NILO-PD

PK and Biomarker Specimen Collection, Processing, and Shipment Manual

NOTE: *BLOOD FOR CLINICAL SAFETY LABS MUST BE COLLECTED PRIOR TO BLOOD FOR PK AND BIOMARKER SAMPLES. Please see ACM Manual for instructions.*

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1.0 Biorepository Information

1.1 Biorepository Contacts

General Study Contact Information
Phone: 317-274-5744
E-mail: nilopd@iu.edu
Fax: 317-278-1100

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Phone: 317-274-2218
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Danielle Smith, BS, Secondary Project Manager
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Specimen Shipment Mailing Address
NILO-PD Biorepository
Indiana University School of Medicine
TK – Room 342
351 W. 10th Street
Indianapolis, IN 46202

Kit Request Website
http://kits.iu.edu/nilopd

1.2 Hours of Operation

Indiana University business hours are from 8 AM to 5 PM Eastern Time, Monday through Friday.

Frozen specimens must be shipped Monday, Tuesday, and Wednesday only. Ambient specimens may be shipped Monday – Thursday.

1.3 Holiday Schedules

Please note that courier services may observe a different set of holidays. Please be sure to verify shipping dates with your courier prior to any holiday.

Holiday Observations
New Year's Day
Martin Luther King, Jr. Day
Memorial Day
Independence Day (observed on Friday if the holiday falls on a Saturday, and observed on Monday if it falls on a Sunday)
Labor Day
Thanksgiving Day and following Friday
Christmas Day (observed on Friday if the holiday falls on a Saturday, and observed on Monday if it falls on a Sunday)

Please note that during the last two weeks of each year, Indiana University will be open Monday through Friday for essential operations **ONLY** and will re-open for normal operations on the first Tuesday of the New Year. If at all possible, biological specimens should **NOT** be collected and shipped to Indiana University during the last two weeks of the year. Should it be necessary to ship blood specimens during this period, please contact the Indiana University staff by e-mailing nilopd@iu.edu so that they can arrange to have staff available to process incoming specimens. If specimens are collected during this holiday period will not be shipped immediately, please store them at -80 degrees Celsius and ship them on dry ice to Indiana University **AFTER** the first of the year.

2.0 Specimen Collection Kits and Supplies

2.1 Specimen Collection Supplies

PK and biomarker specimen collection kits will be provided to sites by Indiana University. Kits will include most of the materials needed for specimen collection, processing, and shipping. Kits will also include tube labels, which will be pre-printed with study information and the type of specimen being drawn. It is important that you check to be sure that all tubes are properly labeled during processing and at the time of shipment. The kits will also include shipping labels and packaging necessary for sending specimens back to the biorepository.

Once the initial enrollment and kit request is submitted to http://kits.iu.edu/nilopd, kits for Screening Visit 2, Baseline, Visit 1, and Visit 2 will be shipped to the site. Kits for subsequent visits will be automatically shipped to the site approximately four weeks prior to the scheduled visit window.

Collection and shipping supplies for the clinical safety labs are provided separately by ACM Medical Laboratories.

Each kit contains the necessary supplies to collect specimens from one subject at one visit. Kit components have been carefully selected to suit the needs of each project. Do not replace or supplement any of the tubes or kit components provided by Indiana University and ACM Medical Laboratory with your own supplies unless you have received approval from the CTCC/Indiana University to do so.

Note that "supplemental" kits will be provided to sites should you require additional supplies in addition to those contained in the visit specific kits.

For a complete list of supplies provided in the kits, please see **Appendix D**.

Additional PK and biomarker supplies can be ordered using Indiana University's online kit module by visiting http://kits.iu.edu/nilopd.

Store all blood and CSF collection supplies at room temperature, 64°F - 77°F (18°C to 25°C) until use.

2.2 Supplies to be Provided/Maintained by Sites

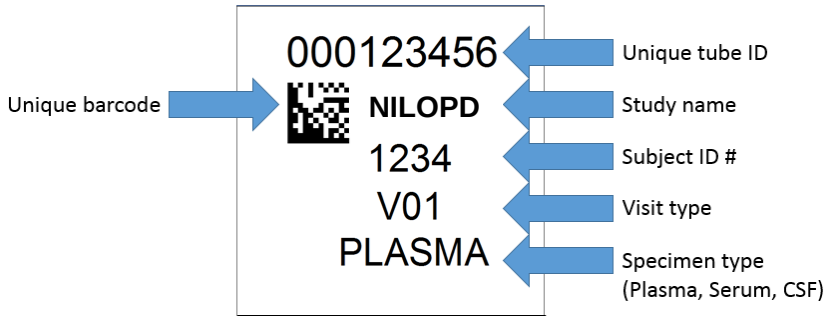
Although blood collection tubes and shipping supplies will be provided by the laboratories, sites will be required to provide certain supplies for biospecimen collection and handling.

Each Site Will Need to Provide:

Dry ice (for shipment)	Crushed ice (for CSF only)
Alcohol prep pads	Gauze pads
Bandages	Butterfly needles and hub
Tourniquets	Tube racks (2 ml to 10 ml)
Gloves	Sharps bin and lid

2.3 Specimen Labels

Labels for each collection, processing, and aliquot tube will be provided with each kit. An extra label is provided for each specimen type. Please discard any unused labels. Each label will contain the information indicated in the sample below:



3.0 Equipment Required at Clinical Sites

In order to process specimens consistently across all sites and ensure the highest quality specimen possible, sites must have access to the following equipment:

- Refrigerated and Room Temperature* Centrifuge
- -80°C Freezer

*CSF only

*****Important Note*****

In order to ensure that the highest quality specimens are collected, processed, and stored, it is essential to follow the specific collection, processing, and shipment procedures detailed in the following pages and appendices. Please read the following instructions prior to collecting any specimens. Have all of your collection and processing supplies and equipment prepared prior to drawing blood.

4.0 Specimen Collection and Processing Procedures

If indicated, record whether or not subject was fasting at the time of collection on the appropriate data form.

4.1 Sample Collection

The following samples will be collected as part of the NILO-PD study visits:

- Whole blood for clinical safety labs
- Serum for pharmacokinetic analysis
- Serum, plasma, buffy coat, whole blood and PBMCs suitable for proteomic, metabolomic, and other analyte studies
- Cerebrospinal fluid for analyte analysis

Not all samples are collected at every visit. Please review the specimen collection schedule in the protocol.

When possible, samples should be collected in the morning between 8 am – 10 am, preferably fasted. If fasting is not feasible, a low fat diet should be followed (**see Appendix L**).

