

Biofluids: Collection, Processing And Shipment Of Samples

The collection of biofluids is central to the goals of the Alzheimer's Disease Neuroimaging Initiative.

The development of valid and reliable biomarkers for AD is needed to:

- ⇒ aid in the recognition of the illness at its earliest clinically recognizable stages
- ⇒ detect the disease before dementia or other symptoms appear
- ⇒ distinguish AD from other causes of dementia

Biomarkers will be especially valuable, together with imaging tests, in the evaluation of disease-modifying therapies.

Promising biomarkers that will be measured in ADNI fluids:

- ⇒ Tau in CSF
- ⇒ Amyloid beta in CSF
- ⇒ Isoprostanes in CSF, plasma, urine
- ⇒ Homocysteine in plasma, CSF
- ⇒ APOE genotyping-blood
- ⇒ DNA from blood cells

This section includes procedures for the collection, processing and shipment of clinical laboratory and APOE samples at screen, cell immortalization samples at baseline, blood and urine samples for biomarkers, and lastly cerebral spinal fluid samples. A glossary of terms is at the end of this section.

SAMPLE IDENTIFICATION:

All samples for ADNI will be identified by a barcode. Clinical Laboratory Samples at Screen will use Covance's barcode system. All other samples (APOE, Cell Immortalization, Blood, Urine and CSF) must be identified using the ADNI barcode (see image)

Barcodes will be sent to sites by the Coordinating Center when the site has completed all certification requirements. Barcodes will be grouped by visit, and should ONLY be used for the visit recorded in print on the barcode.

Please confirm you have barcodes for each visit before scheduling the visit.

Sample Primary Tube Barcode Label	
Subject ID#	_____ S _____
Time	_____ : _____
Date	____ / ____ / ____
Circle: M or F	
ADNI 005 VST SCR BLD EDT	 100100

SAMPLE TRACKING

All samples (except for clinical laboratory samples sent to Covance) will be tracked online using the barcode License Plate and Fedex Tracking number. The APOE Collection Form and Biomarker Collection Form must be completed **on the day of each visit**. This form includes information used to track the sample, confirm receipt of the sample, and information essential to processing and analysis.

Once completed online, a printed copy of the Biomarker Collection Form should be included in the sample box. Please follow specific collection, processing and shipment procedures as detailed in the following pages.

SAMPLE QUALITY CHECKS

In addition to being tracked online, the condition and amount of samples received will be tracked by the Biomarker Core. Sites are responsible to ensure the requested amounts of each fluid are collected, to the best of their ability. If a sample is not obtained at a particular visit, this will be recorded on the Biomarker Collection Form and a reason should be provided.

Sample Amounts Obtained at each visit (mL)

Sample Type	Amount	Visit
Urine	10 mL	All Visits
Plasma from blood	20 mL	All Visits
Serum from blood	20 mL	All Visits
CSF	15 – 20 mL	BL and MI2
Blood for NCRAD	2 x 8.5 mL	BL
Blood for APOE	10 mL	SC

Visit Numbering

Visit	Number	Abbreviation
Screen	1	VST 1
Baseline	2	VST 2
6 months	3	VST 3
12 months	4	VST 4
18 months	5	VST 5
24 months	6	VST 6
36 months	7	VST 7

Number of Tubes Collected per Subject by Visit

ENTITY	TEST TYPE	SC	BL	M6	M12	M18 MCI only	M24	M36 NL & MCI
Upenn	Biomarker Labs		2 BLD EDT PL 2 BLD SER 2 CSF 1 URN	2 BLD EDT PL 2 BLD SER 1 URN	2 BLD EDT PL 2 BLD SER 2 CSF 1 URN	2 BLD EDT PL 2 BLD SER 1 URN	2 BLD EDT PL 2 BLD SER 1 URN	2 BLD EDT PL 2 BLD SER 1 URN
UPenn	ApoE	1 BLD EDT						
NCRAD	Cell Immortalization		2 BLD ACD					
Covance	Clinical Labs	2 BLD SER 1 BLD EDT PL 1 URN						
Total # tubes per visit per subject		5	9	5	7	5	5	5

CLINICAL LABORATORY SAMPLES AT SCREENING

CLINICAL LABORATORY KITS

Covance will send an initial supply of clinical laboratory kits to each site. Instructions on how to correctly complete the Laboratory Requisition form and prepare and ship samples are provided with the kits.

IMPORTANT NOTE: ALL CLINICAL LABORATORY SPECIMENS MUST BE SHIPPED ON THE DAY OF COLLECTION.

