ACTG/IMPAACT LABORATORY STANDARD OPERATING PROCEDURE			
Title:	Cerebral Spinal Fluid Processing Standard Operating Procedure		
SOP number:	LTC-SOP-67	Effective:	06 JAN 2025
Version:	V2.0	Last reviewed:	08 APR 2013
Originator:	ACTG/IMPAACT Lab Technologist Committee	Pages:	7

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1 PURPOSE

The purpose of this SOP is to describe procedures for the processing and storage of cerebrospinal fluid (CSF).

2 BACKGROUND

Sampling the brain is dangerously invasive, but an approximation of the brain compartment is safely available by sampling the cerebrospinal fluid (CSF) via lumbar puncture (LP). Cerebrospinal fluid is an important specimen for monitoring the status of the immune response, drug delivery and viral status close to the brain.

3 SCOPE

This procedure is to be used to standardize the processing and storage of CSF specimens across protocols. Network protocol-specific instructions (e.g., LPC or MOP) supersede those in this SOP.

4 DEFINITIONS

Term	Definition
ACTG	Advancing Clinical Therapeutics Globally
BSC	Biosafety Cabinet
CSF	Cerebrospinal Fluid
IMPAACT	International Maternal Pediatric Adolescent AIDS Clinical Trials Group
LDMS	Laboratory Data Management System
LN2	Liquid Nitrogen
LP	Lumbar Puncture
LPC	Lab Processing Chart
LTC	ACTG/IMPAACT Laboratory Technologist Committee
MOP	Manual of Procedures

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.

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

5.3.4. The site's principal investigator and designees are responsible for understanding and adhering to the patient preparation and specimen collection components.

5.4. IATA/ICAO regulations state that any individual who handles, offers for transport, or transports dangerous goods must be formally trained and certified in Biological Substance, Category B specimen shipping. [8.1]

6 EQUIPMENT AND CONSUMABLES

6.1 Sterile tubes with caps for CSF collection. Polypropylene tubes are recommended to decrease possible binding of protocol analytes to the tube surface.

6.2 15mL disposable centrifuge tubes, sterile, graduated, polypropylene, as needed for research purposes. Note: Depending on the research analyte, alternative collection tube may be required per the MOP or LPC.

6.3 Gloves, powder free

6.4 Class II biosafety cabinet (BSC)

6.5 Centrifuge, low speed (capable of 200 to 1000 x g), with swinging bucket rotor, refrigerated preferred, ambient acceptable. Buckets with caps/lids are required.

6.6 Micropipettes and disposable tips

6.7 Pipet-Aid (cordless preferred)

6.8 Disposable 2mL, 5mL serological pipets

6.9 2 to 8°C refrigerator

6.10 -20°C (or lower) freezer without automatic defrost (for FBS storage)

6.11 -80°C freezer (-65°C to -95°C)

6.12 LN2 freezer (≤ -140°C) or LN2 storage tank

6.13 -150°C mechanical freezer (if LN2 is not available)

6.14 Cryogenic vials (cryovials), 1.8 to 2mL, (per protocol requirements)

6.15 Cryopreservation Media (10% DMSO in 90% FBS) [8.2]

6.16 Cryochambers (e.g., Stratagene StrataCooler® and BioCision® CoolCell are preferred) [8.2]

7 Procedures

Note: Special storage vials and/or additives as well as temperature and light precautions may be necessary to maintain the integrity of CSF for the analyte or biomarker being tested at the time of collection.

7.1 Collection and Transport of CSF

7.1.1 Follow standard local procedures for performing a lumbar puncture.

7.1.2 Collect and transport CSF as required in protocol specific procedures (e.g., MOP).

Note: Special collection vials and/or additives as well as temperature and light precautions may be necessary to maintain specimen integrity at the time of collection.

7.2 Whole CSF

Note: Refrigerate until processing is complete otherwise indicated by protocol.

7.2.1 Mix gently by pipetting and transfer CSF into sterile cryovials as defined in protocol specific procedures (e.g., LPC).

7.2.2 Freeze CSF at -65°C to -95°C unless otherwise specified as defined in protocol specific procedures.

7.3 Fractionation of CSF into Cells and Supernatant

Note: Refrigerate until processing is complete otherwise indicated by protocol.

7.3.1 Nonviable cells and Single Spun CSF supernatant

7.3.1.1 Centrifuge all tubes at 800-1000xg for 15 min at 4°C.

Note: Cell pellet may not be visible after centrifugation.

7.3.1.2 Transfer supernatant into sterile cryovials as defined in protocol specific procedures (e.g., LPC).

Note: If saving nonviable cells leave behind a small volume (e.g., 50 – 100 uL or as outlined in the LPC) of CSF supernatant and proceed to section 7.3.1.4.

7.3.1.3 Freeze supernatant at -65°C to -95°C unless otherwise specified as defined in protocol specific procedures.

7.3.1.4 For nonviable cells, resuspend the cell pellets by gently pipetting the fluid up and down.

7.3.1.5 Transfer cells to cryovials as defined in LPC

7.3.1.6 Freeze nonviable cells at -65°C to -95°C unless otherwise specified as defined in protocol specific procedures.

7.3.2 Viable Cells and Double Spun CSF Supernatant

7.3.2.1 Centrifuge supernatant at 250 – 400xg for 15 min at 4°C.

7.3.2.2 Transfer supernatant to sterile centrifuge tube and re-spin at 800-1000xg for 10 minutes at 4°C.

Note: Cell pellet may not be visible after centrifugation.

7.3.2.3 Transfer supernatant into sterile cryovials as defined in protocol specific procedures. (e.g., LPC)

7.3.2.4 Freeze supernatant at -65°C to -95°C unless otherwise specified as defined in protocol specific procedures.

7.3.2.5 For viable cells, remove all residual supernatant (unless otherwise specified in the LPC) prior to resuspending cell pellet in cryopreservation media and distribute into sterile cryovials as defined in the LPC.

7.3.2.6 Immediately transfer all cryovials to the controlled rate freezing container. Follow manufacturer's instructions for use and transfer aliquots to final storage.

7.3.2.7 Long-term storage should be in gas-phase LN2 or at -150° C or colder (defer to protocol instructions).

8 REFERENCES

8.1 Cross-Network PBMC SOP. Version 7.0, 19Aug2024. https://www.hanc.info/resources/sops-guidelines-resources/laboratory/cross-network-pbmc-processing-sop.html

8.2 Dangerous Goods Training Guidance. Edition 1, 01Jan2023. IATA Dangerous Goods. https://www.iata.org/en/programs/cargo/dgr/

9 INQUIRIES

Contact the ACTG/IMPAACT LTC Leadership at actg.ltcleadership@fstrf.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 CM ACTG/IMPAACT Network Laboratory Principal Investigator		06 JAN 2025

11 REVISION HISTORY OR RECORD RETIREMENT

VERSION #	EFFECTIVE DATE	REPLACES	DATE OF REVISION	RATIONALE FOR [REVISION/RETIREMENT]
1.0	08Apr2013	Original		
2.0	06 JAN 2025	1.0	06 JAN 2025	Lumbar puncture instructions were removed, and procedures were updated to reflect current practices.
3.0				

12 Appendices: Not Applicable

13 LABORATORY SOP REVIEW

LABORATORY STAFF NAME	DATE OF CONFIRMATION OF UNDERSTANDING