



Alzheimer's Biomarker Consortium – Down Syndrome

Lumbar Puncture
Manual of Procedures
Version 2.2
February 23, 2024

(Adapted from the Dominantly Inherited Alzheimer Network (DIAN)

Procedures Manual (August 2015)

(Lumbar Puncture for CSF pages 52-62)

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LUMBAR PUNCTURE FOR CSF (Non-Fasting) – General Information

What	Why	How	When: (M- Th only)	Recipient
CSF	CSF A β and tau analytes	Polypropylene tubes (50 ml tube). 15 ml CSF is required. MINIMUM.	LP @ 1-2 pm	Wash-U CSF Laboratory (International sites batch ship)

The ABC-DS protocol recommends collection of CSF by gravity using an atraumatic 22 gauge Sprotte spinal needle and introducer, although aspiration through this or smaller needles is allowable. Other **deviations from the procedures described in the ABC-DS protocol require prior approval from the ABC-DS Coordinating Center.** Additional exception approvals may be required by local regulatory agencies. Sites must designate the method of CSF collection on the CSF Collection Worksheet for data tracking purposes.

All ABC-DS Lumbar Puncture (LP) supplies will be provided through NCRAD using their online kit request module. We ask all sites to start by ordering five “Lumbar Puncture Tray Kits” and one “CSF Supplemental Supply Kit.” Sites should order additional supplies ahead of time as necessary. Sites should have at least two LP tray kits on hand at all times. In addition, sites are responsible for requesting items to re-stock the “CSF Supplemental Supply Kit” before the original “CSF Supplemental Supply Kit” becomes depleted.

To access the online NCRAD requisition system, please click the following link:

[ABC-DS Kit Request System](#)

This online system allows study staff to request additional kits at any time. Requesting personnel need only follow the prompts to request the desired supplies. Options include ordering specific number of kits; the NCRAD site also includes the option of or simply ordering the desired amount of extra supplies. A NCRAD Coordinator will respond via email with confirmation that the request was submitted and ask additional questions as necessary.

Please allow TWO weeks for kit orders to be processed and delivered to the study site.

Direct questions about your CSF supplies or collection to: klunkwe@upmc.edu.

LP SUPPLIES

1. Supplies Provided by Site:

- ➡ Refrigerated centrifuge
- ➡ Sharps container
- ➡ Dry and wet ice

2. Refrigerated Centrifuge:

All sites will need a refrigerated centrifuge for blood processing. A centrifuge similar to the Sorvall Legend XTR (refrigerated) Cell Culture Package, TX-750 rotor is recommended. Centrifuge must be able to spin samples under the appropriate conditions (2000 x g, 10 min, 4°C with brake off).

3. “Lumbar Puncture Tray Kit” supplies (provided through NCRAD)

Quantity	Lumbar Puncture Tray Kit Components
1	Sprotte needle, 22 gauge X 3.5” (90mm)
1	Introducer needle, 1 mm x 30 mm
1	Hypodermic needle, 22 gauge x 1.5”
1	Plastic syringe, (3 ml, luer lock) with 25G x 5/8” needle attached
4	Polypropylene syringe (6 ml, Luer lock)
1	Needle stick pad
1	Adhesive bandage
1	Drape, fenestrated, 2 tabs, paper, 18” x 26”
2	Towel, 13.5” x 18”
6	Gauze pad, 2” x 2”
3	Sponge stick applicator
1	Lidocaine 1%, 5 ml
1	Povidone-Iodine Topical Solution, 0.75 oz

