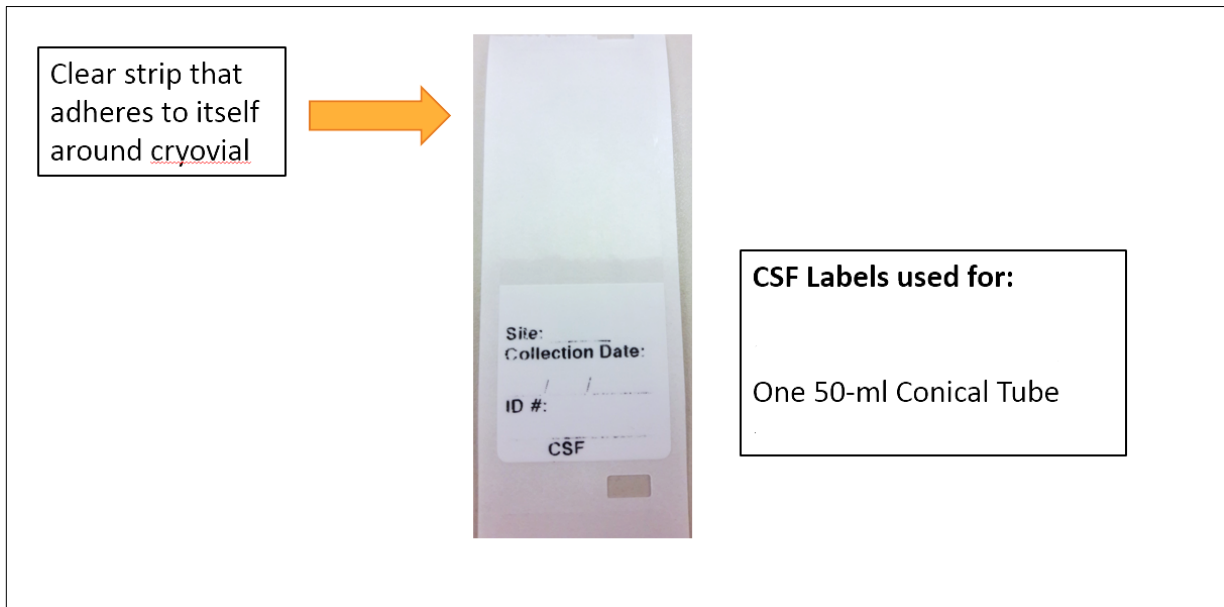


Step by Step Summary of CSF Collection Procedure

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.
- 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 (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.

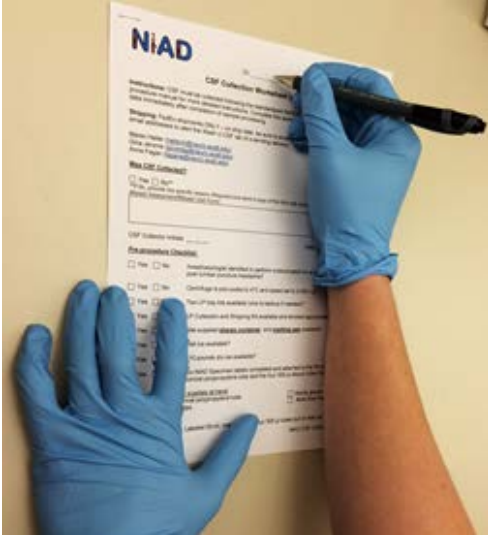
NOTE: Most FedEx offices have a **cutoff time** for dropping off an overnight shipment based on their pickup schedule. Any shipment that is dropped off after the scheduled pickup time, will not be shipped overnight. When scheduling your overnight shipment, **please confirm the deadline for drop off with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

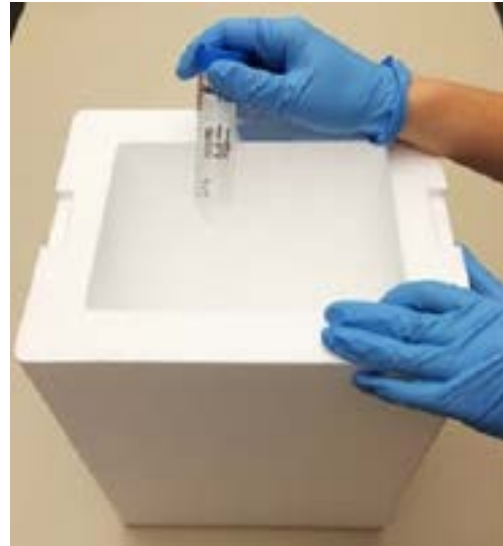
SHIPPING ADDRESS: See page 15.

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.



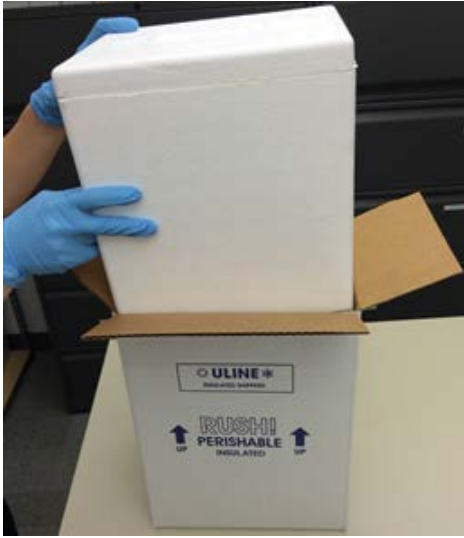
3. Place tube in the bubble wrap bag, ensuring that the absorbent pad is included. Place bubble wrapped tube in sealable plastic bag.



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 CSF Laboratory

Dept. of Neurology (ADRC Biomarker Core/Schindler Lab)

Washington University School of Medicine

425 South Euclid Ave.,

BJCIH 9th Floor, Lab Door #9113

St. Louis, MO 63110

Contact: Rachel Henson

Tel: 314-747-8396

Email:

ABCDSbiomarkerCSF@wustl.edu

International Sites - QUARTERLY BATCH SHIP TO:

Wash-U CSF Laboratory

Dept. of Neurology (ADRC Biomarker Core/Schindler Lab)

Washington University School of Medicine

425 South Euclid Ave.,

BJCIH 9th Floor, Lab Door #9113

St. Louis, MO 63110

USA

Contact: Rachel Henson

Tel: 314-747-8396

Email:

ABCDSbiomarkerCSF@wustl.edu