

ASSESS ALL ALS

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Section	Change
Document Footer	Updated to “Version (June 2026)”
Section 4.2.2, Section 7.1, Appendix I	Adding new visits for Super Control Participants

National Institute of Neurological Disorders and Stroke
Biorepository:

BioSpecimen Exchange for Neurological Disorders, BioSEND

**Biospecimen Collection, Processing, and Shipment Manual for
ASSESS ALL ALS study**

Table of Contents

1.0	Purpose	4
2.0	Abbreviations	4
3.0	BioSEND Information	5
3.1	BioSEND Contacts	
3.2	Hours of Operation	
3.3	Holiday Schedules	
3.4	Holiday Observations	
4.0	BioSEND Sample Requirements	7
4.1	Collection Volumes	
4.2	Protocol Schedule for Biospecimen Submission – ASSESS	
5.0	Specimen Collection Kits, Shipping and Supplies	9
5.1	Kit Supply to Study Sites	
5.2	Specimen Collection Kit Contents – ASSESS	
5.3	Site Required Equipment	
6.0	Specimen Labels	13
6.1	Types of Labels	
6.2	Affixing Labels	
7.0	Specimen Collection and Processing Procedures	15
7.1	Order of Standard Blood Collection	
7.2	Blood Collection Protocols	
7.3	Lumbar Puncture Protocol	
7.4	Filling Aliquot Tubes	
7.5	Blood Processing Timeline	
8.0	Packaging and Shipping Instructions	18
8.1	Sample Collection and Processing Form	
8.2	Shipping Instructions	
8.3	Shipping Address	

9.0 [Data Queries and Reconciliation](#).....**19**

10.0 [Appendices](#)**20**

 Appendix A: Whole Blood Collection for Isolation of Serum

 Appendix B: Whole Blood Collection for PBMC

 Appendix C: Whole Blood Collection for Isolation of Plasma (EDTA) and Buffy Coat

 Appendix D: Whole Blood Collection for Banking

 Appendix G: Cerebrospinal Fluid Collection

 Appendix I: Sample Collection and Processing Form

 Appendix K: Frozen Shipping Instructions

 Appendix L: Ambient Shipping Instructions

 Appendix Q: UPS ShipExec™ Thin Client Instructions

1.0 Purpose

The purpose of this manual is to provide collection site staff (PIs, study coordinators, and the sample collection and processing teams) at various study sites with instructions for collection and submission of biological samples. It includes instructions for biospecimen submission to the BioSpecimen Exchange for Neurological Disorders (BioSEND) located at Indiana University.

This manual includes instructions for the collection, processing, aliquoting and shipping of the following samples:

- Whole Blood for PBMC
- Serum
- Plasma
- Buffy Coat for DNA extraction
- Whole Blood for banking
- CSF

These procedures are relevant to all study personnel responsible for processing blood specimens to be submitted to BioSEND.

2.0 Abbreviations

BioSEND	BioSpecimen Exchange for Neurological Disorders
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic acid
EDTA	Ethylene Diamine Tetra-acetic Acid
IATA	International Air Transport Association
NAHEP	Sodium heparin
PBMC	Peripheral blood mononuclear cells
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute

3.0 BioSEND Information

3.1 BioSEND Contacts

Tatiana Foroud, PhD, Principal Investigator

Claire Wegel, Project Manager

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General BioSEND Contact Information

Phone: 317-278-6158

Email: biosend@iu.edu

Website: www.BioSEND.org

Sample Shipment Mailing Address

BioSEND

Indiana University School of Medicine 351

W. 10th Street. TK-217

Indianapolis, IN 46202-5188

3.2 Hours of Operation

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

Frozen samples must be shipped Monday- Wednesday only.

Ambient samples must be shipped Monday- Thursday only.

For packaging and shipment details, please refer to Appendix K (Frozen Shipping Instructions), Appendix L (Ambient Shipping Instructions) and Appendix Q (UPS ShipExec™ Thin Client Instructions).

Check the weather reports and the shipping courier website to make sure impending weather events (blizzards, hurricanes, etc.) will not impact the shipping or delivery of the samples. Couriers often report anticipated weather delays on their website.

3.3 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/holiday deliveries will not be accepted.**

3.4 Holiday Observations

Date	Holiday
January 1	New Year's Day
3 rd Monday in January	Martin Luther King, Jr Day
4 th Monday in May	Memorial Day
June 19	Juneteenth (observed)
July 4	Independence Day (observed)
1 st Monday in September	Labor Day
4 th Thursday in November	Thanksgiving
4 th Friday in November	Friday after Thanksgiving
December 25	Christmas Day

Please note that BioSEND has extended closures to inbound shipments around the Thanksgiving and Christmas holidays. In addition to sending advance notification of these closures to sites, dates will be posted on the BioSEND website. Frozen specimens collected during this period should be held at your site to ship after the first business day in January. If you are ever unsure whether or not it is safe to ship samples, please email biosend@iu.edu to confirm.

Please see https://biosend.org/holiday_closures.html for additional information.

4.0 BioSEND Sample Requirements

Please make every effort to meet the approved biospecimen collection requirements. The expected sample volume collected and number of aliquots to be returned to BioSEND from each study visit are listed in [sections 4.1-4.2](#). Because the fractionation of blood can vary, the number of plasma and serum aliquots created may deviate slightly from expected.

If a sample is not obtained (or the volume collected is less than standard) at a particular visit, this should be recorded in the notes section of the **Sample Record and Shipment Notification Form (see Appendix I)**. This form is submitted with your sample shipment to BioSEND, and a copy of this form should be retained for your site records.

4.1 Collection Volumes

Sample Type	Collection Tube(s)	Volume
Whole Blood for Plasma and Buffy Coat	2 x 10 ml EDTA	20 ml
Whole Blood for Serum	2 x 10 ml Serum	20 ml
Whole Blood for Banking	2 x 3 ml EDTA	6 ml
Whole Blood for PBMC	1 x 10 ml NaHep	10 ml
Total		56 ml
Cerebrospinal Fluid	N/A	15 ml

*Due to limitations on volume of blood which can be collected for research from pregnant individuals, if your participant is pregnant, reduce the collection for serum by 10ml. **For a pregnant participant, you will collect 1 x 10ml Serum, rather than 2.**

4.2 Protocol Schedule for Biospecimen Submission to BioSEND – ASSESS ALL ALS

4.2.1 Number of Samples to be Submitted for Symptomatic ALS Participants

Sample Type and Volume	Visit 1 Screening, Day 0	Visit 2 120 ± 30 days	Visit 3 240 ± 30 days	Visit 4 360 ± 30 days	Visit 5 480 ± 30 days	Visit 6 600 ± 30 days	Visit 7 720 ± 30 days
Serum aliquots, 0.5 ml	20	20	20	20	20	20	20
Plasma aliquots, 0.5 ml	20	20	20	20	20	20	20
Buffy Coat, 0.75 ml (approx.)	2	2	2	2	2	2	2
Whole blood for banking, 3 ml	2	2	2	2	2	2	2
PBMC, 10ml	1	-	-	-	-	-	-
CSF aliquots, 0.5ml (optional, on-site visits only)	30	30	30	30	30	30	30

4.2.2 Number of Samples to be Submitted for Control and Super Control Participants

Sample Type and Volume	Visit 1 Screening, Day 0	SC Visit 1* 120 ± 30 days	SC Visit 2* 240 ± 30 days	Visit 2 360 + 60 days	SC Visit 3* 480 ± 30 days	SC Visit 4* 600 ± 30 days	Visit 3 720 ± 60 days
Serum aliquots, 0.5 ml	20	20	20	20	20	20	20
Plasma aliquots, 0.5 ml	20	20	20	20	20	20	20
Buffy Coat, 0.75 ml (approx.)	2	2	2	2	2	2	2
Whole blood for banking, 3 ml	2	2	2	2	2	2	2
PBMC, 10 ml	1	-	-	-	-	-	-
CSF aliquots, 0.5ml (optional, on-site visits only)	30	-	-	30	-	-	30

*SC Visit 1, SC Visit 2, SC Visit 3, and SC Visit 4 are applicable only for control participants who consent to the Super Control addendum

5.0 Specimen Collection Kits, Shipping Kits, and Supplies

BioSEND will provide labels and supplies only for those specimens that are to be shipped back to the BioSEND repository. Any tubes that will remain at the collection site or shipped to another laboratory should be labeled accordingly.

All kit bags will be labeled with an ASSESS Control or ASSESS Case label to distinguish the two kit types. The kit bags will also include an expiration date label that matches the earliest expiring tube within the kit bag. Please note that this expiration date does not apply to all tube types and replacement tubes can be ordered through the kit request module.

5.1 Kit Supply to Study Sites

Each individual site will be responsible for ordering collection kits from BioSEND. We advise sites to proactively confirm kits are on hand ahead of study visits.

The link to the kit request module is shown below:

- ALL ALS - ASSESS: <https://redcap.link/assessallals>

Please allow **TWO weeks** for kit orders to be processed and delivered.

