

National Institute of Neurological Disorders and Stroke  
Biorepository:

**BioSpecimen Exchange for Neurological Disorders, BioSEND**

**Biospecimen Collection, Processing, and Shipment Manual  
for PREVENT ALL ALS study - Indiana University Site**

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## 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
- Whole Blood for RNA extraction
- 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
RNA	Ribonucleic acid
RPM	Revolutions Per Minute

## 3.0 BioSEND Information

### 3.1 BioSEND Contacts

**Tatiana Foroud, PhD, Principal Investigator**

**Claire Wegel, Project Manager**

Email: [cwegel@iu.edu](mailto:cwegel@iu.edu)

**Ashley Garwood, Research Coordinator**

Email: [hoviousa@iu.edu](mailto:hoviousa@iu.edu)

#### **General BioSEND Contact Information**

Phone: 317-278-6158

Email: [biosend@iu.edu](mailto:biosend@iu.edu)

Website: [www.BioSEND.org](http://www.BioSEND.org)

#### **Sample Shipment Mailing Address**

BioSEND

Indiana University School of Medicine 351

W. 10<sup>th</sup> 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.

### 3.3 Holiday Schedules

- BioSEND will be closed on the following days (ie, unable to accept samples or to provide kits).
- **Weekend/holiday deliveries will not be accepted.**

### 3.4 Holiday Observations

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

Please note that BioSEND is open only to essential operations the last two weeks of the year. BioSEND will send advance notification of this closure to all study staff and will post a notification on biosend.org

Please see <https://biosend.org/holiday-closures> 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 Collection and Processing 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
Whole Blood for RNA	1 x 2.5 ml PAXgene®	2.5 ml
	<b>Total</b>	<b>58.5 ml</b>
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 – PREVENT ALL ALS

### 4.2.1 Number of Samples to be Submitted for PREVENT

Sample Type	Visit 1 Screening, Day 0 (Clinic)	Visit 2 120 + 30 days (Remote)	Visit 3 240 + 30 days (Remote)	Visit 4 365 + 60 days (Clinic)	Visit 5 485 + 30 days (Remote)	Visit 6 605 + 30 days (Remote)	Visit 7 730 + 60 days (Clinic)	Visit 8 850 + 30 days (Remote)	Visit 9 970 + 30 days (Remote)	Visit 10 1095 + 60 days (Clinic)
Serum aliquots, 0.5ml	20	20	20	20	20	20	20	20	20	20
Plasma aliquots, 0.5ml	20	20	20	20	20	20	20	20	20	20
Buffy Coat	2	2	2	2	2	2	2	2	2	2
Whole blood for banking, 3ml	2	2	2	2	2	2	2	2	2	2
PAXgene® RNA, 2.5ml	1	-	-	-	-	-	-	-	-	-
PBMC, 10ml*	1	-	-	-	-	-	-	-	-	-
CSF aliquots, 0.5ml (optional)	30	-	-	30	-	-	30	-	-	30

\*If the PBMC tube is unable to be collected at the Visit 1 Screening visit, this tube can be collected at one other timepoint. There is a separate PBMC kit that will need to be ordered for any patient that did not have a PBMC collected at their Visit 1 Screening visit.



## 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 blood kits will be sent in a yellow resealable bag. The kit bags will 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 - PREVENT: <https://redcap.link/preventallals>

Please allow **TWO weeks** for kit orders to be processed and ready for pick-up.

Kits may be picked up from BioSEND at the following address:

IU Innovation Center (TK)  
351 W. 10th Street.  
Indianapolis, IN 46202-5188

**5.2 Specimen Collection Kit General 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.

<b>PREVENT Blood Collection Kit – Screening/Baseline</b>	
<b>Component</b>	<b>Quantity</b>
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
PAXgene® (plastic) tube, 2.5ml	1
Bubble-tube sleeve	8
Disposable pipet, 3ml	3
Biohazard bag w/ absorbent sheet	1
Label set (kit & specimen labels)	1

<b>PREVENT Blood Collection Kit – Longitudinal</b>	
<b>Component</b>	<b>Quantity</b>
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	3
Biohazard bag w/ absorbent sheet	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  $\geq 1500$  rcf (1500 x g)

## 6.0 Specimen Labels

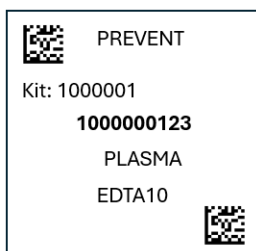
Labels must be affixed on all collection and aliquot tubes to ensure unique specimen identity. BioSEND provides labels for all samples being collected and returned to BioSEND. The site is responsible for providing labels for biospecimens that will be retained at the site. **If a sample is not collected, 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 and L 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. The labels indicate the specimen type, as well as the collection tube for that specimen. For example, the label to the right would be placed on a plasma aliquot generated from a 10ml EDTA tube.