4.1.1 Blood Collection

The following techniques should be used for all blood collection to prevent possible backflow:

a. Place donor's arm in a downward position.
b. Hold tube in a vertical position, below the donor's arm during blood collection.
c. Release tourniquet as soon as blood starts to flow into tube.
d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

See **Appendices E, F, G, H, and J** for specific instructions on the collection and processing of blood for serum, plasma, PBMCs, and buffy coats.

4.1.2 CSF Collection

Maintaining the sterile field is critical when performing a lumbar puncture. To ensure the safety of the patient, the following steps should be performed prior to the LP:

a. On an over-bed table, remove the contents of the LP kit from the outer plastic packaging, leaving the contents wrapped in their sterile drape. Leave everything wrapped until the person performing the LP is seated and begins examining the subject.
b. Feel the outside of the LP tray (still wrapped) to determine which end contains the spongy swabs used for application of topical disinfectant. Turn this end toward the person performing the LP and begin unwrapping the kit.

c. Touch only the outside of the paper wrapper. When you unfold the sterile drape, 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 or outside of the wrapper touches any part of the inside of the wrapper, discard the tray and start over. If you are in doubt as to whether something touched the inside of the paper wrapper, throw the tray away and start over.

d. Be extremely careful not to touch any of the sterile items with a non-sterile gloved hand! Conversely, be sure not to touch anything not sterile with a sterile gloved hand.

See **Appendix K** for tips on patient care during and after the LP.
See **Appendix I** for specific instructions for the processing of CSF.

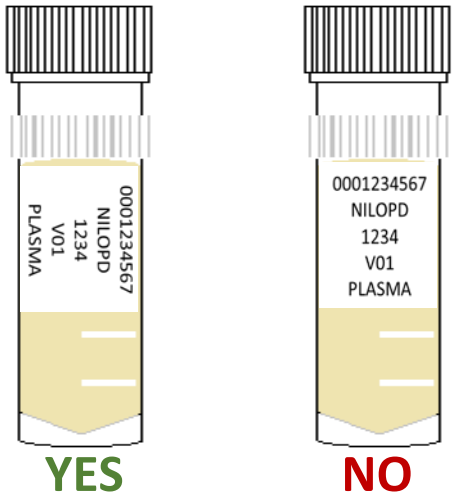
4.2 Labeling Specimens

In order to ensure the label adheres properly and remains on the tube:

- Place labels on **ALL** collection and aliquot tubes **BEFORE** specimen collection, processing, or freezing. This should help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.

- Place label ***horizontally*** on the tube (wrapped around sideways if the tube is upright) and ***just below the ridges*** of the aliquot tubes (see attached labeling diagram). There is enough space on the aliquot tube for the label to be placed without overlapping the ridges.

- Take a moment to ensure the label is ***completely adhered*** to each tube. It may be helpful to roll the tube between your fingers after applying the label.



4.3 Sample Collection Volumes

Sample Type	Tube Type	Amount
PK		
Whole blood for serum (PK)	SST (gold top)	3.5 ml
Biomarkers		
Whole blood for PBMCs	Sodium Heparin (green top)	10 ml
Whole blood for plasma & buffy coat	K3 EDTA (purple top)	20 ml
Whole blood for serum	Serum (red top)	10 ml
Whole blood to store for future research	K2 EDTA (purple top)	6 ml
Cerebrospinal fluid	N/A	15-20 ml
Clinical Safety Labs		
Hematology	K2 EDTA	3 ml
Chemistry, pancreatic enzyme panel, lipid profile, serum pregnancy	SST (red/black tiger top)	7.5 ml
Coagulation panel	Sodium Citrate (light blue)	2.7 ml
Hep B surface antigen, HCV, HIV 1/2	SST (gold top)	5 ml
HCV confirmation	SST (gold top)	3.5 ml
Cerebrospinal Fluid		
CSF for clinical lab	3 ml luer lock syringe/ 2 ml cryovial (purple top)	1-2 ml
CSF for biomarkers	50 ml conical tube/ 2 ml cryovials (clear top)	15-18 ml

4.4 Order of Blood Draw

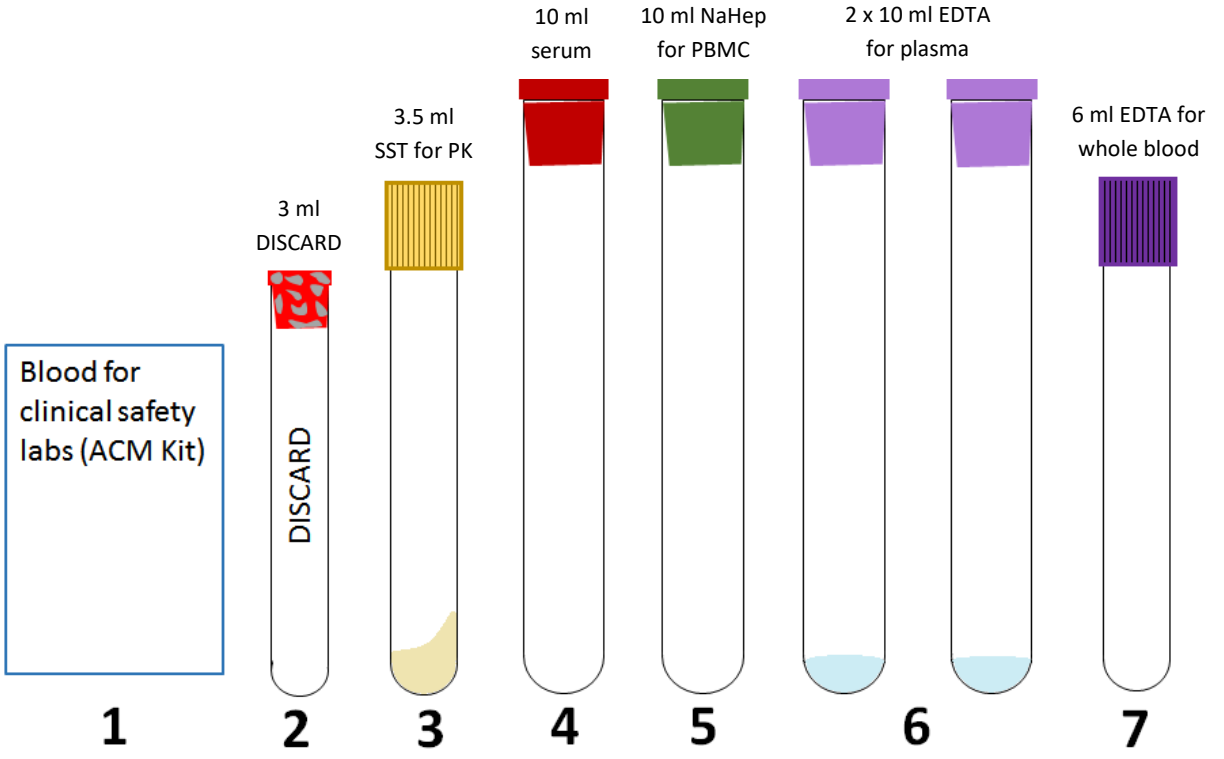
Tubes should be filled in the following order (see diagram on next page):

