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| **Title:** | Dried PBMC Pellet Processing Standard Operating Procedure (LDMS specimen code = BLD/EDTA or ACD/PEL) | | |
| **Origination Date:** | 01 Jun 2004 | **Total Pages:** | 6 |
| **Effective Date:** | 29 Nov 2012 | **SOP Number** | LTC-SOP-59 v4.0 |
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| | Version Effective Date (dd/mmm/yy) | Comments |
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| **Revision History** | | |
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## Table of Contents

# 1 Purpose

Dried Peripheral Blood Mononuclear Cell (PBMC) pellets are typically used as a source of DNA to probe for integrated HIV viral or specific human gene sequences. This document has been prepared to describe the correct procedures to collect, process and store dried PBMC pellets for network protocols.

# 2 Scope

Users of the ACTG/IMPAACT Lab Manual

# 3 Background

Most protocols undertaken by the AIDS Clinical Trials and IMPAACT networks are clinical in nature, with both real time and post-study requirements. Real-time assay results may be used to determine individual patient responses to therapies and determine when changes in protocol steps (i.e. changes in patient management) are warranted. Post study analyses are used to make recommendations for future treatment and management models for HIV-infected patient populations. As such, all specimens including dried PBMC pellets should be processed appropriately to ensure the best results for both individual patient management and completion of the collective protocol objectives.

The success of a protocol depends upon the adequate collection, processing, preservation, storage, transport and retrieval of specimens. Guidelines for sample collection and storage need to anticipate the requirements of future studies that are yet to be designed or technological advances which are in the early stages of development. While this is not always possible, certain basic tenets exist. For example, all specimens should be collected and processed using aseptic techniques and Universal Safety Precautions. This includes the use of sterile tubes, pipette tips and reagents, and a work environment that is designed to prevent contamination of samples and provide adequate safety measures for everyone in the lab.

# 4 Authority and Responsibility

4.1 The Network Laboratory Directors (or his/her designee) have the authority to establish, review and update this procedure.

4.2 The ACTG/IMPAACT Laboratory Technologist Committee (LTC) is responsible for the maintenance and control of SOP documentation.

4.3 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:

4.3.1 Include, without procedural modification, the portions of the current version of the LTC SOP that are used within the network site-affiliated laboratory

4.3.2 Reference the current version of the LTC SOP

4.4 All laboratory technicians are responsible for reading and understanding this SOP prior to performing the procedures described.

4.5 The site PI and designees are responsible for understanding and adhering to the patient preparation and specimen collection components.

# 5 Requirements

5.1 Specimen Requirements

Refer to the protocol-specific Lab Processing Chart (LPC) to determine the type and quantity of specimen(s) required for each collection. Unusual patient preparation or specimen collection requirements will be explained in the assay procedure.

5.2 Specimen Logging, Labeling and Shipping

5.2.1 Log all specimens into the LDMS for specimen tracking, storage and shipping according to the lab processing chart or other protocol related documents.

5.2.2 Use LDMS barcoded labels for all specimen aliquots. Aliquot labels must be LDMS-generated and contain the following information:

5.2.2.1 LDMS Specimen ID# or Other specimen ID#

5.2.2.2 Global Specimen aliquot-unique ID

5.2.2.2.1 Only for specimen dates after September 1, 2005

5.2.2.2.2 Not required for hand-written specimens

5.2.2.3 PID

5.2.2.4 Protocol

5.2.2.5 Specimen Date

5.2.2.6 Specimen Time (24 hour time)

5.2.2.7 Visit Identifiers

5.2.2.8 Primary/Additive/Derivative/SubAddDer

5.2.2.9 Visit

5.2.2.10 Volume

5.2.3 Record aliquot storage locations in the LDMS.

5.2.4 Export data at least weekly to the Data Management Center. More frequent exporting is suggested for testing laboratories to assure timely distribution of assay results.

5.2.5 Specimen shipping must follow the protocol instructions. See the ACTG/IMPAACT Specimen Shipping Guidelines at https://www.hanc.info/labs/labresources/procedures/Pages/actgImpaactLabManual.aspx.

# 6 Equipment, Consumables and PPE

6.1 Laminar flow hood (minimum class 2, type A biosafety hood).

6.2 Gloves (latex, vinyl, nitrile).

6.3 Lab coat or protective gown.

6.4 Microfuge capable of speeds > 10,000xg.

6.5 Sterile pipettes, graduated and transfer.

6.6 Pipetting device.

6.7 Micropipettors of various volumes.

6.8 Sterile cryopreservation vials: 1.8 to 2mL with screw cap, external threads, and o-rings.

***Example***: Sarstedt cat# 72.694.006 (flat), 72.693.005 (conical- PREFERRED)

6.9 -70°/ -80°C freezer (range = -65 to -95°C).

# 7 Reagents and Reagent Preparation

7.1 Phosphate Buffered Saline (PBS) without Ca++ and Mg++, 1X or Hank's Buffered Salt Solution (HBSS).

7.1.1 Store at 18 to 25°C or at the temperature recommended by the manufacturer

7.1.2 Observe manufacturer's outdate.

7.1.3 Label bottle with open date. Shelf life of opened bottle is three months (unless the manufacturer indicates a shorter shelf life).

# 8 Procedure

***Note:*** DMSO is a potent PCR inhibitor. If the PBMCs to be stored as pellets (PEL) have been in contact with DMSO (e.g. cryopreservative media), they must be washed twice with PBS and re-counted prior to storage. In general, the safe assumption is that any stored virology sample pellet is intended for a PCR procedure and should be completely free of DMSO.

***Note:*** Do NOT use cells derived from Heparin tubes for this procedure if pellets are to be used for PCR.

8.1 Isolate PBMC according to Cross-Network PBMC Processing SOP through PBMC Count (section 17.3 of v4.0): http://www.hanc.info/labs/Pages/PBMCSOP.aspx.

8.2 The cell concentration should be per cryovial is 2 x $10^6$ to 10 x $10^6$ cells or as directed by the specific protocol. Adjust the sample with sterile PBS or HBSS to achieve the protocol-specified concentration.

8.3 Aliquot the cell suspension into cryopreservation vials according to protocol instructions.

8.4 Centrifuge for 3 minutes in a microfuge at the highest speed (10,000 to 16,000 x g).

8.5 Aspirate the supernatant from each tube without disturbing the pellet. If “V” bottom tubes are centrifuged in a fixed angle microfuge, the cell pellet will be on the side of the tube, a pipette tip can be placed in the bottom of the tube (bottom of the “V”) and the supernatant can be removed completely without touching the cell pellet.

8.6 If the pellet is not immediately extracted for nucleic acids or other analyte, it should be stored at -70°C to -80°C (-65 to -95°C) and shipped on dry ice according to the network instructions in the protocol Lab Processing Chart.

# 9 Limitations

9.1 PCR inhibitors are of particular concern with dried blood spot preparations:

9.2 Treat PBMCs contaminated with red blood cells with an RBC lytic reagent to remove hemoglobin prior to use.

9.3 Don't use heparin collected PBMCs for pellet preparation.

9.4 Carefully process as stated pellets prepared from previously cryopreserved cells.

9.5 Store Quantity Not Sufficient (QNS) samples that generate pellets containing $<2 \times 10^6$ PBMCs as there may be enough DNA present for specific applications. Note low PBMC numbers in LDMS with QNS comment.