



ADNI3 Biomarker Biofluid Collection, Processing and Shipment

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Biomarkers in Biofluids

Rationale:

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

- Aid in the recognition of the illness at its earliest clinically recognizable stages (prodromal or MCI).
- Detect the disease before dementia or other symptoms appear (Pre-clinical).
- Distinguish AD from other causes of dementia especially valuable, together with imaging tests, in the evaluation of disease-modifying therapies in research settings determination of risk for clinical decline and progression to AD dementia.

AD Biomarkers

Validated biomarkers measured in ADNI fluids include:

- A β 42, t-tau and p-tau181 in CSF using the highly automated Roche Elecsys immunoassays on the Cobas e601 automated system; A β 40 added to this for ADNI3. Replaces the AlzBio3 immunoassay platform.
- A β 42 and A β 40 in CSF using validated UPLC/MSMS
- Isoprostanes in ADNI1 CSF using validated HPLC/MSMS
- A β 42 & A β 40 in ADNI1 plasma
- Homocysteine in ADNI1 plasma using clinical laboratory testing

New biomarkers measured in CSF, plasma and serum:

- Research studies approved by the RARC that use ADNI biofluids
- See regular updated information in RARC-approved studies table

ADNI 3 Sampling Schedule

Newly Enrolled Participants	CSF	Plasma	Serum	Rollover Participants	CSF	Plasma	Serum
Baseline	✓	✓	✓	Initial Visit	✓*	✓	✓
Ongoing Annual Visits	✓*	✓	✓	Ongoing Annual Visits	✓*	✓	✓

* LP performed every two years from Baseline (New Participants) / Initial Visit (Rollover participants)

Collection

- After overnight fast (minimum 6 hours)
- Standard time of day (8 am)
- Order of collection*
 - 2 plain red top tubes (for serum)
 - 2 EDTA purple top (for plasma)
 - CSF by Lumbar Puncture

*Complete order of collection including samples for shipment to the Genetics core are summarized on the Worksheet page.

ADNI Biomarker Labels

- Specific to sample type and visit
- Barcodes identify sample
- License plate same within the visit for all samples
- Record License Plate on worksheet and eCRF.

BLD Serum Label	BLD Plasma Label	CSF Label	CSF Label
Subject ID #: _____S_____			
Date ____/____/____	Date ____/____/____	Date ____/____/____	Date ____/____/____
Time ____:____	Time ____:____	Time ____:____	Time ____:____
Gender: M or F			
Biomarkers	Biomarkers	Biomarkers	Biomarkers
ADNI3	ADNI3	ADNI3 CSF	ADNI3 CSF
Red Cap	Lavender Cap	License Plate	License Plate
BLD SERUM	BLD PLASMA	Number: _____	Number: _____



This is the License Plate Number. Remember to include this number in the CSF label!

Biomarker Supplies

- Screening Lab kits are provided by UPMC
- All Biomarker and Genetic sample collection and shipping materials are provided by ATRI
- Initial Supplies are sent upon ADNI 3 Approval
- Resupply Order forms can be found on line:
 - <https://www.atrihub.info/adni3-ordersupplies>
 - <https://www.upmc.rochester.edu/pathology-labs/clinical.aspx>

LP Supplies Provided by ATRI

USA Sites

Universal (VWR) Medical Lumbar Puncture Trays (all sterile components):		
Tray: 1	Label: 2	Insert: 1
Needle 22G Sprotte: 1	Gauze: 2	Pipette: 3
Needle 24G Sprotte: 1	Blunt filter needles: 2	Towels: 2
Needle 25G: 1	3mL Luer-lok Syringes: 2	Tray 3 (compartment SM molded): 1
RX Lidocaine 1%: 1	Wrap: 1	Bandage: 1
14 mL Collection tubes: 2	Syringes 5 mL: 5	Sponges: 3
2 mL Cryovials: 2	Needle 20G: 1	25G X 5/8 Safety Needles: 2
Drape: 1	Needle stick pad cube: 1	Ampule Cracker: 1

