been collected in NaHep or ACD tubes.

2.3. All CTSL processing SOPs may be superseded by specific written directives from the investigator or protocol as directed in CTSI-CRC-PL-151 Management of Requests for Sample Processing Support. Initial entry into the worksheet will define whether there are specific processing directives applicable to a specimen.

3. RESPONSIBILITIES

3.1. CTSL personnel are responsible for compliance with this procedure when processing for Viable Cell Cryopreservation.

4. DEFINITIONS

4.1. Principle:

4.1.1. Both the cryoprotectant and the process are critical for recovery of viable cells. Therefore, it is imperative that samples are processed uniformly according to protocol specific conditions. This SOP provides standard operating procedures to minimize processing variables

4.1.2. If study specific modifications are requested per CTSI-CRC-PL-151 Management of Requests for Sample Processing Support, these modifications must be recorded

4.1.3. Prior to freezing, the cells are treated with a cryoprotectant solution such as dimethyl sulfoxide (DMSO) to protect the cells and their membranes from damage during the freezing process. After the cells have been exposed to the freezing medium containing the cryoprotectant, they are dehydrated by very slow cooling to minimize formation of ice crystals that damage the cell. Within 24

hours of completion of the slow cooling process the cells are moved to permanent storage as specified by study protocol requirements.

ACD: Acetate Citrate Dextrose	BS: Bovine Serum
CRC: Clinical Research Center	CTSI: Clinical and Translational Sciences Institute
CTSL: Clinical and Translational Support Laboratory	DMSO: Dimethyl sulfoxide
NaHEP: Sodium Heparin	PI: Principal Investigator
PL: Processing Laboratory	SOP: Standard Operating Procedure

5. ASSOCIATED DOCUMENTS

5.1. CTSI-CRC-QA-003 Document Control and Management
5.2. CTSI-CRC-CLN-030 Handling of SOP Deviations
5.3. CTSI-CRC-CLN-031 Handling of Protocol Deviations
5.4. CTSI-CRC-PL-121 General Safety
5.5. CTSI-CRC-PL-301 Mechanical Refrigeration Units
5.6. CTSI-CRC-PL-151 Management of Requests for Sample Processing Support
5.7. CTSI-CRC-PL-209 Sample Processing
5.8. CTSI-CRC-PL-160 Specimen Receipt, Tracking and Distribution
5.9. CTSI-CRC-PL-158 Data Sample Management System
5.10. CTSI-CRC-PL-206 Ficoll Separation of Mononuclear or Total White Cell Fraction from Peripheral Blood

6. PROCEDURE

6.1. Reagents-
  6.1.1. Bovine Serum (BS), (Dot Scientific) Cat. # 44707-500
  6.1.2. Dimethyl sulfoxide (DMSO), (Sigma) Cat. # D2650
  6.1.3. Dulbecco's Phosphate-Buffered Saline (DPBS), sterile (Invtitrogen) Cat # 14190-235
  6.1.4. Isopropyl Alcohol, 70% in a spray bottle and 250ml for Nalgene Cryo 1°C freezing container
6.2. Supplies
  6.2.1. Alcohol wipes
  6.2.2. Conical centrifuge tubes, sterile disposable 5ml, 15ml, 50ml
  6.2.3. Cryovials 2ml
  6.2.4. Nalgene Cryo 1°C Freezing Container (Freezing Container)
  6.2.5. Pipette tips for micropipettes (20 µl, 200µl, 1000µl), sterile

6.3. Equipment
- 6.3.1. 4°C/-20°C/-80°C Mechanical Refrigerators/Freezers (CTSI-CRC-PL-301 Mechanical Refrigeration Units)
- 6.3.2. Biological Safety Cabinet (BSC)
- 6.3.3. Centrifuge
- 6.3.4. Micropipettes (2-20 µl, 10-100µl, 100-1000µl)

6.4. Receive samples per CTSI-CRC-PL-153 Sample Receipt, Log-in and Tracking
- 6.4.1. Record any exceptions or issues on the protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet.
- 6.4.2. If samples cannot be processed immediately, maintain sample temperature conditions as received.
- 6.4.3. Unlabeled/mislabeled specimens will be held until identification can be determined per CTSI-CRC-PL-157 Unlabeled/Mislabeled Specimens.

6.5. Notify study personnel if sample(s) are not received as directed per protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet and attach email or document communication on the form

6.6. Determine the volume of freeze media needed using the chart below:

Total # of Cryovials	Volume BS (ml)	Volume DMSO (ml)	Total Volume (ml)
1-5	2	0.5	2.5
6-10	4	1	5
11-15	6	1.5	7.5
16-20	8	2	10

- 6.6.1. Prepare Freeze media daily based on the chart above.
  - 6.6.1.1. Cool to 4°C prior to addition to the cells.
- 6.6.2. Combine required volume of BS and required volume of DMSO in an appropriate sized sterile tube.
  - 6.6.2.1. Record Manufacturer, lot number, expiration date and amount of BS and DMSO used on Form No. CTSI-CRC-PL-FM591 Viable Cell Preservation Processing Sheet.
    - 6.6.2.1.1. Label tube as Cryo Media and date.