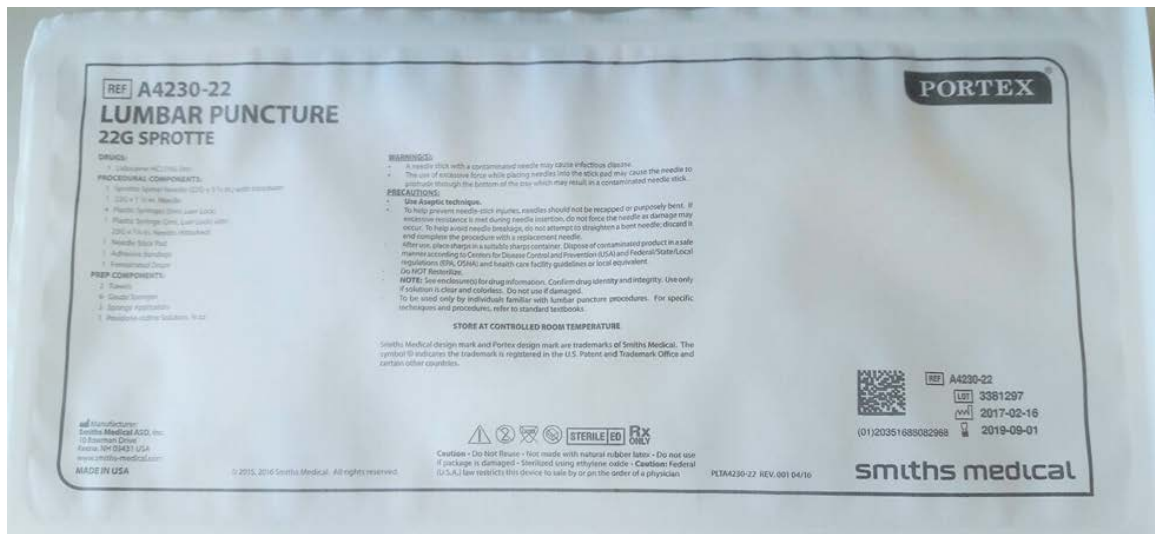
4. “CSF Supplemental Supply Kit” (provided through NCRAD)

Quantity	Lumbar Puncture Collection and Shipping Kit Components
10	50-ml conical polypropylene tube-individually wrapped (Rose Sci. cat #17004)
20	15-ml conical polypropylene tube-individually wrapped (Rose Sci. cat #17022)
3	Styrofoam Shipping Containers (11"x9"x8" 1-½" wall)
3	Pre-printed airbills/shipping labels/plastic protective pouches
3	Dry Ice Shipping Label
3	Exempt Human Specimen Label
3	Resealable Plastic Bag
3	Sealable Bubble Wrap Bag
3	Absorbent Pad (100 ml capacity)
100	Pre-printed blank labels for 50 & 15-ml conical tubes
1 pack of 4	Sharpie Marker Pens
5	3 ½” x 22 gauge Sprotte needle with Introducer (90mm)
3	4 ¾” x 22 gauge Sprotte needle with Introducer (120mm)
1 box	Sterile gloves (5 pair medium and 5 pair large)
10	Adhesive Spot Bandage
1 box	Alcohol swabs
1	Bel-Art™ SP Scienceware™ No-Wire™ Multi-Size Centrifuge Tube Racks

5. “CSF Shipping Supply Kit” (provided by NCRAD)

Quantity	CSF Shipping Supply Kit Components
1	Styrofoam Shipping Containers (11"x9"x8" 1-½" wall)
1	Pre-printed airbills/shipping labels
1	Dry Ice Shipping Label
1	Exempt Human Specimen Label
1	Resealable Plastic Bag
1	Single Use-Sealable Bubble Wrap Bag
1	Absorbent Tube Sleeve

LUMBAR PUNCTURE TRAY KIT IMAGES



Exterior of LP Tray Provided by NCRAD which contains the 22 gauge Sprotte Needle with Introducer



Interior of LP Tray Provided by NCRAD



Close up of Sprotte Spinal Needle (22 gauge x 3 1/2 in.) with Introducer

SCHEDULING FOR AN LP

Before scheduling any ABC-DS LP study visits, please do the following:

1. Identify a physician (e.g., anesthesiologist) able to perform a blood patch for any participant who experiences a post lumbar puncture headache. Find out ahead of time who to call to schedule and perform a blood patch at your center, should the need arise, as well as how the study account will be billed (ABC-DS coordinating center will reimburse the cost).
2. Ensure you have at least two “Lumbar Puncture Tray Kits” and sufficient “CSF Supplemental Supply Kit” provisions on hand prior to scheduling an LP visit. Also ensure adequate site-provided supplies (see above), including pelleted dry ice. Check expiration dates on all supplies, especially lidocaine.
3. Assign the following LP Tasks to site team members (3-4 people recommended):
 - Collect CSF
 - Assist with sample collection (maintain sterile field, unwrap supplies, prepare lidocaine)
 - Ensure patient remains still and is comfortable
 - Label tubes (ahead of time) and freeze bulk CSF on dry ice immediately
 - Record vitals
 - Complete CSF Collection Worksheet, and other source documents
 - Ship CSF specimens priority overnight or store in -80°C freezer until shipment.

