MANUAL OF OPERATING PROCEDURES:

Biological Specimens Collection, Processing and Handling

ACUTE FLACCID MYELITIS WORKING GROUP

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STANDARD OPERATING PROCEDURE	SOP No: #5 Respiratory samples collection, processing and storage
	Version Number: 1.0 (Jan 2019)

1. Purpose: To describe and standardize the procedure for Nasopharyngeal and Oropharyngeal swab collection or alternative nasal wash, 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:

- Mask
- Gloves
- Flocked sterile swab preferred. Sterile Flexible Dacron or Rayon Swabs are acceptable

--NOTE: AVOID calcium alginate swabs or swabs with wooden sticks, as they may contain substances that inactivate some viruses and inhibit some molecular assays.

- Sterile vial containing viral transport media without antibiotics
- Sterile Nasal bulb syringe
- Lactate Ringer solution
- 3 ml sterile syringe
- Paper towelettes
- Sterile specimen cup

5. NP Swab Collection procedures:

1. Put on mask and gloves.
2. Have subject sit with head against a wall or stabilize the head with your hand if a wall is not accessible. A patient who is acutely ill or a young child may lie back against the bed with the head of the bed raised. Subjects tend to pull away during this procedure.
3. Measure the distance from the nose to the ear. This gives an estimate of the distance the swab has to be inserted for an NP sample. Mark this distance on the shaft of the swab.
4. Insert swab into one nostril straight back parallel to the palate (not upwards) and continue along the floor of the nasal passage for several centimeters until reaching the mark on the shaft of the swab (resistance will be met). Do not force swab, if

obstruction is encountered before reaching the nasopharynx, remove swab, and try the other side.
5. Rotate the swab gently for 5-10 seconds to loosen the epithelial cells. Allow swab to remain in place for additional 5-10 seconds to absorb sample materials.
6. Remove swab from nasopharynx and place into a sterile vial containing universal viral transport media without antibiotics or prepackaged culture swab tube.
7. Check the patient´s nasal or throat passage for evidence of trauma, such as bleeding.

-Note: Continue with the following steps if you have capability for sample aliquoting, otherwise, store swab in viral transport medium and ship to referral center and/or CDC.

8. Mix the swab around in the VTM.
9. Ring out the swab by pressing it against the inside of the VTM tube.
10. Remove the swab and discard.
11. Vortex the sample for approximately 30 seconds.
12. Aliquot the VTM into multiple cryovials containing at least 250µl per aliquot.
13. Label the aliquots appropriately, including collection date.
14. Freeze the aliquots at -70 degrees Celsius until ready to ship.
15. Remember to save and ship an aliquot to the CDC per the site's local reportable disease specimen shipping protocol

6. Nasal Wash procedure (alternative to NP swab if participant refuses NP collection or an NP sample is available from a clinical care nasal wash procedure):

1. Perform hand hygiene. Put on mask and gloves
2. Position subject facing forward in sitting position in an adult's lap in chair
3. Have adult hug child with one arm holding both child's arms at his/her sides.
4. Instruct parent/guardian to place other hand on child's forehead and gently position child's head facing forward and back of head against adults' chest (head tilted back 45 degrees)
5. Prepare solution by pouring 15-20 mL of lactate ringer solution into sterile specimen cup
6. Draw entire volume of rinsing solution into sterile bulb syringe by compressing and releasing bulb to create a vacuum.
7. Hold sterile specimen cup under both nostrils
8. Assist child to position head forward minimizing solution draining to back of throat.
9. Gently place tip of bulb syringe into opening of one nostril.
10. Gently compress the bulb syringe to expel the rinsing solution
11. Gently release pressure on bulb syringe to collect effluent from around the bulb syringe and from opposite nostril.
12. Collect effluent into specimen container.
13. Recap specimen cup until ready to process nasal wash sample
14. Wipe child's face and nose with paper tissue
15. Remove gloves and sanitize hands
16. Reward/praise the child

17. Combine Nasal Wash for Virology with viral transport media (VTM) within one hour of collecting specimen.
18. Store NP wash in the refrigerator (2-8°C) until processing and final storage.

