



ACTG LABORATORY STANDARD OPERATING PROCEDURE			
Title:	Leukopak PBMC Processing Standard Operating Procedure		
SOP number:	LTC-SOP-61	Effective:	22Nov2022
Version:	v2.0	Last reviewed:	11Jun2013
Originator:	ACTG/IMPAACT Lab Technologist Committee	Pages:	19

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## 1. PURPOSE

The purpose of this SOP is to provide instruction for consistent collection and processing of leukopaks to obtain a large number of peripheral blood mononuclear cells (PBMC) in support of clinical trials.

## 2. BACKGROUND

Leukapheresis is a safe, well-tolerated, outpatient procedure that has been used in clinical practice for over 25 years whereby leukocytes, or in some instances specific cell fractions (e.g., PBMCs) are selectively harvested but red cells and other blood components are returned to the participant. In a typical 1.5 - 3-hour leukapheresis procedure, 3-10 x10<sup>9</sup> PBMCs can be isolated with only minimal loss of red blood cells [8.1, 8.2]. The purpose of this document is to define the procedure for isolating PBMCs from the leukopak.

## 3. SCOPE

Users of the ACTG/IMPAACT Lab Manual and clinical trial site staff.

## 4. DEFINITIONS

Term	Definition
ACTG	AIDS Clinical Trials Group
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BSC	Biosafety cabinet
CBC	Complete blood count
CPS	Cryopreservation solution
CRF	Case Report Form
DGM	Density Gradient Medium
DMSO	Dimethyl sulfoxide
FBS	Fetal Bovine Serum
HBSS	Hanks' Balanced Salt Solution
HI-FBS	Heat Inactivated Fetal Bovine Serum
IMPAACT	International Maternal Pediatric Adolescent AIDS Clinical Trials Group
INR	International normalized ratio
IQA	Immunology Quality Assessment

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<b>Term</b>	<b>Definition</b>
LDMS	Laboratory Data Management System
Leukopak	The product obtained from the leukapheresis
LN2	Liquid nitrogen
LPC	Lab Processing Chart
NSAID	Non-steroidal anti-inflammatory drug
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate-buffered saline
PID	Participant Identification Number
PT	Prothrombin time
PTT	Partial thromboplastin time
QS	Add sufficient quantity of liquid to bring to specified volume
Room Temperature (RT)	15 to 30°C
RPMI 1640	Roswell Park Memorial Institute culture medium
ULN	Upper limit of normal
Vapor phase storage	The space in the LN2 storage tank that is above the LN2 liquid.
WDR	Wash Diluent Reagent (HBSS, PBS or RPMI 1640)

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## 5. RESPONSIBILITIES

- 5.1. The Network Laboratory Directors (or his/her designee) have the authority to establish, review and update this procedure.
- 5.2. The ACTG/IMPAACT Laboratory Technologist Committee (LTC) is responsible for the maintenance and control of SOP documentation.
- 5.3. The Laboratory Director is responsible for the implementation of this LTC SOP or laboratory-specific SOP and for ensuring that all appropriate personnel are trained. A laboratory SOP must:
  - 5.3.1. Include, without procedural modification, the portions of the current version of the LTC SOP that are used within the network site-affiliated laboratory.
  - 5.3.2. Reference the current version of the LTC SOP
- 5.4. All laboratory technicians are responsible for reading and understanding this SOP prior to performing the procedures described.
- 5.5. The site PI and designees are responsible for understanding and adhering to the patient preparation and specimen collection components.
- 5.6. A laboratory must be approved for leukopak processing prior to processing a specimen for a clinical trial. Refer to [Initial or Annual Renewal Leukopak Qualification and Near Time Quality Control \(QC\) Plan](#). [8.3]
  - 5.6.1. For prequalification a laboratory must process a leukopak and submit specimens to the IQA for evaluation. The laboratory will be notified by the IQA of the results.
  - 5.6.2. If a laboratory does not process a leukopak for more than a 12-month period, they must re-qualify with the IQA using the same process described above.

