Psychiatric Biomarkers Network

PBN

Biospecimen Collection, Processing, and Shipment Manual

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1.0 Biorepository Information

1.1 Biorepository Contacts

1.1.1 Indiana University Study Support

General Study Contact Information
Phone: 317-278-6158
Email: pbn@iu.edu

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Claire Wegel, MPH, Project Manager
Email: cwegel@iu.edu
Phone: 317-278-0594

Holly Reynolds, CCRP, Clinical Research Coordinator
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Phone: 317-278-1217

Sample Shipment Mailing Address
PBN Biorepository
Indiana University School of Medicine
351 W. 10th Street
TK-217
Indianapolis, IN 46202

1.2 Hours of Operation

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

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. *Weekend and/or holiday delivery must be arranged in advance with the biorepository.*

Samples must be shipped Monday through Thursday. Do NOT ship for weekend delivery.

1.3.1 Holiday Observations – United States

Date
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)
Juneteenth (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:

Between December 24 and January 2, Indiana University will be open Monday through Friday for essential operations **ONLY** and will re-open for normal operations on January 2. If possible, biological specimens for submission to Indiana University should **NOT** be collected and shipped to Indiana University between December 24 and January 2. Should it be necessary to ship blood samples for DNA extraction to Indiana University during this period, please contact the Indiana University staff before December 24 by e-mailing pbn@iu.edu so that they can arrange to have staff available to process incoming samples. If samples are collected during this holiday period and cannot be shipped, please store them at -70 or -80 degrees Celsius and ship them on dry ice to Indiana University **AFTER** January 1.

2.0 Research and Clinical Laboratory Collection Schedule

The following samples will be collected according to the visit schedule noted below.

- Serum for analysis at local CLIA pathology laboratory
- Serum, plasma, buffy coat, and whole blood suitable for NMDAR testing, proteomic, metabolomic, genomic, and other analyte studies
- Whole blood for PBMC collection
- RNA for transcriptomic analysis
- Cerebrospinal fluid for clinical, NMDAR testing, and analyte analysis

If a sample is not obtained at a particular visit, this should be recorded on the appropriate data form and a reason should be provided.

2.1 Sample Collection Volumes

Sample Type	Amount
Whole Blood for Clinical Labs	5 ml
Whole Blood for RNA	5 ml
Whole Blood for Plasma and Buffy Coat	10 ml
Whole Blood for Serum	10 ml
Whole Blood for PBMCs **(OPTIONAL)**	20 ml
Cerebrospinal Fluid	13-15 ml

Maximum blood volume: 50 ml (approx. 8 teaspoons)
Maximum CSF volume: 15 ml (approx. 3 teaspoons)

**Anti-NMDA receptor testing will NOT be conducted on healthy control participants. Both serum and CSF for NMDA receptor testing must be submitted at Baseline for all for SCZ/BD/CHR participants. For follow-up visits, only serum must be submitted from SCZ/BD/CHR participants.*

2.2 Study Visit Schedule

All samples listed in the table in Section 2.1 should be collected at each visit, unless otherwise indicated.

2.3 Assigning the PBN Subject ID

Each participant will be assigned a PBN Subject ID upon enrollment. Each site will assign the PBN Subject ID from a list of unique, site-specific IDs generated by the Broad Institute. PBN Subject IDs will also be pre-loaded into the PBN Clinical Data REDCap database. The PBN subject ID will serve as the patient identifier throughout their participation in the PBN.

IUGB will mail a batch of barcoded PBN Subject ID labels (20 labels per PBN Subject ID) to each site with the initial kit shipment. Replacements and additional label sets can be requested from IUGB through the kit request module, https://redcap.link/PBN-kits. These labels can be affixed to local site documents, specimen kits, and biological specimens sent to local analysis labs. These labels should not be used on specimens shipped to IUGB.

The PBN Subject ID should also be entered into the IUGB Sample Submission form when shipping samples to IUGB.

2.4 Assigning the Globally Unique Identifier (GUID)

The GUID is a subject ID that allows researchers to share data specific to a study participant, without exposing personally identifiable information. A GUID is made up of random alpha-numeric characters and does not include any PHI in the identifier. By using GUIDs in your research data, the system can associate a single research participant's genetic, imaging, and clinical assessment data even if the data was collected at different locations or throughout different studies.

To create a GUID, follow these steps:

1. Create an account: Request an account by emailing guidhelp@mail.nih.gov stating your purpose for needing an account. This may be to create GUIDs for a project submitting data to an NIMH Data Archive (NDA) repository, or to use GUIDs as subject identifiers in your own research community.
2. Ensure the latest version of Java is installed on your device.
3. To obtain GUID, click Launch the GUID Tool: https://nda.nih.gov/s/guid/nda-guid.html
4. In order to generate a GUID, the following PHI is required:
    - Complete legal given (first) name of subject at birth
    - If the subject has a middle name
    - Complete legal family (last) name of subject at birth
    - Day of birth
    - Month of birth
    - Year of birth
    - Name of city/municipality in which subject was born
    - Country of birth

3.0 Specimen Collection Kits and Supplies

Clinical Lab Collection Supplies

Collection and aliquot tubes for submission to the local CLIA pathology lab are provided.

Research Biospecimen Collection Kits and supplies

Research specimen collection kits will be provided to sites by Indiana University. Kits will include most of the materials needed for blood and CSF collection. Kits will include tube labels, which will be pre-printed with study information and the type of sample 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 provided include all packaging necessary for shipping samples back to the PBN biorepository.

Some consumables are NOT included in the kits and will need to be supplied by the site. If you do not have the necessary calibrated pipette or Mr. Frosty™ freezing container, IU may be able to provide those items to you in the initial kit shipment.

Each site will need to provide the following consumables and supplies:

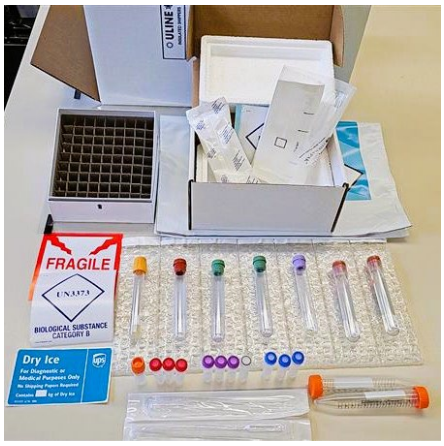
Dry Ice	Crushed Ice
Alcohol Prep Pads	Gauze Pads
Bandages	Butterfly Needles
Tourniquets	Plastic/Wire Tube Racks (2 ml to 10 ml) *No Styrofoam racks, please!*
Gloves	Sharps Bin and Lid
Calibrated Pipette and Pipette Tips	Mr. Frosty™ Freezing Container; Thermo Scientific #5100-0001
CryoStor® (CS10); Stemcell Technologies #7956	Isopropyl alcohol

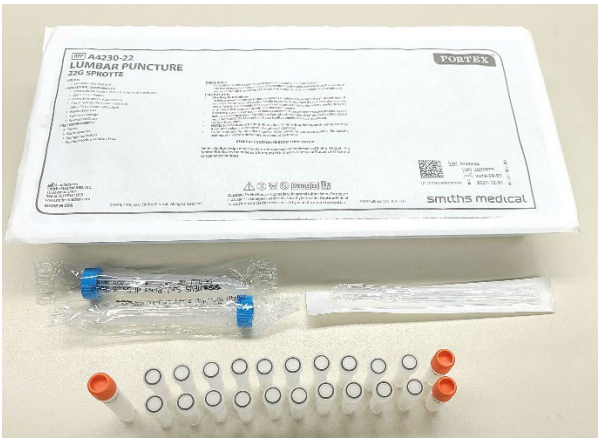
3.1 Indiana University – Specimen Collection Kit Contents

Research collection kits contain the items listed below under each kit type. Each kit provides the necessary supplies to collect samples from **one** subject. PBN 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 Indiana University with your own supplies unless you have received approval from PBN/Indiana University to do so. Note that “supplemental” kits will be provided to sites should you require additional supplies from those contained in the visit specific kits. See Section 6.1 for LP Tray contents.