LP Supplies Provided by ATRI

Canadian Sites

Item	Purpose	QTY
CANADIAN SITES		
LP Trays by Carefusion	CSF collection by gravity/drip or suction/syringe	1
22g Sprotte Needle w/Introducer by Pajunk	For sites that request this type of needle for LP/CSF collection by gravity/drip. Recommended by ATRI	1 (if requested)
24g Sprotte Needle w/Introducer by Pajunk	For sites that request this type of needle for LP/CSF collection by suction.	1 (if requested)
Sterile Syringe, 5cc , Norm-ject Henke Sass Wolf/Air-Tite Products	Lumbar Puncture: Suction Method	5 (if requested)
Lidocaine Needle by BD Vacutainer	Administration of Lidocaine	1
Externally threaded 2 mL Cryogenic Vial by Thermo Scientific	Shipping of CSF to local labs	2
13 mL starstedt polypropylene transfer tubes	Lumbar Puncture: Gravity/Drip Method. Shipping of CSF to UPENN Biomarker Core	2
Samco Sterile pipette	Aliquot 2ml of CSF to cryogenic vials for local lab ONLY	3

Have on hand:

Lumbar Punctures

- Extra lidocaine
- Dry Ice
- Sterile Gloves
- Blue pad
- Extra gauze pads, bandages, alcohol wipes
- Clean washcloths/towels
- Sharps container
- LP Fact Sheet and post-LP instructions

Blood Collection

- Centrifuge
- Dry Ice
- Bandage/Plasters
- Sharps container
- Sterile Gloves
- Gauze pads

Worksheet

ADNI3

Biomarker Samples (Plasma, Serum, CSF)

Site: _____
 Participant Number: _____
 Individual Recording Data:
 Initials: _____ Date: _____

Instructions

Fluids should be collected in the following order:

- Biomarker plain red-top tubes (2 blood collection tubes)
- Biomarker lavender-top (2 blood collection tubes)
- CSF Collection (if applicable) CSF is collected only every two years
- If genetic samples are collected on the same day as Biomarker Samples, Blood RNA tubes should be the last tubes drawn in the phlebotomy procedure

The label must be placed on the transfer tube PRIOR to freezing.

Complete a separate eCRF record for each time a sample is collected on a different date. Complete a single eCRF record for all samples drawn on the same day. Enter eCRF prior to shipping samples. Include a copy of this worksheet with the shipment. FedEx all biomarker samples the SAME DAY on DRY ICE. Refer to the Procedures Manual for more detailed instructions.

****Please use separate worksheet (Genetics Sample Collection) for the Buffy Coat and RBC sample sent to NCRAD**

Date of Collection: _____ [YYYY-MM-DD]
Blood Sample Collection On this date, was blood for biomarkers collected? <input type="checkbox"/> Yes <input type="checkbox"/> No If no, please provide reason why blood was not collected: <input type="checkbox"/> Illness <input type="checkbox"/> Participant unavailable <input type="checkbox"/> Participant unwilling <input type="checkbox"/> Administrative problems <input type="checkbox"/> Withdrawn consent <input type="checkbox"/> Collection done on a different date for this visit <input type="checkbox"/> Other (specify): _____
Was participant fasting for at least 6 hours? <input type="checkbox"/> Yes <input type="checkbox"/> No
Two Tubes of 10 mL PLAIN RED-TOP: Serum Samples Serum: Time Collected: _____ [HHMM] Serum: Amount Collected: _____ mL Serum: Centrifuged Time: _____ [HHMM] Serum: Transfer Time: _____ [HHMM] Serum: Volume of Serum Transferred: _____ mL Serum: Time Frozen: _____ [HHMM]
Two Tubes of 10 mL PLAIN LAVENDER-TOP: Plasma Samples Plasma: Time Collected: _____ [HHMM] Plasma: Amount Collected: _____ mL Plasma: Centrifuged Time: _____ [HHMM] Plasma: Transfer Time: _____ [HHMM] Plasma: Volume of Plasma Transferred: _____ mL Plasma: Time Frozen: _____ [HHMM]