5.2 Specimen Collection Kit Contents

Collection kits contain the following (for each subject) as designated per your protocol and/or NINDS resource development agreement. Kits provide the necessary supplies to collect samples from a given subject. Do not replace or supplement any of the tubes or kit components provided with your own supplies unless you have received approval from the NINDS/BioSEND Study team to do so. Please store all kits at room temperature until use.

ASSESS Blood Collection Kit – Screening/Baseline	
Component	Quantity
Cryogenic vials (2ml) with purple caps	20
Cryogenic vials (2ml) with red caps	20
Cryogenic vials (2ml) with clear caps	2
NaHep (plastic) tube, 10ml	1
Serum (plastic) tube, 10ml	2
EDTA (plastic) tube, 10ml	2
EDTA (plastic) tube, 3ml	2
Bubble-tube sleeve	7
Disposable pipet, 3ml	3
Cryobox, 81 cell	1
Biohazard bag w/ absorbent sheet	2
Fragile label	1
UN3373 label	1
Dry ice label	1
Airway bill envelope	1
Ambient shipper	1
UPS ClinPak	1
Frozen shipper	1
Label set (kit & specimen labels)	1

ASSESS Blood Collection Kit – Longitudinal	
Component	Quantity
Cryogenic vials (2ml) with purple caps	20
Cryogenic vials (2ml) with red caps	20
Cryogenic vials (2ml) with clear caps	2
Serum (plastic) tube, 10ml	2
EDTA (plastic) tube, 10ml	2
EDTA (plastic) tube, 3ml	2
Bubble-tube sleeve	6
Disposable pipet, 3ml	2
Cryobox, 81 cell	1
Biohazard bag w/ absorbent sheet	2
Fragile label	1
UN3373 label	1
Dry ice label	1
Airway bill envelope	1
Frozen shipper	1
Label set (kit & specimen labels)	1

CSF Collection Kit	
Component	Quantity
Cryogenic vials (2ml) with clear caps	30
Individually-wrapped conical tube, 15ml	2
Individually-wrapped conical tube, 50ml	2
Medication transfer filter straw	1
LP tray (22g or 24g available)	1

Lumbar Puncture Tray	
Component	Quantity
Sprotte® needle, 24G x 90mm OR Sprotte® needle, 22G x 90mm	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	1
Polypropylene syringe (6 ml, luer lock)	4
Needle stick pad	1
Adhesive bandage	1
Drape, fenestrated, 2 tabs, paper, 18" x 26"	1
Towel, 13.5" x 18"	2
Gauze pad, 2" x 2"	6
Sponge stick applicator	3
Lidocaine 1%, 5 ml	1
Povidone-Iodine Topical Solution, 0.75 oz	1

5.3 Site Required Equipment

The following materials and equipment are necessary for the processing of specimens at the collection site and are to be **supplied by the local site**:

- Personal Protective Equipment: lab coat, nitrile/latex gloves, safety glasses
- Tourniquets
- Alcohol Prep Pads
- Gauze Pads
- Bandages
- Butterfly needles and hubs
- Microcentrifuge tube rack
- Test tube rack
- Sharps bin and lid
- Wet ice bucket
- Wet ice

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:

- Centrifuge capable of ≥ 1500 rcf (1500 x g)
- -80°C Freezer

In order to ship specimens, you must provide:

- Dry ice (minimum 10 pounds per shipment)

6.0 Specimen Labels

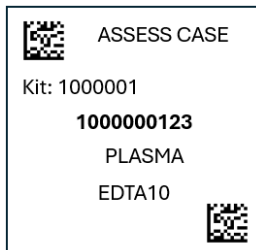
Labels must be affixed on all collection and aliquot tubes to prevent sample mix-ups and ensure chain-of-custody tracking. BioSEND provides labels for all samples being collected and returned to BioSEND. The site is responsible for providing labels for biospecimens that will be retained at the site. **If a sample is not collected, please discard the unused labels.**

6.1 Types of Labels

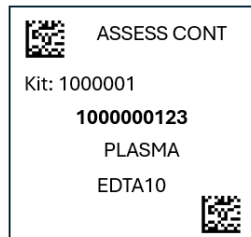
Each kit contains all labels required for the return of biospecimens to BioSEND.



The **Kit Labels** should be affixed on BioSEND forms and on specific packing materials. See Appendix K for further instructions.



The **Specimen Labels** are placed on all sample collection and aliquot tubes. Each Specimen Label has a unique barcode that is tied to the Kit. These labels indicate the specimen type and the collection tube. For example, the label to the left would be placed on a plasma aliquot generated from a 10ml EDTA tube.



Note that specimen labels will also indicate case or control.

6.2 Affixing Labels

To ensure the label adheres properly and remains on the tube, follow these instructions:

- Place specimen labels on **ALL** collection tubes and cryovials **BEFORE** sample collection, sample processing, or freezing. This will help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.
- The blood collection tube labels contain a 2D barcode on the left hand side of the label. When turned horizontally, the barcode should be closer to the top (cap end) of the tube.
- Place label **horizontally** on the tube (wrapped around sideways if the tube is upright) and **just below the ridges** of the aliquot tubes (see attached labeling diagram).



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

7.0 Specimen Collection and Processing Procedures

Consistency in sample collection and processing is essential for biomarker studies. All samples should be drawn in the same order and then processed in a uniform fashion. **Please read the instructions before collecting any specimens. Have all your supplies and equipment out and prepared prior to drawing blood.**

If you are collecting blood for safety labs, collect those samples *prior* to collecting the samples for BioSEND.

7.1 Order of Standard Blood Collection

Draw Order	Collection Tube	Aliquot Volume	Number of Tubes/ Aliquots to Ship	Cryovial Cap Color	Visits
1	2 Serum (red-top) Tubes, 10ml	0.5 ml	20	Red	For Case Participants: Visits 1 (Screening), 2, 3, 4, 5, 6, and 7. Samples should also be collected at the ET visit (as needed). For Control Participants: Visits 1 (Screening), 2, and 3. Samples should also be collected at the ET visit (as needed). For Super Control Participants: Visits 1 (Screening), SC 1, SC 2, 2, SC 3, SC 4, and 3. Samples should also be collected at the ET visit (as needed).
2	1 NaHep (Green-top) Tube, 10ml	N/A	N/A (Entire collection tube to be shipped without aliquoting)	N/A	For Case and Control Participants: Visit 1 (Screening)
3	2 EDTA (purple-top) Tubes, 10ml	0.5 ml	20	Purple	For Case Participants: Visits 1 (Screening), 2, 3, 4, 5, 6, and 7. Samples should also be collected at the ET visit (as needed).
		750 µl (approx)	2	Clear	For Control Participants: Visits 1 (Screening), 2, and 3. Samples should also be collected at the ET visit (as needed).
4	2 EDTA (purple-top) Tube, 3ml	N/A	N/A (Entire collection tubes to be shipped without aliquoting)	N/A	For Super Control Participants: Visits 1 (Screening), SC 1, SC 2, 2, SC 3, SC 4, and 3. Samples should also be collected at the ET visit (as needed).

*Standard control participants who have not consented to the Super control addendum will only complete 1, 2 and 3

7.2 Blood Collection Protocols

- Appendix A: Whole Blood Collection for Isolation of Serum
- Appendix B: Whole Blood Collection for PBMC
- Appendix C: Whole Blood Collection for Isolation of Plasma (EDTA) and Buffy Coat
- Appendix D: Whole Blood Collection for Banking

