Instructions for processing and freezing of the Cerebral Spinal Fluid (CSF) for future isolation of RNA

Materials needed:

- CSF - Provided by the Study Coordinator (expected volume is between 12-30 ml)
- 50 ml conical tube - not provided by ITN. It needs to be ordered by the specimen processing site.
- 10 ml sterile pipette
- ITN033AI CSF Kit/Labels

Reagents needed:

- TRIzol® Reagent (Life Technologies, Cat. No. 15596-026 Size 100ml; Store at 2 to 8°C) -not provided by ITN. Must be ordered by the specimen processing site.
  WARNING: *Toxic in contact with skin and if swallowed. Causes burns. After contact with skin, wash immediately with a generous amount of detergent and water. If you feel ill, seek immediate medical advice (show label where possible). Contains Phenol (108-95-2) and other components (NJTSRN 80100437-5000p).*

  Other Precautions:
  - Use of disposable tubes made of clear polypropylene is recommended when working with less than 2 ml volumes of TRIzol® Reagent.
  - Polypropylene tubes must be capped before centrifugation.

Procedures:

1. Processing of the CSF specimens: **NOTE: All Steps MUST be done in the hood.**

1.1. Write down the Lumbar specimen collection (i.e. the time it is ready to be sent to the lab) and the start time of the CSF processing (by the lab) on the requisition titled “Requisition Form for ITN033AI Visit CSF Collection”.

1.2. Using a 10 ml sterile pipette transfer CSF into a 50 ml conical tube and label it “CSF”.

1.3. Spin CSF at 1000 rpm for 12 minutes at 4˚C.

1.4. Following centrifugation, take CSF tube out of the centrifuge and place it on ice. Note on the requisition in the “Comments” field whether you see any red color in the CSF cell pellet. These are red blood cells, and are an indication of peripheral blood contamination in the tap.

1.5. Take the specimen from ice. Using a 10 ml sterile pipette; transfer the supernatant into the 50 ml conical tube and label it “CSF Sup”.

1.5.1. Place the 50 ml conical tube containing the cell pellet back on ice while you continue processing the supernatant.

1.6. Use CSF supernatant collected in previous steps to prepare the following aliquots:

- Aliquot 100µl of the CSF supernatant into 2 x 1.8ml cryovials each. Cryovials for 100 µl aliquots will contain yellow cap inserts and will be labeled with suffix J17 and J18.
- Aliquot remaining volume in 500 µl increments each into the remaining 1.8 ml cryovials labeled with suffix J01 through J16 (the required number of cryovials is 16; however, if you need additional cryovials please take from bulk supplies and use the extra labels provided inside the kit box. These labels will contain suffix 99A through 99I).

2. Processing of the CSF pellet for future RNA isolations:

2.1. Take the specimen from ice and disturb the pellet by flicking. Add 500 µl of TRIzol® Reagent and lyse the pellet by repetitive pipetting.

NOTE: Washing cells before addition of TRIZOL reagents should be avoided as this increases the possibility of mRNA degradation.

2.2. Upon completion of cell pellet lysis, transfer lyzed cells to the cryovial provided specifically for CSF Pellet (cryovial will be labeled with suffix J19).

3. Freezing and Storage

3.1. Freeze both the CSF lyzed cell pellet and CSF supernatant in -80˚C freezer at least overnight or until ready for shipment.