Complete as you collect and process serum, plasma & CSF samples

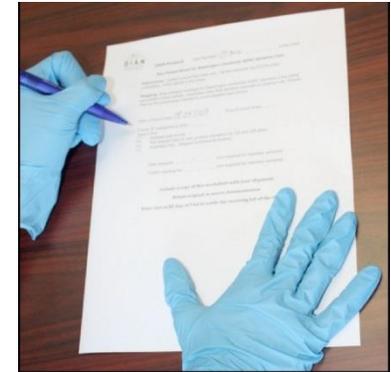
- **Date, Time, Amount of Collection**
- **Centrifugation time(serum, plasma, not CSF)**
- **Transfer time**
- **Aliquot Amount, Time**
- **Time Frozen**
- **Shipping Details**

Include copy in shipment

Enter into eCRF day of collection

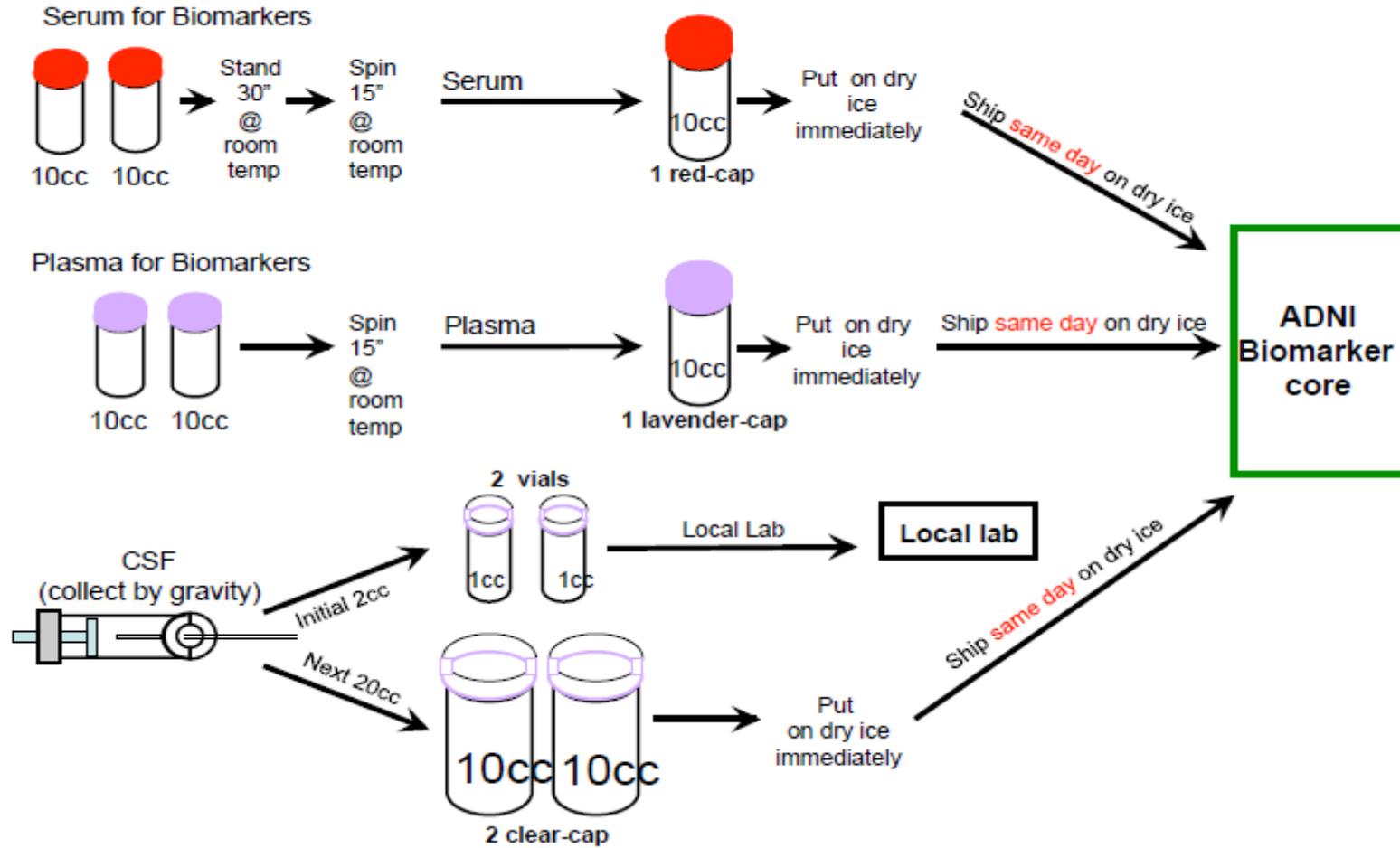
*See end of this document for complete set of 3 worksheet pages

