National Institute of Neurological Disorders and Stroke (NINDS)

National Institutes of Health (NIH)

NINDS Repository Biomarkers Discovery Samples Resource

Research Biomarkers Laboratory Manual Guidelines

Protocol #: 001

11/12/2

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1 NINDS Repository Laboratory Contacts

Contact information for the NINDS Repository and research laboratories at Coriell:

Study Support:

Matthew Self, Repository Core
Phone #: 856-757-9742

Chi Tarn, Repository Core
Phone #: 856-757-9726

After Hours Phone #: 609-709-1373

Study Support E-mail: ninds@coriell.org

Study Support Fax #: 856-966-5067

Mailing Address:

Coriell Institute for Medical Research
403 Haddon Avenue
Camden, NJ 08103

2 NINDS Repository Laboratory Information

2.1 Hours of Operation

Coriell operates from 9 AM to 5 PM Eastern Time, Monday through Friday.

2.2 Holiday Schedules

Please note that courier services may observe a different set of holidays. Please be sure to verify with your courier's schedule prior to any holiday.
Frozen samples must be shipped Monday – Wednesday only.
Frozen samples must be shipped within two weeks of sample collection; if frozen samples are not shipped immediately please ensure adequate storage at -80°C prior to shipment.

Ambient samples may be shipped Monday – Friday (preferably Monday – Thursday) provided they are received at Coriell within 5 days of collection.

Weekend/holiday delivery must be arranged in advance with Coriell.
*Additional information about shipping restrictions will be provided as necessary.

2.3 Holiday Observations* – United States

Date	Holiday
Thursday, Nov 22, 2012	Thanksgiving
Friday, Nov 23, 2012	Day after Thanksgiving
Tuesday, Dec 25, 2012	Christmas Day
Tuesday, Jan 1, 2013	New Year's Day
Monday, Jan 21, 2013	Martin Luther King Day
Monday, May 27, 2013	Memorial Day
Thursday, July 4, 2013	Independence Day
Monday, Sept 2, 2013	Labor Day
Thursday, Nov 28, 2013	Thanksgiving Day
Thursday, Nov 29, 2013	Day after Thanksgiving
Wednesday, Dec 25, 2013	Christmas Day

3 Research and Clinical Laboratory Collection Schedule

3.1 Biospecimens to be sent for Research (NINDS Repository at Coriell) or Clinical (Local) Labs:

Biospecimens

Biospecimens collected may include whole blood, plasma, cerebrospinal fluid (CSF), and may also include serum; all planned submissions to the NINDS Repository, including specific protocols developed following these guidelines, by project (not for each submission instance) are subject to prior approval by the NINDS.

Note: Skin biopsies for fibroblasts may be appropriate for special populations, such as those with known genetic variants causal for disease; collection of these or other biospecimens must be justified in the application and will require programmatic approval.

Consent forms must make it clear that any biological samples and de-identified clinical data will be shared with academics or industry and must be consistent with the NINDS Repository and NINDS data management resource consent requirements. A copy of the consent form for each subject should be kept on file by the investigator, and a blank approved consent at the NINDS Repository, but these do not need to be sent with each sample.

Newly collected room temperature blood samples for DNA extraction must be submitted to the NINDS Repository within 24 hours of each subject visit; new frozen samples are to be submitted ASAP according to the schedule in Section 2.2 above. Existing sample collections may be submitted to the Repository if specific conditions for NINDS approval are met. In general, the following volumes must be submitted to the NINDS Repository for each sample type:

- Plasma/Serum: minimum 6 milliliters
- Whole blood, room temp.: (for DNA extraction) minimum 6 milliners (initial visit only)
- Frozen whole blood for other studies minimum 6 milliliters (in addition to that for DNA at initial and also other than initial visits)
- PAXgene™ tube (for RNA) minimum 8 milliliters (optional - for use if doing RNA studies)
- CSF: minimum 10 milliliters (local analyses to include cell count, total protein, glucose, submitted to DMR)

All fibroblasts must be sent as skin biopsies directly to the NINDS Repository; please contact NINDS program staff for approval if you want to submit this type of material. Fibroblasts already established in culture will not be accepted except under special circumstances, requiring NINDS approval. All biospecimens submitted must be accompanied by a full set of clinical data elements submitted to the Data Management Resource (DMR) as defined above.

Guidelines for the processing, storage location and timing of sample collection are in the order in which they appear in the tables below.

Biannual Biospecimen Collection Visits (Baseline and Follow-up Visits):

Sample Type	Tube Type	Number of Tubes Supplied in Kit	Processing/ Aliquoting	Tubes Shipped to Coriell	Tubes Retained at Site	Tubes Shipped to Local Lab
Whole-blood: for RNA extraction	**2.5 ml PAXgene™ Tube**	**4**	**N/A**	**3**	**1**	0
Whole-blood; no processing	**6 ml Lavender Top EDTA Tube**	**1**	**N/A**	**1**	**0**	0
Whole-blood: for isolation of plasma	**2 ml microcentrifuge tubes**	**18**	**1 ml plasma aliquots in each 2 ml microcentrifuge tube**	**6**	**0-12**	0
	10ml Lavender Top EDTA Tube	**3**	**N/A**	**N/A**	**N/A**	0
Whole-blood: for isolation of serum*	**2 ml microcentrifuge tubes**	**18**	**1 ml serum aliquots in each 2 ml microcentrifuge tube**	**6**	**0-12**	0
	10ml Red Top Tube	**3**	**N/A**	**N/A**	**N/A**	0
Whole-blood: for extraction of DNA**	**8.5 ml Yellow Top ACD Tube**	**1**	**N/A**	**1**	**0**	0
TOTAL		**48†**		**17†**	**1-25†**	

*Serum collection is optional as per site discretion; tubes required for collection/aliquoting of serum will be supplied by the NINDS Repository at Coriell upon request

** Whole-blood for extraction of DNA (yellow-top ACD tube) is required only at the Baseline Visit (Visit 01); all subsequent visits will not include this sample

†Dependent upon inclusion of whole-blood sample for DNA extraction and/or serum collection

Annual CSF collection

CSF collection is to be once a year at visit of the site's choosing (three years total unless otherwise specified):

Sample Type	Tube Type	Number of Tubes Supplied in Kit	Processing/ Aliquoting	Tubes Shipped to Coriell	Tubes Retained at Site	Tubes Shipped to Local Lab
CSF	50 ml Conical Tube	1	Combine and mix total CSF	N/A		
	15 ml conical tubes	2	Divide and spin total CSF			
	2 ml microcentrifuge tubes	20	1 ml CSF aliquots in each 2 ml microcentrifuge tube	10	0-10	0
	2 ml purple-top microcentrifuge tube	1	1 ml CSF aliquots in 2 ml purple-top microcentrifuge tube	0	0	1

If a sample is not obtained at a particular visit, this should be recorded on the **Sample Record Summary and Shipment Notification Form** and submit to the NINDS Repository and a reason should be provided.

4 Specimen Collection Kits and Supplies

Research specimen collection kits as well as clinical lab supplies (except dry ice) will be provided to you by the NINDS Repository at Coriell with materials needed for blood and CSF collection, containers for plasma and CSF aliquots, as well as shipping labels to send materials back to the research laboratory. Tube labels will be provided to you by the NINDS Repository. Labels will be pre-printed with study information specific to the type of sample being drawn. Ensure that all tubes are properly labeled during processing and at the time of shipment (see Appendix J).

4.1 NINDS Repository at Coriell – Specimen Collection Kit Contents

Collection kits contain the following (for each subject) and provide the necessary supplies to collect samples from a given subject. NINDS biomarkers sample discovery kit components have been carefully selected to suit the needs of this project. Do not replace or supplement any of the tubes or kit components provided by Coriell with your own supplies unless you have received approval from NINDS/NINDS Repository at Coriell to do so. Note that "supplemental" kits will be provided to project sites should you require additional supplies from those contained in the visit specific kits. See Section 7.1 for LP Kit contents.