## 6. EQUIPMENT, REAGENTS AND CONSUMABLES

### 6.1. Equipment

#### 6.1.1. Preparation & Processing

- 6.1.1.1. Class II biosafety cabinet (BSC) as set up by laboratory (BSL2, BSL2.5 or BSL3)
- 6.1.1.2. Centrifuge, low speed (capable of 300 to 1000 x g), with swinging bucket rotors and sufficient capacity for twenty 50mL conical centrifuge tubes
- 6.1.1.3. Micropipettes, range 20, 200, 1000µL
- 6.1.1.4. Pipet-Aid (cordless preferred) for disposable, serological pipets
- 6.1.1.5. 2 to 8°C refrigerator
- 6.1.1.6. -20°C (or lower) freezer without automatic defrost (for FBS storage)
- 6.1.1.7. -80°C freezer (e.g., -65 to -95°C); for short-term PBMC storage
- 6.1.1.8. Ice water bath (optional for preparation of CPS)

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6.1.1.9. Bucket or beaker for bleach or other disinfectant, for rinsing pipets if required by local safety practice

6.1.1.10. Scissors (wrapped and sterilized or clean and sprayed with alcohol prior to use)

6.1.1.11. Temperature data logger (if shipping the leukopak to a central processing lab) such as the FlashLink® USB PDF Reusable Data Logger (Model 40510)

#### 6.1.2. Cell Counting (select one of following options)

6.1.2.1. Automated cell counter capable of enumerating viable cells (Beckman-Coulter Vi-Cell, Guava PCA® or equivalent).

6.1.2.2. Automated cell counter not capable of distinguishing viable cells (Coulter Counter, Abbott Cell-Dyn®, Sysmex® or equivalent).

*Note:* If an automated cell counter that is not capable of distinguishing viable cells is used, viability must be determined with a manual cell counting chamber.

6.1.2.3. Manual cell counting chamber (hemacytometer, disposable or re-useable) and light-field or phase contrast microscope.

*Note:* Non-disposable hemacytometers must be used with hemacytometer grade coverslips to ensure accurate volume in the counting chamber. Regular coverslips are not adequate for this function.

#### 6.1.3. Cryopreservation

*Note:* Refer to the Cross Network PBMC Processing SOP [8.4] for additional guidance on cryopreservation.

6.1.3.1. Stratagene StrataCooler® Cryo (preferred option)

6.1.3.2. BioCision® CoolCell

6.1.3.3. NALGENE® Mr. Frosty™, 1°C/minute cryo-freezing container

#### 6.2. Personal Protective Equipment

*Note:* Personal protective equipment suitable for use with bloodborne pathogens is required. Follow local laboratory guidelines and practices for the handling of blood products.

6.2.1. Laboratory coat

6.2.2. Eye protection

6.2.3. Non-powdered, nitrile or equivalent gloves

6.2.4. Cryogenic gloves and face shields (with chin cap optional) are necessary if using LN2.

#### 6.3. Reagents

*Note:* Refer to the Cross-Network PBMC Processing SOP [8.4] for examples of reagents for this procedure.

6.3.1. The use of sterile reagents and aseptic technique are required.

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6.3.1.1. Store opened bottles at the temperature recommended by the manufacturer until used or until manufacturer's expiration date.

6.3.1.2. Discard if visible signs of contamination, such as a cloudy appearance, develop.

6.3.2. 1.077g/ml Density Gradient Media (DGM)

6.3.3. Wash Diluent Reagents (WDR)

Hanks' Balanced Salt Solution (HBSS\*) without calcium or magnesium, ready-to-use.

\*Alternative: 1X Phosphate-Buffered Saline (PBS) without calcium or magnesium, ready-to-use or RPMI 1640 Medium without FBS or antibiotics unless otherwise mandated by the LPC.

6.3.4. Freezing Reagents

6.3.4.1. Heat-Inactivated Fetal Bovine Serum (HI-FBS)

6.3.4.1.1. An Immunology Quality Assessment (IQA) approved lot must be used. Ordering information is posted on the [HANC ACTG/IMPAACT FBS Ordering Procedure page](#) [8.5].

6.3.4.1.2. Obtain a certificate of analysis for local laboratory quality control records.

*Note:* A copy of the current certificate of analysis is located on the [HANC ACTG/IMPAACT FBS Ordering Procedure page](#) [8.5].

*Note:* A copy of the FBS certificate of analysis may be required to export (or import) PBMC aliquots between countries.

6.3.4.2. Dimethylsulfoxide (DMSO), cell-culture grade

6.3.4.2.1. Use cell-culture grade DMSO.

6.3.4.2.2. Store unopened bottles at room temperature (15 to 30°C). Check bottle for expiration date and discard if expired.

6.3.4.2.3. After opening, undiluted DMSO is stable at room temperature (15 to 30°C) for 6 months when protected from light and moisture.

6.3.4.2.4. Use aseptic technique when removing DMSO from the bottle to avoid possible contamination. Discard open bottle if visible signs of contamination are noted.

6.3.4.2.5. Reagent may be aliquoted in small amounts to help preserve sterility. Protect aliquots from light.

*Note:* If aliquots are made, they must be labeled as DMSO, and indicate the lot number, open date, expiration date, storage temperature and tech initials.