EACH CLINICAL LAB KIT CONTAINS:

- ⇒ Tubes (expiration date noted on outside of box)
- ⇒ Requisition (bar coded). Use black or blue ink, and send white copy to Covance
- ⇒ Labels (bar coded). **USE ONLY THE LABELS WITH THE REQUISITION**, or samples could be lost.
- ⇒ Vacutainer holder with needle guard
- ⇒ Needle
- ⇒ Diff-safe (Be sure to REMOVE this from the tube before shipping)
- ⇒ Pipettes
- ⇒ Test tube sleeve
- ⇒ Absorbent material
- ⇒ Bag with sleeve for requisition
- ⇒ Gel-pack (Do not put specimens into the gel; wrap the whole gel pack around the specimens)

YOU WILL NEED TO PROVIDE:

- ⇒ Tourniquet
- ⇒ Alcohol
- ⇒ Gauze
- ⇒ Bandage
- ⇒ Dry ice

COVANCE WILL ALSO SEND:

- ⇒ Investigator Manual (with CAP, CLIA, and Medical Director CV)
- ⇒ Shipping boxes: ambient, frozen, and combination
- ⇒ Airborne labels

REQUISITIONS

- ⇒ Use black or blue ink, as Covance uses other colors for coding
- ⇒ Do not mix up the bar-coded labels, as these are linked to a specific requisition form
- ⇒ Fill in all the blanks
- ⇒ Put the white original copy of the requisition with the ambient specimen. If only a frozen specimen is sent, put the white original with the frozen specimen. Place the requisition in the sleeve of the sample bag
- ⇒ Use military, or 24-hour clock
- ⇒ Dates use the international convention: DD_MMM_YYYY. Example: 01JAN2004

CLINICAL LABORATORY REPORTS

Covance will fax a laboratory report to each center within 36 hours after receipt of the specimens.

- ⇒ For each laboratory test, the participant's test result will be provided, as well as the reference range for that test.
- ⇒ All results that are out of range will be flagged as high or low by Covance.
- ⇒ For all out-of-range results, a clinician at the center must indicate clinical significance (yes or no) by checking the appropriate box on the report.
- ⇒ The clinician must also initial and date each page of the report. All clinically significant out-of-range lab values should be entered as an Adverse Event online.
- ⇒ Freeze tubes upright

**FOR SPECIMENS MAILED ON A FRIDAY, BE SURE TO CHECK
"SATURDAY DELIVERY" ON THE AIRBORNE SHIPPING LABEL**

To order additional clinical laboratory kits, or if you have any questions about how to use the clinical laboratory kits, complete the requisition forms, or ship supplies, please call Covance at: (800) 327-7270

It is the responsibility of each site to monitor the expiration date of each kit.

To call Airborne for a Covance specimen pickup, call (888) 708-8825

To contact Covance, call (800) 327-7270 and listen to the messages.

**PLEASE REFER TO THE COVANCE PROCEDURES MANUAL FOR SPECIFIC
INSTRUCTIONS ON SAMPLE COLLECTION, PROCESSING AND SHIPMENT**

APOE SAMPLES AT SCREENING

APOE sampling is required for final approval in ADNI. For more details on this, please refer to the section on scan category assignment. One blood sample will be collected at the screening visit for APOE genotyping.

Begin by completing the information on the BLD EDT tube label, as described below, and secure the label onto the lavender top tube.

Sample Bar Code Labels will contain the following information:

- ⇒ Bar Code & License Plate
- ⇒ ADNI
- ⇒ Lavender Top
- ⇒ Visit (VST I)
- ⇒ Sample type (BLD EDT)

In addition, write in the subject ID number, the date/time of collection and circle M or F to indicate subject gender, on the bar code label specific for BLD EDT VST I. Secure this label on the BLD EDT tube prior to the blood draw.

Collection Tube: 1 10cc Lavender Top tube; gently mix by inversion, 10 -12 times, to assure that the EDTA anticoagulant is well-mixed with the whole blood sample.

Temperature Requirements: The whole blood sample must be received by the UPenn Biomarker repository within 24 hours of collection. The whole blood sample is maintained at room temperature and shipped at ambient temperature.

Sample Tracking: Complete online form with sampling date, time, bar code License Plate and FedEx tracking number. Print out the completed form and include it with the shipment.

Shipping: APOE sample shipping kits, including blood tubes & blood collection set, will be provided by Covance. Sites must request resupply of these kits. Samples will be shipped by Federal Express – Priority Overnight. For those instances in which a Friday study visit is necessary, be sure to clearly check **Saturday Delivery** on the FedEx form and apply orange “Saturday Delivery” labels prominently to the package.

SHIPPING MATERIALS for APOE at SCREENING -

**SHIPPING THE APOE SAMPLES –
Ambient Room Temperature**



10ML Lavender-top Tube



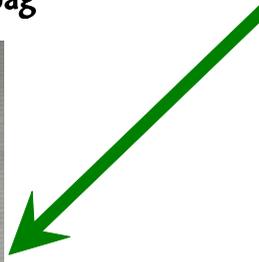
Tubes wrapped in bubble wrap bag



Saf-T-Pak Inner Poly bag



Saf-T-Pak Outer Tyvek bag



Cardboard Shipping Box

CELLS FOR IMMORTALIZATION

Blood will be collected at the Baseline visit that will be sent to the National Cell Repository for AD.

Begin by completing the information on the two BLD ACD tube bar code labels, and secure one label onto each of two yellow top tubes.