7.3 Lumbar Puncture Protocol

- Appendix G: Cerebrospinal Fluid Collection

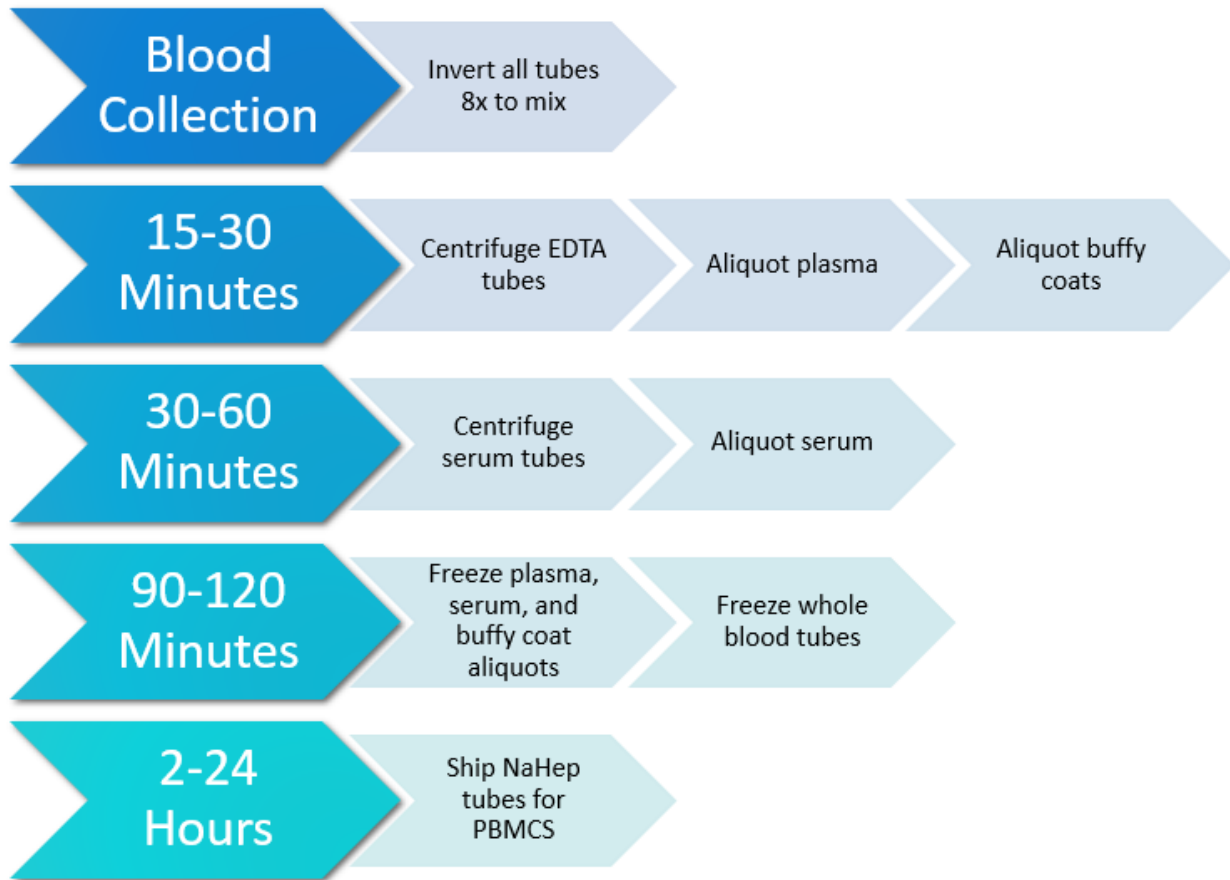
7.4 Filling Aliquot Tubes

In order to ensure that BioSEND receives a sufficient amount of sample for processing and storage, and to avoid cracking of the tubes prior to shipment, each aliquot tube should be filled to the specified volume (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. Each kit includes the appropriate collection tubes to provide the specimen volume described in the Protocol Schedules for Biospecimen Submission ([see Section 4](#)). Specimens collected in addition to those described in Section 4 are collected at the site’s discretion and are not returned to BioSEND.

Cap Color	Specimen Type	Aliquot Volume
Purple	Plasma (EDTA)	0.5 ml
Clear	Buffy Coat	0.75 ml (approx)
Clear	CSF	0.5 ml
Red	Serum	0.5 ml

Please fill as many aliquot tubes as possible to the standard volume, using any remaining sample to generate a “residual” aliquot (i.e., less than standard volume).

7.5 Blood Processing Timeline



8.0 Packaging and Shipping Instructions

ALL study personnel responsible for shipping should be certified in biospecimen shipping. If not available at your University, training and certification is available through the CITI training site (Course titled “Shipping and Transport of Regulated Biological Materials” at <https://www.citiprogram.org/>).

8.1 Sample Record and Shipment Notification Form

All sample shipments to BioSEND must include the BioSEND Sample Form. The completed forms are:

- Completed using the RedCap sample form <https://redcap.link/ASSESSALLALSSampleForm>
- Included in the shipment with the samples

8.2 Shipping Instructions

Please reference Appendix K for frozen shipping instructions, Appendix L for ambient shipping instructions and Appendix Q for generating airbills and scheduling pick-ups.

- Frozen Whole Blood (3ml EDTA tubes)
- Frozen PAXgene® Tube
- Frozen 0.5 ml aliquots of Plasma
- Frozen 0.5 ml aliquots of Serum
- Frozen 0.5 ml aliquots of CSF
- Frozen Buffy Coat
- Ambient PBMC Tube

8.3 Shipping Address

All samples are shipped to the BioSEND laboratory:

BioSEND
Indiana University School of Medicine
351 W. 10th Street. TK-217
Indianapolis, IN 46202-5188

9.0 Data Queries and Reconciliation

Appendix I must be completed the day that samples are collected to capture information related to 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.

BioSEND will contact the site as soon as possible when a discrepancy or issue is found with either the samples or paperwork.

Common non-conformance issues that will result in BioSEND staff contacting your site include:

- Missing samples (samples documented on the sample form that are not physically present in the shipment)
- Incorrect samples collected and shipped
- Damaged or incorrectly prepared samples
- Unlabeled or mislabeled samples
- Samples frozen and stored longer than three months at the site

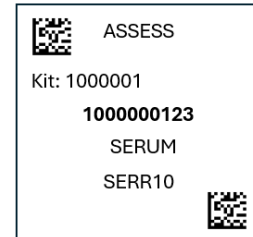
10.0 Appendices

- Appendix A: Whole Blood Collection for Isolation of Serum
- Appendix B: Whole Blood Collection for PBMC
- Appendix C: Whole Blood Collection for Isolation of Plasma (EDTA) and Buffy Coat
- Appendix D: Whole Blood Collection for Banking
- Appendix G: Cerebrospinal Fluid Collection
- Appendix I: Sample Collection and Processing Form
- Appendix K: Frozen Shipping Instructions
- Appendix L: Ambient Shipping Instructions
- Appendix Q: UPS ShipExec™ Thin Client Instructions

Appendix A – Whole Blood Collection for Isolation of Serum

Two 10 ml red-top serum (plastic) tubes and cryovials are provided by BioSEND for the collection and processing of serum.

1. Store kits and supplies at room temperature 64°F - 77°F (18°C to 25°C) before use.
2. Place pre-printed “SERUM” specimen labels on the 10ml red-top serum tubes and on 20 of the red-capped 2 ml cryovials prior to blood draw. 20 cryovials will be shipped to BioSEND.
3. Pre-chill labeled cryovials on wet ice for at least 5 minutes or longer.
4. Using a blood collection set and a holder, collect blood into the 10 ml red-top serum tubes using your institution’s recommended procedure for standard venipuncture technique.

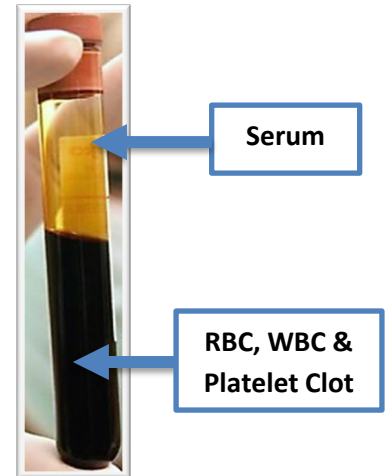


The following techniques shall be used to prevent possible backflow:

- 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 tube with its vacuum is designed to draw 10 ml of blood into the tube.
 6. Immediately after blood collection, gently invert/mix (180 degree turns) the serum determination tube 8-10 times. Do not shake the tubes!
 7. Allow blood to clot at room temperature for **at least 30 minutes**.
 8. Within 30 to 60 minutes from blood collection, centrifuge tubes for 15 minutes at 1500 RCF (x g) at room temperature. It is critical that the tubes be centrifuged at the appropriate speed and temperature to ensure proper serum separation.

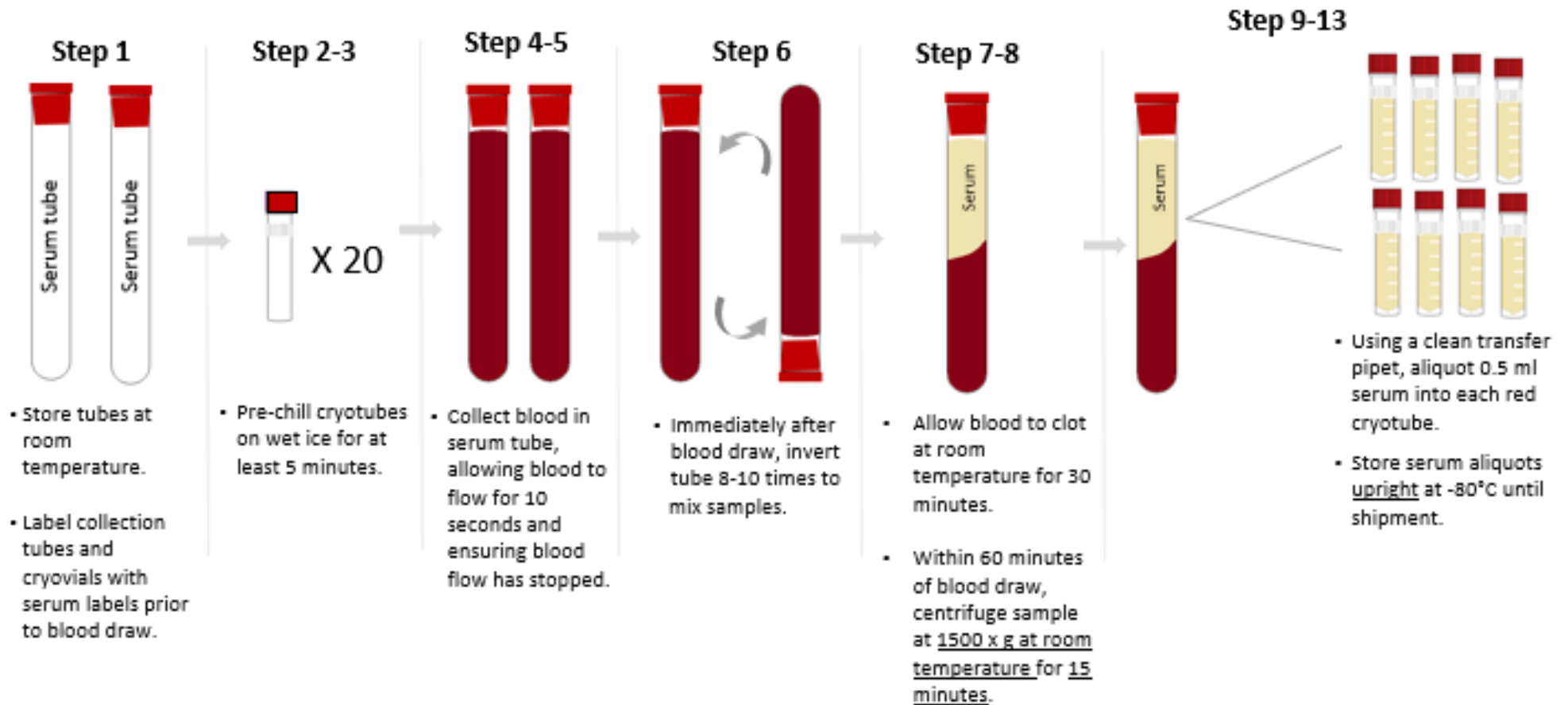
9. Remove the serum by tilting the tube and placing the pipette tip along the lower side of the tube wall. Use caution to pipet only the serum layer and not the red blood cell layer. Using a disposable tipped micropipette, transfer serum into the pre-labeled cryovials. Aliquot 0.5 ml per cryovial. Each red-top serum tube should yield approximately 4-5 ml of serum.