1. Blood for clinical safety labs ***MUST BE COLLECTED FIRST***
2. 1 x 3 ml discard tube
3. 1 (2 at V03) x 3.5 ml SST for serum PK
4. 1 x 10 ml serum for serum biomarkers
5. 1 x 10 ml sodium heparin for PBMCs
6. 2 x 10 ml EDTA for plasma and buffy coat
7. 1 x 6 ml EDTA for whole blood

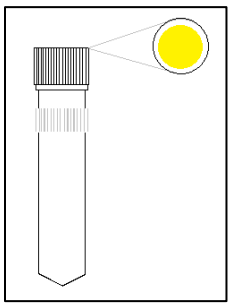
Draw order remains the same even when a visit does not require collection of all sample types. Simply skip the excluded tube(s).

For the complete PK and biomarker specimen collection schedule, please refer to the NILO-PD protocol and Section 8.0 of the Manual.

Correct blood draw order for PK and biomarker blood collection tubes:

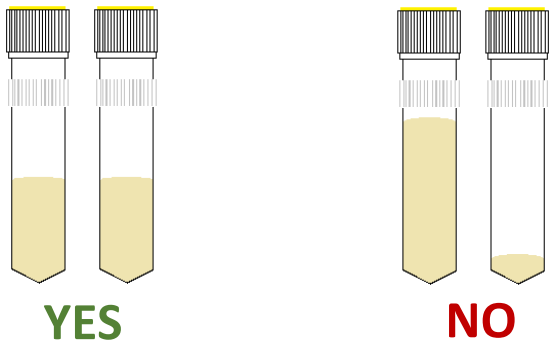


4.5 Filling Aliquot Tubes: PK Serum



The aliquot tubes for serum PK analysis are 2 ml tapered polypropylene cryotubes. The PK aliquot tubes have a round yellow label on the top of the cap (see picture, right). The serum collected for PK analysis should be split evenly between the two tubes PK aliquot tubes, provided. The SST tube used to collect serum for PK analysis should yield approximately 1.5 ml of serum, so each of the two aliquot tubes should be filled to approximately 0.75 milliliters.

Example of correct and incorrect aliquot volumes for PK serum samples:

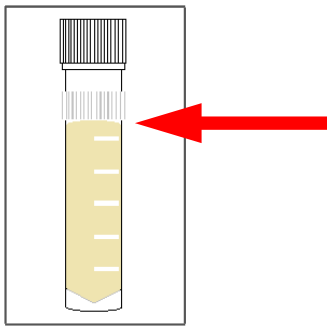


4.6 Filling Aliquot Tubes: Biomarker Specimens (Plasma, Buffy Coat, Serum, and CSF)

To assist in the preparation and aliquoting of the biomarker specimens, colored caps are used for the biomarker aliquot tubes. These aliquot tubes are 2 ml skirted (self-standing) polypropylene cryotubes. The chart below summarizes the correspondence between cap color and type of aliquot, if used.

Cap Color	Specimen Type
Purple	Plasma
Purple	CSF for local lab (optional)
Red	Serum
Clear	CSF
Clear	Buffy coat

In order to ensure that the biorepository receives a sufficient amount of the specimen for processing and storage, and to avoid cracking of the tubes prior to shipment, each aliquot tube should ideally be filled to 1.5 milliliters (see picture, right) after processing is completed (refer to detailed processing instructions per specimen type for average yield per specimen below). A 1.5 ml aliquot will reach the bottom of the ridged section of the cryovial, as shown. Over-filled tubes may burst once placed in the freezer, resulting in a loss of that specimen.



If there is biologic material remaining that will not fill a subsequent aliquot tube to 1.5 ml, that remaining amount should still be kept and sent in a partially filled aliquot tube.

All biomarker specimens should be shipped to the biorepository. Aliquot the recommended maximum volume into as many aliquot tubes as will allow after processing the biospecimen specimen. Fill as many tubes as possible with 1.5 ml of specimen. For example, if 3.7 ml of plasma is obtained, you should fill two cryovial tubes each with 1.5 ml, and one additional cryovial tube with the remaining 0.7 specimen volume (see example below).

Example of correct and incorrect aliquot volumes for biomarker samples:

YES

NO

5.0 Packaging and Shipping Specimens

Please refer to the applicable appendix for detailed shipping instructions.

*****Important Notes*****

Include only **one** subject visit set of specimens per shipping carton in order to have room for a sufficient amount of dry ice to keep specimens frozen for at least 48 hours.

Ship all specimens Monday through Wednesday ONLY! BE AWARE OF HOLIDAYS!!
Specimens must be shipped within TWO WEEKS of collection.

Remember to complete the Sample Record Summary and Shipment Notification Form, include a copy in your shipment AND notify Indiana University IN ADVANCE to confirm the shipment.

6.0 Specimen Quality Checks and Feedback to Sites

In addition to tracking and reconciliation of specimens, the condition and amount of specimens received is tracked by Indiana University for each specimen type received. Sites are responsible to ensure the requested amounts of each fluid are collected to the best of their ability and that specimens are packed well with sufficient amounts of dry ice to avoid thawing in the shipment process. Indiana University will complete a Non-Conformance Report should there be any issues with a shipment and will provide this feedback to the site. Issues of concern that may impact collection, processing, or future analyses of the specimens will be addressed by the study Steering Committee and communicated to sites.

7.0 Data Queries and Reconciliation

The applicable data form(s) regarding laboratory procedures must be completed on the day that specimens are collected, since they capture information related to the details of the specimen collection and processing. These forms include information that will be used to reconcile specimen collection and receipt, as well as information essential to future analyses. All data should be recorded on the data form(s) within 14 days of the subject visit per protocol.

Indiana University will be collaborating with the clinical core to reconcile information captured in the study database compared to specimens received and logged in at Indiana University. Information that appears incorrect in the study database will be queried and additional discrepancies that may be unrelated to data entry will be resolved with sites in a separate follow-up communication.

Data queries or discrepancies with specimens shipped versus received at Indiana University may result from:

- Missing specimens at Indiana University
- Incorrect specimens collected and shipped to Indiana University
- Damaged or incorrectly prepared specimens
- Unlabeled specimens, specimens labeled with incomplete information, or mislabeled specimens
- Discrepant information documented on the Sample Record Summary and Shipment Notification Form and logged in at Indiana University compared to information entered into the study database.

