



**MANUAL OF OPERATING PROCEDURES:**  
**Biological Specimens Collection, Processing and Handling**

**ACUTE FLACCID MYELITIS WORKING GROUP**

**09/01/2020**



<b>STANDARD OPERATING PROCEDURE</b>	<b>SOP No: #1 Serum Collection, processing and storage</b>
	<b>Version Number: 1.0 (Jan 2019)</b>

**1. Purpose:** To describe and standardize the procedure for the collection, processing and handling of acute and convalescent blood (serum) samples for laboratory testing

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

**3. Responsibilities:** Authorized personnel performing the collection and storage of the serum samples must ensure that all procedures are followed correctly. If the clinical staff agrees to collect the study sample, this is permissible as long as it complies with the SOP.

**4. Supplies needed:**

- Biohazard Bag
- 10.0 mL serum separator tubes (SST Tube Red Tiger Top Tube)
- Venipuncture kit
- Gloves
- 2.0 mL Cryogenic Vials
- Cryobox
- Disposable pipette tips 100 -1000  $\mu$ L
- Micropipettes 100 y 1000  $\mu$ L
- Centrifuge
- Laminar flow cabinet

**5. Venipuncture Blood (serum) Collection Procedure:**

-For the purposes of this SOP, the amount of blood to be collected will be determined on age-based quantities, following the 1ml+ 1ml per year of age rule.

-Blood can be collected in serum separator tubes (SST-“Tiger Tops”) or Red-Top tubes

-Up to 2 attempts may be made to draw blood.

-Collect at same time or within 24 hours of CSF if feasible

1. Select a suitable site for venipuncture, by placing the tourniquet 3 to 4 inches above the selected puncture site on the patient.
2. Put on mask and gloves.
3. Apply a tourniquet if necessary and palpate for a vein.
4. When a vein is selected, cleanse the area with alcohol in a circular motion, beginning at the site and working outward. Allow the area to air dry. After the area is cleansed, it should not be touched or palpated again. If you find it necessary to reevaluate the site by palpation, the area needs to be re-cleansed before the venipuncture is performed.



5. Ask the subject to make a fist; avoid “pumping the fist”. Grasp the subject’s arm firmly using your thumb to draw the skin taut and anchor the vein. Swiftly insert the needle through the skin into the lumen of the vein. The needle should form a 15-30-degree angle with the arm surface. Avoid excess probing.
6. When the tubes are filled, remove the tourniquet. Place gauze on the puncture site and remove needle from subject’s arm.
7. After holding pressure for 1 minute, apply a Band-Aid to puncture site.

## 6. Blood (Serum) Processing:

1. Following collection, tubes will be immediately labeled and inverted no less than 5 times, kept upright at room temperature (18-25°C) for a minimum of 30 minutes to allow for clot to form.
2. After 30 minutes the tube should be placed upright in the refrigerator (2-8°C) for storage if further processing is delayed. Efforts should be made for blood to be processed as soon as possible within 1 hour of collection. The time of collection and time of processing will be recorded
3. Centrifuge the tube containing the whole blood specimen at 1100-1300 x g for 10-15 minutes at 4°C

-The following process must be done in a Laminar flow hood.

4. Use a sterile pipette to aliquot the serum into 2 mL cryovials with a volume of 250 µL per tube, trying to obtain as many aliquots as possible.
5. Each serum aliquot will be appropriately labeled with the subject ID number
6. All serum aliquots will be stored at -80°C in the participating center laboratory freezer in a cryobox
7. The authorized personnel will document the number of aliquots, collection date and time in the participating center laboratory freezer inventory log
8. Remember to save and ship an aliquot to the CDC per the site’s local reportable disease specimen shipping protocol



### 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
Serum	>0.4 mL (collect at the same time or within 24hs of CSF)	Tiger/red Top	Spun and serum removed to tiger/red top	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>