6.3.4.3. Disinfectant

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6.3.4.3.1. 70% v/v ethanol disinfectant, spray bottle

6.3.4.3.2. 10% v/v bleach, bucket or beaker and spray bottle

6.3.4.3.3. Other disinfectant as specified by local laboratory policy

#### 6.3.5. Cell Counting Reagents

6.3.5.1. 0.4% trypan blue solution or other automated cell counter viability stains.

#### 6.4. Reagent Preparation

6.4.1. Heat-Inactivated FBS (HI-FBS): Refer to the Cross-Network PBMC Processing SOP [8.4] for freezing, thawing and storage procedures.

#### 6.4.2. Fresh Cryopreservation Solution (CPS)

Note: CPS is a mixture of FBS and DMSO. When prepared, an exothermic reaction occurs. Therefore, it is important to prepare the CPS and equilibrate to 2 to 8°C before adding it to the cells.

6.4.2.1. 100 mL of CPS is needed to process a leukopak (90mL of FBS + 10 mL of DMSO).

6.4.2.2. Combine the DMSO and FBS in a sterile container and mix well.

6.4.2.3. CPS must be prepared in advance and chilled in the refrigerator (2to 8°C) for at least 30 minutes or in an ice bath for at least 15 minutes prior to use.

6.4.2.4. CPS can be stored at 2 to 8°C for 1 working day (<18 hours).

#### 6.5. Disposables

Refer to the Cross-Network PBMC Processing SOP [8.4] for examples of disposables for this procedure.

##### 6.5.1. Plastics

6.5.1.1. Serological pipets, disposable, 1, 5, 10, 25, 50mL, sterile

6.5.1.2. Precision pipet tips, 20, 100, 200, 1000 µL, sterile

6.5.1.3. 50mL disposable centrifuge tubes, sterile, conical bottom, graduated polypropylene

6.5.1.4. Externally threaded cryogenic vials (cryovials), 1.8 to 2mL, screw cap with o-ring, sterile, polypropylene only, self-standing, graduated, leak-proof, formulated for vapor-phase LN2 preservation (approximately -140°C)

Note: Not all cryovial brands are suitable for long-term storage in LN2.

6.5.1.5. Sterile disposable conical tubes or bottles (250mL conical tube, e.g., Corning® #430776, 500mL bottles, e.g., Corning® #430282, or 1L bottles, e.g., Corning® #430518) for pooling and diluting leukapheresis specimen and/or making cryopreservation solution (CPS).

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6.5.1.6. Optional: 5mL sterile, individually wrapped plastic transfer pipets

6.5.2. Markers: Markers for writing on processing tubes and vials should have a fine point, and contain fast drying, indelible ink.

6.5.3. Labels: Cryogenic labels suitable for -80°C and LN2 temperatures.

## 7. PROCEDURES

### 7.1. Preventative Maintenance

7.1.1. Performance specifications for all equipment (centrifuges, refrigerators, freezer, pipettors, BSCs, etc.) must be verified according to the manufacturer and DAIDS GCLP requirements.

7.1.2. Follow the applicable laboratory procedures for the verification of performance using an automated or manual cell count.

### 7.2. Quality Control

#### 7.2.1. Cell Yields:

7.2.1.1. The number of cells obtained from a leukapheresis procedure will depend upon vein size, blood pressure, duration of the procedure, and participant.

#### 7.2.2. Cell Viability

7.2.2.1. Freshly isolated PBMC viability should be >95%. Long processing time, or poor technique may adversely affect the viability. Calculate and record the % viable cells according to laboratory and network requirements.

7.2.2.2. If the fresh PBMC viability is <95%, review the results with the supervisor and document according to network and laboratory requirements.

### 7.3. Reporting Results

7.3.1. Use of a "PBMC Isolation from Leukapheresis – Processing Record" and the Laboratory Data Management System (LDMS) is required to track the timing of processing, calculations and document problems that arise during processing.

7.3.1.1. Enter data into the LDMS for the generation of cryovial labels, storage location documentation and shipping manifest requirements. See the table below for requirement details.

7.3.1.2. Report deviations according to laboratory protocol.

7.3.1.3. The laboratory may use the "PBMC Processing Worksheet" (shown and linked in Appendix B), or the Leukapheresis Processing Worksheet (shown and linked in Appendix A), or a modification appropriate for the laboratory's procedures. If the laboratory chooses to develop its own document and supplementary tracking materials (such as the LDMS, or a separate worksheet or log) the laboratory should comply with the guidelines below.