Note: If a low volume draw occurs, please generate as many 0.5ml aliquots as possible. Fewer standard size aliquots are preferred over 20 aliquots of non-standard size.



10. After serum has been aliquoted into cryovials, **discard** the 10ml serum collection tubes. Do not send these tubes to BioSEND.
11. Place the labeled cryovials in the 81 slot cryobox.
12. **For in-clinic visits:** Transfer cryobox to -80°C freezer as soon as possible and within two hours of collection. Store all samples at -80°C until shipped to BioSEND on dry ice.
13. **For remote visits:** Place cryobox on dry ice. Once all aliquots from the visit have been placed in the cryobox, place box in biohazard bag and seal. Transfer the biohazard bag to dry ice as soon as possible, keeping the box upright and level to help the aliquots freeze in an upright position.
14. Complete the sample form (Appendix I). Please note any issues that may have occurred during collection and processing.
15. Ship the frozen serum aliquots to BioSEND according to Appendix K – Frozen Shipping Instructions.

Serum Preparation –10 ml Serum (Red Top) Tube



Appendix B – Whole Blood Collection for PBMC

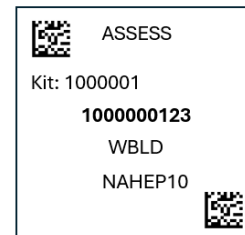
(BASELINE VISIT ONLY)

*Sodium heparin tubes **MUST** be shipped to the BioSEND 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.*

One 10ml Green-top NaHep Tube is provided by BioSEND for PBMC

1. Store kits and supplies at room temperature 64⁰F - 77⁰F (18°C to 25°C) before use.

2. Place pre-printed “PBMC” label on the NaHep tube prior to blood draw.



3. Store kits and supplies at room temperature 64⁰F - 77⁰F (18°C to 25°C) before use.

4. Place pre-printed “PBMC” label on the NaHep tube prior to blood draw.

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

The following techniques shall be used to prevent possible backflow:

Place donor's arm in a downward position.

Hold tube in a vertical position, below the donor's arm during blood collection.

Release tourniquet as soon as blood starts to flow into tube.

Make sure tube additives do not touch stopper or end of the needle during venipuncture.

6. 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 NaHep tube with its vacuum is designed to draw 10 ml of blood into the tube.

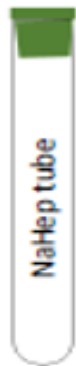
7. Immediately after blood collection, gently invert/mix (180 degree turns) the tube 8 – 10 times. Do not shake the tube!

8. Seal the Sodium Heparin tube in the ambient shipment kit and ship the unprocessed tube ambient to the appropriate BioSEND biorepository, following the instructions in Appendix L. 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 BioSEND biorepository the following day after collection. Do NOT draw or ship this sample on a Friday.

9. Complete the sample form (Appendix I).

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

Step 1 – 2



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

Step 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 sample.

Step 6 - 7

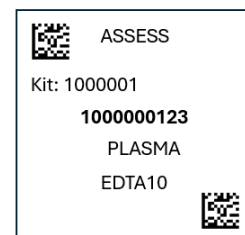
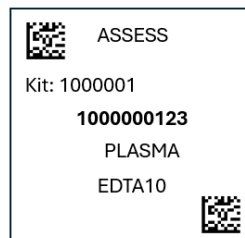


- Store tube at room temperature until shipment.
- ***Must be shipped ambient to IU on day of blood draw!***

Appendix C – Whole Blood Collection and Processing for Plasma and Buffy Coat

Whole Blood Collection for Plasma and Buffy Coat using two 10 ml EDTA (plastic) tubes.

1. Store empty EDTA (plastic) tubes at room temperature 64°F – 77°F (18°C to 25°C) prior to use.
2. Place “PLASMA” specimen labels on 10 ml EDTA tubes and on the 20 purple-capped 2 ml cryovial tubes. Place “BUFFY COAT” specimen labels on the two clear-capped 2ml cryovial tubes.
3. Pre-chill labeled cryovials on wet ice for at least 5 minutes or longer.
4. Using a blood collection set and a holder, collect blood into the purple top 10 ml EDTA (plastic) tubes using your institution's recommended procedure for standard venipuncture technique.

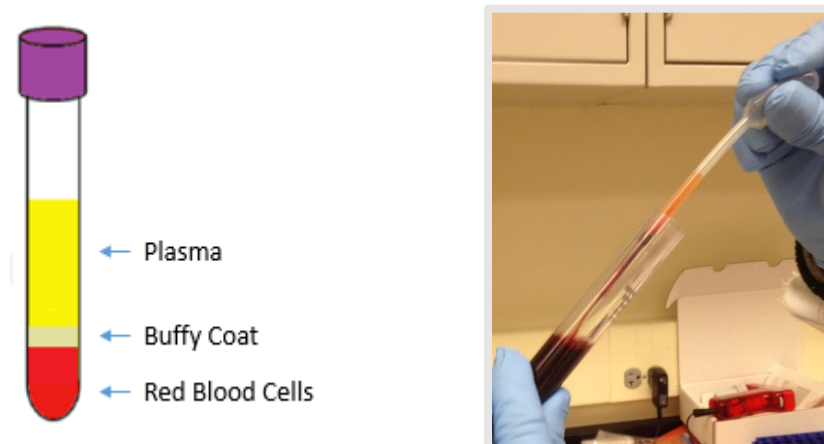


The following techniques shall be used to prevent possible backflow:

- 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 the 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 tube vacuum is designed to draw 10 ml of blood into the tube.
 6. Immediately after blood collection, **gently** invert/mix (180 degree turns) the EDTA tubes 8 – 10 times. Do not shake the tubes!
 7. Within 30 minutes of blood collection, centrifuge tubes at room temperature for 15 minutes at 1500 RCF (x g). If any deviations occur during processing, please note them on the collection and processing form.
 8. Remove the plasma by tilting the tube and placing the pipette tip along the lower side of the wall. **Use caution not to touch the buffy coat or packed red blood cells at the bottom of the tube so that the plasma is not contaminated** (see graphic on next page). Using a disposable tipped micropipette, transfer plasma into the purple-capped cryovials. Aliquot 0.5 ml per cryovial. Each EDTA tube should yield approximately 4-5 ml of plasma.