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

3.1.1 Collection Kit – Biomic Blood, CSF, and Blood for PBMC





Quantity	Component
1	Set protective bubble pouches for tubes
1	Cryobox
21	Cryogenic vials (2 ml) with clear caps
3	Cryogenic vials (2 ml) with red caps
3	Cryogenic vials (2 ml) with purple caps
3	Cryogenic vials (2 ml) with orange caps
3	Cryogenic vials (2 ml) with blue caps
1	Cryogenic vial (4 ml) with orange cap
2	Sterile screw-top centrifuge tubes (15 ml)
2	Screw-top centrifuge tubes (15 ml)
1	Shipping container for dry ice shipments
1	95 kPa biohazard bag with absorbent sheet
2	PAXgene™ tube (2.5 ml)
1	Purple-top EDTA tube (10 ml)
1	Red-top serum tube (10 ml)
2	Green-top sodium heparin tube (10 ml)
1	Gold-top serum separation tube (5ml)
3	Disposable transfer pipets
2	Shipping label packets
2	Shipping instruction sheets
1	Ambient shipping box with coldpack and biohazard bag
1	Overpack
1	Lumbar puncture tray
1	Medication transfer filter straw

3.1.1.2 Anti-NMDAR Test Sample Shipping Kit (for shipping to UPENN)

Quantity	Component
1	Shipping container for dry ice shipments
1	95 kPa biohazard bag with absorbent sheet
1	Shipping label packet

3.1.2 Supplemental Kit Components

Quantity	Supplemental Kit Component
5	250 ml absorbent sheets
10	Protective bubble pouches for tubes
2	Cryoboxes
10	Cryogenic vials (2 ml) with red caps
10	Cryogenic vials (2 ml) with purple caps
20	Cryogenic vials (2 ml) with clear caps
10	Cryogenic vials (2 ml) with blue caps
10	Cryogenic vials (2 ml) with orange caps
10	Cryogenic vial (4 ml) with orange caps
2	Needles – Sprotte® spinal (22G)
2	Medication transfer filter straws
10	Screw-top centrifuge tubes (15 ml)
5	Sterile screw-top centrifuge tubes (15 ml)
5	95 kPa biohazard bag with absorbent sheet
2	PAXgene™ tube (2.5 ml)
2	Purple-top EDTA tubes (10 ml)
2	Red-top serum tubes (10 ml)
2	Green-top sodium heparin tubes (10 ml)
2	Gold-top SST tubes (5 ml)
10	Disposable transfer pipets

3.1.3 Extra Supplies Available on Request

Quantity	Component
5, 10	250 ml absorbent sheets
5, 10	Protective bubble pouches for tubes
2, 5	Cryoboxes
10, 20	Cryogenic vials (2 ml) with red caps
10, 20	Cryogenic vials (2 ml) with purple caps
10, 20, 50	Cryogenic vials (2 ml) with clear caps
10, 20	Cryogenic vials (2 ml) with blue caps
10, 20	Cryogenic vials (2 ml) with orange caps
10, 20	Cryogenic vial (4 ml) with orange caps
2, 5	Overpacks for ambient shipments
1, 2	Lumbar puncture trays (Lidocaine)
1, 2	Needles – Sprotte® spinal (22G, 22G long)
1, 2	Medication transfer filter straws
5, 10	Screw-top centrifuge tubes (15 ml)
5, 10	Sterile screw-top centrifuge tubes (15 ml)
2, 5	Ambient shipping box
2	Shipping container for dry ice shipments
5, 10	95 kPa biohazard bag with absorbent sheet
5, 10	PAXgene™ tube (2.5 ml)
5, 10	Purple-top EDTA tubes (10 ml)
5, 10	Red-top serum tubes (10 ml)
5, 10	Green-top Sodium Heparin tubes (10 ml)
5, 10	Gold-top SST tubes (5 ml)
5, 10	Disposable transfer pipets
5, 10	Shipping label packets

3.2 Indiana University – Kit and Label Ordering

Each site will be responsible for keeping track of the number of kits available at their site and ordering additional kits, as needed. Indiana University's online kit ordering module for this study can be found at https://redcap.link/PBN-kits.

In the electronic kit ordering module, select site name from the drop-down list provided; this will automatically fill the form with the site coordinator's contact and shipping information. Verify that this information is correct (and modify it if necessary), and select the number of kits, supplies, and/or labels needed.

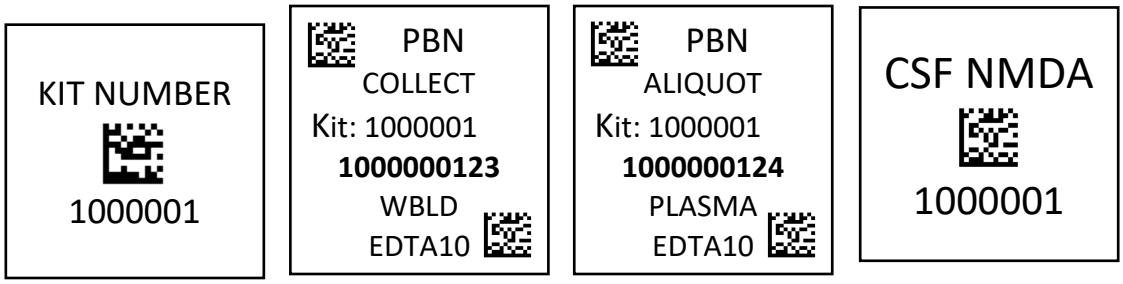
Please note that Indiana University cannot guarantee overnight delivery of kits. Whenever possible, sites should allow 10 business days for kit production and delivery.

3.3 Indiana University – Kit and Specimen Labels

Barcoded kit and specimen labels will be provided with all kits. Each kit will have a unique six- or seven-digit Kit ID. When the site performs a study visit, they will assign a kit to the subject/visit. The unique kit number will then be associated with that subject-visit in the specimen database.

Each specimen label provided with a kit has a unique ten-digit barcode, which is linked to the Kit ID. Study, Kit ID, visit, and specimen type are also printed on the specimen labels. Specimen labels are NOT interchangeable between kits. *Each kit and set of kit labels is designed to be used for one subject-visit.*

Kit Label **Specimen Labels** **UPENN NMDA Labels**



4.0 Equipment Required at Clinical Sites

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

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

5.0. Blood Collection and Processing Procedures

Blood samples should be collected preferably after the subject has fasted. If fasting is not feasible, the subject should follow the low fat diet (see Appendix F). Record if the patient has fasted or followed a low fat diet on the Sample Processing and Collection Form (see Appendix B).

5.1 Order of Blood Draws

Blood collection tubes must be filled in the following order to prevent cross-contamination:

1. 1 x 5 ml Serum Separator Tube (to be sent to local pathology lab)
2. 1 x 10 ml Serum Determination Red Top
3. 2 x 10 ml PBMC Sodium Heparin Green Top
4. 1 x 10 ml EDTA Purple Top
5. 2 x 2.5 ml PAXgene™

Draw order remains the same even when you are not collecting all sample types. Simply skip the excluded tube(s).

5.2 Serum for clinical labs

A 5 ml serum separator tube is included in the specimen collection kit. This tube is intended for processing at your local CLIA pathology laboratory, and it should not be shipped with the specimens to IU. Collect and label this specimen per the requirement for submission to your local pathology lab.

Please requisition the following tests:

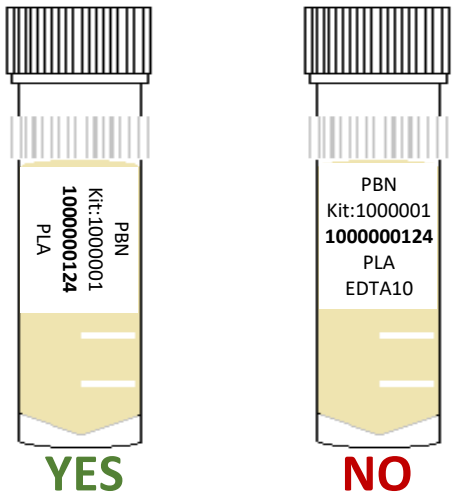
1. Glucose
2. Total IgG*
3. Albumin*
4. Oligoclonal bands

*IgG and Albumin are often measured as part of the “IgG index or CSF index”

5.3 Labeling Samples

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

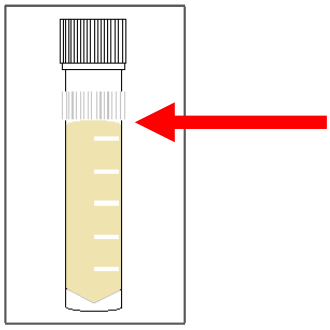
- Place labels on **ALL** collection and aliquot tubes **BEFORE** sample collection, sample processing or freezing. This should help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.
- Place label ***horizontally*** on the tube (wrapped around sideways if the tube is upright) and ***just below the ridges*** of the aliquot tubes (see diagram below). 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.



5.4 Filling Aliquot Tubes (Plasma, Buffy Coat, and Serum)

To assist in the preparation and aliquoting of the plasma, buffy coat, and serum 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.

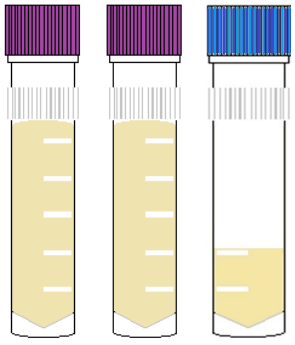
Cap Color	Specimen Type
Purple	Plasma
Red	Serum
Clear	Buffy coat and CSF
Orange	CSF Cell Pellet and tubes for anti-NMDA testing
Blue	Residual volumes



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. **Please place a blue cap on this aliquot to indicate that it is a residual.**

All plasma and serum specimens should be shipped to the biorepository. 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 examples of correct and incorrect aliquot volumes below).



YES

NO

5.5 Serum from Serum Determination Tube

1. **CRITICAL STEP: Store empty Serum Determination Tubes at room temperature 64°F – 77°F (18°C to 25°C) before use.**

2. Place a pre-printed "SERUM" specimen label on the 10 ml Serum tube prior to blood draw (per Section 5.3)

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

   The following techniques should 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. Record the time of draw on the Sample Processing & Collection form (see Appendix B).

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

6. **CRITICAL STEP: Allow blood to clot at room temperature for at least 15 minutes.**

7. Within 60 minutes of serum collection (after 15 minutes of clotting at room temperature), 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.** For assistance, see Appendix A.

   ✓ Equivalent rpm for spin at 1500 x g =

   ✓ While centrifuging, record the time of centrifuge start on the Sample Processing & Collection form (Appendix B). If you are entering this data directly into the online form, the temperature, duration, and speed of centrifugation will default to those specified in this manual.