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7.3.1.4. Electronic and editable versions of these documents are provided in the [HANC ACTG/IMPAACT Lab Manual](#) for download and utilization or modification [8.6].

#### 7.4. Handling Requirements

7.4.1. Leukopak specimens should be collected as early in the day as possible to allow the processing laboratory ample time to process and/or ship the leukopak.

7.4.2. Leukopak specimens should be logged into LDMS and processed as soon as possible upon receipt:

7.4.2.1. Collection time is the time when the leukopak collection has ended.

7.4.2.2. Processing start time is the time when the leukopak is first opened.

7.4.2.3. Freeze time is defined as the time when the last StrataCooler® Cryo, NALGENE® Mr. Frosty™ or BioCision® CoolCell is put into the -80°C freezer.

7.4.2.4. The LDMS code for leukopak PBMCs is LPK/ACD/CEL/DMS.

Note: Verify the anticoagulant in the LPC

7.4.3. All leukopaks should be processed regardless of volume or red blood cell contamination, unless otherwise directed by the protocol. If the cell yield is insufficient to meet the needs of the protocol, contact the clinic and protocol team as soon as possible.

#### 7.5. PBMC Isolation from Leukapheresis using Manual Density Gradient Media Overlay/Underlay Method (Procedure)

Note: Several steps can be completed prior to receipt of the leukopak in the processing laboratory. Prepare reagents and supplies in advance to facilitate timely processing of the leukopak.

7.5.1. Perform all specimen processing in a BSC within a BSL-2 or greater facility using aseptic techniques. The optimal temperature for this procedure is room temperature (15 to 30°C).

7.5.2. Clean all surfaces, racks, and reagent bottles with an appropriate disinfectant prior to using the BSC. Set up BSC and work areas with needed supplies to initiate the processing.

7.5.3. Verify that -80oC space is available for the anticipated step-down freezer units that will be used later in the process. If the laboratory uses Mr. Frosty™ units, verify the isopropanol status.

Note: Only 100% isopropanol should be used with Mr. Frosty™ units.

7.5.4. Prior to processing or sufficiently in advance of mixing with PBMC, prepare and chill the CPS (see section 15-Reagent Preparation).

7.5.5. Allow the DGM to come to room temperature (15 to 30°C). See Section 14 Reagents for more information.

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- 7.5.6. Prepare twenty (20) 50mL conical tubes by labeling each tube with the PID and adding 15mL of DGM in each tube.
- 7.5.7. Label twenty (20) new sterile conical 50 mL centrifuge tubes with the PID. These tubes will be used for Wash 1 (section 20.1).
- 7.5.8. Based on the CRF information, log leukopak into the LDMS, generate labels, affix labels to cryovials and QC the label process. Pre-chilling the cryovials is recommended.
- 7.5.9. Upon receipt, carefully check the PID and other information on the leukopak and accompanying paperwork. It is not advisable to process more than one leukopak at a time. If the leukopak is cold to the touch (due to cold ambient conditions such as transport in cooler months), allow it to reach room temperature (15 to 30°C) before processing.
- 7.5.9.1. Spray the leukapheresis pouch and scissors with 70% ethanol. Hold the pouch in one hand and cut one of the tubing ports with the clean scissors.
- 7.5.9.2. Pour the specimen through the tubing into a sterile flask or bottle. Determine and record the specimen volume (typically 150 – 200mL).
- 7.5.10. Leukopak Dilution and Manual Density Gradient Cell Separation: See flow diagram in Appendix C.
- 7.5.10.1. Add sufficient WDR to dilute the cells to 600mL total. Mix the specimen well by gently swirling or pipetting up and down; avoid creating bubbles. Distribute equal volumes of the diluted leukopak amongst the processing LTs.
- 7.5.10.2. Carefully and slowly overlay/underlay 30mL of the diluted leukopak on top of/below the DGM in each tube. Cap each tube after the diluted leukopak is added.
- Note: Tilting the conical tube to approximately 45° angle often helps with the overlay process. If you have an adjustable pipette aid, set the rate to slow.
- 7.5.11. Lymphocyte density centrifugation and collection
- 7.5.11.1. Hold the tubes in an upright position and gently transfer them to the centrifuge.
- 7.5.11.2. Centrifuge at 400 x g for 30 minutes at 15 to 30°C with the brake OFF, or as outlined in the package insert that accompanies the gradient medium.
- Note: Leaving the brake on will disrupt the layers. The centrifuge brake must be turned OFF to maintain a sharp interface between the gradient media and the PBMC layer. This will maximize retrieval of the PBMCs with minimal contamination by unwanted cell species (i.e., granulocytes).
- Note: Refer to Section 20 in the Cross-Network PBMC Processing SOP [8.4] for calculations to convert g to rpm for your rotor length.