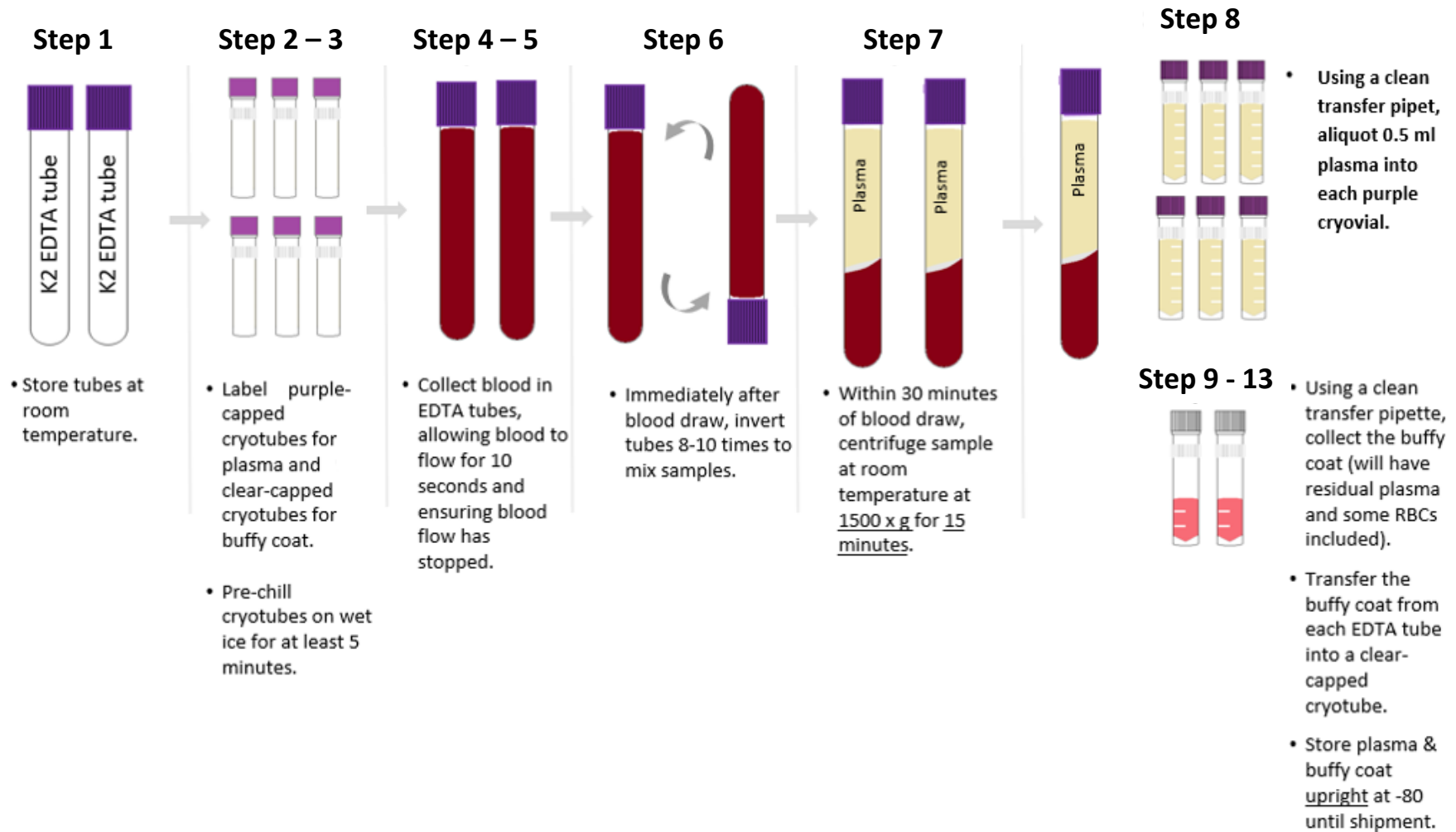
Note: If a low volume draw occurs, please generate as many 0.5ml aliquots as possible. Fewer standard size aliquots are preferred over 20 aliquots of non-standard size.

9. After plasma has been removed from the EDTA tubes, aliquot buffy coat layer from one of the EDTA tubes (see figure below) into a clear-capped cryovial using a disposable graduated micropipette. Repeat for the second EDTA tube. Each 10ml EDTA tube will produce 1 buffy coat aliquot, resulting in a total of two buffy coat aliquots. The buffy coat aliquot is expected to have a reddish color from the red blood cells. If the buffy coat looks like the plasma (ie, yellow), it was not properly captured.



10. After plasma and buffy coat has been aliquoted into cryovials, discard the 10ml EDTA collection tubes. Do not send these tubes to BioSEND.
11. Place the labeled cryovials in the 81 slot cryobox.
12. **For in-clinic visits:** Transfer cryobox to -80°C freezer as soon as possible and within two hours of collection. Store all samples at -80°C until shipped to BioSEND on dry ice.
13. **For remote visits:** Place cryobox on dry ice. Once all aliquots from the visit have been placed in the cryobox, place box in biohazard bag and seal. Transfer the biohazard bag to dry ice as soon as possible, keeping the box upright and level to help the aliquots freeze in an upright position.
14. Complete the sample form (Appendix I). Please note any issues that may have occurred during collection and processing.
15. Ship the aliquots to BioSEND according to Appendix K – Frozen Shipping Instructions.

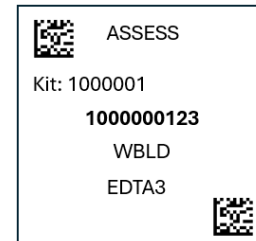
Plasma and Buffy Coat Preparation –10 ml EDTA (Purple Top) Tubes



Appendix D – Whole Blood Collection for Banking

Two 3ml Purple-Top EDTA Tube are provided by BioSEND for Whole Blood collection (to be shipped to BioSEND FROZEN; no processing required).

1. Store kits and supplies at room temperature 640F - 770F (18°C to 25°C) before use.
2. Place pre-printed specimen label (WBLD) on the two 3ml purple top EDTA tubes prior to blood draw.



3. Using a blood collection set and a holder, collect whole blood into the 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.
 5. Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tubes 8-10 times. Do not shake the tubes!
 - 6. For in-clinic visits:** Place the tubes in a wire or plastic rack. Do not use a Styrofoam rack, as this will cause the tubes to crack when frozen. Transfer the tubes in the wire or plastic rack to a -80°C freezer within two hours of collection. Store tube at -80°C until shipment to BioSEND.
 - 7. For remote visits:** Place each tube in the provided bubble wrap tube sleeve and seal. Wedge the tubes in the dry ice so that the tube is upright, to help the tube freeze in an upright position. Before shipping, the tube will be placed in a biohazard bag along with other frozen collection tubes from the visit—see Appendix K for details.
 8. Complete the sample form (Appendix I).
 9. Ship the tube to BioSEND according to Appendix K - Frozen Shipping Instructions.

WBLD Preparation – 2 x 3 ml K2 EDTA (Purple Top) Tube

Step 1 – 2



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

Step 3 – 4



- Collect blood into both 3ml EDTA tubes, 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 - 9



- Transfer to -80°C. Store upright and keep frozen until shipment to BioSEND.

Appendix G — Cerebrospinal Fluid Collection

Important Note

CSF should be collected in the morning between 8am – 10am, preferably fasted.

1. Lumbar Puncture Supplies

The lumbar puncture tray contains the following items, which will be used to perform the lumbar puncture. Check the dates of expiration: these reflect the expiration date of the lidocaine and sterile seal. Supplies for shipment of CSF are sent with the blood collection kit.

Lumbar Puncture Tray	
Component	Quantity
Sprotte® needle, 24G x 90mm OR Sprotte® needle, 22G x 90mm	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	1
Polypropylene syringe (6 ml, luer lock)	4
Needle stick pad	1
Adhesive bandage	1
Drape, fenestrated, 2 tabs, paper, 18" x 26"	1
Towel, 13.5" x 18"	2
Gauze pad, 2" x 2"	6
Sponge stick applicator	3
Lidocaine 1%, 5 ml	1
Povidone-Iodine Topical Solution, 0.75 oz	1

Sterile, individually packaged 50 ml conical tubes are provided for sites who are completing the Lumbar Puncture through the use of the gravitational method. Please ensure that all supplies necessary for a participant draw are available at your site at least two weeks prior to the appointment.

2. Setting Up the LP

- a. 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.
- b. 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.
- c. 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 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.

3. Maintaining the sterile field

- a. Keep in mind that there are usually many 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 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.

4. Tips for Clinicians Performing Lumbar Puncture: Optimizing patient comfort and minimizing the risk of adverse events.

- a. Talk the patient through the procedure so that there are no surprises.
- b. Use of a Sprotte® 24g or 22g atraumatic spinal needle and careful technique are optimal for reducing post-LP headache risk. This Sprotte® 24g or 22g atraumatic spinal needle is included in the BioSEND LP Tray; additional needles may be ordered upon request. A pencil point spinal needle such as Whitacre® 24g, Spinocan® 22g, or other 24g may also be used.
- c. Use adequate local anesthesia. Use the 25g 1/2" needle and inject lidocaine to raise a skin wheal. Then, inject lidocaine using the pattern of a square— first the center, and then to all 4 corners. 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).

- d. Encourage fluid intake immediately after LP is helpful.
- e. Be sure to give post-LP care instructions verbally to the subject (see below).

5. Post-LP Care Instructions

- Advise the subject to refrain from exertion (e.g., exercise, housework, gardening, lifting, or any other strenuous activities) for 24 hours after the LP.
- Advise the subject to continue with increased fluid intake.

6. 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 soft drink (for example) is preferable to coffee, which has some diuretic activity.
 - Acetaminophen should be used for symptomatic relief. If a subject cannot tolerate acetaminophen, ibuprofen should be used. Avoid aspirin. If these do not relieve the headache, acetaminophen with codeine or an equivalent could be considered.