Baseline Kit Supplies

Quantity	Baseline Kit Components
36	Polypropylene microcentrifuge tubes (2 ml)*
4	PAXgeneTM blood collection tube (2.5 ml)
3	EDTA (lavender top) blood collection tube (10 ml)
3	Serum determination tube (red top) (10 ml)**
2	Air waybill (pre-filled)
2	Shipping instruction sheet
2	Plastic Biohazard bag
2	Tyvek envelope
1	EDTA (lavender top) blood collection tube (6 ml)
1	ACD Sol A (yellow top) blood collection tube (8.5 ml)
1	Shipping carton & plastic/foam liner (to be supplied in a separate box; for shipment of yellow-top ACD tube)
1	Overpack: Biological Substance Category B (for shipment of yellow-top ACD tube in shipping carton & plastic/foam liner)
1	Bubble wrap pouch
1	Shipping box/Styrofoam container
1	Microcentrifuge tube box
1	Warning label packet with dry ice sticker

*Number of aliquot tubes dependent upon inclusion of serum collection tubes
**Serum collection tubes supplied only upon request from site

Follow-up Kit Supplies

Quantity	Follow-up Kit Components
36	Polypropylene microcentrifuge tubes, sterile (2 ml)*
4	PAXgeneTM blood collection tube (2.5 ml)
3	EDTA (lavender top) blood collection tube (10 ml)
3	Serum determination tube (red top) (10 ml)**
2	Plastic Biohazard bag
2	Tyvek envelope
1	Air waybill (pre-filled)
1	Bubble wrap pouch
1	Microcentrifuge tube box
1	Shipping box/Styrofoam container
1	Shipping instruction sheet
1	Warning label packet with dry ice sticker

*Number of aliquot tubes dependent upon inclusion of serum collection tubes
**Serum collection tubes supplied only upon request from site

CSF Kit Supplies

Quantity	Follow-up Kit Components
20	Polypropylene microcentrifuge tubes, sterile (2 ml)
2	Conical centrifuge tube (15 ml)
1	CSF lab (purple top) microcentrifuge tubes, sterile (2 ml)
1	Conical centrifuge tube (50 ml)
1	Lumbar puncture tray

Supplemental Supplies

Quantity	Supplemental Kit
200	Polypropylene microcentrifuge tubes (2 ml)
20	PAXgeneTM blood collection tube (2.5 ml)
15	EDTA (lavender top) blood collection tube (10 ml)
15	Serum determination tube (red top) (10 ml)
10	Conical centrifuge tube (15 ml)
5	EDTA (lavender top) blood collection tube (6 ml)
5	ACD Sol A (yellow top) blood collection tube (8.5 ml)
5	CSF lab (purple top) microcentrifuge tubes, sterile (2 ml)
5	Conical centrifuge tube (50 ml)
1	Box of individually wrapped pencil point spinal needles, 24 G x 3.5 in. (0.55 mm x 90 mm)
1	Box of Introducer needles, 20 G x 1.25 in. (0.90mm x 32mm)

Each Site Will Need To Provide:

Dry ice
Tourniquet
Alcohol Prep Pad
Gauze Pad
Bandage
Butterfly needles
Microcentrifuge tube rack
Crushed Ice
Gloves
Sharps bin and lid
Micropipette and 1 ml micropipette tips

4.2 NINDS Repository at Coriell – Initial Supply

Each site will be initially supplied 7 baseline visit kits and 7 CSF kits. This means project sites will have sufficient baseline visit kits (including collection supplies for serum) and CSF kits for seven subjects. Project sites will also be supplied with one package of supplemental supplies. Subsequent kits should be ordered from The NINDS Repository at Coriell when in anticipation of need.

4.3 NINDS Repository at Coriell – Resupply

Each individual site will be responsible for ordering additional kits from The NINDS Repository at Coriell after the initial supply has been sent. QUEUE is The NINDS Repository at Coriell's online database that you will use for ordering collection kits. Once a site is activated by the NINDS, the site coordinator/s will be provided with a username and password to access the database.

1. Log in to QUEUE with your username and password at: https://queue.coriell.org/q/.
    - Under Contracts link in the upper left-hand corner of the screen, select the appropriate project.
    - A menu will appear; click on the Kit Request link.
2. Use the drop-down menu on the Kit Request page to select where the supplies should be shipped. If the shipping address(es) on the list is not the one you need, contact ninds@coriell.org
3. Select the type of kit required from the drop-down menu
4. Enter any special requests/remarks in the Request field.
5. Click the Submit button.

RESUPPLY: Be sure to check your supplies and order additional materials before you run out so you are prepared for both scheduled and unanticipated visits. Please allow **TWO weeks** for kit orders to be processed and delivered.

5 Site Required Equipment

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

4°C Refrigerated and Room Temperature Centrifuge
-80°C Freezer

6 Blood Collection and Processing Procedures

Blood samples at follow-up visits should be collected in the morning between 8 am – 10 am, preferably fasted. If fasting is not feasible, the low fat diet should be followed (see Appendix L). Record time of last meal (and whether low fat diet followed, if applicable) on the Laboratory Procedures data form.

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

In order to ensure the highest quality samples are collected, processed and stored, it is essential to follow the specific collection, processing and shipment procedures detailed in the following pages.

SPECIFIC INSTRUCTIONS FOR COLLECTION AND PROCESSING OF EACH SAMPLE ARE DETAILED ON THE FOLLOWING PAGES. See Appendix K for lab flow worksheet that may be used for processing of all lab samples.

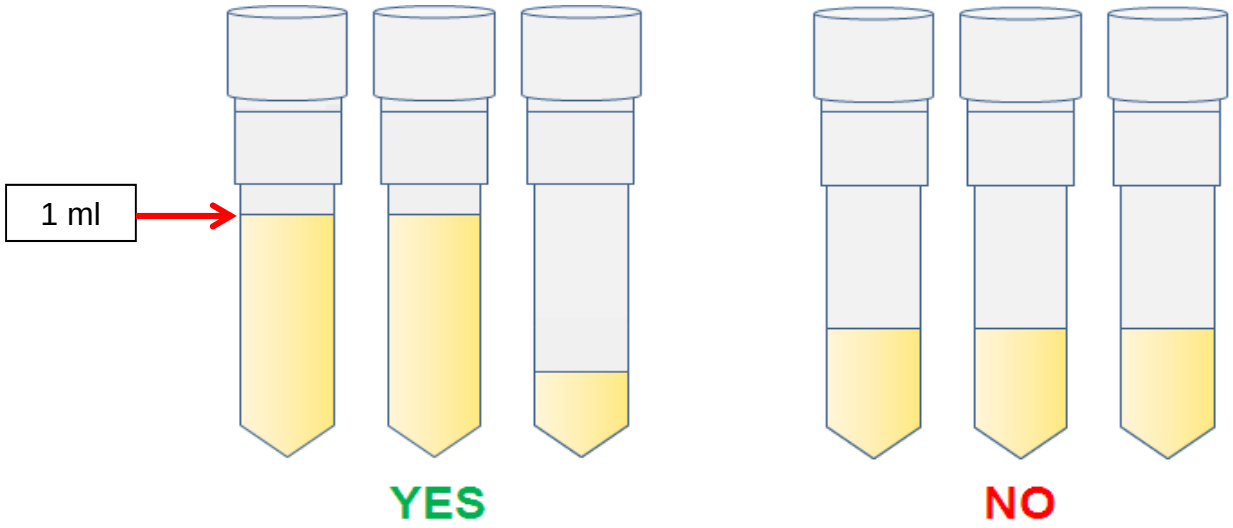
6.1 Labeling Samples

In order to ensure the label adheres properly and remains on the tube, please follow these instructions (see Appendix J diagram):

- Place labels on **ALL** collection and aliquot tubes **BEFORE** any cooling of tubes, sample collection or sample processing/freezing. This should help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.
- Place label **horizontal** on the tube (wrapped around sideways if 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 help to roll the tube between your fingers after applying the label.

6.2 Filling Aliquot Tubes (Plasma, Serum, and CSF)

In order to ensure that The NINDS Repository at Coriell receives a sufficient amount of the sample for processing and storage, and to avoid cracking of the tubes prior to shipment, each aliquot tube should ideally be filled to 1.0 milliliters (see picture below) with the respective biologic material after processing is completed (refer to detailed processing instructions for average yield per sample). Over-filled tubes may burst once placed in the freezer, resulting in a loss of that sample. If there is biologic material remaining that will not fill a subsequent aliquot tube to 1.0 ml, that remaining amount should still be included and shipped to The NINDS Repository at Coriell. Essentially, all material should be shipped to The NINDS Repository at Coriell, ensuring maximum amount in as many aliquot tubes as will allow after processing the sample. You do not have to fill all microcentrifuge tubes provided; you should attempt to fill as many tubes as possible with 1.0 ml of sample. For example, if 3.5 ml of sample is obtained, you should fill three microcentrifuge tubes each with 1.0 ml, and one additional microcentrifuge tube with the remaining 0.5 ml.

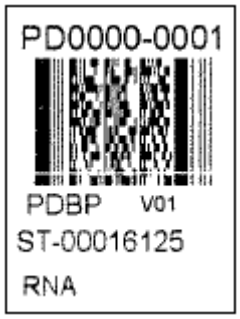


6.3 Whole Blood Collection for Extraction of RNA: PAXgene™ Tubes – optional, to be used if doing RNA studies

See training videos for blood collection (http://www.preanalytix.com/videos/rna-tube-collection-video/) and freezing (http://www.preanalytix.com/videos/rna-tube-freezing-video/).

***NOTE: The preanalytix training video recommends drawing the PAXgene blood tubes last in the draw order however for the purpose of this study, the PAXgene blood tubes should be drawn first.**

1. Place "**RNA**" label on the PAXgene RNA tubes prior to blood draw (per Section 6.1); no processing is required for these tubes, **three tubes are to be shipped as-is to The NINDS Repository at Coriell, one tube is to be retained at the collection site.**



2. CRITICAL STEP: **Store PAXgene™ Blood RNA Tubes at room temperature 64^{0}F - 77^{0}F (18°C to 25°C) before use.**

3. CRITICAL STEP: The **PAXgene™ Blood RNA Tubes should be the first tubes drawn** in the phlebotomy procedure.

4. Using a blood collection set and a holder, collect blood into the **first of the four PAXgene™ Blood RNA Tubes** using your institution's recommended procedure for standard venipuncture technique.

   The following techniques shall be used 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.

5. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The PAXgene™ Blood RNA Tube with its vacuum is designed to draw 2.5ml of blood into the tube. Record time of draw on Laboratory Procedures data form.

6. CRITICAL STEP: **Immediately after blood collection, gently invert/mix (180 degree turns) the PAXgene™ Blood RNA Tube 8 – 10 times**.

7. **REPEAT STEPS 4 TO 6** for the second and third PAXgene™ Blood RNA Tubes to be collected.

8. CRITICAL STEP: Incubate the PAXgene™ Blood RNA Tubes **UPRIGHT at room temperature (18°C to 25°C) for 24 hours**. Record time and date of draw on Laboratory Procedures data form.
   - *If blood is drawn on a Friday and you are unable to return on Saturday to place tubes in the freezer, transfer the tubes as late as possible before leaving on Friday.* ***Samples must sit at room temperature for a minimum of 2 hours.***

9. After **24 hours** at room temperature, transfer the three PAXgene tubes to **-80°C (minus eighty) freezer.** Keep the **PAXgene™ Blood RNA Tubes at -80 °C** until you ship on dry ice (three tubes are to be shipped to The NINDS Repository at Coriell, the fourth will remain at the collection site). Complete remainder of the Laboratory Procedures data form.

6.4 Whole Blood Collection – frozen blood submission: 6 ml EDTA Lavender Top Tube (to be shipped to the NINDS Repository as frozen whole-blood; no processing required)

1. Place pre-printed "**WB**" label on the 6 ml EDTA tube

2. CRITICAL STEP: **Store EDTA 6 ml Lavender Top Tube at room temperature 64^{0}F - 77^{0}F (18°C to 25°C) before use.**

3. Using a blood collection set and a holder, collect blood into the **6 ml EDTA tube** using your institution's recommended procedure for standard venipuncture technique.

   The following techniques shall be used 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.

4. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 6 ml of blood into the tube.

5. CRITICAL STEP: **Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tube 8 – 10 times.**

6. Freeze sample immediately following collection by transferring to **-80°C Freezer**. If sample cannot be immediately transferred to **-80°C Freezer**, prepare a sufficient amount of dry ice for immediate freezing. Store all samples at **-80°C Freezer**. Complete the remainder of the Laboratory Procedures data form and ensure timely entry and submission of data .

6.5 Whole Blood Collection for Isolation of Plasma: 10 ml EDTA Lavender Top Tube (for processing of plasma aliquots)

1. Place pre-printed "**PLSMA**" labels on the 2 ml microcentrifuge tubes

2. CRITICAL STEP: **Store EDTA 10 ml Lavender Top Tubes at room temperature 64^{0}F - 77^{0}F (18°C to 25°C) before use. Place <u>2 ml microcentrifuge aliquot tubes</u> on ice prior to procedure, <u>but after labeling</u>, so they are pre-cooled.**

3. Using a blood collection set and a holder, collect blood into the **10 ml EDTA tube** using your institution's recommended procedure for standard venipuncture technique.

   The following techniques shall be used 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.

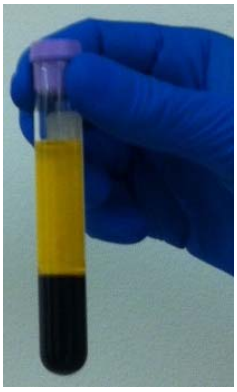
4. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.

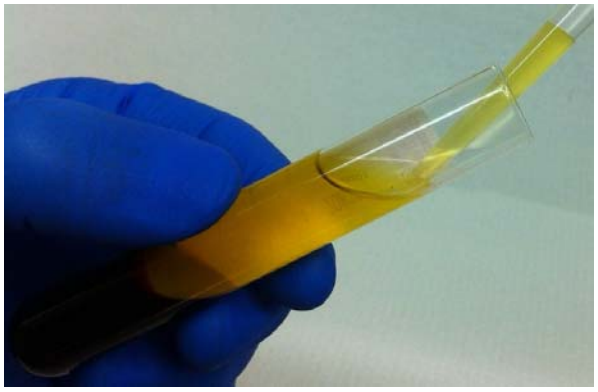
5. CRITICAL STEP: **Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tube 8 – 10 times.**

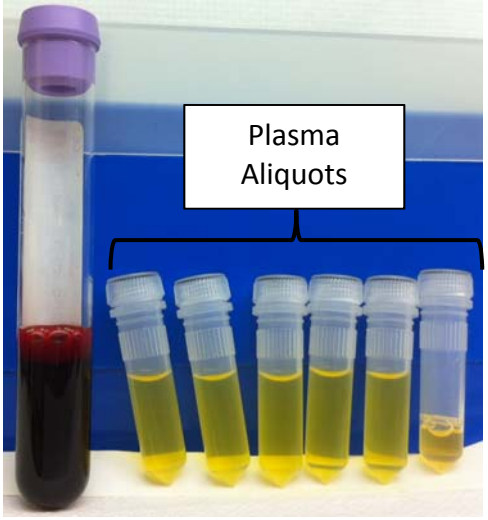
6. Within 30 minutes of blood collection, centrifuge balanced tubes at **4°C** for 15 minutes at 1500 x g. **It is critical that the tubes be centrifuged at the appropriate speed to ensure proper plasma separation.**

   - Equivalent rpm for spin at 1500 x g = _____
   - While centrifuging record the time of centrifuge start on the Laboratory Procedures data form.

7. Using a **micropipette**, transfer 1.0 ml of blood plasma (top layer) into each labeled, pre-cooled, aliquot tube. The EDTA tube should yield, on average, 4.5 ml of blood plasma for a total of 4-6 aliquot tubes per subject. Take caution not to disturb the pellet at the bottom of the tube by tilting the tube and placing the pipette tip along the lower side of the glass wall without touching the pellet so that plasma is not contaminated by pellet material (see below).







8. Freeze samples immediately following processing by transferring to **-80°C Freezer**. If samples cannot be immediately transferred to **-80°C Freezer**, prepare a sufficient amount of dry ice for immediate freezing. Store all samples at **-80°C Freezer**. Complete the remainder of the Laboratory Procedures data form and ensure timely entry of data.

6.6 OPTIONAL COLLECTION:

Whole Blood Collection for Isolation of Serum: 10 ml Red Top Tube (for processing of serum aliquots)

1. Place pre-printed "**SERUM**" labels on the 2 ml microcentrifuge tubes

2. CRITICAL STEP: **Store 10 ml red top tubes at room temperature 64^{0}F - 77^{0}F (18°C to 25°C) before use. Place <u>2 ml microcentrifuge aliquot tubes</u> on ice prior to procedure, <u>but after labeling</u>, so they are pre-cooled.**

3. Using a blood collection set and a holder, collect blood into the **10 ml red top tube** using your institution's recommended procedure for standard venipuncture technique.

   The following techniques shall be used 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.

4. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.

5. CRITICAL STEP: **Immediately after blood collection, gently invert/mix (180 degree turns) the tube 8 – 10 times.**

6. Within 60 minutes of blood collection, centrifuge balanced tubes at **4°C** for 15 minutes at 1500 x g. **It is critical that the tubes be centrifuged at the appropriate speed to ensure proper serum separation.**

   - Equivalent rpm for spin at 1500 x g = _____
   - While centrifuging record the time of centrifuge start on the Laboratory Procedures data form.

7. Using a **micropipette**, transfer 1.0 ml of blood serum (top layer) into each labeled, pre-cooled, aliquot tube. The red top tube should yield, on average, 4.5 ml of blood serum for a total of 4-6 aliquot tubes per subject. Take caution not to disturb the pellet at the bottom of the tube by tilting the tube and placing the pipette tip along the lower side of the glass wall without touching the pellet so that plasma is not contaminated by pellet material (refer to images in section 6.5).

8. Freeze samples immediately following processing by transferring to **-80°C Freezer**. If samples cannot be immediately transferred to **-80°C Freezer**, prepare a sufficient amount of dry ice for immediate freezing. Store all samples at **-80°C Freezer**. Complete the remainder of the Laboratory Procedures data form and ensure timely entry of data.

6.7 BASELINE VISIT ONLY:

Whole Blood Collection for Extraction of DNA: Yellow Top ACD Tube (for ambient shipment to The NINDS Repository at Coriell)

Blood sample must be received at The NINDS Repository at Coriell within 5 days of being collected. Samples not received within 5 days of collection must be re-drawn at the site.

1. Place pre-printed "**DNA**" label on the ACD tube prior to blood draw (per Section 6.1); no processing is required for this tube, it will be sent as-is to **The NINDS Repository at Coriell**.

2. Using a blood collection set and a holder, collect blood into the ACD tube using your institution's recommended procedure for standard venipuncture technique.

   The following techniques shall be used 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.

3. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 8.5 ml of blood into the tube.

4. CRITICAL STEP: **Immediately after blood collection, gently invert/mix (180 degree turns) the ACD tube 8 – 10 times**

5. Seal the 1 ACD tube in the ambient shipment kit and complete the NINDS Sample Record and Summary Shipment Notification Form.