Lab Notification

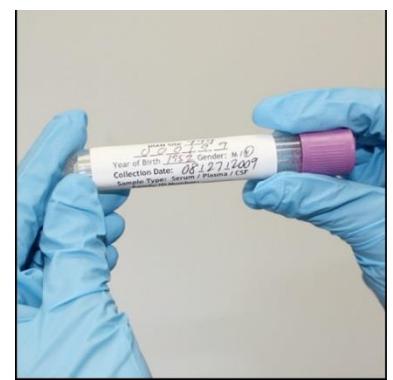


- Notify the UPenn laboratory of your shipment by entering the Biomarker collection eCRF day of collection/shipment
- Email a copy of the completed worksheet to UPENN at ADNI@uphs.upenn.edu.

Fasted Biomarker Samples



Plasma Samples

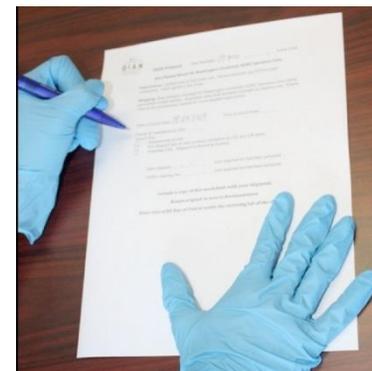


Use 2 x 10 mL **lavender-top** EDTA tubes

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 ADNI worksheet
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. Complete ADNI Biomarker Label for BLD PLASMA, place on 13 mL Polypropylene Transfer Tube. Stand in tube rack.
6. Using a STERILE transfer pipette, carefully transfer plasma from blood tubes into the Polypropylene Transfer Tube; firmly cap with the **lavender screw cap**.
7. Place upright in dry ice and allow it to completely freeze.
8. Complete the Biomarker Samples worksheet (serum/plasma).

NOTE: a review of actual processing times for plasma, serum and CSF, achieved across all ADNI sites during the ADNIGO/2 phase is provided in a separate report(LINK NEEDED).

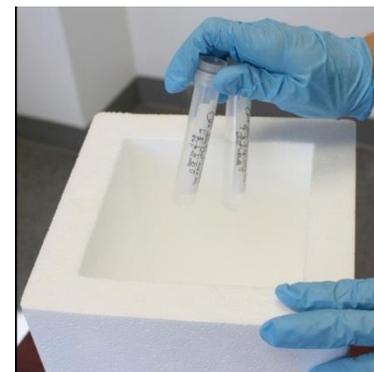
Serum Samples



Use 2 x 10 mL plain **red-top tubes**

1. Write the ADNI subject ID on tubes prior to drawing blood.
2. Collect blood until each tube is full.
3. Estimate blood volume and record on ADNI worksheet.
4. Allow to clot for 30 minutes (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. Complete ADNI Biomarker Label for BLD SERUM, place on 13 mL Polypropylene Transfer Tube. Stand in tube rack.
7. Using a STERILE transfer pipette carefully transfer serum from each of the two red-top tubes into the 13 mL Polypropylene Transfer Tube, then firmly cap with the **red screw cap**.
8. Place upright in dry ice and allow it to completely freeze.
9. Complete the Biomarker Samples worksheet (serum/plasma).