8. Place pre-printed "SERUM" specimen labels on the 15 ml centrifuge and 2 ml red-capped aliquot tubes. Remove the serum, being careful not to disturb the clot at the bottom of the tube by tilting the tube and placing the disposable graduated pipette tip along the lower side of the tube wall without touching the pellet. Using a disposable graduated transfer pipette, transfer all blood serum

(top layer) into the 15 ml centrifuge tube at room temperature, and mix gently by inverting 3-4 times.

NOTE: When pipetting serum, be very careful to pipette the serum top layer only, leaving the cell pellet untouched.

9. Place pre-printed "SERUM NMDA" specimen label on the 2 ml orange-capped aliquot tube. Serum should be submitted for NMDA testing for *all SCZ/BD/CHR visits, including follow-up.*

10. Pipette *1.0 ml of serum* into the SERUM NMDA labelled orange-capped 2 ml aliquot tube. **Freeze this sample per the instructions below; ship this sample to UPENN on dry ice using the provided kit (Appendix I).**

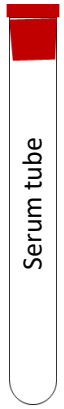
11. Pipette *1.5 ml of serum* into each SERUM labeled red-capped 2 ml aliquot tube. The serum tube should yield, on average, 4.5 ml of serum, for a total of 2-3 aliquot tubes per subject. Seal each aliquot tube with a red cap. *If a residual aliquot (<1.5 ml) is created, place a blue cap on that tube and document the estimated residual volume on the Sample Processing and Collection form (Appendix B).*

12. Discard the used red-top collection tube and transfer pipette according to site guidelines for disposing of biomedical waste.

13. Within 90-120 minutes of serum collection, freeze and store samples at **-80°C.** Samples should be frozen and stored **UPRIGHT**. A cryobox is provided for this purpose. Complete the remainder of the Sample Processing & Collection form (Appendix B). Samples must be shipped within two weeks of collection, following the instructions in Appendix E.

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

Steps 1-2

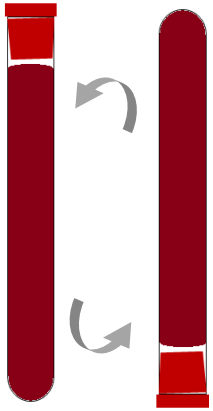


- Store tubes at room temperature.
- Label tubes with preprinted PBN SERUM label prior to blood draw.

Steps 3-4

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

Step 5

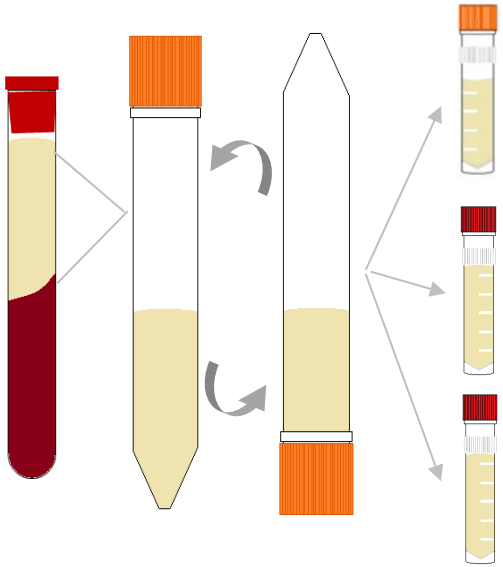


- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step 6

- 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.

Steps 7-13



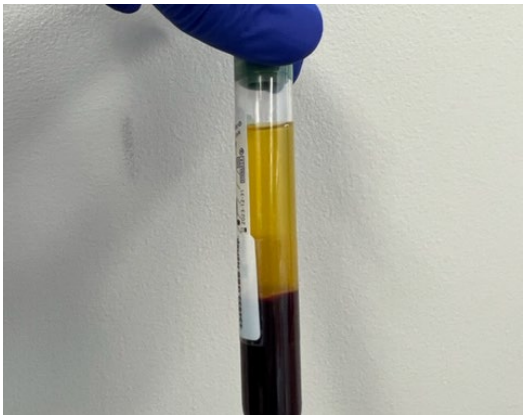
- Label red-capped cryotubes with SERUM labels and orange-capped cryotube with SERUM NMDA label.
- Using a clean transfer pipet, transfer all serum into a 15 ml conical tube and mix gently by inverting 3-4 times.
- Aliquot 1 ml of serum into the orange-capped SERUM NMDA labelled tube (*SCZ/BD/CHR subjects*). **Ship to UPENN on dry ice.**
- Aliquot 1.5 ml into each SERUM cryotube.
- If a residual aliquot (<1.5 ml) is created, place a blue cap on this aliquot.
- Store red-capped serum aliquots upright at -80°C until shipment.

5.6 Peripheral Blood Mononuclear Cells (PBMC) from Sodium Heparin Tubes

*Sodium heparin tubes **MUST** be shipped to the PBN biorepository on the day of collection via UPS overnight delivery in order to ensure the specimen has the most viable cells available at extraction. These samples should only be collected Monday-Thursday. Please DO NOT collect these samples on Fridays.*

1. **CRITICAL STEPS: Store empty Sodium Heparin tube at room temperature, 64°F - 77°F (18°C to 25°C) before use. Freeze refrigerant pack for at least 24 hours prior to use.**

2. Place pre-printed "PBMC" specimen label on the two 10 ml Sodium Heparin tubes prior to blood draw.
   - ❖ Place the label toward the bottom of the tubes and positioned to allow a viewing window down the entire length of the tube (*as shown in the picture below*)



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

   The following techniques should 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. Record the time of draw on the Sample Processing & Collection form (see Appendix B).

5. Immediately after blood collection, gently invert the tube 8-10 times to mix.

6. **REPEAT STEPS 3 TO 5** for the second Sodium Heparin tube to be collected.

7. Seal the Sodium Heparin tube in the ambient shipment kit and ship the unprocessed tube ambient to the appropriate PBN biorepository, following the instructions in Appendix D. Sample must be shipped the same day as collection using the refrigerant pack provided. *Do not refrigerate or freeze the actual sample.* **Sample must be received at the PBN biorepository the following day after collection. Do NOT draw or ship this sample on a Friday.**

8. Complete the Sample Processing & Collection Form (see Appendix B).

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

(BASELINE VISIT ONLY)

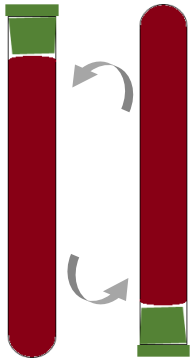
Steps 1-2

- Store tubes at room temperature.
- Label tubes with preprinted PBN PBMC specimen label prior to blood draw, *preferably toward the bottom of the tubes and positioned to allow a viewing window along the whole length of the tubes*.

Steps 3-4

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

Step 5



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

Step 7

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

5.7 Plasma/Buffy Coat from EDTA Tube

1. **CRITICAL STEP: Store empty EDTA tubes at room temperature, 64°F - 77°F (18°C to 25°C) before use.**

2. Place a pre-printed "PLASMA" specimen label on the 10 ml EDTA tube prior to blood draw (per Section 5.3).

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

   The following techniques should 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. Record the time of draw on the Sample Processing & Collection form (Appendix B).

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

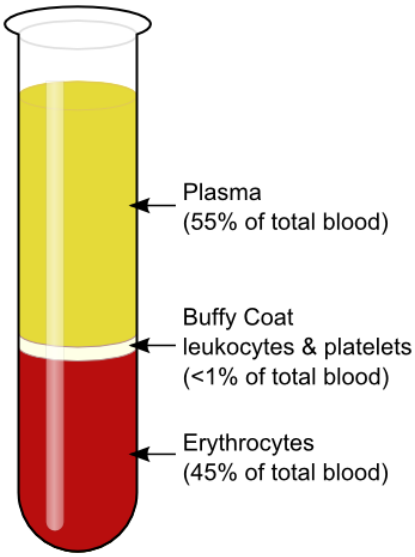
6. Within 30 minutes of plasma collection, centrifuge balanced tubes at 4°C for 15 minutes at 1500 x g. **It is critical that the tubes be centrifuged at the appropriate speed to ensure proper plasma separation.** For assistance, see Appendix A.

   ✓ Equivalent rpm for spin at 1500 x g =
   ✓ While centrifuging, record the time of centrifuge start on the Sample Processing & Collection form (Appendix B). If you are entering this data directly into the online form, the temperature, duration, and speed of centrifugation will default to those specified in this manual.

7. Place pre-printed "PLASMA" specimen labels on the 15 ml centrifuge and 2 ml aliquot tubes. Remove the plasma, being careful not to agitate the packed blood cells at the bottom of the purple top tube, by tilting the tube and placing the pipette tip along the lower side of the tube wall without touching the pellet so that plasma is not contaminated by pellet material. Using a disposable graduated transfer pipette, transfer plasma into the 15 ml centrifuge tube at room temperature and mix gently by inverting 3-4 times.

8. Pipette at least 1.5 ml of plasma into each labeled 2 ml aliquot tube. The EDTA tube should yield, on average, 4.5 ml of blood plasma, for a total of 2-3 aliquot tubes per subject. Seal each aliquot tube with a purple cap. *If a residual aliquot (<1.5 ml) is created, place a blue cap on that tube and document the estimated residual volume on the Sample Processing and Collection form (Appendix B).*

   NOTE: When pipetting plasma from the EDTA tube, be very careful to pipet the plasma top layer only, leaving the buffy coat and the red blood cell layers untouched.