6. Ship the sample back to The NINDS Repository at Coriell at **room temperature** according to kit instructions within 12 hours of collection. If sample cannot be shipped the same day as collected, hold at room temperature until shipping can be arranged. **Sample must be received at The NINDS Repository at Coriell within 5 days of being collected.**

7. Complete the DNA Sample source worksheet and ensure timely entry of data .

7 Cerebrospinal Fluid Collection:

CSF collection is to be once a year at visit of the sites choosing (three years total unless otherwise specified)

CSF should be collected in the morning between 8 am – 10 am, preferably fasted. If fasting is not feasible, the low fat diet should be followed (see Appendix L). Record time of last meal (and whether low fat diet followed, if applicable) on the Lumbar Puncture data form.

7.1 Lumbar Puncture Supplies

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. Supplies for collection of CSF are sent from The NINDS Repository at Coriell to project sites in a separate kit. .

Lumbar Puncture Tray

Quantity	Kit Component
1	Sprotte Spinal Needle, 24G x 90 mm
1	Introducer needle, 1mm x 30mm
1	22G x 1.5 in. Needle
4	Plastic Syringes (5 ml, Luer Lock)
1	Plastic Syringe (3 ml, Luer Lock) with 25G x 5/8 in. Needle (Attached)
1	Needle stick pad
1	Adhesive bandage
1	Fenestrated Drape
2	Towels
6	Gauze Sponges
3	Sponge Applicators
1	Lidocaine HCl (1%), 5 ml
1	Povidone-Iodine Solution, 0.75 oz

7.2 CSF Kits - Initial and Resupply

Each site will be initially supplied 7 CSF kits. Subsequent CSF kits should be ordered from The NINDS Repository at Coriell when needed (see Section 4.3 for instructions to access Queue).

7.3 Setting up for the LP

On an overbed table, remove the contents of the LP kit from 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.

Feel the outside of the LP kit (still wrapped up) to determine which end contains the spongy swabs. Turn this end toward the person performing the LP 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.

Maintaining the sterile field
Keep in mind that there is usually a lot of staff in the room during an LP, and a big part of assisting with the LP is keeping the field sterile, and 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 LP 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.

7.4 Tips for clinicians performing lumbar puncture

Optimizing patient comfort and minimizing risk of adverse events.

- Talk the patient through the procedure - no surprises.

- Use of a Sprotte 24G x 90 mm atraumatic spinal needle and careful technique are optimal for reducing post-LP headache risk. A pencil point spinal needle such as Spinocan, 24G x 90 mm is used.

- Use adequate local anesthesia. Use the 22G x 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. 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 24G pencil point spinal needle).

- Increasing fluid intake immediately after LP is helpful.

- Be sure to give post-LP care instructions verbally to subject (see below).

7.4.1 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 subject to continue with increased fluid intake.

<u>Mild to Moderate headache after a lumbar puncture:</u>

- Mild to moderate headache following lumbar puncture usually resolves within 3-4 days.
- 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.

<u>Severe headache after a lumbar puncture:</u>

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

7.5 Detailed Lumbar Puncture Procedure

CSF is processed at Room Temperature [64^0F - 77^0F (18°C to 25°C)]. Also, a portion of the CSF must be sent to your clinical lab and analyzed within 4 hours of collection.

1. Place "CSF" label on the 2 ml microcentrifuge aliquot tubes (per section 6.1). Prepare at least 20 aliquot tubes (including purple-top tube for local lab analysis) based on the collection of 15-20 mls of CSF. **Unlike the plasma and serum aliquot tubes, the CSF aliquot tubes should remain at room temperature, not pre-cooled**.

2. Perform lumbar puncture using the atraumatic technique.

3. Collect CSF into syringes (if a noticeably bloody tap, discard first 1-2 mls). After the LP has begun and fluid is being collected, take the first 1 ml of CSF from the first syringe and place in the **CSF labs tube (1 ml in purple-top microcentrifuge tube)**, and send it to the **local lab** for routine diagnostic tests. **Do not freeze this sample.**

   - Send at room temperature to local clinical lab for basic CSF analyses.
     NOTE: Sample must be analyzed within 4 hours of collection.
     1. Cell count (erythrocytes first)
     2. Total protein
     3. Glucose

4. Collect an additional 15-20 mls of CSF and transfer to 50 ml conical polypropylene tube; mix gently by inverting 3-4 times. Record time of draw (i.e., once collection is complete) on the Lumbar Puncture data form.

5. Within 15 minutes of collection, transfer the CSF from the 50 ml conical tube to the two 15 ml conical tubes ensuring that there is equal volume in each 15 ml conical tube. Spin the CSF sample at 2000 x g for 10 minutes at **Room Temperature** [64^0F - 77^0F (18°C to 25°C)].
   - Equivalent rpm for spin at 2000 x g = _____
   - While centrifuging record the time of centrifuge start on the Laboratory Procedures data form.

6. Using a **micropipette**, transfer 1.0 ml of supernatant directly into the polypropylene CSF collection aliquot tubes (clear-top, 2 ml microcentrifuge tubes). This will yield, on average, 15-20 aliquot tubes per subject.

8. Freeze samples immediately following processing by transferring to -80°C Freezer. If samples cannot be immediately transferred to -80°C Freezer, prepare a sufficient amount of dry ice for immediate freezing. Store all samples at -80°C Freezer until you ship on dry ice. Complete the remainder of the Laboratory Procedures data form and ensure timely entry and submission of data.

7. 8 Packaging Instructions

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

Ambient shipments (yellow-top ACD tubes) must be received at The NINDS Repository at Coriell within five days of collection. Up to three yellow-top ACD tubes can be shipped in one ambient shipping container.

For frozen shipments, include no more than two packing envelopes per shipping container in order to have room for a sufficient amount of dry ice to keep samples frozen up to 24 hours.

Include no more than one subject-set of samples per shipping container

Shipping Instructions

Baseline Shipments Only

- **DNA Yellow Top ACD Blood Tube (AMBIENT SHIPMENT)**

Baseline and Follow-up Shipments

- **Frozen PAXgene Tubes (FROZEN SHIPMENT)**
- **Frozen lavender top whole-blood tube (FROZEN SHIPMENT)**
- **Frozen 1 ml aliquots of plasma (FROZEN SHIPMENT)**
- **Frozen 1 ml aliquots of serum (FROZEN SHIPMENT) - optional**

The following items may be part of frozen shipments

- **Frozen 1 ml aliquots of CSF (FROZEN SHIPMENT)**

IMPORTANT!
FROZEN SAMPLES MUST BE SHIPPED MONDAY-WEDNESDAY ONLY! **Include no more than ONE set of samples per shipping carton.** **Include no more than TWO packing envelopes per shipping container.**
AMBIENT SAMPLES MAY BE SHIPPED MONDAY-FRIDAY (PREFERABLY MONDAY-THURSDAY) PROVIDED THEY ARE RECEIVED AT THE NINDS REPOSITORY AT CORIELL WITHIN 5 DAYS OF COLLECTION

Sample Packaging and Shipment Instructions

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

2. Notify The NINDS Repository at Coriell of shipment by emailing the NINDS Repository at ninds@coriell.org (preferred) or faxing (856) 966-5067 a copy of the completed Sample Record Summary and Shipment Notification Form.

- **DNA Yellow Top ACD Blood Tube (AMBIENT SHIPMENT; BASELINE ONLY)**

3. Insert yellow top ACD tube in the foam-lined plastic box and close securely.

4. Place the plastic container into the clear plastic bag and seal. Enclose this sealed bag in the cardboard shipping container.

5. Apply the UN3373 label to the outside of the cardboard container.

6. Place the cardboard shipping container 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.

7. 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.

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

9. Ship the sample to The NINDS Repository at Coriell on the day of collection. If sample cannot be shipped the same day as collected, hold at room temperature until shipping can be arranged. Sample must be received at The NINDS Repository at Coriell within 5 days of collection.

- **Frozen PAXgene Tubes (FROZEN SHIPMENT)**
- **Frozen lavender top whole-blood tube (FROZEN SHIPMENT)**
- **Frozen 1 ml aliquots of plasma (FROZEN SHIPMENT)**
- **Frozen 1 ml aliquots of serum (FROZEN SHIPMENT) - optional**

10. Insert PAXgene tubes, and lavender top whole blood tube into bubble-wrap slots.

11. Place bubble-wrapped tubes into the clear plastic biohazard bag (do NOT remove the absorbent material found in the bag) and seal according to the instructions on the bag. Insert this into the white Tyvek biohazard envelope and seal according to the instructions on the envelope.

12. Place the envelope upright in the provided Styrofoam-lined shipping carton, as shown below:

13. Place all frozen 1ml aliquots of plasma, serum (optional), and CSF (if obtained) in the provided cardboard cryobox. Label the outside of the cryobox with the subject ID (eight-digit “ST” number).

14. Place the cryobox in the 2nd clear plastic biohazard bag (do NOT remove the absorbent material found in the bag) and seal according to the instructions on the bag. Insert this into the 2nd white Tyvek biohazard envelope and seal according to the instructions on the envelope.

15. Place both envelopes upright and side-by-side in the provided Styrofoam-lined shipping carton, as shown below:

16. **<u>FILL</u> the remaining space in the shipping carton with approximately 10 lbs of dry ice, ensuring ice surrounds the envelope(s) and reaches the <u>top</u> of the carton, as shown below:**

17. Place the completed Sample Record Summary and Shipment Notification Form in the package, replace the lid on the Styrofoam 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.