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7.5.11.3. Carefully remove the tubes from the centrifuge.

*Note:* If the cell layers are not visible, confirm that the centrifuge is operating properly. Correct any problems you find. Re-centrifuge the tube(s). Document the problem and actions taken according to network and laboratory requirements.

7.5.11.4. Inspect the tubes for small clots visible at the cell interface that were not previously noted and document them. Record your observations in LDMS or other documentation.

7.5.11.5. Use a sterile pipet (serological or transfer pipet), to remove the upper, "plasma"-WDR fraction in each tube down to within (above) approximately 2 cm of the cloudy white PBMC band located at the interface between the "plasma"-WDR fraction and the clear separation medium solution. Discard the "plasma"-WDR fraction per laboratory policy.

*Note:* Alternatively, the upper "plasma"-WDR fraction may be left in place and the cloudy white PBMC band may be removed by carefully inserting the pipet through the upper layer to the PBMC band.

7.5.11.6. Use a sterile serological or transfer pipet to collect all cells at the cloudy white interface. Take care not to aspirate any more separation medium solution than necessary. Do NOT scrape cells that are adhering to the plastic tube; these adherent cells are generally not PBMCs.

7.5.11.7. Transfer the collected cells from one conical gradient tube into one 50 mL conical wash tube. Repeat these steps for the remaining tubes.

7.5.11.8. After removing the cell layer, re-cap the conical centrifuge tubes containing the residual DGM and discard according to laboratory policy.

7.5.12. Make sure all appropriate information is documented according to network and laboratory requirements (See Section 13, Reporting Results).

## 7.6. Washing, Counting, Resuspension, Concentration, and Overnight Controlled-Rate Freezing

### 7.6.1. Wash 1

7.6.1.1. Q.S. each 50 mL centrifuge tube containing the PBMC fraction to approximately 45mL by adding WDR. Mix gently to resuspend the cells.

7.6.1.2. Re-cap the harvested cell tubes and place the 20 tubes in the centrifuge.

7.6.1.3. Centrifuge the 20 tubes containing diluted cells at 200 to 400 x g for 10 minutes at 15 to 30°C (brake optional).

7.6.1.4. Remove the 20 tubes from the centrifuge and check for the cell pellets.

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7.6.1.5. If the cell pellets are not visible, confirm that the centrifuge is operating properly. Correct any problems you find. Re-centrifuge the tubes. Document the problem and actions taken according to network and laboratory requirements. If the cell pellets are still not visible after re-centrifuging the tube, document.

7.6.1.6. Carefully remove and discard the supernatants without disturbing the cell pellets.

Note: These pellets will be quite large and relatively “loose” compared to routine PBMC pellets.

#### 7.6.2. Wash 2

7.6.2.1. Resuspend each pellet in 5mL of WDR, mixing gently but thoroughly, into a homogenous cell suspension.

7.6.2.2. Combine the pellet suspensions from four (4) 50mL conical tubes into one tube. Rinse the 4 “empty” tubes with 5 mL WDR and transfer to the collective tube. Discard the empty tubes after this rinse. This step condenses the cells from 20 tubes to five (5) tubes.

Note: Laboratories may opt to perform this step in the 20 original wash tubes. A condition where this might be advisable is a sticky cell pellet.

7.6.2.3. In each 50 mL centrifuge tube, QS the PBMC fraction to approximately 45mL by adding WDR, re-cap the tubes and mix gently.

7.6.2.4. Centrifuge diluted cells at 200 to 400 x g for 10 minutes at 15 to 30°C (brake optional).

7.6.2.5. Remove the tubes from the centrifuge and check for the cell pellets.

7.6.2.6. Remove and discard the supernatants without disturbing the cell pellets.

7.6.2.7. Resuspend each of the cell pellets in 10mL of WDR and combine into one sterile 250 or 500mL bottle and document the exact volume transferred.

7.6.2.8. Q.S. to 200mL with WDR and mix gently. It is important to be as accurate as possible because the cell count will be based on a resuspension volume of 200mL.

#### 7.6.3. PBMC Cell Count

7.6.3.1. Mix cells gently, but thoroughly, before sampling for the cell count.

7.6.3.2. Follow the SOP for the cell counting method approved at the processing laboratory to determine the total cell number and viability.

Note: Duplicate counts are preferred.

#### 7.6.4. Distribution of Cells for Final Centrifugation

7.6.4.1. Determine the appropriate batch size for centrifuging and aliquoting the cells.

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7.6.4.2. Prepare 85 vials with a maximum concentration of 50 x 10<sup>6</sup> PBMC / mL per aliquot.