8.0 Specimen Collection Schedule

8.1 Cohort 1 collection schedule with blood volumes

	Cohort 1	Screening		Baseline	Titration Phase				Maintenance Period			Final visit on drug	Post-Drug Evaluation		Unschedul Visit	Premature W/D
		SC1	SC2	Visit BL	Safety visit	Visit 1	Visit 2	Safety visit	Visit 3	Safety visit	Safety visit	Visit 4	Visit 5	Visit 6		
				Week 0	SV 1	V01	V02	SV 2	V03	SV 3	SV 4	V04	V05	V06 (FNL)	(U01, U02, etc.)	PW
									3 month			6 month	1 month	3 month		
	Blood collection tubes	Day -28-7d	Day -14-7d	Day 0	Day 7±3d	Day 14±3d	Day 30±3d	Day 60±7d	Day 90±7d	Day 120±7d	Day 150± 7d	Day 180±7d	POST +4w ±7d	POST +12w ±7d		
PK	3.5 ml SST (serum PK)					1	1	1	2	1	1	1				1
Biomarkers	10 ml Serum tube (serum)			1					1			1	1			1
	10 ml EDTA (plasma & DNA)			2					2			2	2			2
	10 ml NaHep (PBMC)			1					1			1	1			1
	6 ml EDTA (whole blood to bank)			1					1			1	1			1
ACM Samples	3 ml EDTA (hematology)	1				1	1	1	1	1	1	1	1	1	1	1
	7.5 ml SST (chemistry, lipids)	1				1	1	1	1	1	1	1	1	1	1	1
	2.7 ml NaCit (coagulation)	1						1				1				
	5 ml SST (serology, Hep B & C, HIV)	1														
	3.5 ml SST (HCV confirmation)	1														
	Total blood draw volume	**21.7**	**0**	**46**	**0**	**14**	**14**	**16.7**	**63.5**	**14**	**14**	**62.7**	**56.5**	**10.5**	**10.5**	**60**
	CSF collection		**Yes**						**Yes**				**Optional**			**Yes**

8.2 Cohort 2 collection schedule with blood volumes

	Cohort 2	Screening		Baseline	Titration Phase				Maintenance Period							Final visit on drug	Symtomati Tx	Post-Drug Evaluation		Unschedul Visit	Premature W/D
		SC1	SC2	Visit BL	Safety visit	Visit 1	Visit 2	Safety visit	Visit 3	Safety visit	Safety visit	Visit 4	Safety visit	Safety visit	Visit 5	Visit 6		Visit 7	Visit 8		
				Week 0	SV1	V01	V02	SV02	V03	SV03	SV04	V04	SV5	SV6	V05	V06		V07	V08 (FNL)	(U01, U02, etc.)	PW
									3 month			6 month			9 month	12 month		1 month	3 month		
	Blood collection tubes	Day -28-7d	Day -14-7d	Day 0	Day 7+3d	Day 14±3d	Day 30±3d	Day 60±7d	Day 90±7d	Day 120±7d	Day 150± 7d	Day 180±7d	Day 210±7d	Day 240±7d	Day 270±7d	Day 365±7d		POST +4w ±7d	POST +12w ±7d		
PK	3.5 ml SST (serum PK)					1	1	1	2	1	1	1	1	1	1	1	1				1
Biomarkers	10 ml Serum tube (serum)			1					1			1				1	1	1			1
	10 ml EDTA (plasma & DNA)			2					2			2				2	2	2			2
	10 ml NaHep (PBMC)			1					1			1				1	1	1			1
	6 ml EDTA (whole blood to bank)			1					1			1				1	1	1			1
ACM Samples	3 ml EDTA (hematology)	1				1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	7.5 ml SST (chemistry, lipids)	1				1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	2.7 ml NaCit (coagulation)	1						1								1	1				1
	5 ml SST (serology, Hep B & C, HIV)	1																			
	3.5 ml SST (HCV confirmation)	1																			
	Total blood draw volume	**21.7**	**0**	**46**	**0**	**14**	**14**	**16.7**	**63.5**	**14**	**14**	**60**	**14**	**14**	**14**	**62.7**	**62.7**	**56.5**	**10.5**	**10.5**	**62.7**
	CSF collection		**Yes**						**Yes**								**Optional**	**Optional**			**Yes**

9.0 Appendices

Please see the applicable appendices for your specific project for information on sample collection, kit components and ordering, nonconformance, and shipping.

APPENDIX A

Rate of Centrifugation Worksheet

Please complete and return this form by fax or e-mail to the biorepository if you have any questions regarding sample processing. The correct RPM will be sent back to you. Make note of this in your Biologics Manual.

Submitter Information

Name: Site #:

Submitter e-mail:

Centrifuge information

Please answer the following questions about your centrifuge.

Centrifuge Type:

Fixed Angle Rotor Swing Bucket Rotor

Radius of Rotation (mm):

Determine the centrifuge's radius of rotation (in mm) by measuring distance from the center of the centrifuge spindle to the bottom of the device when inserted into the rotor (if measuring a swing bucket rotor, measure to the middle of the bucket).

Calculating RPM from G-force:

$$\boldsymbol{RPM} = \sqrt{\frac{\boldsymbol{RCF}}{\boldsymbol{r} \times 1.118}} \times \mathbf{1,000}$$

RCF = relative centrifugal force (G-force)

RPM = rotational speed (revolutions per minute)

R = centrifugal radius in mm = distance from the center of the turning axis to the bottom of the centrifuge

Comments:

Please send this form to the biorepository:

nilopd@iu.edu

APPENDIX B

NILO-PD Sample Record Summary and Shipment Notification Form

Site: _____ **Site Investigator:** _____

Coordinator: _____ **Telephone:** _____ **E-mail:** _____

Instructions: **Ship frozen samples Monday – Wednesday ONLY**. This form must be completed for all biorepository sample shipments. Notify Indiana University (e-mail preferred) prior to shipment using the contact information below. Place a copy of the completed form in the shipment box and also file a copy in the site study binder. The site will be contacted if any issues with the samples/form are noted upon receipt.