Scheduling

All LPs should be performed as close to 1-2 pm as possible on Monday-Wednesday if the sample is to be shipped overnight the same day it is collected. CSF from LPs performed on Thursday or Friday will need to be stored until Monday shipment. Availability of staff and facilities for next day blood patch should be considered when scheduling LPs. Thursday's and Friday's are not recommended unless arrangements are made for a potential weekend blood patch procedure. CSF amyloid levels can vary depending upon the time of day the sample is collected. We can accommodate shipments sent on Thursday on a case by case basis but strongly prefer shipments to be sent Monday - Wednesday in case there is a delay in transit and delivery gets postponed. **We can not accommodate shipments that get sent out on Friday or Saturday under any circumstances.**

It is important for the time of day of collection to remain consistent across study visits. The recommended collection time is 1:00 PM local time.

The LP should be rescheduled if the participant does not feel well or is febrile.

!! The FDG PET should be performed before the LP at the 16-month visit. If the LP needs to be done before the FDG PET, there should be at least 24 hours between the 2 measures, and the participant should not have symptoms of low CSF volume at the time of the FDG PET (i.e., headache, dizziness, vomiting, visual changes)!!

PERFORMING THE LP

Assisting with Lumbar Puncture Set-up:

The ABC-DS-recommended position is sitting. The same position should be used at follow-up LPs. It is critical to try to optimize positioning, and usually requires an assistant. **The standard needle is the Sprotte 22 gauge × 3½" needle (90mm; with 1 × 30mm introducer needle), however, the CSF Supplemental Supply Kit also contains 22 gauge × 4¾" needles (120mm) with introducer needles if needed for use on obese participants. Additional 120mm needles are also available by request.** Other positions and needles are allowed (e.g., when using fluoroscopy) but this should be recorded on the CSF Collection Worksheet.

On the bedside table nearest where the person performing the lumbar puncture will sit, place a pair of sterile gloves (in their packaging) and a blue pad. Remove the contents of the lumbar puncture tray from the outer plastic packaging, leaving the contents wrapped in their sterile drape. Leave everything wrapped until the person performing the lumbar puncture is seated.

Feel the outside of the lumbar puncture kit (still wrapped up) to determine which end contains the spongy swabs. Turn this end toward the person performing the lumbar puncture and begin unwrapping the kit.

TOUCH ONLY THE OUTSIDE OF THE PAPER WRAPPER.

When you grab an edge to unfold it, touch only the folded under portions of the outside of the wrapper. Also, don't let the outside of the wrapper touch any part of the inside.

- ➡ If you touch any part of the paper wrapper, or if any non-sterile object outside of the wrapper touches any part of the inside of the wrapper, throw the kit away and start over.
- ➡ If you are in any doubt as to whether the inside of the wrapper has been touched, throw the kit away and start over.

Cleaning the Lumbar Puncture Site

The lumbar puncture site is cleaned with Povidone-Iodine Topical Solution according to best standard medical practices.

Once the kit is successfully unwrapped, open the bottle of Povidone-Iodine Topical Solution somewhere away from the kit. Use an alcohol swab to remove any loose chunks of dried material off of the bottle top. You don't want anything to fall onto the open and sterile lumbar puncture kit. Pour enough Povidone-Iodine Topical Solution into the prep well to cover the bottom, about ¼ inch deep.

Maintaining the Sterile Field

An important aspect of assisting with a successful lumbar puncture is keeping the field sterile. If there are a number of staff members in the room, please be sure they do not accidentally contaminate the sterile field. Once the person performing the lumbar puncture has donned sterile gloves, additional help may be needed to obtain or un-wrap any new tubes, needles, or supplies.