Sample Bar Code Labels will contain the following information:

- ⇒ Bar Code & License Plate
- ⇒ ADNI
- ⇒ Yellow Top
- ⇒ Visit (VST 2)
- ⇒ Sample type (BLD ACD)

In addition, write in the subject ID number, date/time of collection and circle M or F to indicate subject gender, on each of two bar code labels specific for BLD ACD and place each on the yellow top blood collection tubes.

Collection Tube: Two 8.5 cc Yellow Top tubes; fill each bar code labeled tube with blood and gently mix by inversion 6-10 times to assure complete mixing of the blood sample with the anticoagulant Acid Citrate Dextrose.

Temperature Requirements: The whole blood sample must be received by the National Cell Repository within 24 hrs of collection. The whole blood sample is maintained at room temperature and shipped at room temperature.

Sample Tracking: Complete online form with sampling date, time, bar code License Plate and FedEx tracking number. Print out the completed form and include it with the shipment.

Shipping: Sample shipping kits for shipment to the National Cell Repository for AD will be provided by Covance. Sites must request resupply of these kits. Shipping of all samples will be done by Federal Express, priority overnight. All shipping materials including yellow top tubes (8.5 mL), instructions, pre-made shipping labels with the NCRAD address, shipping kit, shipping stickers for biological samples, will be provided by Covance. For those instances in which a Friday study visit is necessary, store the tubes at room temperature and ship on Monday by Federal Express, priority overnight.

SHIPPING THE CELL IMMORTALIZATION SAMPLES – Ambient Room Temperature



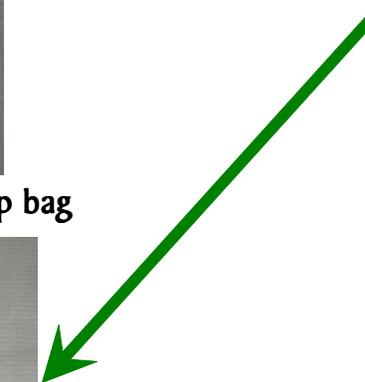
8.5ML Yellow-top tube



Tubes wrapped in bubble wrap bag



Saf-T-Pak Inner Poly bag



Saf-T-Pak Outer Tyvek Bag



Cardboard Shipping Box

BIOMARKERS: BLOOD AND URINE SAMPLES

Blood and Urine Samples will be collected at every study visit from Baseline.

An initial supply of Biomarker Lab Kits will be sent to each site upon approval to screen participants. Detailed processing information will be required for these samples.

Begin by printing out a PDF of the Biomarkers Samples Form from the ADCS distributed data entry system and completing the Sample Identification labels . The bar code label must be placed on the transfer tube prior to freezing!

ORDER OF FLUIDS TO BE COLLECTED:

1. Biomarker plain red-top tubes (2 blood collection tubes)
2. Biomarker lavender-top (2 blood collection tubes)
3. Urine collection container
4. CSF Collection (if applicable)

FOLLOW SAFE BLOOD-HANDLING PROCEDURES THROUGHOUT!

Tubes 1 and 2 - 10 mL plain Red-top tubes for serum samples

1. Write the Subject Identification Number on the side of the tubes prior to drawing blood.
2. Collect blood until each tube is full.
3. Estimate blood volume and record on the ADNI Biomarker Samples form.
4. Allow the blood to clot for 30 minutes at room temperature in a vertical position.
5. Centrifuge the tube at room temperature within one (1) hour of collection. Spin for 15 minutes using the Sorvall T 6000D Centrifuge (rotor H-1000B swinging bucket rotor) at 3000 rpm (1500 rcf) with the brake on, or in another centrifuge at a comparable rcf.
6. Write in the Subject Identification Number, the time and date of collection and circle M or F to indicate subject gender, on the bar code label specific for BLD SER and place this on one 14 mL plastic transfer tube (red screw cap) standing in a tube rack in the vertical position.
7. Using a clean sterile transfer pipette carefully transfer serum from each of the two red-top tubes into the bar code-labeled 14 mL plastic transfer tube, then firmly cap with the red screw cap.
8. After the plasma has been transferred to the plastic bar-code labeled tube and capped, place the red screw-capped BLD SER-labeled tube upright in dry ice and allow to completely freeze.

Tubes 3 and 4 - 10 mL Lavender-top tubes for plasma samples

1. Write the Subject Identification Number on the side of the tubes prior to drawing blood.
2. Collect blood until each tube is full; gently mix by inversion, 10-12 times.
3. Estimate blood volume and record on the ADNI Biomarker Samples form.
4. Centrifuge the tube at room temperature within one (1) hour of collection. Spin for 15 minutes using the Sorvall T 6000D Centrifuge (rotor H-1000B swinging bucket rotor) at 3000 rpm (1500 rcf) with the brake on, or in another centrifuge and rotor at a comparable rcf.
5. Write in the Subject Identification Number, the time and date of collection and circle M or F to indicate subject gender, on the bar code label specific for BLD EDT PL and place this on one 14 mL plastic transfer tube (lavender top screw cap) standing in a tube rack in the vertical position.
6. Using a clean sterile transfer pipette carefully transfer plasma from each of the two lavender-top blood tubes into the bar code-labeled 14 mL plastic transfer tube, and firmly cap with the lavender screw cap.
7. After the plasma has been transferred to the plastic labeled tube and capped, place the lavender screw-capped BLD EDT PL-labeled tube upright in dry ice and allow to completely freeze.