To be Completed by Submitter/Site List Subject ID that corresponds to pre-printed labels. List only one Specimen Type per row.						**To be Completed by Biorepository**
Subject ID	*Specimen Type (Plasma, Serum, buffy coat, CSF)*	*# of Tubes*	*Sex*	*Date of Draw*	*Tube Volume (if less than standard)*	*Notation of problems*
Total number of tubes:						

Date shipped: _____

FedEx Tracking #: _____-_____-_____

IMPORTANT!
BEFORE SHIPPING, EMAIL (PREFERRED) OR FAX A COPY OF THE COMPLETED FORM TO INDIANA UNIVERSITY:

E-mail: nilopd@iu.edu **Fax: 317-278-1100**

NILO-PD V03 Serum PK Time Point Form

Site: _____ Site Investigator: _____

Coordinator: _____ E-mail: _____

Instructions: **Please complete this form for V03 Serum PK samples.** Please complete only one form per subject. List the last 4 digits of the barcode on each Serum PK aliquot included in the shipment.

To be Completed by Submitter/Site							**To be Completed by Biorepository**
Subject ID	*Serum PK Collection Time Point*	*Time of Draw*	*Date of Draw*	*Tube Volume (if < 0.75 ml)*	*# of tubes*	*Last 4 digits of barcode*	*Notation of problems*
	PRE-DOSE TROUGH					Tube 1: Tube 2:	
	2 ± 0.5 HOURS POST OBSERVED DOSE					Tube 1: Tube 2:	

IMPORTANT!

BEFORE SHIPPING, EMAIL (PREFERRED) OR FAX A COPY OF THE COMPLETED FORM WITH THE *NILO-PD Sample Record Summary and Shipment Notification Form* TO INDIANA UNIVERSITY:

E-mail: nilopd@iu.edu **Fax: 317-278-1100**

APPENDIX C

NILO-PD Shipping Instructions

Frozen PK and Biomarker Specimen Shipping Instructions - Domestic

Samples Shipped on Dry Ice:

- Frozen plasma in 2ml polypropylene tubes
- Frozen serum in 2ml polypropylene tubes
- Frozen buffy coat in 2ml polypropylene tube
- Frozen CSF in 2ml polypropylene tubes
- Frozen whole blood in 6ml EDTA tubes

IMPORTANT!
FROZEN SAMPLES MAY BE SHIPPED MONDAY-WEDNESDAY ONLY! **Only ONE set of samples may be shipped in a single package.**

1. Contact FedEx to confirm service is available and schedule package to be picked up.

2. Notify Indiana University of shipment by e-mailing nilopd@iu.edu (preferred) or faxing (317-278-1100) a copy of the completed Sample Record Summary and Shipment Notification Form (Appendix B).

3. Place all frozen 2ml aliquot vials in the provided cardboard cryobox. Label the outside of the cryobox with the subject ID and visit number.

4. Place the cryobox into a clear plastic biohazard bag with the absorbent sheet and seal according to the instructions on the bag.

5. Place approximately 2-3 inches of dry ice in the bottom of the Styrofoam-lined shipping carton.

6. Place the biohazard bag containing the cyrobox into the Styrofoam-lined shipping carton, on top of the dry ice. Please ensure that the cryobox is placed so that the cryovials are upright in the shipping container.

7. Fill the remaining space in the shipping carton with dry ice, ensuring ice surrounds the bag and reaches the top of the carton, as shown below:

8. Replace the lid on the Styrofoam carton, place the completed Sample Record Summary and Shipment Notification Form on top of the carton, and close and seal the outer cardboard shipping carton with packing tape.

IMPORTANT!
Complete the required fields on the FedEx air waybill and Class 9 Dry Ice label, or FedEx may reject or return your package.

9. Complete the FedEx air waybill with the following information:
    a. Section 1, “From”: fill in your name, address, and phone number
    b. Section 6, “Special Handling and Delivery Signature Options”: under “Does this shipment contain dangerous goods?” check the boxes for “Yes, Shipper’s Declaration not required” and “Dry Ice”. Enter the number of packages (1) x the net weight of the dry ice in kg.

10. Complete the Class 9 UN 1845 Dry Ice label (black and white diamond) with the following information:
    a. Your name and return address
    b. Net weight of dry ice in kg
    c. Consignee name and address:

        NILO-PD Biorepository
        IU School of Medicine
        351 W. 10th St., Rm 342
        Indianapolis, IN 46202

    d. Do not cover any part of this label with other stickers, including pre-printed address labels.

11. Apply all provided warning labels and the completed FedEx air waybill to the outside of the package, taking care not to overlap labels.

12. Hold packaged samples in a -80°C freezer until the time of FedEx pickup.

Ambient Biomarker Specimen Shipping Instructions – Domestic

Samples Shipped Ambient:

- PBMC Green Top 10ml Sodium Heparin Blood Tube

IMPORTANT!
PBMC SAMPLES MUST BE COLLECTED AND SHIPPED MONDAY-THURSDAY ONLY!

1. Contact FedEx to confirm service is available and schedule package to be picked up.

2. Notify Indiana University of shipment by e-mailing nilopd@iu.edu (preferred) or faxing (317-278-1100) a copy of the completed Sample Record Summary and Shipment Notification Form (Appendix B).

3. Insert the green top Sodium Heparin tube in the absorbent tube sleeve. Place the sleeve into the canister and close securely. Wrap the canister in the enclosed bubble wrap and place into the cardboard box.

4. Apply the UN3373 label to the outside of the cardboard box.

5. Place the cardboard box and the completed Sample Record Summary and Shipment Notification Form in the FedEx Clinical Pak, making sure the UN3373 label is visible through the Clinical Pak, and seal according to the instructions on the envelope.

6. Complete the “From” portion of the provided FedEx air waybill by filling in your name, address, and phone number. FedEx is likely to reject or return your shipment without this information.

7. Apply the completed FedEx air waybill to the outside of package and arrange for FedEx pick up.

Sample collection and shipment **only** Monday – Thursday with same day shipment. Do NOT draw or ship ambient samples on Friday. Ship the sample(s) to Indiana University on the day of collection. Sample must be received at Indiana University the day after collection.

APPENDIX D

NILO-PD PK and Biomarker Kit Components

1.0 Blood Biomarker Collection and Shipping

Item	Quantity
Vacutainer® – Red/gray-top additive free discard tube (3 ml)	1
Monoject™ – Purple-top K3 EDTA tube (10 ml)	2
Vacutainer® – Red-top serum tube (10 ml)	1
Vacutainer® – Purple-top K2 EDTA tube (6 ml)	1
Vacutainer® – Green-top Sodium Heparin tube (10 ml)	1
Screw-top centrifuge tube (15 ml)	2
Transfer pipets (3 and 1 ml)	5
Cryogenic vials (2 ml) – 6 purple cap, 3 red cap, 2 clear cap	11
Cryobox, 25 cell	1
Plastic biohazard bag with absorbent sheet	1
IATA/DOT shipping label packet	1
Shipping instruction sheet	1
FedEx return airbill	2
Shipping container for dry ice shipments	1
Shipping container for ambient shipments	1
FedEx ClinPak	1