CSF Samples



1. Complete ADNI CSF labels and place each of these on two Polypropylene Tubes.
2. Follow the LP Procedure for collection of CSF by gravity using 22G Sprotte needle.
3. Collect about 20 mL of CSF into two labeled Polypropylene Tubes.
4. When the “syringe pull” method is used together with a 24G needle transfer the CSF from syringe into two labeled Polypropylene Tubes.
5. Send the first 2 mL of CSF for cell counts, glucose and total protein, to a local lab
6. Place the Polypropylene Tubes upright on dry ice and allow to completely freeze.
7. Complete the Biomarker Samples worksheet (CSF).

Ship it!



Freeze samples completely on dry ice before packaging.

1. Place polypropylene tubes with frozen specimen into segmented absorbent sleeve.
2. Place segmented sleeve and copy of collection worksheet into the 95kPa shipping bag. Place into bubble wrap bag.
3. Place bag directly on dry ice in Styrofoam shipper and fill rest of box with dry ice.
4. Cover Styrofoam box and place into cardboard box.
5. Seal cardboard box firmly with packing tape.
6. Affix preprinted included FedEx label and call for pickup.

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

NEVER ship a sample that was not first frozen upright in dry ice.

Shipping Reminders



- All individuals packaging 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.
- Ship fasted serum and plasma with CSF
- Complete the Biomarker Samples worksheet available online before shipping samples
- Print a pdf of the completed form and include a copy with the shipment.

More Shipping Reminders

- FedEx all biomarker samples the SAME DAY on DRY ICE priority overnight shipping (Monday-Thursday). When Friday visit is necessary CSF, plasma and serum samples should be placed in a -80°Celsius freezer until Monday and shipped on dry ice to UPENN. If a -80°Celsius freezer is not available, a -20°Celsius freezer is acceptable.
- FEDEX Air-bills are provided by ATRI for shipment to:
ADNI Biomarker Core Laboratory
University of Pennsylvania Medical Center
7 Maloney South
3400 Spruce Street
Philadelphia, PA 19104
Attention: Magdalena Korecka

Would you like some help?

- Email UPenn Laboratory at:
ADNI@uphs.upenn.edu

- Phone UPenn Laboratory:
215-662-6266
- Supply and protocol questions can be sent to **adni-study@usc.edu**

APPENDIX

BIOFLUID WORKSHEET
PAGES

Instructions

Fluids should be collected in the following order:

- Biomarker plain red-top tubes (2 blood collection tubes)
- Biomarker lavender-top (2 blood collection tubes)
- CSF Collection (if applicable) CSF is collected only every two years
- If genetic samples are collected on the same day as Biomarker Samples, Blood RNA tubes should be the last tubes drawn in the phlebotomy procedure

The label must be placed on the transfer tube PRIOR to freezing.

Complete a separate eCRF record for each time a sample is collected on a different date. Complete a single eCRF record for all samples drawn on the same day. Enter eCRF prior to shipping samples. Include a copy of this worksheet with the shipment. FedEx all biomarker samples the SAME DAY on DRY ICE. Refer to the Procedures Manual for more detailed instructions.

****Please use separate worksheet (Genetics Sample Collection) for the Buffy Coat and RBC sample sent to NCRAD**

Date of Collection: _____ [YYYY-MM-DD]

Blood Sample Collection

On this date, was blood for biomarkers collected? Yes No

If no, please provide reason why blood was not collected:

- Illness
- Participant unavailable
- Participant unwilling
- Administrative problems
- Withdrawn consent
- Collection done on a different date for this visit
- Other (specify): _____

Was participant fasting for at least 6 hours? Yes No

Two Tubes of 10 mL PLAIN RED-TOP: Serum Samples

Serum: Time Collected: _____ [HHMM]

Serum: Amount Collected: _____ mL

Serum: Centrifuged Time: _____ [HHMM]

Serum: Transfer Time: _____ [HHMM]

Serum: Volume of Serum Transferred: _____ mL

Serum: Time Frozen: _____ [HHMM]

Two Tubes of 10 mL PLAIN LAVENDER-TOP: Plasma Samples

Plasma: Time Collected: _____ [HHMM]

Plasma: Amount Collected: _____ mL

Plasma: Centrifuged Time: _____ [HHMM]