18. Complete the FedEx air waybill with the following info-
    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 dry ice in kg.

19. 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: Coriell Institute, 403 Haddon Ave, Camden, NJ 08103
    d. Do not cover any part of this label with other stickers, including pre-printed address labels.

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

21. Hold packaged samples in -80°C freezer until time of FedEx pick-up/drop-off.

9 Shipping and Tracking Instructions

SHIP ALL FROZEN SAMPLES MONDAY - WEDNESDAY ONLY! AMBIENT SAMPLES MAY BE SHIPPED MONDAY-FRIDAY (PREFERABLY MONDAY-THURSDAY) PROVIDED THEY ARE RECEIVED AT CORIELL WITHIN 5 DAYS OF COLLECTION.
BE AWARE OF HOLIDAYS!!

Remember to complete the Sample Record Summary and Shipment Notification (Appendix B), include a copy in your shipment AND notify Coriell IN ADVANCE to confirm the shipment.

1. Complete the Sample Record Summary and Shipment Notification form. Only one specimen type per row should be listed. Multiple sample types may be included on this form.
2. Once completed, package the samples in the return box and include a copy of the Sample Record Summary and Shipment Notification form in the package.
3. Provide copy of Shipment Notification to Coriell via email (ninds@coriell.org) or fax (856-966-5067) to give advance notice that you are sending samples. Ensure tracking number is indicated.
4. Ship samples via FedEx Priority Overnight. Frozen samples packed on dry ice should be held in a -80°C freezer until the time of FedEx pickup.

10 Sample Quality Checks and Feedback to Projects

In addition to tracking and reconciliation of samples, the condition and amount of samples received is tracked by The NINDS Repository at Coriell for each sample type. Investigators and clinical coordinators for each project are responsible to ensure the requested amounts of each fluid are collected to the best of their ability and that samples are packed with sufficient amounts of dry ice to avoid thawing in the shipment process. The NINDS Repository at Coriell will complete a Non-Conformance Report (Appendix M) 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 samples will be addressed by the NINDS and communicated to the Principal Investigator.

11 Data Queries and Reconciliation

The Laboratory Procedures and Lumbar Puncture source worksheets must be completed on the day that samples are collected since they capture information related to the details of the sample collection and processing. These forms include information that will be used to reconcile sample collection and receipt, as well as information essential to future analyses. All data should be recorded on the DNA Sample, Laboratory Procedures and LP worksheets within 14 days of the subject visit per protocol.

Data queries or discrepancies with samples shipped versus received at Coriell may result from:

- Missing samples at Coriell
- Incorrect samples collected and shipped to Coriell
- Damaged or incorrectly prepared samples
- Unlabeled samples, samples labeled with incomplete information, or mislabeled samples
- Discrepant information documented on the Sample Record Summary and Shipment Notification Form and logged at Coriell compared to information entered into DMR.

12 Appendices

Appendix A: Rate of Centrifugation Worksheet

Appendix B: Sample Record Summary and Shipment Notification Form

Appendix C: Coriell Detailed Domestic Shipping Instructions

Appendix D: PAXgene™ RNA Processing Diagram

Appendix E: 6 ml Lavender top EDTA for frozen whole-blood

Appendix F: 10 ml Lavender top EDTA for Plasma Processing Diagram

Appendix G: 10 ml Red top tube for Serum Processing Diagram

Appendix H: 8.5 ml Yellow top ACD for DNA Processing Diagram

Appendix I: CSF Processing Diagram

Appendix J: Aliquot Tube Label Diagram

Appendix K: NINDS Lab Worksheet

Appendix L: NINDS Low Fat Diet Menu Suggestions

Appendix M: NINDS Sample Submission Non-Conformance Report

APPENDIX A

Rate of Centrifugation Worksheet

Please complete and return this form (by email or fax) to the NINDS Project Manager if you have any questions regarding sample processing. The correct RPM will be sent back to you. Make note of this in your NINDS Biologics Manual.

Submitter Information

Name:

Site Number:

Submitter Email:

Centrifuge Information

Please answer the following questions about your centrifuge.

Centrifuge Type:

☐ Fixed Angle Rotor
☐ Swing Bucket Rotor

Radius of Rotation (mm):

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

Comments

Email this form to:
NINDS Project Manager

It is very important to this study that all samples be processed correctly.

Please call with any questions at 703-908-7126

APPENDIX B – PDBP Example

PDBP Sample Record Summary and Shipment Notification

Site Name/Number: **Principal Investigator:**

Coordinator: **Telephone:** **Email:**

Please list only ONE subject per Sample Record Summary and Shipment Notification Form

GUID : **Subject ID (ST# from pre-printed labels):**

Gender: **Visit ID:**

Date Sample(s) Shipped: **FedEx Tracking Number:**

Instructions: Ship Frozen Shipments Monday – Wednesday ONLY! Ambient Shipments (yellow-top ACD tube) may be shipped Monday – Friday (preferably Monday – Thursday) provided they are received at Coriell within five days of collection. This form must be completed for shipment of all research samples. Notify Coriell (email preferred) and the DMR in advance of shipment using contact information below. Place a copy in the shipment box and file a copy of the completed form in the study binder. **Ensure all frozen shipments are filled with dry ice.**

In the table below, please indicate the date of specimen collection, and the Tube ID (PD# from pre-printed labels)

Completed by Submitter/Site			Completed by Repository
Date of Draw	Specimen Type	Tube ID Number (PD#)	Notation of Problems
	DNA		
	RNA		
	WB		
	Plasma		
	Serum		
	CSF		

Contact Information: Coriell Institute for Medical Research; Email: ninds@coriell.org Fax: 856-966-5067 Ph: 856-757-9742
Data Management Resource (DMR); Email: PDBP-OPS@mail.nih.gov

APPENDIX C – PDBP Example

NINDS PDBP SITES: Sample Packaging and Shipment to Coriell

Baseline Shipments Only

- **DNA Yellow Top ACD Blood Tube (AMBIENT SHIPMENT)**

Baseline and Follow-up Shipments

- **Frozen PAXgene Tubes (FROZEN SHIPMENT)**
- **Frozen lavender top whole-blood tube (FROZEN SHIPMENT)**
- **Frozen 1 ml aliquots of plasma (FROZEN SHIPMENT)**
- **Frozen 1 ml aliquots of serum (FROZEN SHIPMENT) - optional**

The following items may be part of frozen shipments

- **Frozen 1 ml aliquots of CSF (FROZEN SHIPMENT)**

IMPORTANT!
FROZEN SAMPLES MUST BE SHIPPED MONDAY-WEDNESDAY ONLY! **Include no more than ONE set of samples per shipping carton.** **Include no more than TWO packing envelopes per shipping container.**
AMBIENT SAMPLES MAY BE SHIPPED MONDAY-FRIDAY (PREFERABLY MONDAY-THURSDAY) PROVIDED THEY ARE RECEIVED AT THE NINDS REPOSITORY AT CORIELL WITHIN 5 DAYS OF COLLECTION

Sample Packaging and Shipment Instructions

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

2. Notify The NINDS Repository at Coriell of shipment by emailing ninds@the NINDS Repository at Coriell.org (preferred) or faxing (856) 966-5067 a copy of the completed Sample Record Summary and Shipment Notification Form.

- **DNA Yellow Top ACD Blood Tube (AMBIENT SHIPMENT; BASELINE ONLY)**

3. Insert yellow top ACD tube in the foam-lined plastic box and close securely.

4. Place the plastic container into the clear plastic bag and seal. Enclose this sealed bag in the cardboard shipping container.

5. Apply the UN3373 label to the outside of the cardboard container.

6. Place the cardboard shipping container 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.

CORIELL INSTITUTE FOR MEDICAL RESEARCH NINDS PDBP Shipping Instructions / Domestic

7. 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.

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

9. Ship the sample to The NINDS Repository at Coriell on the day of collection. If sample cannot be shipped the same day as collected, hold at room temperature until shipping can be arranged. Sample must be received at The NINDS Repository at Coriell within 5 days of collection.

- **Frozen PAXgene Tubes (FROZEN SHIPMENT)**
- **Frozen lavender top whole-blood tube (FROZEN SHIPMENT)**
- **Frozen 1 ml aliquots of plasma (FROZEN SHIPMENT)**
- **Frozen 1 ml aliquots of serum (FROZEN SHIPMENT) - optional**

10. Insert PAXgene tubes, and lavender top whole blood tube into bubble-wrap slots.

11. Place bubble-wrapped tubes into the clear plastic biohazard bag (do NOT remove the absorbent material found in the bag) and seal according to the instructions on the bag. Insert this into the white Tyvek biohazard envelope and seal according to the instructions on the envelope.

12. Place the envelope upright in the provided Styrofoam-lined shipping carton, as shown below:

13. Place all frozen 1ml aliquots of plasma, serum (optional), and CSF (if obtained) in the provided cardboard cryobox. Label the outside of the cryobox with the subject ID (eight-digit "ST" number).

14. Place the cryobox in the 2nd clear plastic biohazard bag (do NOT remove the absorbent material found in the bag) and seal according to the instructions on the bag. Insert this into the 2nd white Tyvek biohazard envelope and seal according to the instructions on the envelope.