2.0 PK Serum Collection and Shipping

Item	Quantity
Vacutainer® – Red/gray-top additive free discard tube (3 ml)	1
Vacutainer® – Gold-top serum separator tube (SST, 3.5 ml)	1*
Transfer pipet (1 ml)	1
Cryogenic vials (2 ml) – clear cap with yellow label, conical bottom	2‡
Plastic biohazard bag with absorbent sheet	1
IATA/DOT shipping label packet	1
Shipping instruction sheet	1
FedEx return airbill	2
Shipping container for small dry ice shipments	1

* 2 provided for Visit 3 (V03)

‡ 4 provided for Visit 3 (V03)

3.0 CSF Collection

Item	Quantity
LP tray with 24 G Sprotte needle	1
Screw-top centrifuge tube (sterile, 50 ml)	1
Screw-top centrifuge tube (50 ml)	1
Screw-top centrifuge tube (15 ml)	2
Cryogenic vials (2 ml) – clear cap	12
Cryogenic vials (2 ml) – purple cap	1
Transfer pipet (3 ml)	2

4.0 Clinical Safety Labs Kit

To be provided by ACM Global Laboratories

5.0 Supplemental Kit

One Supplemental Kit will be provided to each site at study start up. This kit will provide replacement supplies, should they be needed.

Item	Quantity
Vacutainer® – Red/gray-top additive free discard tube (3 ml)	5
Monoject™ – Purple-top K3 EDTA tube (10 ml)	10
Vacutainer® – Red-top serum tube (10 ml)	5
Vacutainer® – Purple-top K2 EDTA tube (6 ml)	5
Vacutainer® – Green-top Sodium Heparin tube (10 ml)	5
Screw-top centrifuge tube (15 ml)	25
Transfer pipets (3 and 1 ml)	20
Cryogenic vials (2 ml) – purple cap	20
Cryogenic vials (2 ml) – red cap	20
Cryogenic vials (2 ml) – clear cap	20
Cryobox, 25 cell	5
Plastic biohazard bag with absorbent sheet	5
IATA/DOT shipping label packet	5
FedEx return airbill	5
Shipping container for small dry ice shipments	1
Shipping container for ambient shipments	2
FedEx ClinPak	2
Vacutainer® – Gold-top serum separator tube (SST, 3.5 ml)	10
Sprotte 24G x 3.5” needle with introducer	2
Screw-top centrifuge tube (sterile, 50 ml)	10
Screw-top centrifuge tube (50 ml)	10

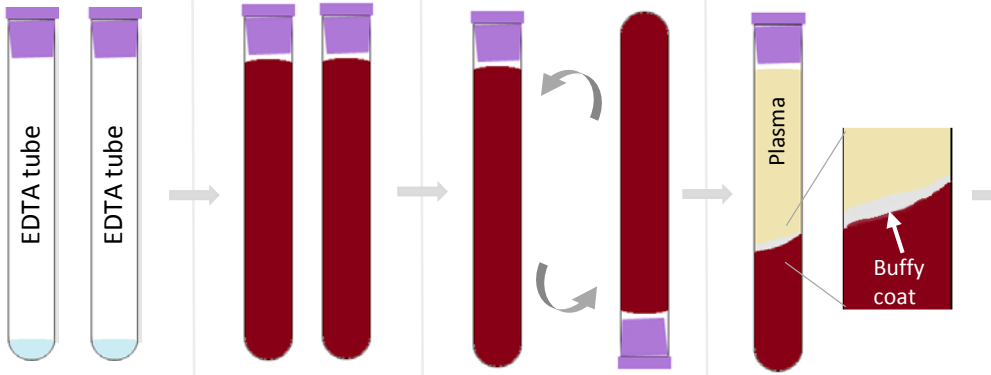
6.0 Kit schedule

The chart below shows the kits which will be supplied by Indiana University and ACM for each visit.

Collection kits by visit (Cohort 1):	*Screening visit 2* **SC2** Day -14±7d	*Baseline visit* **BL** Day 0	*Safety visit 1* **SV1** Day 7±3d	*Visit 1* **V01** Day 14±3d	*Visit 2* **V02** Day 30±3d	*Safety visit 2* **SV2** Day 60±7d	*Visit 3* **V03** Day 90±7d 3 month	*Safety visit 3* **SV3** Day 120±7d	*Safety visit 4* **SV4** Day 150±7d	*Visit 4* **V04** Day 180±7d 6 month	*Visit 5* **V05** Day 210±7d 1 mo. post	*Visit 6* **FNL** Day 270±7d	*Premature withdrawal* **PW**
Serum PK							x 2						
Blood Biomarkers													
CSF											Optional		Optional
ACM Kits	A	N/A	N/A	B	C	D	C	C	C	D	N/A	N/A	F

APPENDIX E

Plasma and Buffy Coat Collection and Preparation – 2 x 10 ml K3 EDTA (purple top) Tube

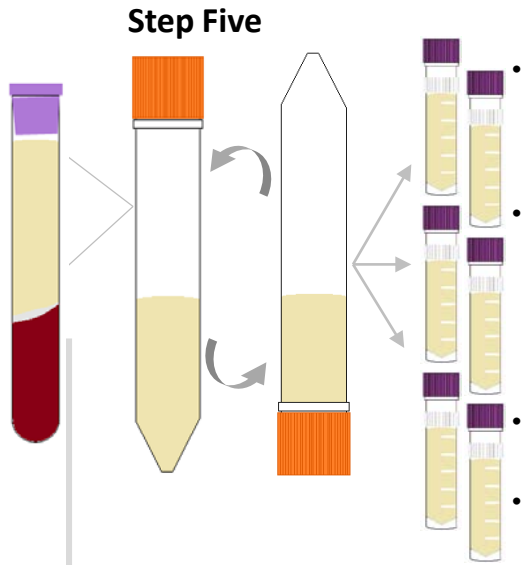


- Store tubes at room temperature.
- Label tubes with preprinted NILO-PD plasma label prior to blood draw.

- Collect blood in EDTA tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

- Immediately after blood draw, invert tubes 8-10 times to mix samples.

- Within 30 minutes of blood draw, centrifuge sample at 4°C at 1500 x g for 15 minutes.



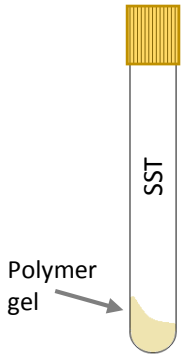
- Label purple-capped cryotubes with preprinted NILO-PD plasma labels.
- Using a clean transfer pipet, transfer *all* plasma from both tubes into a 15 ml conical tube and mix gently by inverting 3-4 times.
- Aliquot 1.5 ml plasma into each cryotube.
- Store plasma aliquots upright at -80°C until shipment.