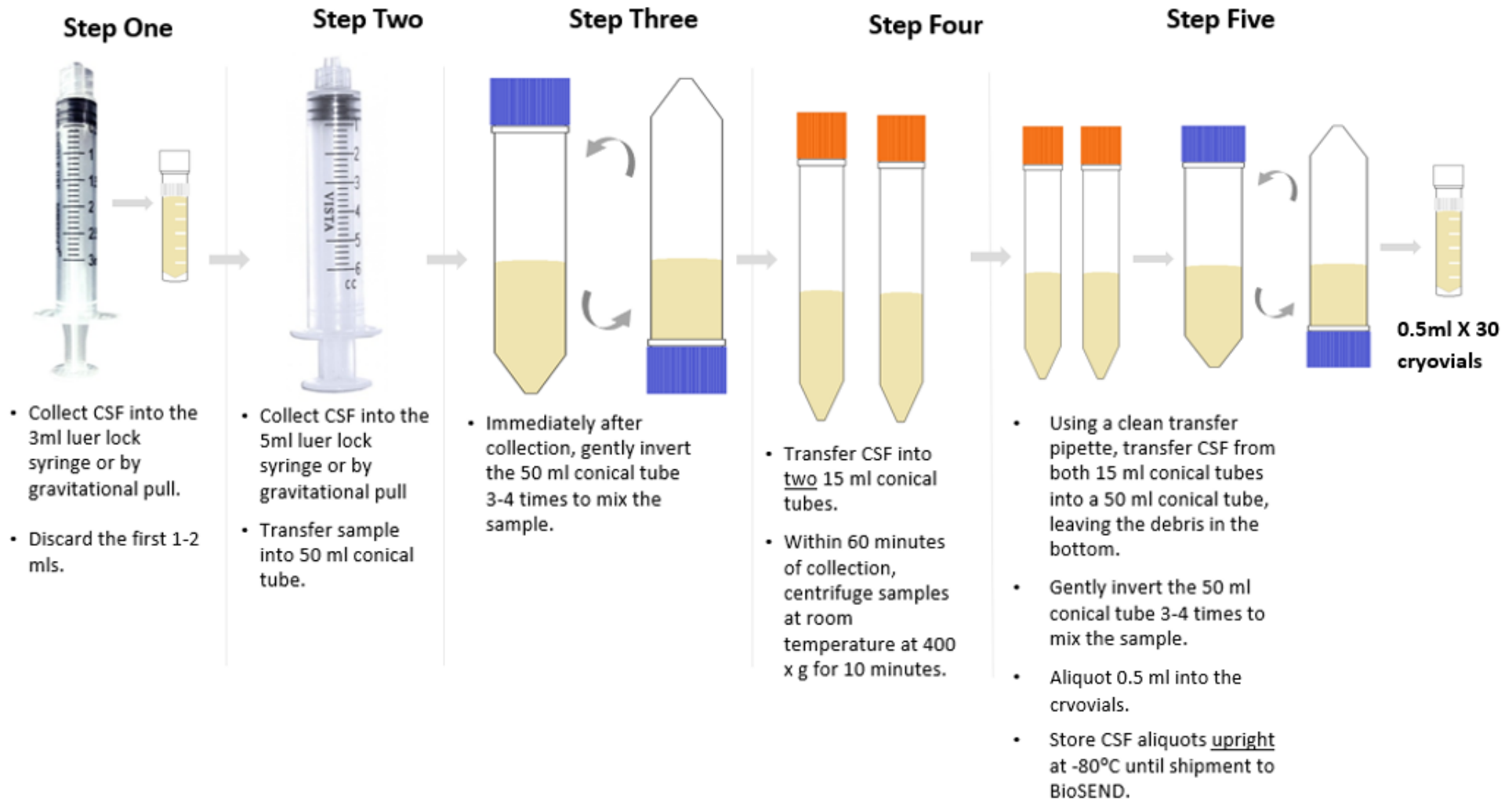
7. Severe headache after a lumbar puncture

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. Detailed Lumbar Puncture Procedure

- a. Place the preprinted Collection and Aliquot “**CSF**” labels on the collection and thirty 2 ml aliquot tubes. These 30 tubes will be shipped to BioSEND. BioSEND only provides labels and supplies for specimens intended for the BioSEND repository.
- b. **CSF cryotubes should remain at room temperature; do not pre-chill these tubes.**
- c. Perform lumbar puncture using the atraumatic technique.
- d. Collect CSF into syringes or sterile conical tube. Discard the first 1-2 mls. After the LP has begun and fluid is being collected, aliquot the first 1-2 mls of CSF from the first syringe into one of the additional cryovials provided by BioSEND, and send it to the local lab for routine diagnostic tests, if applicable to your protocol.
- e. Collect additional CSF per your site’s protocol and transfer to 50 ml conical polypropylene tube at room temperature. Firmly cap and mix gently by inverting 3-4 times. Record the time of draw (once collection is complete) on the samples form (Appendix I). Also ensure that the time of last meal consumed by participant has been documented.
- f. Within 60 minutes of collection, transfer the CSF from the 50 ml conical tube to two 15 ml conical tubes ensuring that there is equal volume in each. Spin the CSF samples down at 400 x g for 10 minutes at **room temperature**, 64°F – 77°F (18°C to 25°C).
- g. After centrifugation, pipette the supernatant from both 15 ml conical tubes and transfer to a new 50 ml conical tube. Ensure that debris at the bottom of the 15 ml conical tubes are not disturbed. Firmly cap the 50 ml conical tube and mix gently by inverting 3-4 times.
- h. Pipette (micropipette preferred) 0.5 ml of CSF directly into each of the pre-labeled aliquot tubes to be sent to BioSEND.
- i. Place the labeled cryovials in the 81-slot cryobox and place **upright** on dry ice. Transfer to **-80°C Freezer**. Store all samples at -80°C until shipped to BioSEND on dry ice.
- j. Ship the CSF aliquots to BioSEND according to Appendix K along with a copy of the sample form (Appendix I).

CSF Collection and Preparation



Appendix I – Sample Collection and Processing Form

A Sample Collection and Processing Form must be completed for each subject-visit submitted to BioSEND. This form includes Frozen and Ambient Shipping Manifests that should be completed in advance of shipping to BioSEND. A copy of the relevant shipping manifest should also be included in the shipper. A copy of the form will be emailed to you upon completion. The form can be completed via REDCap by following the bellow link:

- **Link to Sample Collection and Processing Form:**
<https://redcap.link/ASSESSALLALSSampleForm>

Please note that there is a Save & Return option at the bottom of the survey. This may be used if, for example, you are ready to complete the Collection and Processing portion of the form, but not yet ready to complete the shipping Manifests.

Clicking “Submit” at the end of the Frozen or Ambient Shipping Manifest portion of the form will send an automatic notification to BioSEND of your shipment.

If you are unable to complete the form via REDCap, a copy of the form is on the following pages. If you complete the form by hand, a copy of the form **must** be emailed to biosend@iu.edu in advance of shipment.

Appendix I: ASSESS Blood Collection and Processing Recording Form

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

ALL ALS Subject ID: _____

Visit: V1 V2 V3 V4 V5 V6 V7

Kit Number (6-7 digits): _____

SC V1 SC V2 SC V3 SC V4

Sex: M F Other

Participant Type: Case Control

Phlebotomy Details:

Date of Blood Draw:

Time of Draw:

Date Participant last ate:

Time subject last ate:

[DD/MMM/YYYY]

24 HR clock

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 BioSEND MOP for details)
Total volume collected for serum (mL):		From two 10ml serum tubes; 20ml expected. <i>If participant is pregnant, only one serum tube should be collected.</i>
Time spin started (24 HR):		Tubes should be incubated upright at room temperature for at least 30 minutes prior to centrifugation. 10ml serum tubes should be spun within 60 minutes of collection
Duration of centrifugation (min):	<input type="checkbox"/>	SOP – spin for 15 minutes
Temperature of centrifuge (°C):	<input type="checkbox"/>	SOP – spin at room temperature
Force of centrifugation (x g):	<input type="checkbox"/>	SOP – spin at 1500 x g
# of serum aliquots created: <i>Use red-capped cryovials</i>		Twenty 0.5ml serum cryovials expected. If low volume draw occurs, please generate as many 0.5ml aliquots as possible
Time aliquots frozen (24 HR):		Cryovials should be frozen upright within 2 hours of collection
Storage temperature (°C):	<input type="checkbox"/>	SOP – store at -80°C (±10°) Samples <i>may</i> be stored at -20°C for 2-4 hours.

10ml NaHep (green-top) Tube		Standard Procedures (See BioSEND MOP for details)
Total volume collected for PBMCs (mL):		Tube collected at V1 only. Tube should be shipped ambient on the day of collection

10ml EDTA (purple-top) tubes		Standard Procedures (See BioSEND MOP for details)
Total volume collected for plasma and buffy coat (mL):		From two 10ml EDTA tubes; 20ml expected
Time spin started (24 HR):		10ml EDTA tubes should be spun within 30 minutes of collection
Duration of centrifugation (min):	<input type="checkbox"/>	SOP – spin for 15 minutes
Temperature of centrifuge (°C):	<input type="checkbox"/>	SOP – spin at room temperature
Force of centrifugation (x g):	<input type="checkbox"/>	SOP – spin at 1500 x g
# of plasma aliquots created: <i>Use purple-capped cryovials</i>		From 10ml EDTA tubes, twenty 0.5ml plasma cryovials expected. If low volume draw occurs, please generate as many 0.5ml aliquots as possible
# of buffy coat aliquots created: <i>Use clear-capped cryovials</i>		From 10ml EDTA tubes, two buffy coat cryovials 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):	<input type="checkbox"/>	SOP – store at -80°C (±10°) Samples <i>may</i> be stored at -20°C for 2-4 hours.