Urine collection:

1. Write the Subject Identification Number on the side of one urine collection cup prior to urine collection.
2. Ask study subject to collect urine specimen in the collection cup.
3. Pour enough of the urine specimen to at least half-fill one 50 mL centrifuge tube and cap with the screw-cap.
4. Centrifuge the tube at room temperature within one (1) hour of collection. Spin for 15 minutes to remove sediment and cells using the Sorvall T 6000D Centrifuge (rotor H-1000B swinging bucket rotor) at 2500 rpm (1050 rcf) with the brake on, or in another centrifuge and rotor at a comparable rcf.
5. Write in the Subject Identification Number, the time and date of collection and circle M or F to indicate subject gender, on the bar code label specific for URN and place this on one 14 mL plastic transfer tube (yellow screw cap), place in a tube rack in the vertical position.
6. Using a clean sterile transfer pipette carefully transfer urine from the 50 mL centrifuge tube into the bar code-labeled 14 mL plastic transfer tube, then firmly cap with the yellow screw cap.
7. After the urine aliquot has been transferred to the plastic labeled tube and capped, place the yellow screw-capped URN-labeled tube upright in dry ice and allow to completely freeze the urine sample.

Cerebrospinal fluid collection:

1. Write in the Subject Identification Number, the time and date of collection and circle M or F to indicate subject gender, on two bar code labels specific for CSF and place each of these on two 14 mL plastic transfer tubes (clear screw cap) prior to transfer of the CSF samples.
2. Follow the detailed procedure as described in the Instructions for Assisting with the LP Procedure section of the Procedures Manual for the CSF collection (**end of this section**).
3. Aliquoting the first 2 mL of CSF for cell counts, glucose and total protein, transfer the remainder of the CSF collection into two 14 mL plastic transfer tubes, firmly cap and gently invert 3-4 times to assure the uniformity of the CSF sample.
4. Place the clear screw-capped CSF-labeled tubes upright in dry ice and allow to completely freeze the CSF sample.

Packaging of frozen biofluid specimens for shipment on dry ice:

1. Place small pieces of dry ice in a bubble-wrap bag. Quickly transfer the labeled sample transfer tubes from dry ice to the bubble wrap bag and seal the bag with the self-adhesive flap.
2. Quickly place the bubble bag with contents into the leak-proof inner poly bag and seal. Place this bag into the Tyvek envelope and seal.
3. Place the envelope and contents directly on dry ice in Styrofoam shipping box. Fill the box with dry ice and cover. Place container into cardboard box; affix Federal Express shipping label and call for pick up. Ensure box is labeled with 'biohazard' and 'dry ice' labels prior to shipment.

DO NOT ALLOW SAMPLES TO THAW AT ANY POINT AFTER THEY HAVE BEEN FROZEN.

Sample Tracking:

Enter the sample collection data on the ADCS distributed data entry system (<http://adni.ucsd.edu>). Enter the Bar Code License Plate (one per visit) and FedEx tracking number. Print a copy of the completed form and include it with the shipment.

Shipping:

FedEx all biomarker biofluid samples the SAME DAY on DRY ICE by Federal Express, Priority Overnight shipping (Monday-Thursday). For those instances in which a Friday study visit is necessary, be sure to clearly check Saturday Delivery on the FedEx form and apply orange "Saturday Delivery" labels prominently to the package. Use the Federal Express Airbill that has the ADNI Biomarker Core Laboratory shipping address information printed on it:

**ADNI Biomarker Core Laboratory
7 Maloney South
University of Pennsylvania Medical Center
3400 Spruce Street
Philadelphia, PA 1910**

DISCARD APPROPRIATELY ALL GLOVES, TUBES, DISPOSABLE TRANSFER PIPETTES AND WASTE CONTAINING BLOOD OR BLOOD PRODUCTS.

**IMPORTANT! Complete the Biomarker Samples Online Form before shipping samples.
Print a pdf of the completed form and include a copy with the shipment.**

SUPPLIES FOR BIOMARKER SAMPLES FROM COVANCE:

1. 10-ml, plain red top plastic Vacutainer blood tubes
2. 10-ml, lavender top plastic Vacutainer blood tubes
3. Urine collection containers
4. 50-mL screw-cap centrifuge tube
5. 14-mL polypropylene transfer tubes with colored screw caps
6. Disposable sterile transfer pipettes
7. Bubble-wrap bags
8. Blood collection set with 21-gauge butterfly needle
9. Vacutainer tube holder
10. Inner Saf-T-Pak plastic bag with absorbent
11. Outer Saf-T-Pak Tyvek bag
12. Styrofoam inner shipping container
13. Cardboard shipping box

⇒ To order additional Biomarker kits, please contact Covance at (800) 327-7270.