Plasma: Transfer Time: _____ [HHMM]

Plasma: Volume of Plasma Transferred: _____ mL

Plasma: Time Frozen: _____ [HHMM]

Site: _____
 Participant Number: _____
 Individual Recording Data:
 Initials: _____ Date: _____

CSF Collection

On this date, was LP collected? Yes No

If no, please provide reason why the LP was not conducted:

- Illness
- Participant unavailable
- Participant unwilling
- Administrative problems
- Withdrawn consent
- Not called for by protocol at this visit
- Collection done on a different date for this visit
- Other (specify): _____

Was participant fasting for at least 6 hours? Yes No

Time of LP: _____ [HHMM]

LP Performed by (Initials): _____

Must enter 3 characters. Use '-' if no middle name initial

Method of Collection:

- gravity
- syringe suction

Needle Gauge:

- 18g Quincke (sharp beveled) needle
- 19g Quincke (sharp beveled) needle
- 20g Quincke (sharp beveled) needle
- 21g Quincke (sharp beveled) needle
- 22g Quincke (sharp beveled) needle
- 23g Quincke (sharp beveled) needle
- 24g Quincke (sharp beveled) needle
- 25g Quincke (sharp beveled) needle
- 18g Sprotte (atraumatic) needle
- 19g Sprotte (atraumatic) needle
- 20g Sprotte (atraumatic) needle
- 21g Sprotte (atraumatic) needle
- 22g Sprotte (atraumatic) needle
- 23g Sprotte (atraumatic) needle
- 24g Sprotte (atraumatic) needle
- 25g Sprotte (atraumatic) needle
- Other (specify): _____

LP performed at the:

- L1-L2 inter space
- L2-L3 inter space
- L3-L4 inter space
- L4-L5 inter space

Patient Position:

- Sitting, leaned over (preferred)
- Lying, curled up on side
- Other (specify): _____

Biomarker Samples (Plasma, Serum, CSF)

Site: _____
 Participant Number: _____
 Individual Recording Data:
 Initials: _____ Date: _____

Type of collection tube used:

- Polypropylene.
 Polystyrene

Only Polypropylene tubes should be used for collection and shipment of CSF. If Polystyrene tubes are used, this is a protocol violation and must be noted in the protocol deviation log.

Type of tubes used for shipping:

- Polypropylene

If collected in polystyrene and shipped in polypropylene, please provide estimated amount of time CSF remained in polystyrene collection tube: _____ [minutes]

- Polystyrene

Only Polypropylene tubes should be used for collection and shipment of CSF. If Polystyrene tubes are used, this is a protocol violation and must be noted in the protocol deviation log.

CSF: Amount collected: _____ mL
 CSF: Transfer time: _____ [HHMM]
 CSF: Volume of CSF transferred: _____ mL
 CSF: Time Frozen: _____ [HHMM]

Check if any of the following were performed:

If Month/Day or Day is unknown, enter 'xx'. A valid year must be provided.

- Lumbar Puncture Blood Patch:

Date of Lumbar Puncture Blood Patch: _____ [YYYY-MM-DD]

- Fluoroscopy:

Date of Fluoroscopy: _____ [YYYY-MM-DD]

- Lumbar Spine Film:

Date of Lumbar Spine Film: _____ [YYYY-MM-DD]

All Samples

FedEx Tracking Number: _____ Date FedExed: _____ [YYYY-MM-DD]
 License Plate Number: _____ from ADNI Barcode Label (NOT from Screening Labs) – see Procedures Manual for further clarification.

Sample Collected and Sent By (print full name): _____

Phone and e-mail address: _____

Comments: (Document any items to note regarding lab draw, packaging, or shipping. Please ensure these comments are entered in the "Visit Comment" eCRF for this visit).

*Notify the UPENN (ADNI Biomarker Core Laboratory) the day samples are shipped.
 First, send a copy of this worksheet to:*

Email: ADNI@uphs.upenn.edu

Be sure to include a copy of this worksheet with the sample shipment. UPENN will not be able to receive any shipment on Saturday or Sunday