Unwrapping the Sterile 15- and 50-ml Conical Tubes

Note that the 15-ml and 50-ml tubes into which CSF is collected come individually wrapped and are sterile inside and out. These wrappers should be peeled open by an assistant (not touching the tube) and the tube carefully dropped onto the LP tray or elsewhere in the sterile field in a manner that avoids contamination. Any additional needles or other individually-wrapped sterile items can be handled the same way.

- ➡ Do not drop any packaging onto the tray or sterile field.
- ➡ Do not let the item touch the outside of the packaging on its way to the tray.

Lidocaine, Syringe with Needle, Gauze Pads

Anesthesia is usually achieved within 2 minutes after injecting the lidocaine. Occasionally, the person performing the lumbar puncture will need to use more lidocaine to numb up a particular spot, or they may need to move to another spot entirely.

Next, hold the lidocaine bottle upside down and at a slight angle toward the person performing the lumbar puncture so that they can plunge the needle into the bottle and extract some lidocaine without touching you or the bottle. Use two hands to stabilize the bottle. If the person performing the LP requires additional sterile gauze, open the gauze pad the same way as the syringe and needle, by holding open the package so the person performing the lumbar puncture can grab the gauze without touching you or the package.

General CSF Collection Methods

Prior to conducting the LP, collect blood for serum biomarkers (two 5-ml Gold-Top SST tubes), plasma/DNA biomarkers (two 10-ml Lavender-Top EDTA tubes), RNA (one 2.5ml PAXgene™ RNA tube) and NaHep tube for karyotyping (as needed) **following instructions in the NCRAD Blood-Based Biospecimens Collection, Processing, and Shipment Manual of Procedures**. It is **required** that the blood-based samples are collected prior to the LP, and under the same condition at each in-person visit.

LPs for CSF collection should be performed using a small caliber atraumatic needle. CSF should be obtained via gravity flow using the 22 gauge Sprotte needle, although aspiration through this or smaller needles is allowable. It is recommended that CSF be obtained from participants in a sitting position. Alternate needles, positions or methods (e.g., use of fluoroscopy) should be noted on the CSF Collection Worksheet.

Collection of CSF by Gravity

After the spinal needle is placed in the intrathecal space and the stylet is withdrawn, CSF should flow freely. **COLLECT THE FIRST 1-2 ml OF CSF INTO A STERILE 15-ml POLYPROPYLENE TUBE.** If there is any blood tinged CSF, let the CSF drip into the 15-ml tube until clear, then discard the 15-ml tube and switch to the sterile 50-ml collection tube. If there is no sign of blood from the beginning, the 15-ml tube can be discarded after 1-ml of CSF is collected and then switch to the 50-ml collection tube. Collect the remaining CSF (15-20 ml) in the 50-ml polypropylene tube. All collection should be performed by letting the CSF drip feely from the needle into the collection tube.

Reminder: If the CSF is blood-tinged, the first 1-2 ml of CSF should be discarded (or more if needed) to clear the blood before collecting the 15-20 ml for ABC-DS CSF analysis. **15 ml is the required MINIMUM for CSF biomarker analysis.** If 15 ml is not obtained and provided to the Wash-U CSF Laboratory, document the reason for under-collection on the comments section of the CSF Collection Worksheet.

Washcloths, Band-Aids, and Clean Up

After the person performing the lumbar puncture collects the last of the CSF, remove the needle and introducer and wash the Povidone-Iodine Topical Solution off the participant. A warm, wet washcloth can be used. A Band- Aid should be applied to the puncture site. Next, discard the LP kit following local guidelines, and dispose of sharp components in an appropriate sharps container.

Step by Step Summary of CSF Collection Procedure

Ensure all samples collected as part of ABC-DS are appropriately labeled using the sample label provided by NCRAD in the “CSF Supplemental Supply Kit” (see “step-by-step summary #3 below).