CORIELL INSTITUTE FOR MEDICAL RESEARCH NINDS PDBP Shipping Instructions / Domestic

15. Place both envelopes upright and side-by-side in the provided Styrofoam-lined shipping carton, as shown below:

16. **FILL the remaining space in the shipping carton with approximately 10 lbs of dry ice, ensuring ice surrounds the envelope(s) and reaches the top of the carton, as shown below:**

17. Place the completed Sample Record Summary and Shipment Notification Form in the package, replace the lid on the Styrofoam 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.

18. Complete the FedEx air waybill with the following info-
    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 dry ice in kg.

APPENDIX C – PDBP Example

CORIELL INSTITUTE FOR MEDICAL RESEARCH NINDS PDBP Shipping Instructions / Domestic

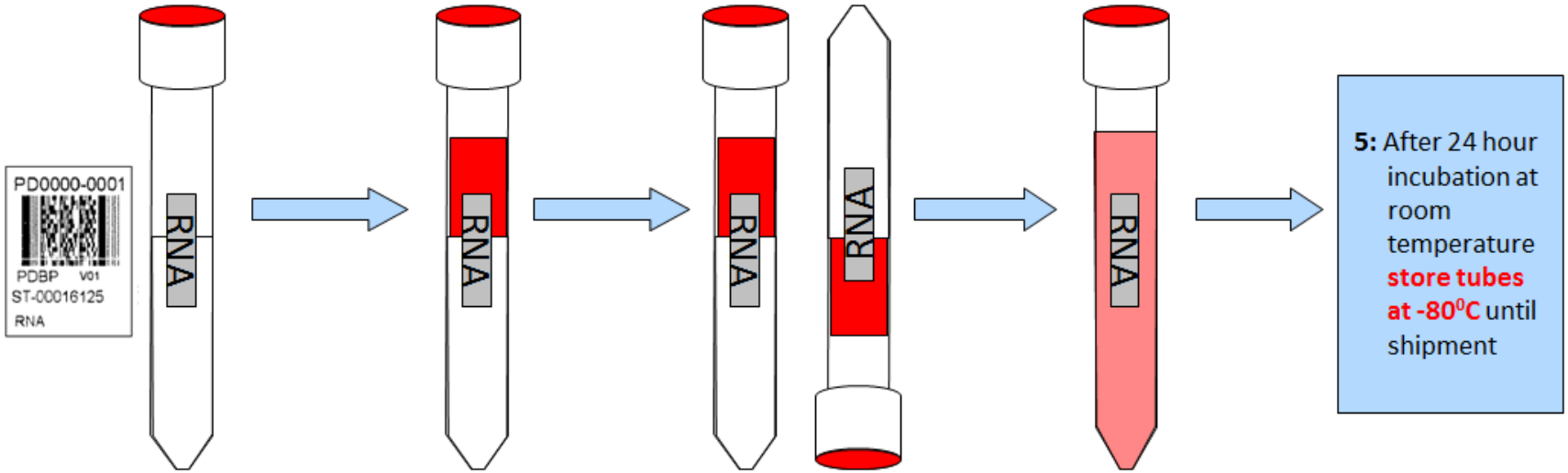
19. Complete the Class 9 UN 1845 Dry Ice label (black and white diamond) with the following information:
    a. Your name and return address
    b. Net weight of dry ice in kg
    c. Consignee name and address: Coriell Institute, 403 Haddon Ave, Camden, NJ 08103
    d. Do not cover any part of this label with other stickers, including pre-printed address labels.

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

21. Hold packaged samples in -80°C freezer until time of FedEx pick-up/drop-off.

APPENDIX D

2.5ml PAXgene™ Tube for RNA



1: Store tubes at room temperature, label with pre-printed "RNA" labels prior to blood draw.

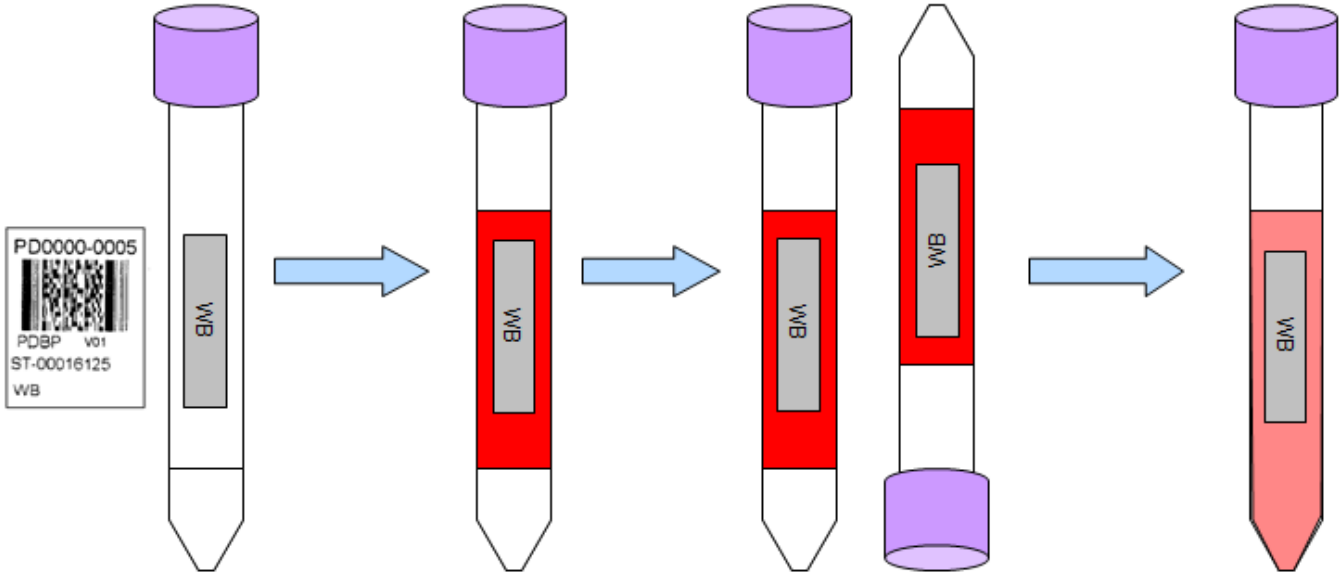
2: Collect blood into <u>one</u> PAXgene tube, allowing blood to flow 10 seconds and ensuring blood has stopped flowing each time.

3: Immediately after blood draw, **invert tube gently 8-10 times** to mix samples. Repeat steps 2 and 3 for remaining tubes.

4: Incubate tubes **upright at room temperature for 24 hours** before freezing samples.

5: After 24 hour incubation at room temperature **store tubes at -80^{0}C** until shipment

6 ml Lavender Top EDTA Tube for Frozen Whole Blood



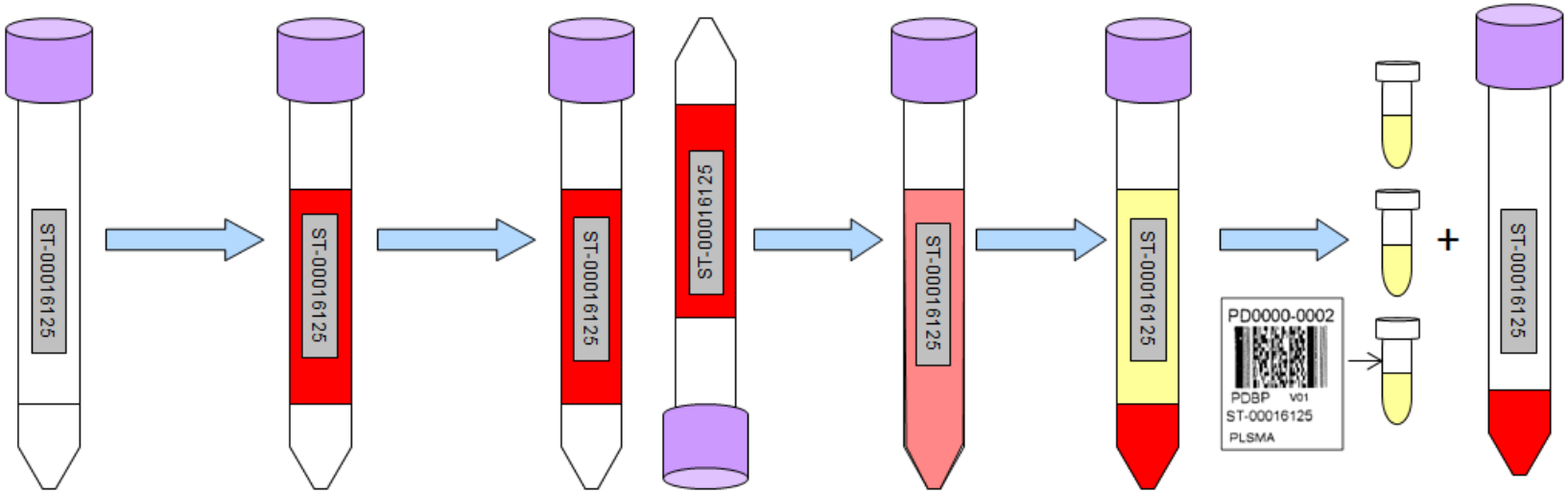
1: Store tubes at room temperature, label tube with pre-printed "WB" label prior to blood draw

2: Collect blood in 6 ml EDTA tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

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

4: Immediately after inversion, freeze sample at -80^{0}C Store sample at -80^{0}C until shipment.