SUPPLIES FOR BIOMARKER SAMPLES FROM ADCS:

1. Bar code labels packs
2. Shipping labels

SHIPPING THE BIOFLUID SAMPLES ON DRY ICE



Tubes wrapped with dry ice in bubble-wrap bag



Saf-T-Pak Inner Poly Bag



Saf-T-Pak Outer Tyvek Bag



Cardboard Shipping Box with inner Styrofoam container packed with dry ice

BIOMARKERS: CEREBRAL SPINAL FLUID

CSF samples will be collected from all subjects who are willing at the Baseline and MI2 Visit. A video describing the LP procedure will be provided to all sites.

Prevention of Post-Lumbar Puncture headache:

1. Use of a **Sprotte 24g atraumatic spinal needle** and careful technique are optimal for reducing post-LP headache risk.
2. Having the subject refrain from exertion (e.g., exercise, housework, gardening, lifting, sexual activity or any other strenuous activities) for 24 hours after the LP is helpful.
3. Increasing fluid intake for 24 hours after LP is helpful.

Mild to Moderate headache after a lumbar puncture:

Mild to moderate headache following lumbar puncture usually resolves within 3-4 days with the above treatment. If the headache becomes severe, posturally sensitive (relieved by supine posture), or is accompanied by nausea, vomiting, tinnitus and/or visual disturbances, it will likely require additional treatment with an epidural blood patch. This usually relieves the headache immediately. The epidural blood patch is typically performed by an anesthesiologist.

Treatment of Mild to Moderate headache:

- ⇒ Limit physical activity as much as possible.
- ⇒ Oral fluids and caffeine are helpful. Drinking a can of Mountain Dew soft drink (for example) is preferable to coffee (which has some diuretic activity).
- ⇒ Tylenol should be used for symptomatic relief. If a subject cannot tolerate Tylenol, ibuprofen should be used. Avoid aspirin. If these do not relieve the headache, Tylenol with codeine or equivalent could be considered.

Severe headache after a lumbar puncture:

Severe headache which may be accompanied by nausea, vomiting, tinnitus, and/or visual disturbances and which is relieved by supine posture requires epidural blood patch to provide rapid relief. This type of headache most commonly occurs on the morning following LP, when the person arises from bed. Posturally-sensitive severe post-LP headache can last as long as a week even with bedrest and analgesics if not treated with epidural blood patch. **IT IS HIGHLY RECOMMENDED** that each site identify an anesthesiologist who is agreeable to performing an EPIDURAL BLOOD PATCH for any subject who experiences severe post lumbar puncture headache. Sites should find out ahead of time who to call to schedule and perform a blood patch at their center, should the need arise, as well as how their study account will be billed.

LUMBAR PUNCTURE SUPPLIES

Supplies for collecting and for shipping the CSF are sent separately to the site.

- I. Initial supply of Lumbar Puncture Materials (provided by the ADCS):
 - Lumbar Puncture Trays (5)
 - 24g Sprotte Atraumatic Spinal Needles and Introducers (10)
 - 25g 1 1/2" needles for deep local anesthesia (25)
 - 5 cc sterile syringes (25)
 - 13 mL transfer tubes with clear caps (20)

Additional Lumbar Puncture kits and needles can be ordered by contacting: support@adni.ucsd.edu

The lumbar puncture tray contains the following items which will be used to perform lumbar puncture. Check the dates of expiration: these reflect the expiration date of the lidocaine.

- Three Sponge Sticks
- Three 2 X 2 Gauze pads
- Paper drape
- Fenestrated Paper Drape
- Lidocaine Hydrochloride USP, 1%, 2 ml ampule
- 20g needle for drawing up lidocaine into syringe (will not be used to inject lidocaine)
- Infiltration needle (25g x 1/2") for skin infiltration for local anesthesia
- Prep Well
- Four pre-labeled Specimen vials with Caps, 10 ml (for cell count and glucose)
- Adhesive Bandage (Band-Aid)

The lumbar puncture tray contains the following items which will **NOT** be used when following the ADNI guidelines for Sprotte Needle use:

- ↔ 20g spinal needle, plastic manometer and extension tube.

The following “stock” items will also be used.