3ml EDTA (purple-top) tubes		Standard Procedures (See BioSEND MOP for details)
Total volume collected for whole blood (mL):		From two 3ml EDTA tubes; 6ml 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):	<input type="checkbox"/>	SOP – store at -80°C (±10°) Samples <i>may</i> be stored at -20°C for 2-4 hours.

Notes (including any deviations from protocol):

Appendix I: ASSESS CSF Collection and Processing Recording Form

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

ALL ALS Subject ID: _____

Visit: V1 V4 V7 V10

Kit Number (6-7 digits): _____

Sex: M F Other

Lumbar Puncture Details:

Date of CSF Collection:
 Date Participant last ate:
[DD/MMM/YYYY]

Time of CSF Collection:
 Time subject last ate:
24 HR clock

CSF Processing:

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

Processing Details		Standard Procedures (See BioSEND MOP for details)
Total volume collected for CSF (mL):	<input style="width: 100%; height: 20px;" type="text"/>	15ml expected
Time spin started (24 HR):	<input style="width: 100%; height: 20px;" type="text"/>	CSF should be spun within 60 minutes of collection
Duration of centrifugation (min):	<input style="width: 100%; height: 20px;" type="text"/>	<input type="checkbox"/> SOP – spin for 10 minutes
Temperature of centrifuge (°C):	<input style="width: 100%; height: 20px;" type="text"/>	<input type="checkbox"/> SOP – spin at room temperature
Force of centrifugation (x g):	<input style="width: 100%; height: 20px;" type="text"/>	<input type="checkbox"/> SOP – spin at 400 x g
# of CSF aliquots created: <i>Use clear-capped cryovials</i>	<input style="width: 100%; height: 20px;" type="text"/>	Thirty 0.5ml serum cryovials expected.
Time aliquots frozen (24 HR):	<input style="width: 100%; height: 20px;" type="text"/>	Cryovials should be frozen upright within 2 hours of collection
Storage temperature (°C):	<input style="width: 100%; height: 20px;" type="text"/>	<input type="checkbox"/> SOP – store at -80°C (±10°) Samples <i>may</i> be stored at -20°C for 2-4 hours.

Notes (including any deviations from protocol):

ASSESS ALL ALS 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:

ALL ALS Subject ID:

Visit:

IU Kit Number:

Number of NaHep tubes collected for PBMC:

Total volume of blood collected for PBMC:

(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 biosend@iu.edu if you unsure whether or not it is safe to ship.

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?

ASSESS ALL ALS 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:

ALL ALS Subject ID:

Visit:

IU Kit Number:

Date of blood collection:

SERUM

Number of SERUM aliquots shipped:

PLASMA EDTA

Number of PLASMA EDTA aliquots shipped:

Number of BUFFY COAT aliquots shipped:

WHOLE BLOOD

Number of Whole Blood tubes shipped:

CSF

Number of CSF aliquots shipped

Shipping Information - Please complete.

Frozen shipments should be sent Monday-Wednesday only. Please check for holiday closures prior to shipping. Contact us at biosend@iu.edu if you unsure whether or not it is safe to ship. NOTE: Samples collected at remote visits may be shipped Monday-Thursday. Weekend deliveries are not accepted.

To generate an air waybill and schedule a UPS pickup, please go to the BioSEND UPS Portal.

Date of shipment:

Appendix K – Frozen Shipping Instructions

IMPORTANT!

Frozen samples must be shipped Monday – Wednesday only,
using Next Day Air delivery

Please be aware of holidays and inclement weather and plan your shipments accordingly. Reach out to biosend@iu.edu if you have any questions

Specimens being shipped to BioSEND are Category B UN3373 specimens and as such must be triple packaged and compliant with IATA Packing Instructions. *See the latest eEdition of the IATA regulations for complete documentation.*

Triple packaging consists of a primary receptacle(s), a secondary packaging, and a rigid outer packaging. The primary receptacles must be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured, or leak their contents into the secondary packaging. Secondary packaging must be secured in outer packaging with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging.

IATA Packing and Labeling Guidelines

- The primary receptacle (cryovials or blood collection tubes) must be leak proof and must not contain more than 1 L total.
- The secondary packaging (plastic canister or biohazard bag) must be leak proof and if multiple blood tubes are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent direct contact with adjacent blood tubes.
- Absorbent material must be placed between the primary receptacle (cryovials or blood collection tubes) and the secondary packaging. The absorbent material must be of sufficient quantity to absorb the entire contents of the specimens being shipped. Examples of absorbent material are paper towels, absorbent pads, cotton balls, or cellulose wadding.
- A shipping manifest listing the specimens being shipped must be included between the secondary and outer packaging.
- The outer shipping container must display the following labels:
 - ✓ Sender's name and address
 - ✓ Recipient's name and address
 - ✓ Responsible persons (shipper and recipient)
 - ✓ The words "Biological Substance, Category B"
 - ✓ UN3373
 - ✓ Class 9 label including UN 1845, and net weight of dry ice contained

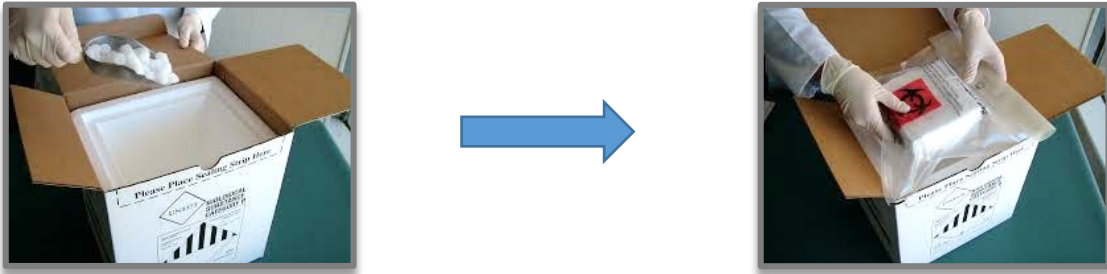
BioSEND Packaging and Shipment Instructions – Frozen Shipments

1. **For in-clinic visits:** Generate airway bill and schedule courier pick-up, as needed.
 - For instructions on generating airway bills and scheduling using the UPS ShipExec™ Thin Client system, see Appendix Q.
2. **For remote visits:** A pre-printed UPS airway bill is included with your kit.
3. Record the tracking number onto the Frozen Shipping Manifest portion of the Specimen Collection and Processing Form (Appendix I).
4. Make a copy of the Frozen Shipping Manifest to include in the shipment.
5. Place all frozen labeled cryovials in the cryobox. Only include specimens from one subject-visit in each cryobox.
6. Place the cryobox in a clear plastic biohazard bag (do NOT remove the absorbent material found in the bag) and seal the biohazard bag according to the instructions on the bag. Affix a Kit Label to the outside of the biohazard bag.



7. Place approximately 2-3 inches of dry ice in the bottom of the Styrofoam® shipping container.
8. Place the biohazard bag containing the cryobox into the provided Styrofoam® shipping container on top of the dry ice. Please ensure that the cryobox is placed so that the cryovials are upright in the shipping container (as pictured on next page).
9. Fully cover the cryobox with approximately 2 inches of dry ice.
10. If collected, place 3ml EDTA tubes for whole blood and 10ml NaHep into bubble-wrap tube sleeves and seal.
11. Place the tubes in a second clear plastic biohazard bag (do NOT remove the absorbent material found in the bag) and seal the biohazard bag according to the instructions on the bag. Affix a Kit Label to the outside of the biohazard bag.

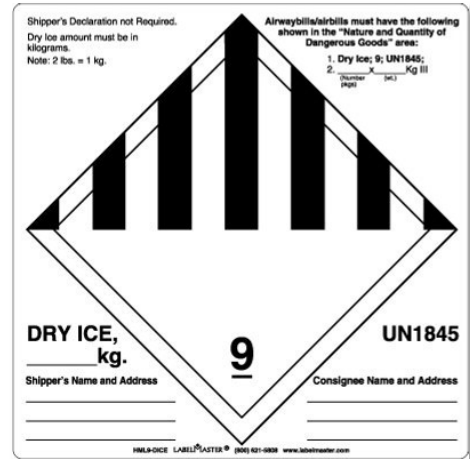
12. If collected, place 2.5ml PAXgene® tubes into bubble-wrap tube sleeves and seal.
13. Place the tubes in a third clear plastic biohazard bag (do NOT remove the absorbent material found in the bag) and seal the biohazard bag according to the instructions on the bag. Affix a Kit Label to the outside of the biohazard bag.