10ml Lavender Top EDTA Tube for Plasma



1: Store tubes at room temperature, label tube with subject ID prior to draw; pre-printed labels are not supplied for these tubes

2: Collect blood in 10 ml EDTA tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

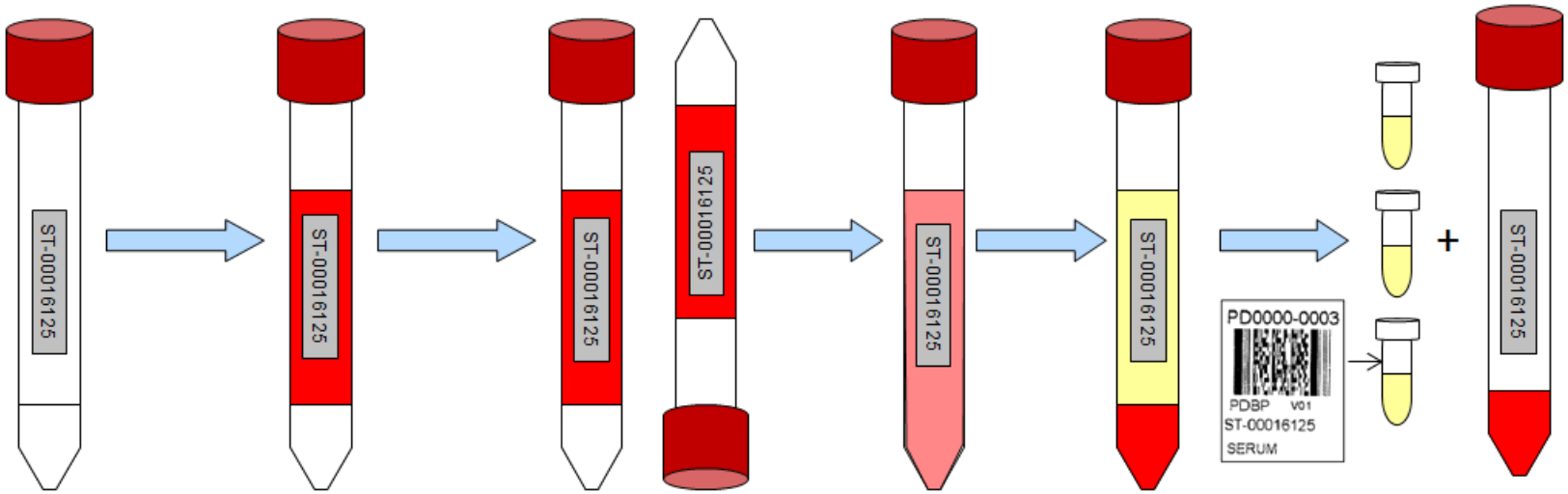
3: Immediately after blood draw, **invert tubes 8-10 times** to mix samples.

4: **Within 30 minutes of blood draw**, centrifuge samples at 4^0C, 1500 x g for 15 minutes.

5: Label microcentrifuge tubes with preprinted "Plsma" labels. Use pipette to aliquot 1 ml samples of plasma. Store plasma aliquots frozen at -80^0C until shipment.

APPENDIX G

10ml Red Top Tube for Serum



1: Store tubes at room temperature, label tube with subject ID prior to draw; pre-printed labels are not supplied for these tubes

2: Collect blood in 10 ml red-top tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

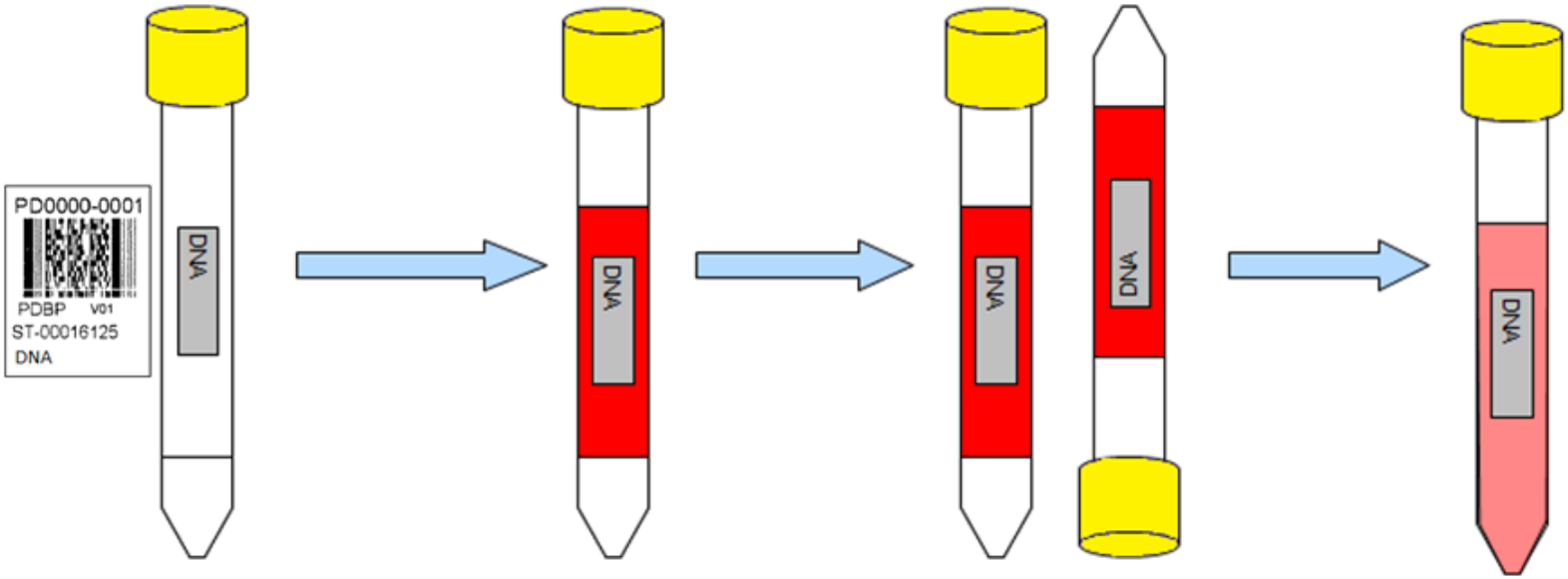
3: Immediately after blood draw, **invert tubes 8-10 times** to mix samples.

4: **Within 60 minutes of blood draw**, centrifuge samples at 4^0C, 1500 x g for 15 minutes.

5: Label microcentrifuge tubes with preprinted "Serum" labels. Use pipette to aliquot 1 ml samples of serum. Store serum aliquots frozen at -80^0C until shipment.

APPENDIX H

8.5ml Yellow Top ACD Tube for DNA Extraction



1: Store tube at room temperature, label with pre-printed "DNA" label prior to blood draw.

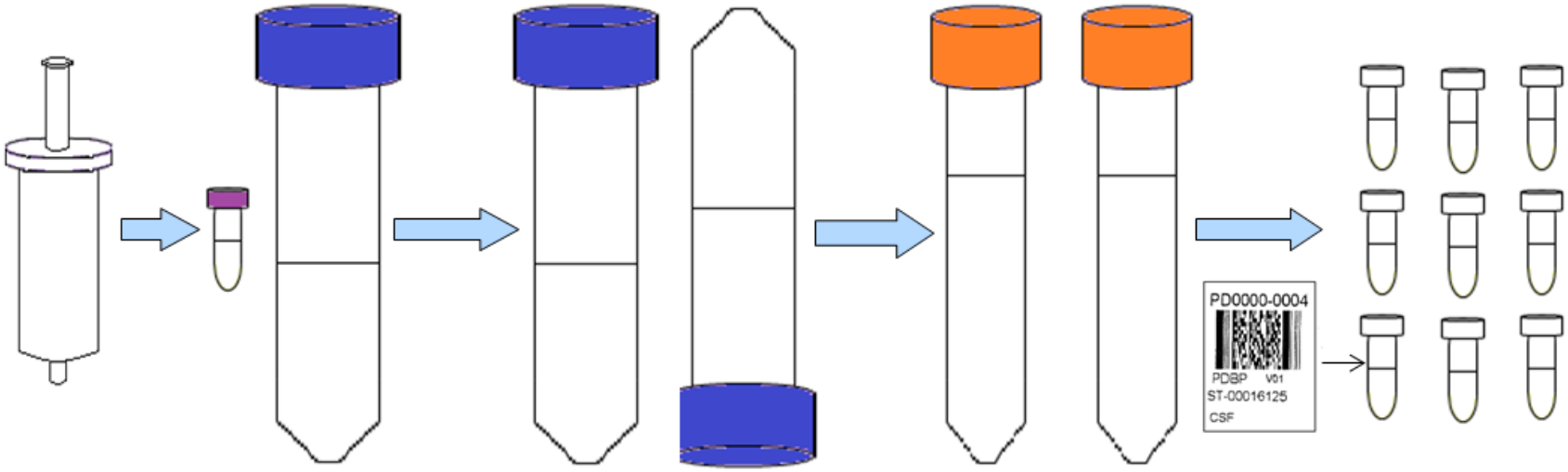
2: Collect blood, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

3: Immediately after blood draw, **invert tube 8-10 times** to mix sample.