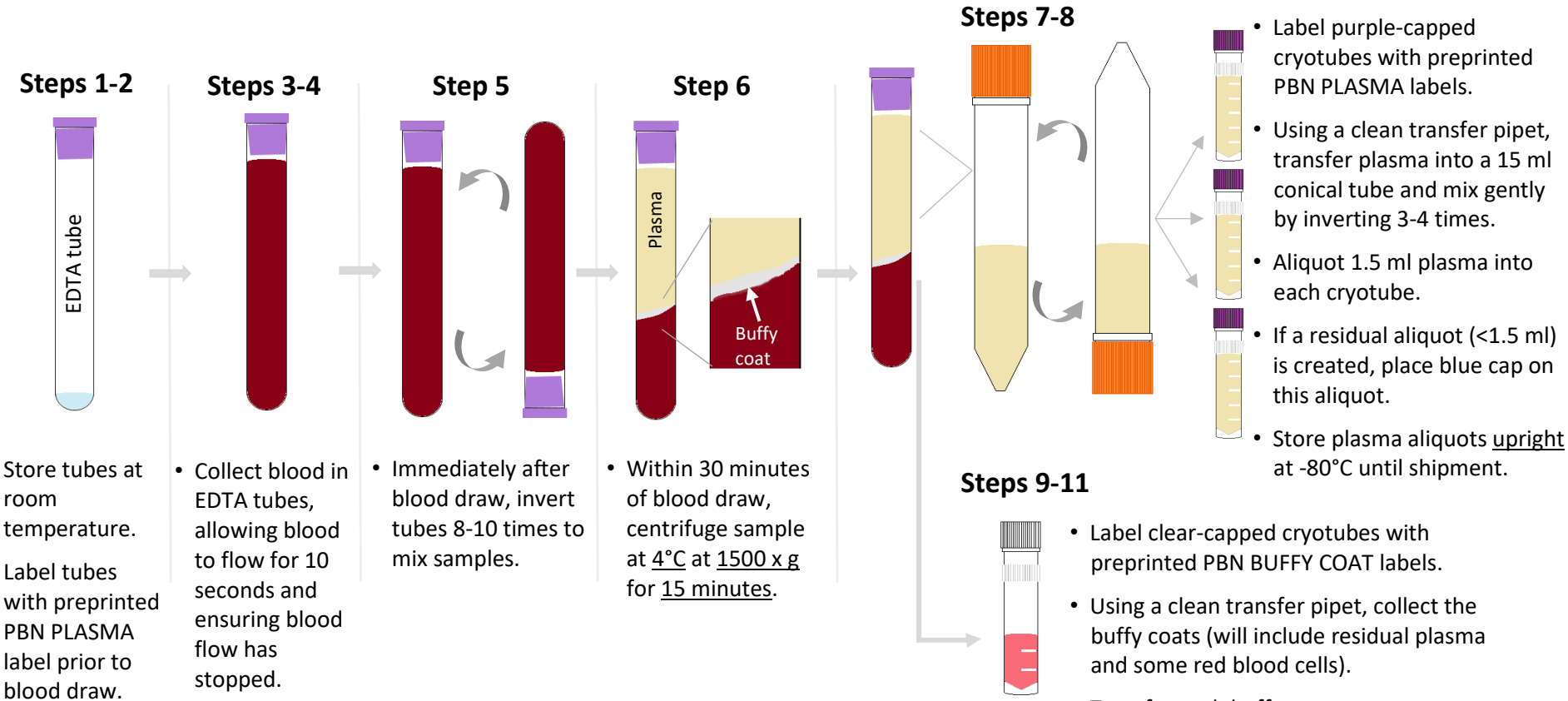


9. Place a pre-printed “BUFFY COAT” label on the 2 ml aliquot tube. Using a clean transfer pipette, transfer the buffy coat layer (middle layer) into an aliquot tube. Seal the aliquot tube with a clear cap. **Residual plasma and RBCs may be collected during this isolation process.**

10. Discard the used EDTA tube and pipette according to site guidelines for disposing of biomedical waste.

11. Within 90-120 minutes of plasma collection, freeze and store samples at **-80°C.** Samples should be frozen and stored **UPRIGHT**. A cryobox is provided for this purpose. Complete the remainder of the Sample Processing & Collection form (Appendix B). Samples should be shipped within two weeks of collection, following the instructions in Appendix E.

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



- Store tubes at room temperature.
- Label tubes with preprinted PBN 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 PBN PLASMA labels.
- Using a clean transfer pipet, transfer plasma into a 15 ml conical tube and mix gently by inverting 3-4 times.
- Aliquot 1.5 ml plasma into each cryotube.
- If a residual aliquot (<1.5 ml) is created, place blue cap on this aliquot.
- Store plasma aliquots upright at -80°C until shipment.

- Label clear-capped cryotubes with preprinted PBN 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.

5.8 PAXgene™ RNA

1. **CRITICAL STEP: Store PAXgene™ Blood RNA Tubes at room temperature, 64°F - 77°F (18°C to 25°C) before use.**

2. Place "RNA" specimen label on the PAXgene™ RNA tubes prior to blood draw (per Section 5.3).

3. Using a blood collection set and a holder, collect blood into the **first of the two 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.

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 PAXgene™ Blood RNA Tube with its vacuum is designed to draw 2.5 ml of blood into the tube. Record time of draw on Sample Processing & Collection form (see Appendix B).

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

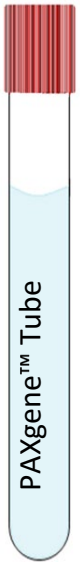
6. **REPEAT STEPS 3 TO 5** for the second PAXgene™ Blood RNA Tube to be collected.

7. CRITICAL STEP: Incubate the PAXgene™ Blood RNA Tubes **UPRIGHT** at room temperature, 64°F - 77°F (18°C to 25°C) **for 2 to 24 hours.** Record time and date of draw on the Sample Processing & Collection form (see Appendix B). ***Samples must sit at room temperature for a minimum of 2 hours.***

8. After **24 hours** at room temperature, place the two PAXgene™ tubes **UPRIGHT** into a WIRE or PLASTIC type test tube rack (DO NOT use a solid Styrofoam test tube holder) and transfer into a **-80°C (minus eighty) freezer.** Keep the two **PAXgene™ Blood RNA Tubes at -80°C** until you ship on dry ice. Complete remainder of the Sample Processing & Collection form (Appendix B). Samples should be shipped within two weeks of collection, following the instructions in Appendix E.

Whole Blood Collection and Preparation – 2.5 ml PAXgene™ RNA Tube

Steps 1-2

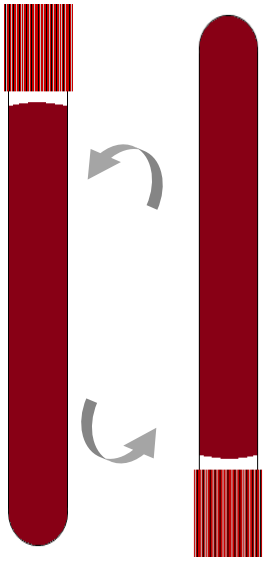


- Store tubes at room temperature.
- Label tubes with preprinted PBN RNA label prior to blood draw.

Steps 3-4

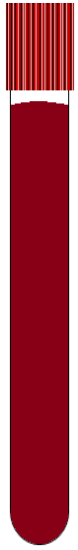
- Collect blood in PAXgene™ tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step 5



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

Steps 7-8



- Incubate the tubes at room temperature for **2 to 24 hours**.
- Freeze tubes upright at -80°C in a WIRE or PLASTIC type rack until shipment. (DO NOT freeze in solid styrofoam tube holder)

6.0 Cerebrospinal Collection and Processing Procedures

To reduce variability due to known diurnal metabolic fluctuations, CSF should be collected between 12:00 PM and 4:00 PM, whenever possible. Recommend that the subject consume a light meal from the low fat diet list (see Appendix F).

The following tests may be ordered on a clinical blood sample collected prior to the lumbar puncture to rule out blood clotting prior to performing the lumbar puncture, if necessary:

- PT/PTT
- PT/INR
- CBC (CBC PLT & DIFF including: WBC, RBC, HGB, HCT, Platelet Count, MCV, MCH, MHCH, WBC differential)

6.1 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, if included. Supplies for collection and shipment of CSF are sent to sites in a separate kit from Indiana University. A filtered medication straw to draw up the lidocaine from the glass ampule is included with these supplies. Sites will receive 22G trays.

6.1.1 Lumbar Puncture Tray Components

Quantity	Lumbar Puncture Tray Kit Component
1	Pencil point spinal needle, 22G x 3.5”
1	Introducer needle, 1 mm x 30 mm
1	Hypodermic needle, 22G x 1.5”
1	Plastic syringe, (3 ml, luer lock) with 25G x 5/8” needle attached
4	Polypropylene syringe (6 ml, luer lock)
1	Needle stick pad
1	Adhesive bandage
1	Drape, fenestrated, 2 tabs, paper, 18” x 26”
2	Towel, 13.5” x 18”
6	Gauze pad, 2” x 2”
3	Sponge stick applicator
2	Lidocaine 1%, 5 ml
1	Povidone-Iodine Topical Solution, 0.75 oz

6.2 Setting up the LP

1. On an overbed 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.

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

3. 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 paper wrapper, or if any non-sterile object or outside of the wrapper touches any part of the inside of the wrapper, discard the kit 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.

