MANUAL OF OPERATING PROCEDURES:

Biological Specimens Collection, Processing and Handling

ACUTE FLACCID MYELITIS WORKING GROUP

09/01/2020

STANDARD OPERATING PROCEDURE	**SOP No: #4** **Cerebrospinal Fluid (CSF) processing and storage**
	Version Number: 1.0 (Jan 2019)

1. Purpose: To describe and standardize the procedure for CSF processing and storage for laboratory testing

2. Scope: Authorized personnel from the AFM consensus participating institutions

3. Responsibilities: Authorized personnel performing the processing and handling of samples must ensure that all procedures are followed correctly.

4. Supplies needed:

- Biohazard Bag
- Sterile screw-cap plastic containers
- Gloves
- Disposable pipette tips 100 -1000 µL
- Micropipettes 100 y 1000 µL
- Centrifuge
- Laminar flow cabinet
- 2.0 mL Cryogenic Vials
- Cryobox

5. Procedure:

-Cerebrospinal fluid sample collected in a sterile container and obtained from a lumbar puncture procedure deemed clinically necessary
-Can also be retrieved as residual CSF sample from hospital lab.
-Minimum amount to be collected: 1 mL

1. CSF will be processed within 30 minutes of collection. The time of collection and time of processing will be recorded
2. Centrifuge tubes at the corresponding lab at 1500 rpm for 10 mins at 4°C.
3. Use a sterile pipette to aliquot the cell free CSF into 2 mL cryovials with a volume of 100 µL per tube, trying to obtain as many aliquots as possible.
4. Each aliquot will be appropriately labeled with the subject ID number
5. All CSF aliquots will be stored at -80°C in the participating center laboratory freezer in a cardbox cryobox

6. The authorized personnel will document the number of aliquots, collection date and time in the participating center laboratory freezer inventory log
7. Remember to save and ship an aliquot to the CDC per the site's local reportable disease specimen shipping protocol

To preserve cellular pellet:

a. Transfer cellular pellet to a 1.5mL cryovial and resuspend in RPMI 1640 medium
b. Centrifuge the cellular suspension at 1200 rpm for 5-10 min at 4ºC and discard the supernatant
c. In another cryovial, add 450µl of FBS + 100 µl of DMSO and immediately place in the fridge or in ice for 5-10 min. This will be the freezing media (90% Fetal Bovine Serum / 10% DMSO)
d. Resuspend the cellular pellet again with 450 µl of FBS and slowly add the cooled freezing medium. Mix gently by pipetting
e. Store cryovial at -80C for 24 hs and then transfer to liquid nitrogen

Submission of specimens to the CDC

Collect specimens as close to onset of limb weakness as possible and store as directed:

Sample	Amount	Tube Type	Processing	Storage	Shipping
Cerebrospinal Fluid (CSF)	1mL (collect at the same time or within 24hs of CSF)	Cryovial	Spun and CSF removed to cryovial	Freeze at -70°C	Ship on dry ice overnight

References

Johns Hopkins School of Medicine (2018). –Johns Hopkins Neuroimmunology and neurological infectious disease sample repository SOP-Revised by Beatriz Parra, PhD. And Carlos Pardo-Villamizar, MD.

Johns Hopkins University (2018). Johns Hopkins Medical Microbiology Specimen Collection Guidelines [PDF file]. Retrieved January 22, 2019, from https://www.hopkinsmedicine.org/microbiology/specimen/index.html

Centers for Disease Control and Prevention (2018). Acute Flaccid Myelitis: Specimen Collection Instructions. Retrieved January 27, 2019, from https://www.cdc.gov/acute-flaccid-myelitis/hcp/instructions.html