- Label clear-capped cryotubes with preprinted NILO-PD buffy coat labels.
- Using a clean transfer pipet, collect the buffy coats (will include residual plasma and some red blood cells).
- Transfer each buffy coat to separate cryotubes.
- Store plasma and buffy coat aliquots upright at -80°C until shipment.

APPENDIX F

PK Serum Collection and Preparation – 3.5 ml SST (gold top) Tube

Step One

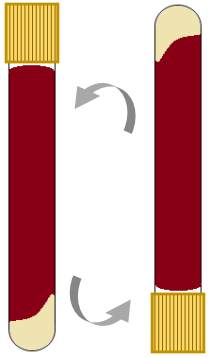


- Store tubes at room temperature.
- Label tubes with preprinted NILO-PD PK label prior to blood draw.

Step Two

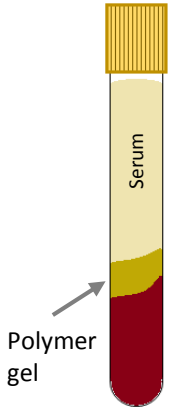
- Collect blood in serum tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



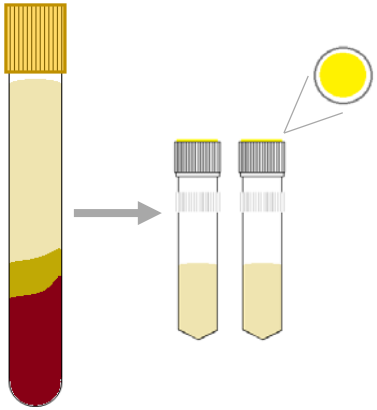
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Four



- ***Stand vertically*** and allow blood to clot at room temp. for 30 minutes
- Within 60 minutes of blood draw, centrifuge sample at <u>5°C</u> at <u>1100 x g</u> for <u>10 minutes</u>.

Step Five



- Label conical-bottom cryotubes (yellow dot on cap) with preprinted NILO-PD labels.
- Using a clean transfer pipet, aliquot approximately 0.75 ml into each cryotube.
- Within 90 minutes of blood draw, store serum aliquots <u>upright</u> at -80°C until shipment.

APPENDIX G

Biomarker Serum Collection and Preparation – 10 ml Serum (red top) Tube

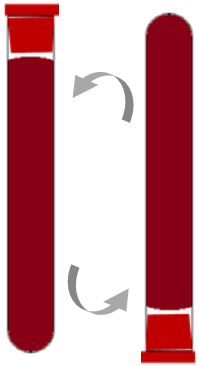
Step One

- Store tubes at room temperature.
- Label tubes with preprinted NILO-PD serum label prior to blood draw.

Step Two

- Collect blood in serum tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three

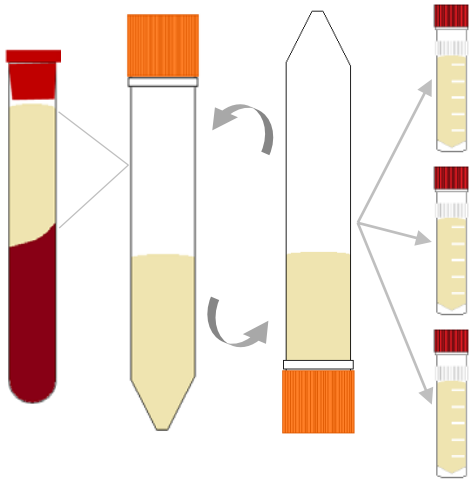


- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Four

- Allow blood to clot at room temp. for at least 15 minutes
- Within 60 minutes of blood draw, centrifuge sample at 4°C at 1500 x g for 15 minutes.

Step Five



- Label red-capped cryotubes with preprinted NILO-PD serum labels.
- Using a clean transfer pipet, transfer all serum into a 15 ml conical tube and mix gently by inverting 3-4 times.
- Aliquot 1.5 ml into each cryotube.
- Store serum aliquots upright at -80°C until shipment.

APPENDIX H

PBMC Collection and Preparation – 10 ml Sodium Heparin (green top) Tube

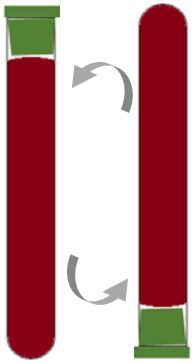
Step One

- Store tubes at room temperature.
- Label tubes with preprinted NILO-PD PBMC label prior to blood draw.

Step Two

- Collect blood in Sodium Heparin tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



- Immediately after blood draw, invert tubes 8-10 times to mix samples.

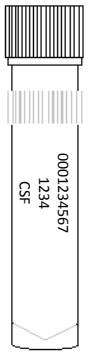
Step Four

- Store tubes at room temperature until shipment.
- ***Must be shipped ambient to IU on day of blood draw!***

APPENDIX I

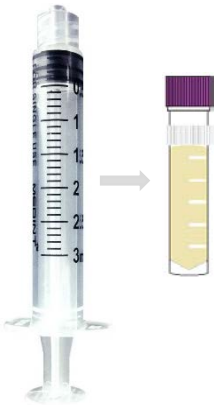
CSF Collection and Preparation (15-20 ml total)

Step One



- Label cryotubes with preprinted NILO-PD CSF labels prior to collection.
- ***Pre-chill all cryovials on wet ice.***

Step Two



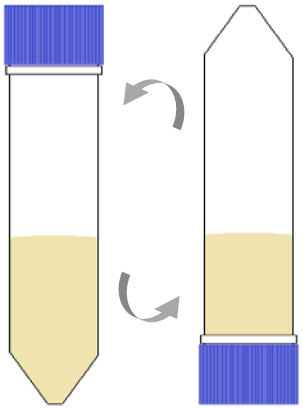
- Collect CSF into the 3ml luer lock syringe.
- Dispense 1-2 ml into the purple cap cryovial.
- Send to local lab for testing.

Step Three

- Collect CSF into the 6ml luer lock syringe.*
- Collect 15-20 ml total, including the 1-2 ml sent to the local lab.
- Transfer sample into 50 ml conical tube.

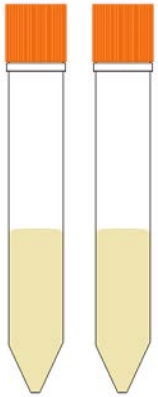
*Alternatively, collect CSF directly into 50 ml conical tube.