6.3 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—keeping people away from it and reminding them to be careful around it. If anyone touches the inside of the paper wrapper or any part of the contents of the kit, throw away 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 speak-up and inform people if need be. Be assertive.

6.4 Tips for Clinicians Performing Lumbar Puncture

Optimizing patient comfort and minimizing the risk of adverse events.

1. Talk the patient through the procedure so that there are no surprises.
2. Use of a Sprotte® 22g atraumatic spinal needle and careful technique are optimal for reducing post-LP headache risk. A pencil point spinal needle such as Spinocan® 22g may also be used.
3. Use adequate local anesthesia. Use the provided Filter Straw to draw up lidocaine from the glass ampule into the syringe. Discard the Filter Straw. Use the 1/2" Luer lock 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 22g pencil point spinal needle).
4. Increasing fluid intake immediately after LP is helpful.
5. Be sure to give post-LP care instructions verbally to the subject (see below).

6.5 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.

6.5.1 Mild to Moderate headache after a lumbar puncture

- 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 an equivalent could be considered.

6.5.2 Severe headache after a lumbar puncture

- 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.

6.6 CSF Processing Procedure

1. **CRITICIAL STEP: The isopropyl alcohol in the Mr. Frosty™ must be replaced every other use. Replace the isopropyl alcohol in the Mr. Frosty™, if needed. Pre-chill the Mr. Frosty™ device in a -80°C freezer.**

2. Chill the 15 ml centrifuge tube(s) in their wrappers on wet ice prior to the procedure. *Take note of the 0.5 ml marking at the tip of the centrifuge tube. This is the amount of CSF that will be aliquoted with the cell pellet.*

3. Place pre-printed CSF specimen labels on the **twenty** 2 ml aliquot tubes (per section 5.3). Label one orange-capped 2 ml cryovial with the CSF PELLET specimen label. Label one orange-capped 2 ml cryotube with CSF NMDA specimen label for *all SCZ/BD/CHR Baseline visits only.*

4. Perform lumbar puncture using the atraumatic technique.

5. **If there is blood in the initial tap, discard the first 1-2 ml of CSF.** Collect a total volume of 13-15 ml of CSF into the pre-chilled 15 ml centrifuge tube. *Note: If CSF flow is too slow, the provided syringes may be utilized to collect the CSF. If the syringes are used, you must note this on the Sample Processing & Collection form.*

   Record the approximate duration of the CSF collection, specifically the length of time the collection needle is in the subarachnoid space, on the Sample Processing & Collection form (Appendix B).

6. **SCZ/BD/CHR BASELINE VISIT ONLY:** Transfer 1 ml of CSF into the CSF NMDA-labelled orange-capped 2ml cryovial provided in the CSF kit. **This vial will be shipped to UPENN for anti-NMDA receptor testing.** See Appendix I.

7. Dispense 2-4 ml of CSF into the 4 ml vial provided in the CSF kit. Label and handle sample per your local pathology lab's instructions. **Send this tube at room temperature to the local CLIA pathology lab** for CSF analysis, including:
   1. Cell count: including RBC (first) and WBC
   2. Total protein
   3. Glucose
   4. IgG*
   5. Albumin*
   6. Oligoclonal bands

   ** IgG and Albumin are often measured as part of the "IgG index or CSF index"*

8. Gently mix the remaining 10ml of CSF by inverting 3-4 times. Record the time of LP (once collection is complete) on the Sample Processing & Collection form (Appendix B).

9. **CSF processing for IU:** Within 30 minutes of collection, spin the CSF in the conical tube at **300 X g for 10 minutes at 4°**C. Using a calibrated pipette, aliquot 500 ul of supernatant directly into each of the **twenty** prepared 2 ml aliquot tubes. Use more aliquot tubes if needed. Be careful to pipette from the top of the supernatant so as not to disturb the pellet. *The pellet will not be visible by eye! Leave at least 500 ul of CSF in the bottom of the 15 ml conical tube.* Seal each CSF tube with a clear cap and store CSF tubes at **-80°C.** Samples should be frozen and stored **UPRIGHT.**

10. Add 500 ul CryoStor® to the bottom of the 15 ml conical and resuspend the pellet by pipetting up and down; transfer the entire volume (approx. 1 ml) to the pre-labeled CSF PELLET cryovial.

11. Within 60 minutes of CSF collection, place CSF PELLET tube in the Mr. Frosty™ and store at **-80°C overnight** to ensure the cells freeze at the correct rate.

12. Complete the remainder of the Sample Processing & Collection form. CSF PELLET should be removed from the Mr. Frosty™ and transferred to the cryobox containing the CSF aliquots. The CSF pellet should be shipped within **TWO DAYS** of collection *(Monday-Thursday only)*, following the instructions in Appendix E.

    CSF pellets not shipped within two days must be transferred to liquid nitrogen storage within three days of collection.

CSF Collection and Preparation

Steps 1-

- Prepare Mr. Frosty™

- Chill 15 ml conical tube on wet ice.
- Label **twenty** *clear*-capped 2 ml cryotubes with preprinted PBN CSF labels.
- Label **one** *orange*-capped 2 ml cryotube with CSF PELLET label.
- Label **one** *orange*-capped 2 ml cryotube with CSF NMDA label.

Steps 4-5

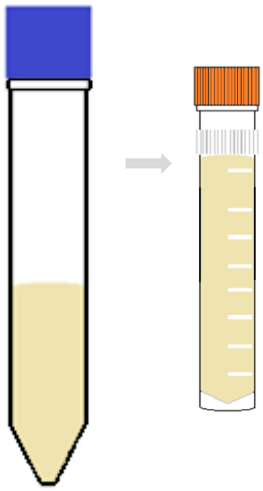
- *If there is blood in the initial tap, discard the first 1-2 ml of CSF.*
- Collect 13-15 ml of CSF into the chilled centrifuge tube

Step 6

BASELINE ONLY

- **SCZ/BD/CHR BASELINE VISIT ONLY:** Dispense 1 ml into the CSF NMDA-labelled orange capped-cryovial
- Ship this tube to UPENN on dry ice.

Step 7

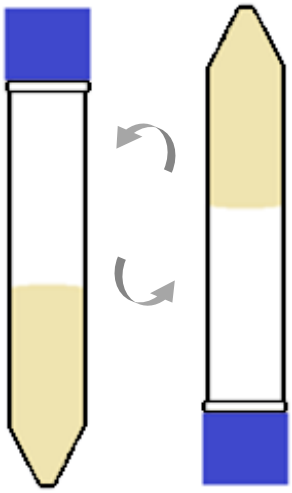


- Dispense 2-4 ml into the 4 ml orange cap cryovial
- Send to local pathology lab for testing. Label and handle this sample per your local path lab's instructions.

Continue to next page for remaining CSF collection instructions.

CSF Collection and Preparation continued…

Step 8



- Gently invert the remaining 10 ml of CSF in the centrifuge tube 3-4 times to mix the sample.

Step 9

- Within 30 minutes of collection, centrifuge samples at <u>300 x g</u> for <u>10 minutes at 4° C</u>.
- Aliquot 500 ul of supernatant directly into each of the prepared cryotubes, being careful not to disturb the pellet at the bottom of the conical tube. *Leave 500 ul of CSF in the conical tube.*

Steps 10-12

CSF PELLET

- Add 500 ul CryoStor® to remaining 500 ul of CSF and cell pellet in the 15 ml conical tube.
- Resuspend pellet using pipetting technique.
- Transfer the resuspended CSF pellet to the pre-labeled CSF PELLET orange cryotube.
- Within 60 minutes of CSF collection, freeze all CSF aliquots **upright** in rack or cryobox at -80° C.
- Place CSF PELLET aliquot in the prepared Mr. Frosty™ and store at -80° C overnight.

7.0 Packaging and Shipping Instructions

Please refer to **Appendix D, Appendix E, and Appendix I** for detailed shipping instructions regarding:

- PBN Ambient Shipping Instructions
- PBN Frozen Shipping Instructions
- PBN Anti-NMDAR Shipping Instructions

Important Notes

1. Include only **one** subject visit set of samples per shipping carton in order to have room for a sufficient amount of dry ice to keep samples frozen up to 48 hours unless otherwise noted by Indiana University.

2. **Do NOT ship samples on Fridays!**
   - CSF pellet must be frozen in Mr. Frosty™ / -80° C freezer overnight before shipped to IU the next day

   If samples are collected on a Thursday, CSF pellet must be frozen in Mr. Frosty/-80 freezer overnight and then placed in LN2 tank until shipment scheduled for the following Monday.

3. Ambient PBMC samples must be **shipped on the day of collection**.

4. BE AWARE OF HOLIDAYS!

5. Frozen serum, plasma, buffy coat, and samples in PAXgene™ tubes must be shipped within TWO WEEKS of collection. Frozen CSF pellet must be shipped within TWO DAYS of collection or transferred to liquid nitrogen storage.

6. Remember to complete the Sample Processing & Collection form (Appendix B). The Ambient and Frozen Shipping Manifests (see Appendix C) and the NMDAR Shipping Manifest (see Appendix I) will pre-populate with the number of tubes/aliquots in each shipment once the Sample Processing & Collection form is completed in REDCap. If required, enter the shipment tracking information in the REDCap. Once the Sample Processing & Collection form and the shipping manifests are submitted, copies of all three forms will be automatically emailed to you. Please print a copy of the manifests to include in your shipment.