1. Print CSF Collection Worksheet (3 pages).
2. Complete Pre-procedure Checklist on Worksheet to confirm all supplies, including dry ice (~10 lbs) and wet ice, are available.
3. Complete (i.e., fill in) 1 ABC-DS CSF Label. Do **NOT** open and label the 15-ml and 50-ml tubes that will be kept sterile to collect the CSF.



4. Pre-cool the labeled 50 mL conical tube on wet ice. Do **NOT** pre-cool the 15-ml and 50-ml tubes that will be kept sterile to collect the CSF
5. Measure vitals (patient lying down).
6. Record the time of LP and associated information on the CSF Collection Worksheets.
7. Collect 15-20 ml CSF at the L3/L4 position (or adjacent position) using a 22 gauge Sprotte spinal needle via gravity flow with subject in upright position (or document alternate method on CSF Collection Worksheet) following these steps:
 - a. Collect initial 1-2 ml (if bloody, collect CSF until cleared of blood) using the **UNLABELED-STERILE** 15-ml polypropylene tube from the “Lumbar Puncture Collection Kit”.
 - b. Collect an additional 15-20 ml CSF into the **UNLABELED-STERILE** 50-ml polypropylene tube from the “CSF Supplemental Supply Kit”. 15 ml is the required **MINIMUM**.
 - c. If using aspiration, use **ONLY** the polypropylene syringes included in the “Lumbar Puncture Collection Kit” and transfer **DIRECTLY** into the **UNLABELED-STERILE** 50-ml polypropylene tube from the “CSF Supplemental Supply Kit”. There are four 6 ml Luer lock polypropylene syringes in the “Lumbar Puncture Collection Kit.” Note this on the CSF Collection Worksheet.
8. When finished collecting the CSF, immediately cap the 50-ml tube, apply the previously prepared label and put the CSF sample upright on wet ice and process as described below **within 1 hour of collection**.
9. As one person takes the immediate post procedure vital signs, a second person should process the CSF as follows:
 - a. Flash-freeze the 50 mL conical tube containing the CSF upright on pelleted dry ice for at

least 20 min prior to same-day shipping on dry ice (or prior to transferring to a -80°C freezer for batched sample shipments).

- b. Record the time of freezing the 50-ml tube on the CSF Collection Worksheets.
- 10. Provide food and drink to participant (participant may lay flat to minimize the chance of a post-LP headache).
- 11. Measure vital signs again one hour post-LP.
- 12. If vital signs are stable and participant feels OK one hour post-procedure, participant may sit upright, stand, and walk.
- 13. Enter collection data into the EDC website on day of visit.
- 14. **Notify the ABC-DS CSF Lab/WUSTL ADRC Biomarker Core (Schindler Lab)** at Washington University as described on page 12.
- 15. See shipping information on Pages 12-15.

LUMBAR PUNCTURE FOLLOW-UP PHONE CALL

This should be done the day after the lumbar puncture for all participants who had the procedure. Enter the information into the “Lumbar Puncture Follow-up Phone Call” Worksheet and EDC website and include any adverse events or resulting therapies (if applicable). Any adverse events should be recorded in the Adverse Events/Hospitalizations Log. Any resulting treatments should be recorded on the ABC-DS Medications Log.

SUGGESTED MANAGEMENT OF POST-LUMBAR PUNCTURE HEADACHE

Classic post-lumbar puncture (low pressure) headache is worse when the participant is upright (sits or stands), and improves when the participant is recumbent with the head **no higher** than the spinal cord.

Safety and comfort of the ABC-DS LP is maximized by the use of atraumatic needles. The ABC-DS protocol strongly encourages use of a 22 gauge Sprotte needle. Lumbar puncture is a standard procedure for collection of CSF but may be associated with pain during the performance of the procedure, comparable to the level of pain experienced during a blood draw. This is usually temporary and confined to the lower back. A persistent low-pressure headache may develop after lumbar puncture, probably due to leakage of CSF. If a post-LP headache persists it may need additional treatment, e.g. with fluids and analgesics. Uncommonly, a blood patch (injection of some of the participant’s blood to patch the CSF leak) may be needed.