*Note:* If fewer cells are recovered adjust the concentration per aliquot, with a minimum of 20 x 10<sup>6</sup> PBMC / mL per aliquot. Only adjust the number of aliquots if the minimum number of cells per aliquot is not attainable.

7.6.4.3. Prepare the conical centrifuge tubes for final centrifugation. There will be one conical centrifuge tube for each batch.

7.6.4.4. Thoroughly, but gently, resuspend the harvested cells prior to distribution.

7.6.4.5. Distribute the harvested cells among the prepared conical centrifuge tubes.

7.6.4.6. Set aside the remaining cells in case more aliquots need to be cryopreserved.

7.6.5. Final Centrifugation, Resuspension in CPS, Aliquoting for Cryopreservation, and Overnight Controlled-Rate Freezing

*Note:* The centrifugation of batches will be dependent on the workflow within each laboratory. Do not allow cells to sit in the centrifuge once spinning has stopped.

*Note:* Work quickly once the CPS has been added. Do not allow the cells to be in contact with CPS for longer than 10 minutes before placing in the freezer.

7.6.5.1. Place the conical centrifuge tube(s) containing the cells in the centrifuge and spin at 200 to 400 x g for 10 minutes at 15 to 30°C.

7.6.5.2. Resuspension in CPS

7.6.5.2.1. Remove and discard the WDR supernatant. Keep the pellet(s).

7.6.5.2.2. Gently resuspend the cell pellet(s) by flicking or pipetting.

7.6.5.2.3. Gently resuspend each pellet(s) in the volume of prepared CPS.

7.6.5.3. Aliquot the PBMC into the cryovials.

7.6.5.3.1. Laboratories may opt to use a repeater pipettor to expedite the aliquoting of the cells into the cryovials.

7.6.5.3.2. Be sure to mix the cells well before refilling the repeater pipettor or in between every 10 aliquots.

7.6.5.4. Immediately transfer all cryovials to a controlled-rate freezing vessel.

7.6.5.5. Close the container and place it in a -80°C freezer, in a location that is not disturbed by repeated freezer access (i.e., away from the door, in the back of the freezer). Follow the cryochamber manufacturer instructions for freezing time.

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Note: Record the time that the last batch is placed in the -80°C freezer in the LDMS and on the PBMC isolation form, Leukapheresis- Processing Record, or equivalent.

7.6.6. Discard extra cells according to laboratory and institutional requirements.

## 7.7. Onsite Storage and Near-Time Quality Assurance

### 7.7.1. Onsite temporary storage in a -80°C freezer

7.7.1.1. Work rapidly and efficiently to minimize cryovial exposure to ambient temperature. Refer to the [Cross-Network Cold Chain Guidelines](#) [8.7].

7.7.1.2. Transfer the cryovials from the controlled-rate cooling system to the designated storage location in a -80°C freezer.

7.7.1.3. Ship cells on dry ice to the testing lab or biorepository within 4 weeks of cryopreservation. Contact the protocol team if there will be delays in shipping.

7.7.1.4. Do not temporarily store specimens in LN2 unless instructed to do so in the LPC.

7.7.1.4.1. If cells are stored in LN2 they must be shipped in an LN2 dry shipper.

7.7.1.4.2. Do not transfer specimens from LN2 back to -80°C freezers.

### 7.7.2. Near-time shipment to IQA

7.7.2.1. Four aliquots (two from the beginning and two from the end) will be shipped to the IQA for near-time quality assurance.

7.7.2.2. Ship cells on dry ice to the IQA within 4 weeks of cryopreservation. Contact the IQA and protocol team if there will be delays in shipping.

## 8. REFERENCES

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- 8.6. *ACTG/IMPAACT Laboratory Manual*. (n.d.). Office of HIV/AIDS Network Coordination.  
<https://www.hanc.info/resources/sops-guidelines-resources/laboratory/actg-impaaact-laboratory-resources.html>
- 8.7. *Cross-Network Cold Chain Guidelines*. (2018, April 26). Office of HIV/AIDS Network Coordination. <https://www.hanc.info/resources/sops-guidelines-resources/laboratory.html>

## 9. INQUIRIES

Contact the ACTG/IMPAACT LTC Leadership at [actg.ltcleadership@fstf.org](mailto:actg.ltcleadership@fstf.org) for questions and comments related to these procedures.