8.0 Sample Quality Checks and Feedback to Sites

In addition to tracking and reconciliation of samples, the condition and number of samples received is recorded by Indiana University for each sample type received. Sites are responsible for ensuring the requested amounts of each fluid are collected to the best of their ability and that samples are packed well with sufficient amounts of dry ice to avoid thawing in the shipment process. Indiana University will complete a Non-Conformance Report (Appendix F) 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 PBN Steering Committee and communicated to sites.

9.0 Data Queries and Reconciliation

Ideally, the online Sample Processing & Collection Form (Appendix B) should be completed on the day that samples are collected, since it captures information related to the details of the sample collection and processing. This form includes information that will be used to reconcile sample collection and receipt, as well as information essential to future analyses. All data must be recorded on the Sample Processing & Collection form (Appendix B) prior to shipping the samples.

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

- Missing samples at Indiana University
- Incorrect samples collected and shipped to Indiana University
- Damaged or incorrectly prepared samples
- Unlabeled samples, samples labeled with incomplete information, or mislabeled samples

10.0 Appendices

Appendix A: Rate of Centrifugation Worksheet
Appendix B: PBN Sample Processing & Collection Forms
Appendix C: Shipping Manifests
Appendix D: PBN Ambient Shipping Instructions
Appendix E: PBN Frozen Shipping Instructions
Appendix F: Low-Fat Diet Suggestions
Appendix G: Sample Submission Non-Conformance Report
Appendix H: Sample Flow Schematic
Appendix I: PBN Anti-NMDAR Shipping Instructions (to UPENN)

APPENDIX A: Rate of Centrifugation Worksheet

Please complete and return this form by e-mail to the PBN 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 PBN 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:

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

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 pbn@iu.edu.

Appendix B: PBN Sample Collection and Processing Recording Form

This template is intended for your convenience, only. This information must be submitted to PBN via the REDCap Sample Collection and Processing Form at https://redcap.link/PBNSampleForm. If you have any questions about sample processing for this study, visit psychiatricbiomarkers.org or email pbn@iu.edu.

PBN Subject ID: _____

Kit Number (6-7 digits): _____

Visit: ☐ BL ☐ 12M ☐ 24M ☐ 36M ☐ 48M ☐ 60M

Participant Type: ☐ SCZ ☐ BD ☐ CHR ☐ Control

Sex (genetic sex, not gender): ☐ M ☐ F ☐ Other

Phlebotomy Details:

Date of Blood Draw: _____ [MM/DD/YYYY]

Time of Draw: _____ 24 HR clock

Fasting Status: _____

Blood Processing:

If processing was performed per SOP, checkbox may be used in place of text field, where provided.

10ml Serum (red-top) Tubes		**Standard Procedures (See PBN MOP for details)**
Total volume collected for serum (mL):		From one 10ml serum tubes; 10ml expected.
Time spin started (24 HR):		Tube should be incubated upright at room temperature for at least 30 (but <60) minutes prior to centrifugation.
Duration of centrifugation (min):		☐ **SOP – spin for 15 minutes**
Temperature of centrifuge (°C):		☐ **SOP – spin at 4°C**
Force of centrifugation (x g):		☐ **SOP – spin at 1500 x g**
Was Serum NMDA aliquots created?: *Use orange-capped cryovial*		One 1ml serum NMDA cryovial expected, *only if subject is SCZ, BD or CHR*
# of serum aliquots created: *Use red-capped cryovials*		Two-three 1.5ml serum cryovials expected. If low volume draw occurs, please generate as many 1.5ml aliquots as possible
Time aliquots frozen (24 HR):		Cryovials should be frozen upright within 2 hours of collection
Storage temperature (°C):		☐ **SOP – store at -80°C (±10°)** Samples *may* be stored at -20°C for 2-4 hours.

10ml NaHep (green-top) Tube		Standard Procedures (See PBN MOP for details)
Total volume collected for PBMCs (mL):		Tube collected at each visit. Tube should be shipped ambient on the day of collection

10ml EDTA (purple-top) tubes		Standard Procedures (See PBN MOP for details)
Total volume collected for plasma and buffy coat (mL):		From one 10ml EDTA tubes; 10ml expected
Time spin started (24 HR):		10ml EDTA tube should be spun within 30 minutes of collection
Duration of centrifugation (min):		☐ **SOP – spin for 15 minutes**
Temperature of centrifuge (°C):		☐ **SOP – spin at 4°C**
Force of centrifugation (x g):		☐ **SOP – spin at 1500 x g**
# of plasma aliquots created: *Use purple-capped cryovials*		From 10ml EDTA tube, three-four 1.5ml plasma cryovials expected. If low volume draw occurs, please generate as many 1.5ml aliquots as possible
# of buffy coat aliquots created: *Use clear-capped cryovials*		From 10ml EDTA tube, one buffy coat cryovial expected. Each 10ml EDTA tube will produce 1 buffy coat. Buffy coats are ~750ul
Time aliquots frozen (24 HR):		Cryovials should be frozen upright within 2 hours of collection
Storage temperature (°C):		☐ **SOP – store at -80°C (±10°)** Samples *may* be stored at -20°C for 2-4 hours.

2.5ml RNA PAXgene™ tubes		Standard Procedures (See PBN MOP for details)
Total volume collected for whole blood (mL):		From two 2.5ml RNA PAXgene™ tubes; 5ml expected
Number of tubes collected:		Two tubes expected
Time tubes frozen (24 HR):		Tubes should be frozen upright within 2 hours of collection
Storage temperature (°C):		☐ **SOP – store at -80°C (±10°)** Samples *may* be stored at -20°C for 2-4 hours.

Notes (including any deviations from protocol):

Lumbar Puncture Details:

Date of Blood Draw: [MM/DD/YYYY]

Time of Draw: 24 HR clock

Fasting Status:

Duration of LP: Minutes

CSF Processing:

If processing was performed per SOP, checkbox may be used in place of text field, where provided.

Processing Details		Standard Procedures (See PBN MOP for details)
Total volume collected for CSF (mL):		15ml expected
Time spin started (24 HR):		CSF should be spun within 30 minutes of collection
Duration of centrifugation (min):		☐ **SOP – spin for 10 minutes**
Temperature of centrifuge (°C):		☐ **SOP – spin at 4°C**
Force of centrifugation (x g):		☐ **SOP – spin at 300 x g**
Was a CSF NMDA aliquot created? *Use orange-capped cryovial*		One 1ml CSF NMDA cryovial expected for BL visit, *only if subject is SCZ, BD or CHR*
# of CSF aliquots created: *Use clear-capped cryovials*		Twenty 0.5ml serum cryovials expected.
# of CSF Cell Pellet aliquots created: *Use orange-capped cryovial*		One 0.5ml CSF Cell Pellet cryovial expected
Time aliquots frozen (24 HR):		Cryovials should be frozen upright within 2 hours of collection
Storage temperature (°C):		☐ **SOP – store at -80°C (±10°)** Samples *may* be stored at -20°C for 2-4 hours.

Notes (including any deviations from protocol):

Specimen Collection and Processing Form

Please complete the Specimen Collection and Processing Form, below.

Study Site	◯ Icahn School of Medicine at Mount Sinai ◯ Perelman School of Medicine at UPenn ◯ Yale School of Medicine ◯ Northwell Health
Email address of staff member completing this form Note: A copy of the completed sample form and the shipping manifests will be sent to this address.	_____
PBN Subject ID:	_____
Is this participant a case or control?	◯ SCZ ◯ BD ◯ CHR ◯ Control (CHR allowed for follow-up ONLY)
Subject's biological sex (used for DNA quality control)	◯ Male ◯ Female ◯ Other
Visit	◯ Baseline Visit ◯ 12 Month Visit ◯ 24 Month Visit ◯ 36 Month Visit ◯ 48 Month Visit ◯ 60 Month Visit ◯ Unscheduled Visit
Kit Number	_____

Blood Collection and Processing

Date of blood collection

_____

Time of blood collection

_____
(Use 24 Hour clock.)

Patient's fasting status at time of blood collection

◯ Fasted
◯ Followed low-fat diet
◯ Not fasted/no dietary limitation

1. SERUM (one red-top 10mL serum tube)

Was blood collected and processed for SERUM?

◯ Yes
◯ No

Blood volume collected for SERUM

_____
(mL)

Reason volume was less than standard

◯ Difficult stick/poor veins
◯ Patient dehydrated
◯ Bad tube vacuum
◯ Other

Time of SERUM tube centrifugation

_____
(Use 24 Hour clock.)

Rate of SERUM tube centrifugation

_____
(x g)

Duration of SERUM tube centrifugation

_____
(minutes)

Temperature of SERUM tube centrifugation

_____
(degrees Celsius)

Total volume of SERUM collected

_____
(mL)

Was a 1 mL aliquot of serum created for NMDA receptor testing?

If yes, set this aliquot aside to be shipped to UPENN.

◯ Yes
◯ No
(Required for all SCZ/BP/CHR visits.)