4: Immediately after inversion, insert into shipping container. Ship ambient to Coriell.

APPENDIX I

CSF Preparation



1: Collect CSF in syringes supplied in LP Tray Kit. Transfer first 1 ml into purple-top microcentrifuge tube for lab analysis. Transfer remaining CSF into 50 ml conical tube

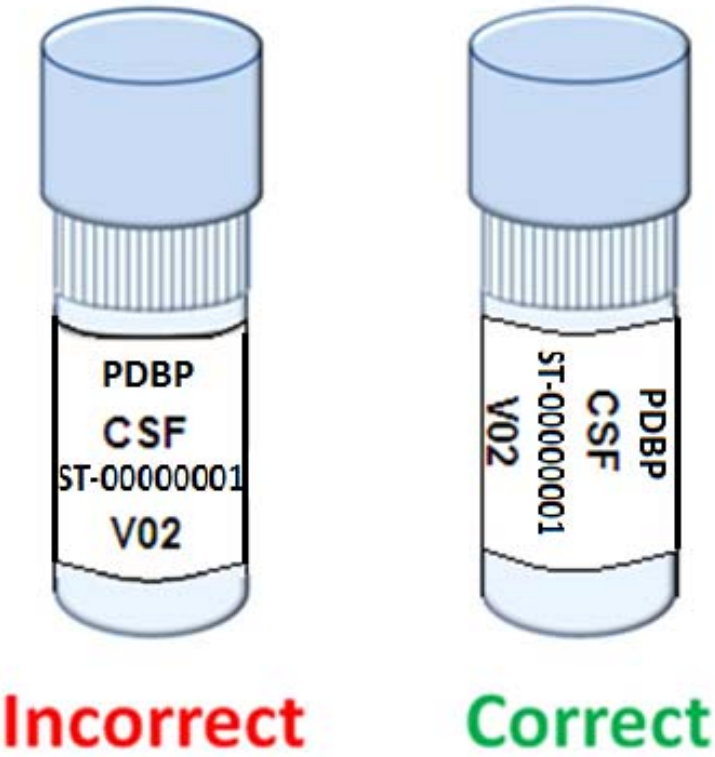
2: Gently invert 50 ml conical tube 3-4 times.

3: **Within 15 minutes of CSF collection**, transfer total CSF from 50 ml conical tube into two 15 ml conical tubes, ensuring that there is an equal volume in each tube. Centrifuge tubes at room temperature, 2000 x g for 10 minutes.

4: Label microcentrifuge tubes with preprinted "CSF" labels. Use micropipette to aliquot 1 ml samples of CSF. Freeze immediately; store CSF aliquots at -80^{0}C until shipment.

APPENDIX J

ALIQUOT TUBE LABELING DIAGRAM



Subject ID: _____ Visit: _____ Date: _____

PDBP Example Lab Worksheet

Blood Draw Order

Baseline Visit

1) **RNA**: Paxgene tubes (x4)
2) **Whole-blood**: 6 ml Lavender top EDTA tube
3) **Plasma:** 10 ml Lavender top EDTA tube (x3)
4) **Serum:** 10 ml Red top tube (x3) – **optional**
5) **DNA**: Yellow top ACD tube – **baseline only**
6) **CSF**: Lumbar Puncture **(if collected at visit)**

Follow-up Visits

1) **RNA**: Paxgene tubes (x4)
2) **Whole-blood**: 6 ml Lavender top EDTA tube
3) **Plasma:** 10 ml Lavender top EDTA tube (x3)
4) **Serum:** 10 ml Red top tube (x3) – **optional**
5) **CSF**: Lumbar Puncture **(if collected at visit)**

Sample Collection Flow Chart

Sample	0 min	15 min	30 min	45 min	60 min		24 hrs
RNA Whole-blood: PAXgene tubes	…… Sit ambient 24 hrs (at minimum >2 hrs)						**Freeze at -80°C**
Whole-blood Whole-blood: 6 ml Lavender top EDTA tube	**Freeze at -80°C**						
Plasma Whole-blood: 10 ml Lavender top EDTA tube	Centrifuge within 30 min. of collection						
	……		Aliquot		**Freeze at -80°C**		
	1500g ×15 min 4 °C, transfer plasma to aliquot tubes; freeze immediately at -80°C						
Serum* Whole-blood: 10 ml Red top tubes	Centrifuge within 60 min. of collection						
	……				Aliquot	**Freeze at -80°C**	
	1500g ×15 min 4 °C, transfer serum to aliquot tubes; freeze immediately at -80°C						
DNA** Whole-blood: Yellow top ACD tube	Ship ambient to Coriell; must be received within 5 days of collection						
CSF*** Lumbar Puncture	Transfer initial 1 ml to purple-top aliquot tube; ship ambient to local lab						
	Centrifuge within 15 min. of collection						
	….		Aliquot	**Freeze at -80°C**			
	2000g x 10 min. 18-25 °C, transfer CSF to aliquot tubes; freeze immediately at -80°C						

*serum collection is optional
**DNA tube only collected at baseline visit
***may or may not be collected depending on visit

Subject ID: _____ Visit: _____ Date: _____

Data Recording

Food ***Intake, Date*** _____ ***Pre-Lab Time*** _____ ***Pre-LP Time***_____ ***Low-Fat Diet Menu*** Yes / No / Fasted
PD Med **Last** ***Intake, Date*** _____ ***Time*** _____

Sample	Collected		Time at Collection	Volume Obtained (mL)	Time at Centrifugation	Time at Aliquoting	No. of Aliquots	Time at Freezing		Freezing Method	
	Yes	No						Date	Time	Dry Ice	-80°C Freezer
RNA											
Whole-blood											
Plasma											
Serum											
DNA								*Ambient*			
CSF											

APPENDIX L

Low-Fat Diet Menu Suggestions

Due to the interference of lipid content in blood specimens collected for biomarker evaluation in the NINDS study, 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 blood collection. These lists are not all inclusive and Investigators should use their best judgment in this process.

Foods that are allowed prior to blood collection:

Sample Breakfast Items:

Dry whole-wheat toast
Fruit salad – no dressing
Clear tea or coffee (no milk or cream)
Fruit or vegetable juice

Dry cereal – (without nuts/ no granola; no milk)
Clear tea or coffee (no milk or cream)
Fruit or vegetable juice

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

Dry whole-wheat toast
Poached egg-whites or egg substitute
Clear tea or coffee (no milk or cream)
Fruit or vegetable juice

Sample Lunch Items:

Turkey breast sandwich on whole wheat bread
Lettuce and Tomato and Mustard
Clear beverage
Flavored gelatin

Plain pasta with plain marinara sauce– no butter or cheese
Side of steamed vegetables or green salad
Clear beverage
Flavored gelatin

Steamed chicken breast (lean, without skin)
Side of steamed vegetables or green salad
Clear beverage
Flavored gelatin

Large tossed green salad with assorted vegetables (no dressing or cheese)
Clear beverage
Flavored gelatin

Cucumber sandwich on whole-wheat bread
Lettuce, tomatoes, shredded carrots, onions or other vegetables
Clear beverage
Flavored gelatin

Clear broth with vegetables and pasta
Fruit salad – no dressing
Clear beverage
Flavored gelatin

APPENDIX L

Low-Fat Diet Menu Suggestions (continued)

Foods to avoid prior to blood 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 cheeses
- All products containing cheese
- Cheeses 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 & frosting

APPENDIX M

CORIELL INSTITUTE FOR MEDICAL RESEARCH

NINDS Biomarker Sample Submission Non-Conformance Report

This form is to be completed by the NINDS Repository personnel when a sample has been received and issues are noted. Completed form is to be emailed or faxed to submission site coordinators and Coordination Centers.

Site #/Name: _____

Subject ID: _____ Visit Type: _____

Received by: _____ Date: _____

Your shipment was received with the observed problem(s) checked below. Please take note so that your future shipments are received without incident.

	Ambient temperature samples (e.g. Yellow Top ACD blood tube for DNA extraction) shipped on Friday or Saturday (domestic sites only)
	Ambient temperature samples (e.g. Yellow Top ACD blood tube for DNA extraction) received after 5-days of collection (domestic and international sites)
	Low volume (< 4ml) in Yellow Top ACD blood tube
	Frozen samples shipped on Thursday, Friday or Saturday (domestic sites only)
	Frozen samples arrived on Saturday or Sunday (International sites only)
	Advanced notice of shipment not provided
	Shipment notification does not match Shipment Notification form received with samples
	No Shipment Notification form included in package
	Shipment Notification form incomplete
	Package contents do not match Shipment Notification form
	Package received has little/no dry ice
	Signs of sample thawing present
	Samples submitted in non-standard tubes
	Sample tubes damaged/cracked
	Samples not labeled appropriately/labels peeling off
	CSF/SER/PL samples pink in color
	Unexpected sample(s) received (specify in Comments section below)
	Other (specify in Comments section below)

Comments:

_____

_____

This form is ( ) emailed ( ) faxed to _____ on _____

Name **Date**