Prevention: Use of a small and atraumatic needle with careful technique are helpful in preventing lumbar puncture headache. Having the participant refrain from exercise or strenuous activities (especially heavy lifting) for 24 hours after the LP may minimize the chance of a lumbar puncture headache.

Treatment of headache after a lumbar puncture:

- Limit physical activity as much as possible for at least 24 hours post-procedure.
- Increase oral fluid intake. Caffeine may be helpful.
- Routine analgesics such as acetaminophen may be used.

Post-lumbar puncture headache often resolves with the above treatment. If the headache persists after 24 hours of this management, it will likely require a blood patch. A blood patch *typically* relieves the headache instantly. Any adverse events or treatments related to the lumbar puncture should be recorded on the applicable worksheet(s) and entered into the on-line database.

Prior approval from the ABC-DS coordinating Center is not necessary to perform a blood patch. However, depending on the site, local IRB approval may be required. Costs related to the performance of a blood patch should be submitted to the ABC-DS Coordinating Center at the University of Pittsburgh.

PACKAGING AND SHIPPING-FROZEN CSF SPECIMENS

The most important issue for shipping is to maintain the temperature of the samples. The frozen samples must never thaw; not even the outside of the tubes should be allowed to defrost. This is best accomplished by making sure the styrofoam container is filled completely with pelleted dry ice.

All individuals packing and shipping biological materials must be certified following local institutional requirements. File any relevant certifications in your site Regulatory Binder for monitoring.

- All shipments for this study are priority overnight.
- **NEVER** send a shipment on any day other than Monday-Thursday. However, Monday - Wednesday is strongly preferred.
- Complete and copy **PAGES 1 & 2** of the CSF Collection Worksheets to include in shipment
- If not shipping same-day: **Place frozen samples in -80°C freezer for batch shipping.**
- Enter collection data on the day of visit into the ATRI Data Capture Form.
- **Notify the ADRC Biomarker Core/Schindler Lab** at Washington University as described below.

In addition to being tracked online, the condition and amount of samples received will be tracked by the receiving laboratories. Sites are responsible to ensure the requested amounts of CSF are collected, to the best of their ability. If a CSF sample is not obtained at a particular visit, record this on the CSF Collection Worksheet and on the Missed Visits/Missed Assessments form.

SHIPPING

PRIOR TO SENDING THE SAMPLES, all sites should email:

Washington University ABC-DS Biomarker CSF Lab/
Schindler Lab (ABCDSbiomarkerCSF@wustl.edu)

The email should include:

1. the expected delivery date
2. the inventory list including:
 - a. Participant ID#
 - b. LP date
 - c. # of 50-ml conical tubes
3. Tracking number of shipment.

US sites should ship frozen samples the day of collection via FedEx on dry ice (~7 lb/ 3 kg) for overnight delivery to the Wash-U CSF Laboratory at Washington University. If same-day shipment is not possible, flash-frozen samples should be transferred to and stored in a -80°C freezer until the next day.

Do not ship samples on Fridays or Saturdays.

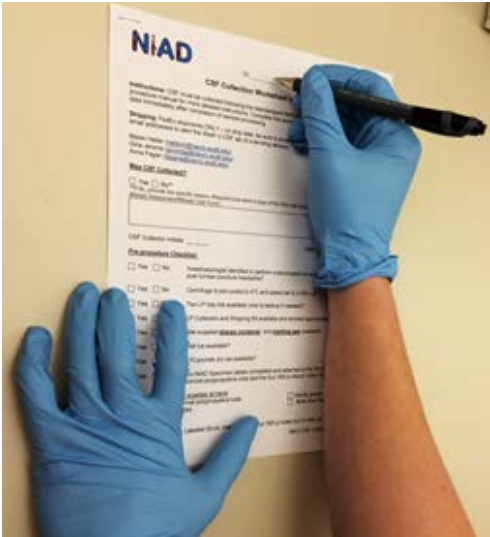
NOTE: Most FedEx offices have a **cutoff time** for dropping off an overnight shipment based on their pickup schedule. Any shipment that is dropped off after the scheduled pickup time, will not be shipped overnight. When scheduling your overnight shipment, **please confirm the deadline for drop off with your local FedEx office.**

Non-US sites are permitted to batch-ship samples on a quarterly basis via World Courier as long as -80°C freezers are available at the sites for storing samples after flash freezing on dry ice.