Number of SERUM aliquots created to ship to the repository at IU

_____
(Aliquot to 1.5 mL, if possible.)

Was a residual SERUM aliquot (less than 1.5 mL) created?	◯ Yes ◯ No (If YES, please ensure the residual aliquot is capped with a blue top.)
What is the approximate volume of the residual SERUM aliquot?	_____ (mL)
Time SERUM was placed in freezer	_____ (Use 24 Hour clock.)
SERUM storage temperature	_____ (degrees Celsius)
SERUM notes	_____
2. PBMCs (two green-top 10mL sodium heparin tubes)	
Confirm participant consented to PBMC collection:	◯ Yes ◯ No
Was blood collected for PBMCs?	◯ Yes ◯ No (PBMCs should be collected at all visits.)
Number of tubes collected for PBMCs	_____
Total blood volume collected for PBMCs	_____ (mL)
Reason volume was less than standard	◯ Difficult stick/poor veins ◯ Patient dehydrated ◯ Bad tube vacuum ◯ Other
PBMC notes	_____
3. PLASMA (one purple-top 10mL EDTA tube)	
Was blood collected and processed for PLASMA?	◯ Yes ◯ No
Blood volume collected for PLASMA	_____ (mL)

Reason volume was less than standard	◯ Difficult stick/poor veins ◯ Patient dehydrated ◯ Bad tube vacuum ◯ Other
Time of PLASMA tube centrifugation	_____ (Use 24 Hour clock.)
Rate of PLASMA tube centrifugation	_____ (x g)
Duration of PLASMA tube centrifugation	_____ (minutes)
Temperature of PLASMA tube centrifugation	_____ (degrees Celsius)
Total volume of PLASMA collected	_____ (mL)
Number of PLASMA aliquots created	_____ (Aliquot to 1.5 mL, if possible.)
Was a residual PLASMA aliquot (less than 1.5 mL) created?	◯ Yes ◯ No (If YES, please ensure the residual aliquot is capped with a blue top.)
What is the approximate volume of the residual PLASMA aliquot?	_____ (mL)
Was the BUFFY COAT collected?	◯ Yes ◯ No
Time PLASMA and BUFFY COAT were placed in freezer	_____ (Use 24 Hour clock.)
PLASMA and BUFFY COAT storage temperature	_____ (degrees Celsius)
PLASMA notes	_____

4. RNA (two 2.5mL PAXGene™ tubes)

Was blood collected for RNA?	◯ Yes ◯ No

Number of PAXGene™ tubes collected for RNA	_____
Blood volume collected for RNA Note: Max collection volume per tube is 2.5 ml.	_____ (mL)
Reason volume was less than standard	◯ Difficult stick/poor veins ◯ Patient dehydrated ◯ Bad tube vacuum ◯ Other
Date RNA were frozen	_____
Time RNA were placed in freezer	_____ (Use 24 Hour clock.)
RNA storage temperature	_____ (degrees Celsius)
RNA notes	_____

CSF Collection and Processing

Was CSF collected?
- ◯ Yes
- ◯ No

Why was CSF not collected?

_____

Date of CSF collection

_____

Time of CSF collection

_____
(Use 24 Hour clock.)

Approximate duration of CSF collection (in minutes)

Note: We are interested in the duration of the CSF collection, specifically the length of time the collection needle is in the subarachnoid space.

_____
(minutes)

Patient's fasting status at time of CSF collection
- ◯ Fasted
- ◯ Followed low-fat diet
- ◯ Not fasted/no dietary limitation

Was CSF submitted for clinical labs?
- ◯ Yes
- ◯ No

Was CSF collected using a syringe(s)?
- ◯ Yes
- ◯ No

Note: Drip method is preferred.

Total volume of CSF collected (incl. CSF submitted for clinical labs)

_____
(mL)

Reason volume collected was less than standard
- ◯ Difficult collection/patient physiology
- ◯ Patient dehydrated
- ◯ Other

BASELINE SCZ/BP/CHR ONLY: Was a 1 mL aliquot of CSF created for NMDA receptor testing?
- ◯ Yes
- ◯ No

If yes, set this aliquot aside to be shipped to UPENN.

Time of CSF tube centrifugation

_____
(Use 24 Hour clock.)

Rate of CSF tube centrifugation

_____
(x g)

Duration of CSF tube centrifugation

_____
(minutes)

Temperature of CSF tube centrifugation	_____ (degrees Celsius)
Number of CSF aliquots created to ship to repository at IU	_____ (Aliquot to 500uL, if possible.)
Was the CSF PELLET collected?	◯ Yes ◯ No
Was the CSF PELLET frozen in a prepared Mr. Frosty™?	◯ Yes ◯ No
Time CSF, CSF NMDA (if collected), and CSF PELLET were placed in freezer	_____ (Use 24 Hour clock.)
CSF, CSF NMDA (if collected), and CSF PELLET initial storage temperature	_____ (degrees Celsius)
Was the CSF PELLET transferred to liquid nitrogen storage?	◯ Yes ◯ No (Required if samples held on site >48 hours.)
CSF notes	_____

PBN Ambient Shipping Manifest

Please verify/update the information below. When you click the "Submit" button below, a PDF copy of the Ambient Shipping Manifest will be emailed to you for Subject [subj_id]. Please print a copy of that document and include it in the shipping container with Kit #[kit_num].If you did NOT collect PBMCs, this form should be blank.

Because blood for PBMCs was not collected from this subject, please skip this form.

Study Site: _____

PBN Subject ID: _____

Visit: _____

IU Kit Number: _____

Number of sodium heparin tubes collected for PBMC extraction: _____

Total volume of blood collected for PBMC extraction: _____ (mL)

Date of collection: _____

Time of collection: _____ (24-hour clock)

Shipping Information - Please complete.

Ambient shipments should be sent Monday-Thursday only. Please check for holiday closures prior to shipping. Contact us at pbn@iu.edu if you unsure whether or not it is safe to ship. To generate an air waybill and schedule a UPS pickup, please go to the IU Repository UPS Portal.

Date of shipment: _____

Did/will you use the IU UPS interface to generate the shipping label?
- ◯ Yes
- ◯ No

Which shipping service did you use?
- ◯ UPS
- ◯ FedEx
- ◯ World Courier
- ◯ Other

What is the shipment tracking number? _____

PBN Frozen Shipping Manifest

Please verify/update the information below. When you click the "Submit" button below, a PDF copy of the Frozen Shipping Manifest will be emailed to you for Subject [subj_id], Visit [visit].Please print a copy of that document and include it in the Kit [kit_num] shipping container.

Study Site: _____

PBN Subject ID: _____

Visit: _____

IU Kit Number: _____

Date of blood collection: _____

SERUM

Number of SERUM aliquots shipped: _____

Volume of residual SERUM aliquot: _____ (mL)

PLASMA

Number of PLASMA aliquots shipped: _____

Volume of residual PLASMA aliquot: _____ (mL)

Number of BUFFY COAT aliquots shipped: _____

RNA

Number of PAXGene™ tubes shipped _____

CSF

Date of CSF collection:

_____

Number of CSF aliquots shipped:

_____

Number of CSF CELL PELLET shipped:

_____

Shipping Information - Please complete.

Frozen shipments may be sent Monday-Thursday, only (M-W preferred). Please check for holiday closures prior to shipping. Contact us at pbn@iu.edu if you unsure whether or not it is safe to ship. To generate an air waybill and schedule a UPS pickup, please go to the IU Repository UPS Portal.

Date of shipment:

_____

Did/will you use the IU UPS interface to generate the shipping label?

◯ Yes
◯ No

Which shipping service did you use?

◯ UPS
◯ FedEx
◯ World Courier
◯ Other

What is the shipment tracking number?

_____

PBN NMDAR Shipping Manifest

Please verify/update the information below. When you click the "Submit" button below, a PDF copy of the NMDAR Shipping Manifest will be emailed to you for Subject [subj_id], Visit [visit].Please print a copy of that document and include it in the Kit [kit_num] shipping container for UPenn.If [subj_id] is a healthy control participant, disregard this manifest.

Study Site: _____

Email address of site contact: _____

PBN Subject ID: _____

Visit: _____

IU Kit Number: _____

SERUM

Number of 1 ml SERUM aliquots for anti-NMDAR testing: _____

Date of serum collection: _____

CSF

Number of 1 ml CSF aliquots for anti-NMDAR testing: _____

Date of CSF collection: _____

Shipping Information

Date of shipment: _____

Estimated date of arrival at Penn _____

What is the tracking number on the preprinted UPS label provided with the NMDAR shipping kit? _____

Estimated date and time of hand delivery (UPENN site only): _____

Please call the laboratory (215-898-0181) or text Junxian (mobile: 610-368-2425) and Eric (mobile: 215-200-7646) to let them know you are on your way.

Samples shipping to:
Junxian Zhang
University of Pennsylvania
3610 Hamilton Walk
165 Johnson Pavilion/Neurology
Philadelphia, PA 19104

Phone: 215-898-0181

Email: junxian@pennmedicine.upenn.edu

APPENDIX D: Ambient Specimen Shipping Instructions

Samples Shipped Ambient:

- PBMC - Green Top 10ml Sodium Heparin Blood Tubes

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

1. **Place refrigerant pack in the freezer at least 24 hours prior to shipment.**

2. Notify Indiana University of shipment by completing the online Sample Processing and Collection form (Appendix B) and the Ambient Shipping Manifest (Appendix C). Print a copy of the Ambient Shipping Manifest (Appendix C) form to include in the shipment.

3. Insert the filled and labeled Sodium Heparin (green top) tubes in the absorbent tube sleeve. Place the sleeve and tubes into the biohazard specimen transport bag.

4. Remove as much air as possible from the plastic biohazard bag, and seal the bag according to the directions printed on the bag. Label the bag with both a kit number and an PBN Subject ID label.