- Sterile gloves in correct size for person performing the LP (one plus extras for backup)
- Blue pad (one, plus extras for backup)
- Bottle of Betadine solution (not Betadine scrub)
- Individually wrapped alcohol wipes
- Sterile 25g, 1 1/2" needle for deep infiltration of lidocaine (one, plus extras for backup)
- Sterile 3 cc syringe with 20g needle attached (in case more lidocaine is needed). Attached needle is used for drawing up lidocaine, but NOT for injecting it.
- Bottle of lidocaine (in case more lidocaine is needed)
- Sterile 4 by 4 gauze pads (extras)
- Extra adhesive bandages (Band-Aids)
- clean washcloths and towels
- Sharps container
- Lumbar Puncture Fact Sheet and Post-LP instructions for subjects

Instructions for Assisting with the LP Procedure

SETTING UP FOR THE LP

- ⇒ 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. They will need these at the beginning of the Lumbar Puncture. Have all other supplies on hand.
- ⇒ The Lumbar Puncture may be done with the subject either lying down on their side, or sitting.
- ⇒ It is critical to try to optimize this positioning, and usually requires an assistant.
- ⇒ On an over-bed table, remove the contents of the Lumbar Puncture kit 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, and begins examining the subject.
- ⇒ 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 inside of the paper wrapper, or if any non-sterile object or outside of the wrapper touches any part of the inside of the wrapper, throw the kit away and start over.
- ⇒ If you are in doubt as to whether something touched the inside of the paper wrapper, throw the kit away and start over.
- ⇒ If you reach a point where the kit is pretty much unwrapped but there is a tricky spot that won't unwrap, the person performing the Lumbar Puncture may be able to help, once they are gloved up.
- ⇒ Remember, once they are gloved they can only touch the inside of the paper wrapper, and you can only touch the outside.

ADDING THE BETADINE

- ⇒ Once the kit is successfully unwrapped, very carefully open the bottle of Betadine solution, somewhere away from the kit. Use an alcohol wipe to remove any loose chunks of dried Betadine off of the bottle top. You don't want anything to fall onto the open and sterile Lumbar Puncture kit.
- ⇒ Pour enough Betadine into the well to cover the bottom, about 1/4 inch deep. Spend as little time as possible lingering over the kit.

MAINTAINING THE STERILE FIELD

- ⇒ Keep in mind that there may be a lot of staff in the room during a Lumbar Puncture, and a big part of assisting with the Lumbar Puncture is keeping the field sterile, keeping people away from it, and reminding people to be careful around it.
- ⇒ If anybody touches the inside of the paper wrapper or any part of the contents of the kit, throw the kit away and start over. If you are in doubt as to whether someone touched the kit, throw it away and start over.
- ⇒ Also, you are the monitor for whether the person performing the Lumbar Puncture has broken sterility - usually by touching something not sterile with a sterile gloved hand. Feel free to be the boss of people if need be. Be assertive.

SPINAL INTRODUCER, SPINAL NEEDLE, AND SYRINGES

- ⇒ During this time, the person performing the Lumbar Puncture is usually preparing the kit, pulling out sterile drapes, getting out the lidocaine, and familiarizing themselves with the kit. If you need a tube to collect the CSF for labs, ask them to "toss" you one from the kit and carefully pick it up from the end of the kit where they have placed it, being careful not to touch the kit. Do not touch the gloved hand of the person performing the Lumbar Puncture. Hand the tube to the person who will be aliquoting the CSF after the Lumbar Puncture. Label it or have them label it.
- ⇒ Wait until the person performing the Lumbar Puncture is finished preparing the kit and has started administering the lidocaine to the subject before you begin dropping items on the tray.
- ⇒ This makes it easier for them to get the items they need first before you add more items to the tray, and they won't accidentally fling a syringe into the Betadine or something else.
- ⇒ After they start numbing up the subject, carefully, and maintaining sterility, unwrap and drop the 25g 1 1/2" deep infiltration needle, spinal introducer and the Sprotte spinal needle onto the Lumbar Puncture tray. Everyone has their own special technique to accomplish this. With the spinal needle and introducer, I find it works best to pinch the item through the clear plastic portion of the package firmly, while removing the paper strip from the other side. Then drop the item onto the tray while holding onto the packaging. Do not drop any packaging onto the tray. Do not let the item touch the outside of the packaging on its way to the tray.

- ⇒ The 25 g 1 1/2" needle and sterile syringes are a little different. The packaging is more flexible. One way is to take hold of the two sides of the packaging with the thumb and forefinger of each hand and pull them apart making sure the opening is facing down toward the tray. Again remember, do not drop any packaging onto the tray, do not touch the tray with your hand, and do not let the item touch the outside of the packaging on its way to the tray. Start with 3 syringes, but be ready to add more if the person performing the Lumbar Puncture needs them.
- ⇒ Occasionally, the person performing the Lumbar Puncture will need to use more lidocaine to numb up a particular spot a little more or if they need to move to another spot entirely. In either case, they will need another 3 cc syringe and needle (packaged together and sterile).
- ⇒ Open the package as you would a sterile syringe by pulling open the two sides of the packaging without touching the inside or the syringe, but hold it upright instead, so that the person performing the Lumbar Puncture can grab the syringe without touching the outside of the packaging. There's no need to do this over the tray. Then, you will need to take a bottle of lidocaine (check the expiration date) and swab the top of it with an alcohol wipe. Show the bottle label to the person performing the Lumbar Puncture.
- ⇒ Next, hold the 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 Lumbar Puncture needs to change the site of the Lumbar Puncture (a different lumbar interspace), they will also need a new 25g needle for injecting lidocaine, a new introducer, and a new spinal needle. They will let you know if they do. Open them the same way as before, by dropping them onto the tray.