-Note: Continue with the following steps if you have capability for sample aliquoting, otherwise, store swab in viral transport medium and ship to referral center and/or CDC.

Nasal Wash Processing and Storage:

1. Dilute the NP wash specimen 1:1 with room temperature MEM media (MEM with L- glutamine and Phenol Red without HEPES; Life Technologies Catalog # 11095-080, or similar item) using a sterile pipette.
2. Transfer the diluted NP wash specimen to an appropriately labeled 15mL conical tube.
    a. If necessary, break cell clumps apart by gently pipetting the NP wash specimen up and down using a sterile pipette.
3. Centrifuge the diluted NP wash specimen at 450 x g for 10 minutes at 4°C.
4. Occasionally lots of mucus is present. If this is the case, a second centrifugation may be necessary after more pipetting up and down.
5. Transfer NP wash supernatant into 1mL aliquots using a sterile pipette and place them into an appropriately labeled 2mL cryovial
6. Store NP wash supernatant aliquots at -80°C
7. Remove any remaining supernatant (take supernatant level down to around the 1mL mark on the 15mL conical tube) and discard.
8. Resuspend the NP wash cell pellet in 5mL of cold (2-8°C) PBS (1X DPBS without calcium, magnesium or phenol red; Life Technologies Catalog # 14190-144, or similar item).
9. Remove 200µL of resuspended NP wash cell pellet and perform cell count procedure.
10. Centrifuge the NP wash cell pellet at 450 x g for 5 minutes at 4°C
11. If the mucus/cells are not “packing” following the centrifugation, add 5mL PBS (cold is better; 2-8°C), pipetting up and down. Repeat the centrifuge step. The additional PBS wash is often sufficient.
12. Remove supernatant and resuspend the NP wash cell pellet in freezing medium (Recovery Cell Culture Freezing Medium; Life Technologies Catalog 12648-010, or similar item) by storing 1x106 cells (or less) per 1mL of freezing medium. Document cell count.
13. Transfer NP wash cells resuspended in freezing medium into appropriately labeled 2mL cryovials as 1mL aliquots.
14. Place cryovials containing resuspended NP wash cells in a freezing container and store at -70°C.
15. Remember to save and ship an aliquot to the CDC per the site’s local reportable disease specimen shipping protocol

7. OP Swab Collection procedures:

1. Put on mask and gloves.
2. Have subject sit with head against a wall or stabilize the head with your hand if a wall is not accessible.
3. Insert swab into the posterior pharynx and tonsillar areas. Rub swab over both tonsillar pillars and posterior oropharynx and avoid touching the tongue, teeth, and gums.
4. Remove swab from pharynx and place into a sterile vial containing universal viral transport media without antibiotics.
5. Check the patient´ throat passage for evidence of trauma, such as bleeding.

-Note: Continue with the following steps if you have capability for sample aliquoting, otherwise, store swab in viral transport medium and ship to referral center and/or CDC.

6. Mix the swab around in the VTM.
7. Ring out the swab by pressing it against the inside of the VTM tube.
8. Remove the swab and discard.
9. Vortex the sample for approximately 30 seconds.
10. Aliquot the VTM into multiple cryovials containing at least 250µl per aliquot.
11. Label the aliquots appropriately, including collection date.
12. Freeze the aliquots at -70 degrees Celsius until ready to ship.
13. Remember to save and ship an aliquot to the CDC per the site's local reportable disease specimen shipping protocol

-Additional educational video demo on the procedures can be found here: http://www.copanusa.com/education/videos/

7. Processing and Storage:

1. If specimens will be processed within 48 hours after collection, keep specimen at 4ºC and ship on wet ice or refrigerant gel-packs.
2. If specimens are not anticipated to be processed soon, they should be stored frozen at -70ºC and eventually shipped on dry ice.

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
Respiratory nasopharyngeal or oropharyngeal swab	1mL	n/a	Store in viral transport medium	Freeze at -20°C**	Ship on dry ice overnight

**All specimens may be stored at -70°C for ease of shipping.

References

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Johns Hopkins Center for Immunization Research-CIR (2016). SOP Nasal Wash- Pediatrics