14. Place the biohazard bag(s) with the collections into the shipper and fully cover with approximately 2 inches of dry ice.
 - **For remote visits**, please wedge the tubes into the ice so that the tube is upright. This will help ensure the tubes remain upright as they freeze.
15. If including additional biohazard bags in package, include a layer of dry ice (approximately 2 inches) between each biohazard bag.
 - Do not package more than four total biohazard bags in a single shipper.
16. The inner Styrofoam® shipping container must contain a minimum 10 lbs (or 4.5 kg) of dry ice. The dry ice should entirely fill the inner box to ensure the frozen state of the specimens.
17. Replace the lid on the Styrofoam® container. Place the completed Frozen Manifest form in the package on top of the Styrofoam® lid for each visit included in the shipper. Close and seal the outer cardboard shipping carton with packing tape.
18. **For in-clinic visits:** Print a copy of your UPS® airway bill generated through the UPS ShipExec™ Thin Client system (see Appendix Q). Place airway bill into the provided airway bill envelope and affix envelope to package.
19. **For remote visits:** Affix the pre-printed UPS® airway bill to the outside of the package.

20. Complete the Class 9 UN 1845 Dry Ice Label with the following information:

- Your name and return address
- Net weight of dry ice in kg (this amount must match the amount recorded on the airway bill)
- Consignee name and address:



BioSEND
 IU School of Medicine
 351 W. 10th Street
 TK-217
 Indianapolis, IN 46202

IMPORTANT!

Complete the required fields on your airway bill and Class 9 Dry Ice labels, or courier may reject or return your package.

21. Apply all provided UN3373 and Fragile labels, taking care not to overlap labels with each other or with airway bill.

22. **For in-clinic visits**, hold packaged samples in -80°C freezer until time of courier pick-up/drop-off.

23. Specimens should be sent to the address below. Frozen shipments should be sent Monday through Wednesday only to avoid shipping delays on Thursday or Friday.

BioSEND
 IU School of Medicine
 351 W. 10th Street
 TK-217
 Indianapolis, IN 46202

24. Notify BioSEND of shipment by completing the sample form in REDCap® at <https://redcap.link/ASSESSALLALSSampleForm> (see Appendix I for details). Submission of this form will send an automatic notification of your shipment to the BioSEND team.
25. Use courier tracking system to ensure the delivery occurs as scheduled and is received by BioSEND.

In addition to tracking and reconciliation of samples, the condition and amount of samples received are tracked by BioSEND for each sample type. Investigators and clinical coordinators for each project are responsible for ensuring that 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.

Appendix L – Ambient Shipping Instructions

IMPORTANT!

Ambient samples must be shipped Monday – Thursday only
using Next Day Air delivery

Please be aware of holidays and inclement weather and plan your shipments accordingly.
Reach out to biosend@iu.edu if you have any questions

Ambient whole blood tube shipments are Category B UN3373 and as such must be triple packaged and compliant with IATA Packing Instructions. *See the latest edition of the IATA regulations for complete documentation.*

Triple packaging consists of a primary receptacle(s), a secondary packaging, and a rigid outer packaging. The primary receptacles must be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured or leak their contents into the secondary packaging. Secondary packaging must be secured in outer packaging with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging.

IATA Packing and Labeling Guidelines

- The primary receptacle (cryovials or blood collection tubes) must be leak proof and must not contain more than 1 L total.
- The secondary packaging (plastic canister or biohazard bag) must be leak proof and if multiple blood tubes are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent direct contact with adjacent blood tubes.
- Absorbent material must be placed between the primary receptacle (cryovials or blood collection tubes) and the secondary packaging. The absorbent material should be of sufficient quantity to absorb the entire contents of the specimens being shipped. Examples of absorbent material are paper towels, absorbent pads, cotton balls, or cellulose wadding.
- A shipping manifest listing the specimens being shipped must be included between the secondary and outer packaging.
- The outer shipping container must display the following labels:
 - ✓ Sender's name and address
 - ✓ Recipient's name and address
 - ✓ Responsible persons (shipper and recipient)
 - ✓ The words "Biological Substance, Category B"
 - ✓ UN3373

BioSEND Packaging and Shipment Instructions – Ambient Shipments

1. Place refrigerant pack in the freezer 24 hours before shipment.
2. Generate airway bill and schedule courier pick-up, as needed.
 - For instructions on generating airway bills and scheduling using the UPS ShipExec™ Thin Client system, see Appendix Q.
3. Record the tracking number onto the Ambient Shipping Manifest portion of the Specimen Collection and Processing Form (Appendix I).
4. Make a copy of the Ambient Shipping Manifest to include in the shipment.
5. Place filled and labeled NaHep tube in the biohazard bag with the absorbent material.
6. Remove as much air as possible from the biohazard bag and seal the bag according to the directions on the bag. Place kitLabel on outside of biohazard bag.



7. Place the specimen into the Styrofoam cooler, and then place the refrigerant pack on top of the specimen.
8. Place a copy of the Sample Record and Shipment Notification form and a completed IATA List of Contents sheet (provided in kit) on top of the cooler and close the cardboard box. Please do NOT tape the cardboard box closed.
9. Close shipping box and place within the provided overpack and seal.
10. Affix airway bill to the sealed overpack.
11. Specimens should be sent to the address below. Shipments should be sent Monday through Thursday.

BioSEND
IU School of Medicine
351 W. 10th Street, TK-217
Indianapolis, IN 46202

12. Notify BioSEND of shipment by completing the Specimen Collection and Processing Form and Ambient Shipping Manifest in REDCap (see Appendix I for details). Submission of this form will send an automatic notification of your shipment to the BioSEND team.

13. Use courier tracking number to ensure the delivery occurs as scheduled and is received by BioSEND.

In addition to tracking and reconciliation of samples, the condition and amount of samples received are tracked by BioSEND for each sample type. Investigators and clinical coordinators for each project are responsible for ensuring that the requested amounts of each fluid are collected to the best of their ability.

Appendix Q - UPS ShipExec™ Thin Client Instructions

***** The shipment label in ShipExec should not be created until the day of shipment *****

- 1) Log in to the UPS ShipExec™ Thin Client website: <https://kits.iu.edu/UPS> or <https://kits.iu.edu/ups>.
 - a. To request an account, complete the following survey:
<https://redcap.uits.iu.edu/surveys/?s=88TTWY3KAF>
- 2) Find the “Shipping” dropdown menu in the top left corner of the screen and click on “Shipping and Rating”.
- 3) Once the Indiana University page loads, look for the “Study Group” dropdown menu under “Shipment Information” on the right side of the screen. Choose your study from the dropdown menu.
- 4) After selecting your study, click on the magnifying glass icon on the left side of the screen under “Ship From”.
- 5) An address book and filters will populate the screen. On the right side of the screen, a list of all the site addresses within the study you selected should populate.
 - a. Filter the list down more by looking to the left side of the screen and searching for their address by filling in the “Company”, “Contact”, or “Address 1” fields. Click on the Search button when ready.
 - b. Once you have found your site address, click on the “Select” button to the left of the address.
- 6) Make sure your address populated in the fields under “Ship From” on the main page.
 - a. If you accidentally selected the wrong address, click on the “Reset” button on the bottom right of the screen. After the page reloads and clears the information, select your study again from the “Study Group” menu and click on the magnifying glass icon again to search for your correct address.
 - b. To change the address for your site and study group, please complete the following survey:
<https://redcap.uits.iu.edu/surveys/?s=88TTWY3KAF>
- 7) Enter the total weight of your package in the “Weight” field on the right side of screen under the name of your study.
 - a. Leave the “Dry Ice Weight” field empty or enter “0” if shipping an ambient sample.
- 8) Enter the weight of the dry ice for frozen shipments in the “Dry Ice Weight” field.
 - a. The “Dry Ice Weight” field can *never* be higher than the “Weight” field.
 - b. **(Steps 9-10 can be skipped if you do not need to schedule a pickup)**
- 9) After entering the weights, click on the blue “Pickup Request” button.
- 10) When the Create Pickup Request box pops up, enter information into all the fields provided.
 - a. Enter the “Earliest Time Ready” and “Latest Time Ready” in 24-hour format.
 - i. Schedule pickup at a minimum 1 hour *before* the “Earliest Time Ready”
 - b. Choose a name and phone number that is the best contact if the UPS driver has question related to picking up your package
 - c. Entering the “Room Number” and “Floor” will help the UPS driver locate your package
 - i. The “Floor” field only allows numerical characters while the “Room Number” field is free text.
 - d. Click “Save” when done.
- 11) Once you are certain that all the correct information has been entered, click the “Ship” button in the bottom right corner of the screen.
- 12) If no red error messages pop up at the top of your screen after clicking on “Ship”, then you should have 2 downloaded PDF files: Shipment Receipt & UPS Package Label

- a. Shipment Receipt will list a “Pickup No.” that references your specific package if there is ever an issue with UPS picking up your package
- 13) Print out the UPS airway bill to any printer at your location.
- a. Fold the UPS airway bill and slide it inside the plastic UPS sleeve.
 - b. Peel the back off the plastic UPS sleeve and stick the sleeve to your package, making sure it is laying as flat as possible along the surface of the package.
- 14) Place your package in the spot designated in your pickup request, or wherever your daily UPS pickups occur.
- 15) If you need to reprint your airway bill or void your shipment, click on “History” at the top of the main screen.
- a. If your shipment does not automatically pop up, enter the date of shipment and then click “Search”.
 - b. To reprint your airway bill, click on the printer icon to the far left under “Action”
 - c. To void your shipment, click on the “X” icon to the far left under “Action”
 - i. If you created an airway bill that you no longer need, you must void the shipment to ensure your study will not be charged for the shipment.