- ⇒ Often they will need an extra sterile 4 x 4 gauze pad. Again, they'll let you know. Open it the same way as the syringe and needle example above, by holding open the package so the person performing the Lumbar Puncture can grab the gauze without touching you or the package.

ALIQOTTING THE CSF

- ⇒ At this point, you will need to glove up. I usually wait until this time because I find it is easier to supply all of the sterile items without gloves on.
- ⇒ As the person performing the Lumbar Puncture fills the 5 cc syringes, they will place them at the far end of the Lumbar Puncture kit, far from them, near to you. Pick each one up carefully, avoiding touching the kit. This is a gray area as far as sterility goes; however, we try to keep it as sterile as possible.

Hand the first full syringe to the aliquoter, have them aliquot 1ml for cell count (local lab) and 1ml for total protein and glucose (Covance tube) into the tubes taken from the kit.. Cap the barcode-labeled transfer tubes with clear screw caps and place them in the upright position on dry ice. Transfer each 5 cc syringe-ful of CSF into the barcode-labeled transfer tubes. Each transfer tube can hold the volume from two 5 cc syringes so the first two 5 cc syringe-fuls are transferred into one transfer tube, labeled with a CSF bar code, and the subsequent 5 cc syringe-fuls are transferred into the second CSF bar code-labeled tube.

- ⇒ It is important to get them on dry ice as soon as possible, and in the upright position, so that the CSF freezes at the bottom of the tube. Please refer to the Biofluid Processing Procedure for full details for the labeling, freezing on dry ice, packaging and shipment of CSF samples.

WASHCLOTHS, BAND-AID, CLEAN UP

- ⇒ After the person performing the Lumbar Puncture collects the last of the CSF, they will remove the needle and introducer and wash the Betadine off the subject.
- ⇒ They can use two warm, wet washcloths and a dry washcloth or towel. Have these ready for them, or appoint someone to retrieve them for them. All visible traces of iodine should be cleaned from the skin (use 2 wet washcloths), the skin dried, and the Band-Aid applied over the puncture site. The Band-Aid is in the lumbar puncture kit. After they have made the subject more comfortable, they will remove the sharps from the kit. They do this because they are more familiar with the kit and where they put all the sharps and how many there are, etc. Acquire a sharps container in which to dispose of them all.
- ⇒ Next, throw all the rest of the kit away and toss the washcloths/towel (you should wear gloves for this - there may be some blood or CSF on the washcloths) into the laundry. After the study ends and the subject has left, wash the over-bed table down with a bleach wipe.

Tips for clinicians performing lumbar puncture: Optimizing patient comfort and minimizing risk of adverse events.

- ⇒ Talk the patient through the procedure - no surprises.
- ⇒ Use adequate local anesthesia. Use the 25g 1/2" needle and inject lidocaine to raise a skin wheal. Then inject lidocaine using the pattern of a square - first the center and then to all 4 corners. Advance the needle approximately 1/2 its length in two stages. Then change to the longer 1 1/2" 25g needle. Again, use the same pattern - be sure to draw the needle back out nearly all the way out to change direction. Advance the needle approximately 1/2" per "pass". **Be sure to draw back on the syringe before injecting every single time to make sure you are not in a blood vessel.** The subject may feel pressure but should feel no sharp pain from the introducer or spinal needle if adequate local anesthesia is used.
- ⇒ If the subject is thin, do not insert the deep infiltration needle OR the spinal introducer all the way. Use only about 2/3 of their length (to prevent entering the subarachnoid space with anything other than the Sprotte 24g spinal needle).
- ⇒ Be sure to give post-LP care instructions verbally to subject.

PROCESSING CSF

Ensure that all necessary equipment and supplies are available ahead of time.

When a Sprotte needle (ADNI recommended procedure) is used for the lumbar puncture, the CSF is drawn in four or more 5 ml sterile syringes.

If a conventional needle was used, then CSF is dripped into the large collection tubes in the Lumbar Puncture kit, and the transfer pipet is used to transfer CSF to the transfer tubes with the clear top.

1. Place 1 ml of CSF in the tube for cell count, send at room temperature to local lab.
2. Place 1ml of CSF for protein and glucose into the tube from the LP tray and ship to your local lab.
3. Aliquoting the first 2 mL of CSF for cell counts, glucose and total protein, transfer the remainder of the CSF collection into two 14 mL plastic transfer tubes, firmly cap and gently invert 3-4 times to assure the uniformity of the CSF sample.
4. Place the clear screw-capped CSF-labeled tubes upright in dry ice and allow to completely freeze the CSF sample.
5. Package and ship along with Biomarker Blood and Urine samples as directed previously in this section.

NOTE: If the initial CSF was blood-tinged, discard the first 1-2 ml until later CSF begins to clear.