## 10. NETWORK LAB CENTER SOP APPROVAL

NAME AND TITLE	SIGNATURE	DATE OF APPROVAL
Grace Aldrovandi, MD ACTG/IMPAACT Network Laboratory Principal Investigator		08Dec2022

## 11. REVISION HISTORY OR RECORD RETIREMENT

VERSION #	EFFECTIVE DATE	REPLACES	DATE OF REVISION	RATIONALE FOR [REVISION/RETIREMENT]
1.0	11Jun2013	NA	01Sep2022	NA
2.0	03Oct2022	Version 1.0	NA	All sections reviewed and updated to reflect current practice.

## 12. APPENDICES (see following pages)

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12.1. APPENDIX 1: PBMC Processing Worksheet Example 1 (download on HANC [Lab Manual page](#))

REAGENTS	Lot Number	Exp. Date	OPENED/ THAWED DATE	VISUAL QC  OK	TECH INITIALS:
					PROCESS DATE:
HBSS			/ /	YES or NO	SITE:
LSM			/ /	YES or NO	
Trypan Blue			/ /	YES or NO	
DMSO			/ /	YES or NO	
FBS			/ /	YES or NO	
PID:					
PROTOCOL:					
ADDITIVE:	EDT / HEP / ACD				
Leukopak VOL:	mL				
Anticoagulant VOL (If avail.):	mL				
<b>PROCESSING</b>					
START TIME:	:				
LSM TIME:	:				
FREEZING TIME(**):	Time of Last Mr. Frosty™ in freezer				
Mr. Frosty™ #/THAW #:	see right side				
<b>CELL COUNT</b>					
COUNT VOLUME:	mL				
Trypan Blue Dilution					
Tech Initial:					
Sq.#1 (alive/dead):	/	/			
Sq.#2 (alive/dead):	/	/			
Sq.#3 (alive/dead):	/	/			
Sq.#4 (alive/dead):	/	/			
TOTAL (alive/dead):	/	/			
<b>Average</b>					
# OF CELLS/mL:	x 10 <sup>6</sup>				
TOTAL # OF cells (alive)	x 10 <sup>6</sup>				
VIABILITY %:					
# VIALS- # OF CELLS	<b>85 vials @ 50x10<sup>6</sup></b>				
PER VIAL NEEDED:					
Prepare 100mL CPS: 90mL FBS + 10mL DMSO					

# OF CELLS/mL:	x 10 <sup>6</sup>	
# OF CELLS/mL: -10%	x 10 <sup>6</sup>	<<<<

4 Mr. Frosty™ = 72 vials  
Remnant to freeze = 87-72 = 15 vials

Cells for 1 Mr. Frosty™: (18 vials + 1 = 19 vials)	19mL CPS
50 x 10 <sup>6</sup> per vial	950 x 10 <sup>6</sup> cells
950 x 10 <sup>6</sup> cells /	mL X4
<<<<	

Remnant to freeze = 85-72 = 13 vials

Cells for last Mr. Frosty™: (13 vials + 1 = 14 vials)	16mL CPS
50 x 10 <sup>6</sup> per vial	700 x 10 <sup>6</sup> cells
700 x 10 <sup>6</sup> cells /	mL
<<<<	

Mr. Frosty™ # /THAW #:	Tech	Global Spec ID	Time Frozen
/		-	
/		-	
/		-	
/		-	
/		-	(**)