5. Place the specimen into the bottom of the Styrofoam cooler, and then place the refrigerant pack on top. Please do not put the sample form in the cooler, as condensation will develop inside the cooler during transport. Put the lid onto the cooler.

6. Place a printed copy of the Ambient Shipping Manifest Form (Appendix C) along with a completed list of contents card on top of the cooler and close the cardboard box. Do NOT tape the cardboard box closed.

7. Place the box in the provided UPS® clinical overpack, and seal the overpack according to the instructions printed on the package. Stick the clear waybill sleeve to the package where indicated.

8. Login to the Indiana University UPS® portal (https://kits.iu.edu/ups). Click on the Shipping drop down menu and choose Shipping and Rating.

9. Choose your study from the Study Reference drop down menu. Click on the magnifying glass icon to search for your site in the address book, and click the Select button to populate your site's shipping address into the label.

10. Enter the weight of the package in the Package Weight field (leave the Dry Ice Weight field blank).
    (Skip step 11 if you do not need to schedule a pickup)

11. To schedule a pickup, click on the blue Pickup Request button, fill out the pickup information needed, and click Save.

12. Click the blue Ship button and print the downloaded PDF copy of the air waybill. **(Turn instruction page over ->)**

13. Place the printed waybill in the clear sleeve, peel the back and stick the sleeve to the UPS® clinical overpack, and place the package at the UPS pickup location you entered into the Pickup Request fields or an already established UPS pickup location at your site.

Do NOT draw or ship blood for PBMCs on Friday. Ship the sample to Indiana University on the day of collection. Sample must be received by Indiana University the day after collection.

APPENDIX E: Frozen Specimen Shipping Instructions

Samples Shipped on Dry Ice:

- Frozen serum in polypropylene cryotubes
- Frozen plasma in polypropylene cryotubes
- Frozen buffy coat in polypropylene cryotubes
- Frozen whole blood in PAXgene™ tubes
- Frozen CSF and CSF pellet in polypropylene cryotubes

IMPORTANT!
DO NOT SHIP SAMPLES ON FRIDAY! **Only ship samples from ONE subject-visit in each package.**

1. Notify Indiana University of shipment by completing the online Sample Processing and Collection Form (Appendix B) and the Frozen Shipping Manifest (Appendix C). Print a copy of the Frozen Shipping Manifest (Appendix C) to include in the shipment.

2. Place all frozen aliquots in the provided cryobox.

3. Place the cryobox into the clear plastic biohazard shipping bag with the absorbent sheet. Label the outside of the biohazard bag with a kit label and an PBN Subject ID label.

4. Insert frozen PAXgene™ into the bubble tube sleeve provided and place in the biohazard bag with the cryobox.
5. Seal the bag according to the instructions printed on the bag.
6. Put a layer of dry ice in the bottom of the insulated shipper, 3-4 inches deep, and place the bagged samples on top of that layer.

7. Fill the remaining space in the shipping carton with dry ice, ensuring ice surrounds the bag and reaches the top of the carton. The total weight of dry ice should be approximately 10-15 lbs.

8. Replace the lid on the Styrofoam carton, place the printed copy of the Frozen Shipping Manifest (Appendix C) on top of the carton, and close and seal the outer cardboard shipping carton with packing tape.

9. **CRITICAL STEP: Complete the provided Dry Ice label with the correct quantity of dry ice.**

10. Login to the Indiana University UPS® portal (https://kits.iu.edu/ups). Click on the Shipping drop down menu and choose Shipping and Rating.

11. Choose your study from the Study Reference drop down menu. Click on the magnifying glass icon to search for your site in the address book, and click the select button to populate your site's shipping address into the label.

12. Enter the total weight of the package in the Package Weight field. Enter the weight of the dry ice into the Dry Ice Weight field. It is important that the weight of dry ice entered in this field matches the weight on the dry ice label. **(Skip step 13 if you do not need to schedule a pickup)**

13. Click on the blue Pickup Request button, fill out the pickup information needed, and click Save.

14. Click the blue Ship button and print the air waybill. **(Turn instructions page over ->)**

15. Place the printed waybill in the clear sleeve, peel the back off and stick to the carton, and place the package at the UPS pickup location you entered into the Pickup Request fields or at an already established UPS pickup location at your site.

APPENDIX F: Low-Fat Diet Menu Suggestions

Due to the interference of lipid content in blood specimens collected for biomarker evaluation in the SSBC 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 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 F: 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 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

APPENDIX G: PBN Sample Submission Non-Conformance Report

Site #:	Site Name:
PBN Subject ID:	Visit:
Received Date:	Received By:
Kit ID:	Submission Type: Ambient Frozen

Shipping Issues Noted:

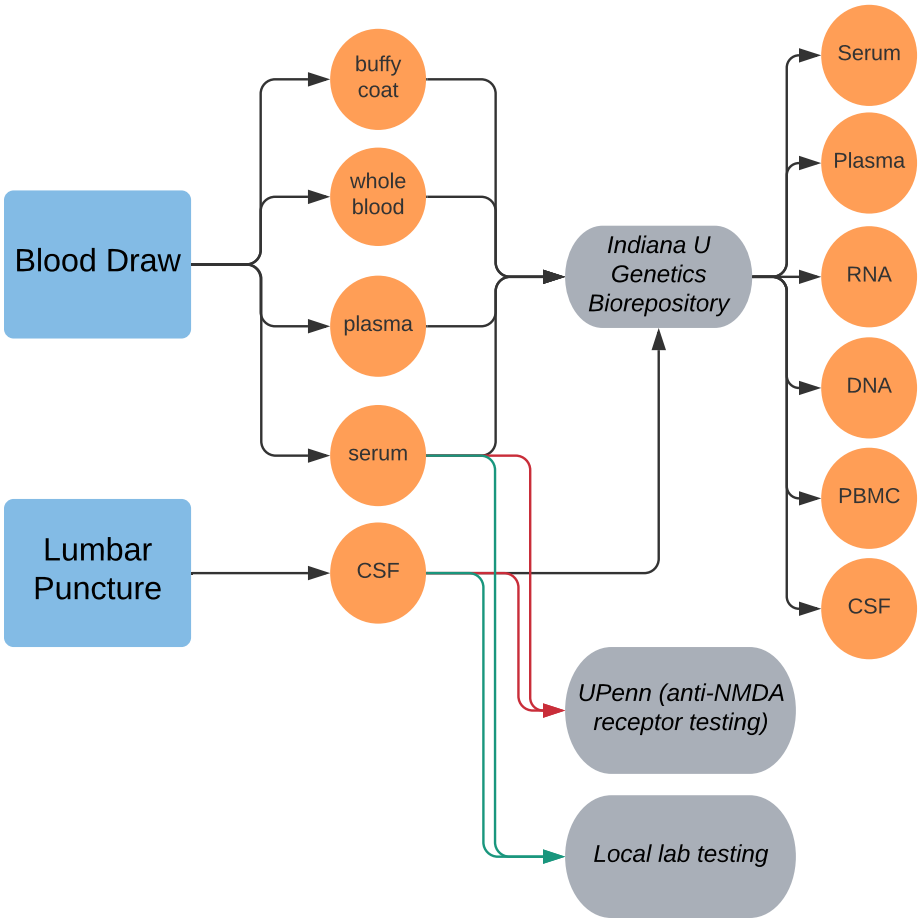
- Shipment notification not received
- Submission form not included in package, incomplete, or inaccurate
- Samples shipped for weekend or holiday delivery
- Samples improperly packaged
- Samples received damaged
- Frozen submission received thawed
- Samples received outside of shipment window
- Other

Sample Collection Issues Noted:

- Submitted in non-standard tube(s)
- Unlabeled or mislabeled tube(s)
- Low volume received
- Sample discolored
- Other

Details/Comments:

Appendix H: Sample Flow Schematic



APPENDIX I: Anti-NMDAR Testing Specimen Shipping Instructions

Samples Shipped on Dry Ice:

- **SCZ/BD/CHR BASELINE & FOLLOW UP:** Serum in polypropylene cryotubes
- **SCZ/BD/CHR BASELINE ONLY:** CSF in polypropylene cryotubes

IMPORTANT!
Only ship samples from ONE subject-visit in each package.

**Anti-NMDA receptor testing will NOT be conducted on healthy control participants. Serum and CSF for NMDA receptor testing should only be collected for SCZ, BD and CHR participants.*

1. Place all NMDA labeled aliquots in the provided biohazard shipping bag with the absorbent sheet.
2. Label the outside of the biohazard bag with a kit label AND an PBN Subject ID label.
3. Seal the bag according to the instructions printed on the bag.
4. Put a layer of dry ice in the bottom of the insulated shipper, 1-2 inches deep, and place the bagged samples on top of that layer.
5. Fill the remaining space in the shipping carton with dry ice, ensuring ice surrounds the bag and reaches the top of the carton. The total weight of dry ice should be approximately 5 lbs.
6. Replace the lid on the Styrofoam carton, place the printed copy of the NMDAR Shipping Manifest (Appendix C) on top of the carton, and close and seal the outer cardboard shipping carton with packing tape.
7. **CRITICAL STEP: Complete the provided Dry Ice label with the correct quantity of dry ice.**
8. On the pre-printed UPS airbill provided by IU, fill out the weight of the package in section 3 and sign and date in section 10.
9. Peel the backing off the airbill and place it on the carton.
10. If you do not already have a pickup scheduled with UPS, call 1-888-742-5877.