Step Four



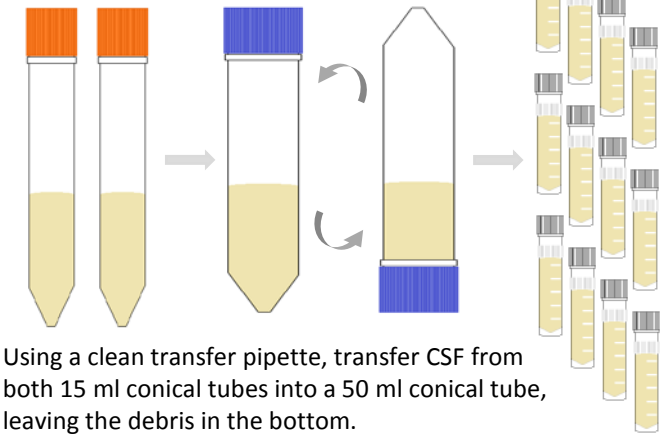
- Immediately after collection, gently invert the 50 ml conical tube 3-4 times to mix the sample.

Step Five



- Transfer CSF into <u>two</u> 15 ml conical tubes.
- Within 15 minutes of collection, centrifuge samples at room temperature at 2000 x g for 10 minutes.

Step Six



- Using a clean transfer pipette, transfer CSF from both 15 ml conical tubes into a 50 ml conical tube, leaving the debris in the bottom.
- Gently invert the 50 ml conical tube 3-4 times to mix the sample.
- Aliquot 1.5 ml into the clear cap cryotubes.
- Store CSF aliquots <u>upright</u> at -80^{o}C until shipment.

APPENDIX J

Whole Blood Collection and Preparation – 6 ml K2 EDTA (purple top) Tube

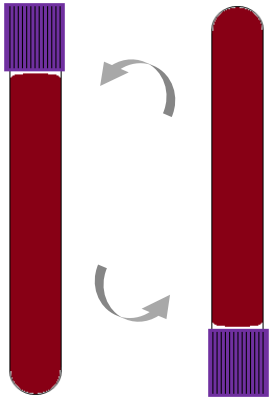
Step One

- Store tubes at room temperature.
- Label tubes with preprinted NILO-PD WB label prior to blood draw.

Step Two

- Collect blood in K2 EDTA tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Four

- Store tube upright at -80°C until shipment.

APPENDIX K

Lumbar Puncture Patient Care Tips

Please review the following recommendations for optimizing patient comfort and minimizing the risk of adverse events.

Tips for Clinicians Performing Lumbar Puncture

1. Talk the patient through the procedure so that there are no surprises.
2. Use of a Sprotte 24g atraumatic spinal needle and careful technique are optimal for reducing post-LP headache risk. A Sprotte 22g atraumatic spinal needle may also be used at the physician's discretion.
3. 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 four corners. If the subject is thin, do not insert the deep infiltration needle OR the spinal introducer all the way. Use only about two thirds of their length to prevent entering the subarachnoid space with anything other than the atraumatic spinal needle.
4. Encourage the patient to increase fluid intake immediately following the procedure.
5. Be sure to give post-LP care instructions verbally to the subject (see below).

Post-LP Care Instructions

Advise the subject to refrain from exertion (e.g., exercise, housework, gardening, lifting, sexual activity, or any other strenuous activities) for 24 hours after the LP.

Advise the patient to continue with increased fluid intake.

Mild to Moderate headache is relatively common after a lumbar puncture and usually resolves within 3-4 days. To treat mild to moderate headache following the LP, the following may be recommended:

1. Limit physical activity as much as possible.
2. 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.
3. Acetaminophen should be used for symptomatic relief. If a subject cannot tolerate acetaminophen, ibuprofen should be used. Avoid aspirin. If these do not relieve the headache, acetaminophen with codeine or an equivalent could be considered.

If the headache becomes severe, posturally sensitive (relieved by supine posture), or is accompanied by nausea, vomiting, tinnitus, and/or visual disturbances, the patient should contact the site study staff for further instruction per standard clinical care.

APPENDIX L

Low Fat Diet Recommendations Prior to Sample Collection

Due to the interference of lipid content in specimens collected for biomarker evaluation, it is **strongly advised that samples be collected after an 8 hour fast (no food or drink except fluids such as water, tea, black coffee).** If fasting is not achievable, a subject should be on a low-fat diet for at least 8 hours prior to blood collection.

Below is a list of suggested sample menus that could be consumed prior to sample collection. These lists are not all inclusive and sites should use their best judgment in this process.

Sample Breakfast Items:	**Sample Lunch Items:**
Dry whole wheat toast Fruit salad • no dressing Clear tea or coffee • no milk or cream Fruit or vegetable juice	Turkey breast sandwich on whole wheat bread Lettuce, Tomato, and Mustard Clear beverage Flavored gelatin
Dry cereal • without nuts/no granola; no milk Clear tea or coffee • no milk or cream Fruit or vegetable juice	Plain pasta with plain marinara sauce • no butter or cheese Side of steamed vegetables or green salad Clear beverage Flavored gelatin
Plain oatmeal or other cooked whole grain cereal • topped with fresh or dried fruit • no butter, milk, or cream Clear tea or coffee • no milk or cream Fruit or vegetable juice	Steamed chicken breast • lean, without skin Side of steamed vegetables or green salad Clear beverage Flavored gelatin
Dry whole wheat toast Poached egg white or egg substitute Clear tea or coffee • no milk or cream Fruit or vegetable juice	Large tossed green salad, assorted vegetables • no dressing or cheese Clear beverage Flavored gelatin
	Cucumber sandwich on whole wheat bread Lettuce, tomatoes, shredded carrots, onions, etc. Clear beverage Flavored gelatin
	Clear broth with vegetables and pasta Fruit salad • no dressing Clear beverage Flavored gelatin

APPENDIX L

Low Fat Diet Recommendations

Foods to avoid prior to sample collection:

Avoid: All fats and nuts such as:

- Butter
- Cream
- Bacon fat
- Lard
- All oils
- All margarine
- All nuts
- Peanut butter
- Coconut
- Whole seeds such as pumpkin and sunflower

Avoid: All milk and dairy products such as:

- All whole milk products
- All cheese
- All products containing cheese
- Cheese spreads such as cream cheese
- Sour cream
- All ice cream
- Milk chocolate

Avoid: High fat prepared foods and foods naturally high in fat:

- All red meats or meats containing fat such as pork
- Fatty meats such as:
    - Luncheon meats
    - Organ meats
    - Bacon
- Fatty fish such as:
    - Salmon
    - Mackerel
- Salad dressing and mayonnaise
- Buttered, au gratin, creamed, or fried vegetables
- Fried foods
- Fried snacks such as:
    - Chips
    - Crackers
    - French fries
- Gravies and sauces
- Baked goods and frosting