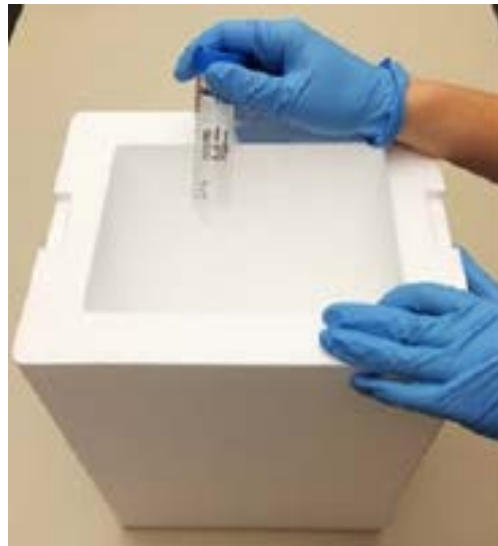
SHIPPING ADDRESS: See page 15.

PACKAGING AND SHIPPING PROCEDURE FOR FROZEN CSF

1. Complete and photocopy **PAGES 1 & 2** CSF Collection Worksheets.



2. Remove the **PRE-LABELED** 50-ml conical tube from the container in which it was frozen.



3. Place tube in the bubble wrap bag, ensuring that the absorbent pad is included. Place bubble wrapped tube in sealable plastic bag.



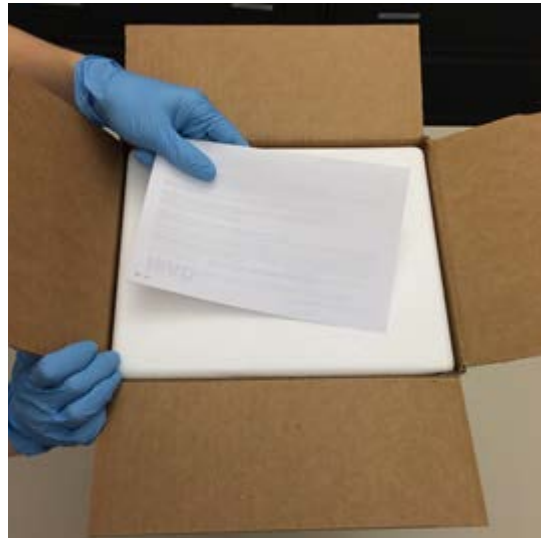
4. Place sealed plastic bag in Styrofoam container half-filled with dry ice. Cover the samples with the rest of dry ice.



5. Place Styrofoam container into shipping box with arrows pointing "UP". DO NOT TAPE INTERIOR STYROFOAM BOX.



6. Place PAGES 1 & 2 of the original CSF Collection Worksheets in-between Styrofoam container and outer shipping box.



7. Tape outside of shipping box allowing ventilation of the package.



8. Record amount of dry ice in box and Sender/Recipient addresses on UN1845 sticker. Affix dry ice (UN1845) and "Exempt Human Specimen" stickers. Affix FedEx overnight shipping airbill to box using a protective clear pouch (required).



Call for pick up.

US SHIPPING ADDRESS:

Wash-U Schindler Lab

ADRC Biomarker Core/Schindler Lab - ATTN: Rachel Henson
Washington University School of Medicine
4370 Duncan Ave
Neurosciences Research Building, Room 6350
St. Louis, MO 63110

Contact: Rachel Henson

Tel: (314) 273-8224

Email:

ABCDSbiomarkerCSF@wustl.edu

International Sites - QUARTERLY BATCH SHIP TO:

Wash-U Schindler Lab

ADRC Biomarker Core/Schindler Lab - ATTN: Rachel Henson
Washington University School of Medicine
4370 Duncan Ave
Neurosciences Research Building, Room 6350
St. Louis, MO 63110

Contact: Rachel Henson

Tel: (314) 273-8224

Email:

ABCDSbiomarkerCSF@wustl.edu