      NOTE: This is a 20% DMSO solution
- 6.6.3. Place Cryo Media tube at 4°C (ice is acceptable) until needed.
- 6.6.4. Prepare cell pellet(s) according to CTSI-CRC-PL-203 Buffy Layer processing, CTSI-CRC-PL-206 Ficoll Separation of Mononuclear or Total White Cell Fraction from Peripheral Blood or the Form No. CTSI-CRC-PL-FM507 CTSL Sample Management and Protocol Specific Training Form and protocol specific Form No. CTSI-CRC-PL-FM508 Lab Processing Sheet, (Appendices

03 and 04 of CTSI-CRC-PL-151 Management of Requests for Sample Processing Support) noting that only Sodium Heparin or ACD anticoagulants are acceptable for this procedure.

6.6.5. Determine the volume of BS needed to suspend the cell pellet(s) using the chart below: Freeze three cryovials for each sample received unless otherwise specified per Form No CTSI-CRC-PL-FM507 CTSL Sample Management and Protocol Specific Training Form and protocol specific Form No. CTSI-CRC-PL-FM508 Lab Processing Sheet, (Appendices 03 and 04 of CTSI-CRC-PL-151 for Management of Requests for Sample Processing Support).

# of Cryovials per Specimen	Volume BS (ml)
1	0.5
2	1
3	1.5
4	2
5	2.5
6	3
7	3.5
8	4
9	4.5
10	5

6.6.6. Add the required volume of BS (determined from chart above) to suspend the cells. Record volume used on Form No. CTSI-CRC-PL-FM591 Viable Cell Preservation Processing Sheet.

6.7. Transfer cells into a labeled conical centrifuge tube (that can hold the total volume) using a disposable sterile pipette.

6.8. Place the cell suspension at 4°C (ice is acceptable) until thoroughly cooled (minimum 15 minutes).

6.9. Preparation of freezing materials

6.9.1. Label all cryovials using labels provided by the study protocol per CTSI-CRC-PL-151 Management of Requests for Sample Processing Support.

6.9.2. Ensure an alcohol Freezing Container is available for use.

6.9.3. Open Freezing Container.

6.9.4. Remove tube rack from Freezing Container.

6.9.5. Verify Isopropanol is at 250ml

6.9.6. If less than 250ml, add Isopropanol to bring the alcohol level to the 250ml mark on the container.

6.9.7. Discard Isopropanol after fifth use.

6.9.8. Insert the tube rack back into the Freezing Container.

6.9.9. Place labeled cryovials in the alcohol Freezing Container.
6.9.10. Close Freezing Container.
6.9.11. Store Freezing Container with cryovials in refrigerator until proceeding to next step.

6.10. Final cell suspension
6.10.1. Determine the volume of Cryo Media to add to the cell suspension using the chart below:

# of Cryovials per Specimen	Volume of Cryo Media (ml)
1	0.5
2	1
3	1.5
4	2
5	2.5
6	3
7	3.5
8	4
9	4.5
10	5

6.10.2. Obtain the 4° C Cryo Media and cell suspension.
6.10.3. **SLOWLY** add the listed volume of Cryo Media to the cold cell suspension with continual mixing by gently vortexing.
6.10.4. RAPIDLY transfer ~1ml (0.5-1.8 mL) of the cell suspension to each of the cooled, labeled cryovials.
6.10.5. Place the Freezing Container in a -80°C freezer for 3-24 hours. If required, freezing containers may be left at -80° C over a weekend or holiday.
6.10.6. Make appropriate data entries on Form No. CTSI-CRC-PL-FM591 Viable Cell Preservation Processing Sheet.

6.11. Transfer the cryovials to a permanent location designated according to CTSI-CRC-PL-151 Management of Requests for Sample Processing Support.
6.12. Record storage location per CTSI-CRC-PL-158 Data Sample Management System in the comments section of the protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet.
6.13. Protocol deviations are managed per CTSI-CRC-CLN-031 Handling of Protocol Deviations.
6.14. SOP deviations are managed per CTSI-CRC-CLN-030 Handling of SOP Deviations

6.15. Of special note, per scope of this SOP, complying with special investigator specific directives that may or may not be prescribed on the protocol specific CTSI-CRC-PL-FM508 Lab Processing Sheet is not a deviation to this SOP but is noted on the applicable worksheet and documentation of the special directives is maintained in the appropriate study folder in the CTSL.

7. REFERENCES

7.1. Rowlands DT, Whiteside TL, Daniele RP, Cells of the Immune System, in Clinical Diagnosis and Management by Laboratory Methods (ed. J.B. Henry), 17th edition, W. B. Saunders, 1984, p. 833.

7.2. N.C. Gorin, Cryopreservation and Storage of Stem Cells, Bone Marrow and StemCell Processing: A Manual of Current Techniques, Eds., Areman, E., Deeg, H.J., Sacher, R.A., F.A. Davis Co., 1992, pp.292-308.

7.3. W.E. Janssen, C. Lee, Cryopreservation of Bone Marrow in Standardized Medium, Bone Marrow and Stem Cell Processing: A Manual of Current Techniques, Eds., Areman, E., Deeg, H.J., Sacher, R.A., F.A. Davis Co., 1992, pp. 317-319.

8. APPENDICES

None

9. AMENDMENT HISTORY

Date of Amendment:	05 Jan 2017
Amendment Request by:	Robert Orr
Change Control No, if applicable:	CTSI-CRC-CLN-DC-2016-082
Details of Amendment:	Updated with new template Version 02; Revised for clarity; Updated SOP references in section 5 and steps 6.13 and 6.14; removed reference to obsolete SOP in step 6.5