LUMBAR PUNCTURE FACT SHEET

Frequently Asked Questions

Q: What if I'm unable to flex my back and legs?

A: The test can be done without bending or while sitting.

Q: Is the entire needle put into my back?

A: No, but the needle must be long enough to pass through the muscles of the lower back.

Q: Can I be paralyzed if the needle hits the spinal cord?

A: No, there is no need to worry about spinal cord damage. The needle is inserted well below the spinal cord.

What is it?

A Lumbar Puncture (Spinal Tap) Test is a procedure to remove a small sample of cerebral spinal fluid from the lower spine. A needle is inserted between the vertebrae (backbones) in the lower back and into the space containing the spinal fluid which surrounds and cushions the brain and spinal cord.

How long does it take?

About 20 to 30 minutes. There is a recovery period of about 30 minutes after the test, which will take place at the Clinic.

Why is the Lumbar Puncture test performed?

To obtain a specimen for testing.

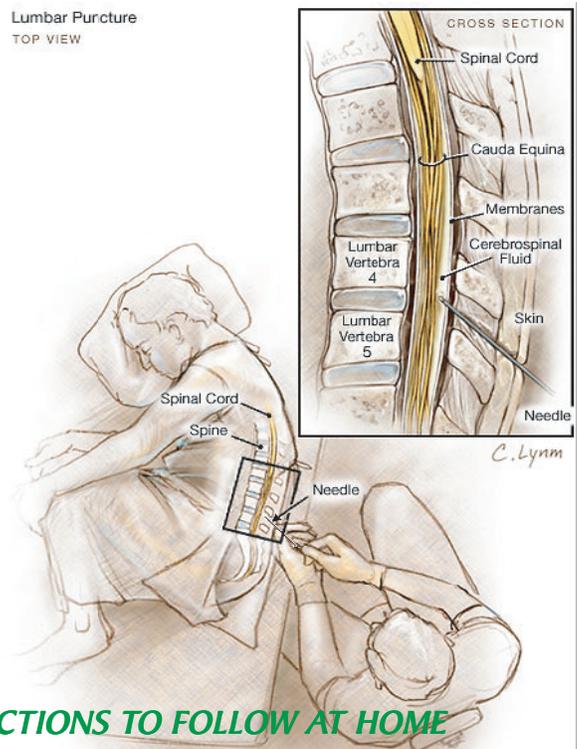
Does it hurt?

You may feel a sharp "bee sting" type pain, then mild burning sensation, when the numbing medication is first given. You may experience pressure when the needle is inserted. You may also feel some very brief leg pain while the needle is positioned because it may briefly touch a floating nerve ending.

How is it performed?

- ⇒ You will lie on your side with your knees drawn up toward your chin as far as possible *OR* you will sit on the edge of a table, in a hunched forward position.
- ⇒ The doctor will cleanse the skin over your spinal column with iodine.
- ⇒ An injection of local anesthetic is be given at the puncture site to numb the site (like getting novocaine at the dentist).
- ⇒ A needle is inserted into your spinal fluid space.
- ⇒ Spinal fluid is collected into specimen tubes for laboratory testing.

Lumbar Puncture
TOP VIEW



AFTER THE TEST INSTRUCTIONS AND INSTRUCTIONS TO FOLLOW AT HOME ARE ON THE REVERSE OF THIS SHEET

After the test

You will be asked to lie down for about 30 minutes.

Report any of the following symptoms to the doctor or nurse:

- ⇒ **Headache**
- ⇒ **Tingling**
- ⇒ **Numbness or pain in your lower back and legs**
- ⇒ **Problems with urination**

You may return home.

Instructions to follow at home

- ⇒ Drink at least 6 glasses of fluid (no alcohol) in the next 12 hours.
- ⇒ Take it easy for the next 24 hours..
- ⇒ Avoid any strenuous physical activity for 48 hours. This includes exercise, housework, gardening, lifting, and sexual activity.
- ⇒ If you develop a headache, call the study coordinator.
- ⇒ A mild to moderate headache is often relieved by Tylenol and caffeine. For caffeine, use Mountain Dew soft drink instead of coffee.
- ⇒ If your headache is severe or if it lasts more than 4 days, call the physician at the hospital that performed the Lumbar Puncture test. You may need a blood patch procedure to relieve the headache.

Biofluids Glossary

ADNI	Alzheimer's Disease Neuroimaging Initiative
AD	Alzheimer's disease subject
NC	Normal Control subject
MCI	Mildly Cognitively Impaired subject
BLD	Blood (Whole)
CSF	Cerebrospinal Fluid
PL	Plasma
URN	Urine
ACD	Acid Citrate Dextrose
EDT	EDTA (Ethylenediaminetetraacetic acid)
SER	Serum
BLD EDT	Whole blood collected in a lavender-top tube
BLD ACD	Whole blood collected in a yellow-top tube (ACD-A)
BLD SER	Whole blood collected in a plain red-top tube
NCRAD	National Cell Repository for Alzheimer's Disease
LP	Lumbar Puncture