\*\*Freezing Time in LDMS is freezing time of the last Mr Frosty

Guide, vials#
01-18
19-36
37-54
56-72
73-85

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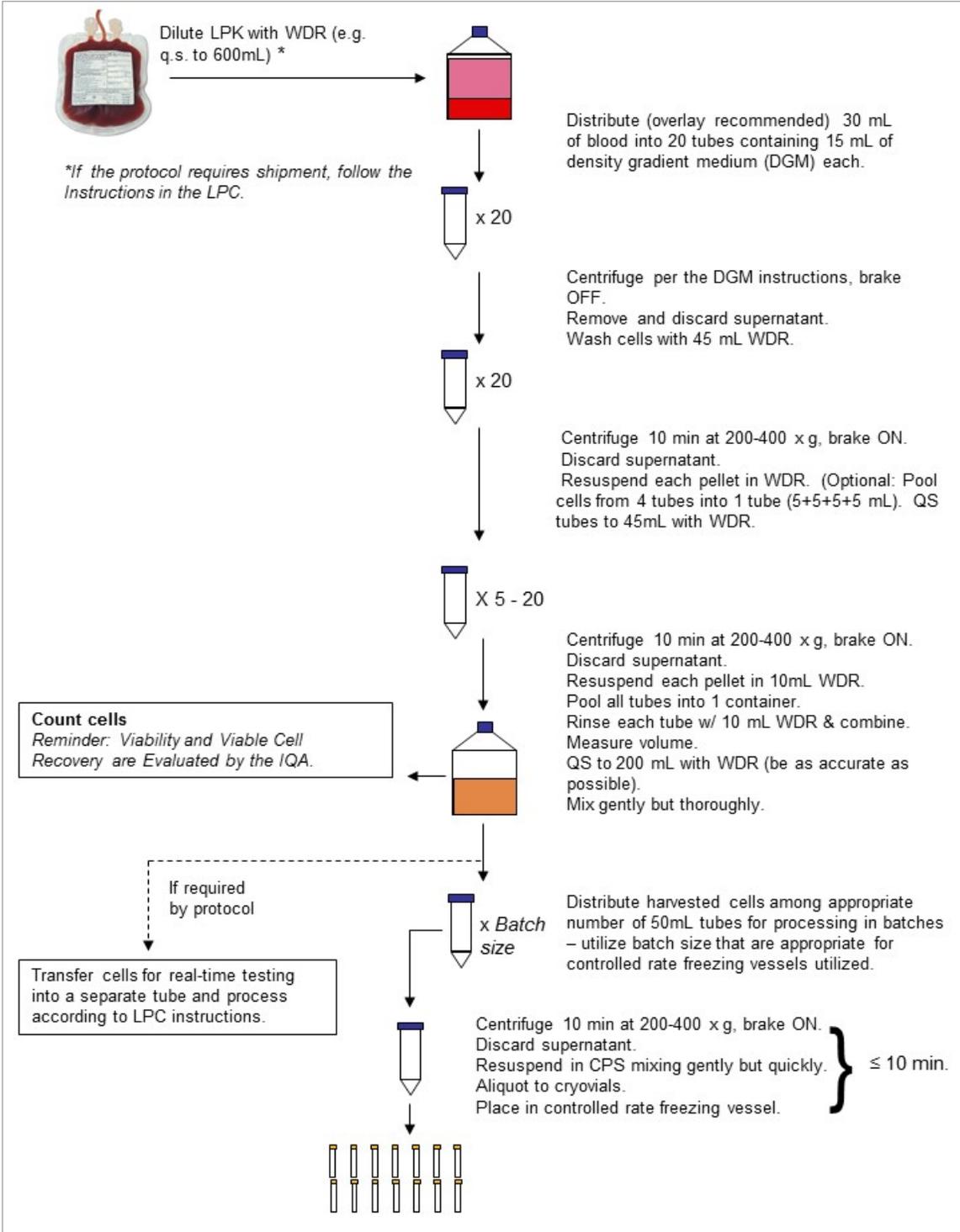
12.2. APPENDIX 2: PBMC Processing Worksheet Example 2 (download on HANC [Lab Manual page](#))

A	B	C	D	E
3	Laboratory Name:	3		
4	LDMS Number:	4		
5	Protocol	5		
6	Participant ID (PID)	6		
7	Visit	7		
8	Collection Date	8		
9	Collection Time	9		
10	Processing Date	10		
11	Processing Start Time	11		
12	Participating Technologists	12		
13	Freeze Time	13		
14	LPK Volume (mL)	14		
15	LPK Condition	15		
16	Cell Count #1	16		
17	Cell Count #2	17		
18	Cell Count #3	18		
19	Cell Count #4	19		
20	Average Cell Count	20		$(D16+D17+D18+D19)/4$
21	Viability	21		
22	Viable Cell count	22		$(D20*D21)$
23	Dilution Factor	23	50	
24	Cell count per mL of diluted cells	24		$(D22*D23)$
25	Total Volume of Diluted cells	25	200	
26	Total Cell Count for LPK	26		$(D24*D25)$
27	Total Number of Aliquots*	27	100	
28	Number of Cells per aliquot	28	50,000,000	50,000,000
29	Total Number of Cells Needed	29		$(D27*D28)$
30	Number of Batches	30	4	
31	Number of cryovials per Batch	31	25	$(D27/D30)$
32	Number of cells per Batch	32		$(D29/D30)$
33	Volume (mL) of Cells Per Batch	33		$(D32/D24)$
34	CPS Volume (mL) per Batch	34	25	D31

*Note:* The standard requirement for LPK certification is 81 aliquots for storage + 8 aliquots for IQA

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12.3. APPENDIX 3: Processing Flow Diagram



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**LABORATORY SOP REVIEW**

LABORATORY STAFF NAME	DATE OF CONFIRMATION OF UNDERSTANDING
	[ddMmmyyyy]
	[ddMmmyyyy]
	[ddMmmyyyy]
	[ddMmmyyyy]