## 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	Collected at Visits
1	2 Serum (red-top) Tubes, 10ml	0.5 ml	20	Red	Visits 1 (Screening), 2, 3, 4, 5, 6, 7, 8, 9, 10. Samples should also be collected at the ET and Ad Hoc visits (as needed).
2	1 NaHep (Green-top) Tube, 10ml	N/A	N/A (Entire collection tube to be shipped without aliquoting)	N/A	Visit 1 (Screening) **if this sample is not collected at Visit 1, it can be collected at one other timepoint
3	2 EDTA (purple-top) Tubes, 10ml	0.5 ml	20	Purple	Visits 1 (Screening), 2, 3, 4, 5, 6, 7, 8, 9, 10. Samples should also be collected at the ET and Ad Hoc visits (as needed).
		750 µl (approx)	2	Clear	
4	2 EDTA (purple-top) Tube, 3ml	N/A	N/A (Entire collection tubes to be shipped without aliquoting)	N/A	
5	1 PAXgene® Tube, 2.5ml	N/A	N/A (Entire collection tube to be shipped without aliquoting)	N/A	Visit 1 (Screening)

## 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
- Appendix F: Whole Blood Collection for RNA

## 7.3 Lumbar Puncture Protocol

- Appendix G: Cerebrospinal Fluid Collection

## 7.4 Filling Aliquot Tubes

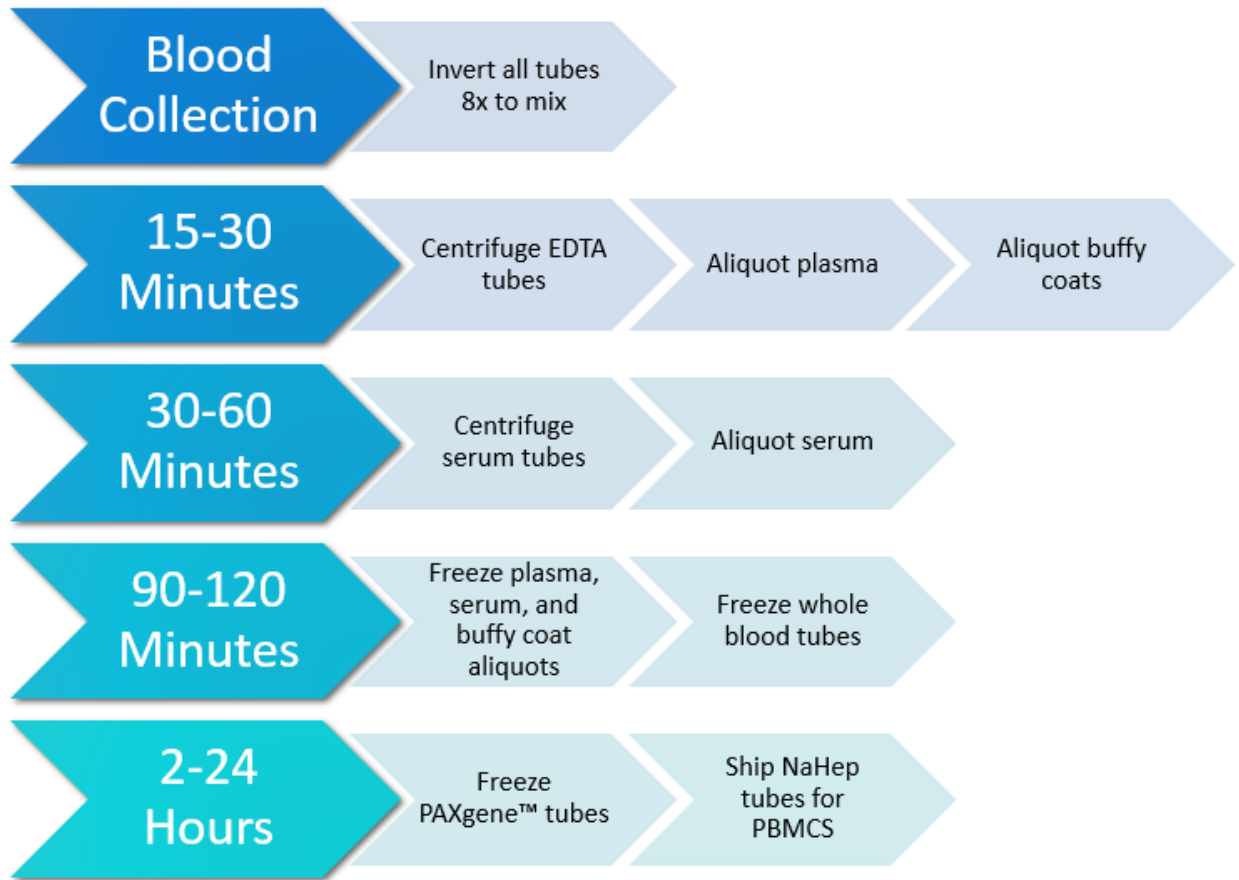
In order to ensure that BioSEND receives a sufficient amount of sample for processing and storage, and to avoid cracking of the tubes prior to shipment, each aliquot tube should be filled to the specified volume (refer to detailed processing instructions for average yield per sample). Over-filled tubes 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

After collection, tubes should be packaged in the provided biohazard bag with absorbent sheet. Do not include samples from more than one subject or visit in a single biohazard bag. Tubes should be sent to the processing lab within two hours of collection.

### 8.1 Sample Collection and Processing Form

All sample shipments to BioSEND must have the BioSEND Sample Form completed.

- Completed using the REDCap sample form <https://redcap.link/PREVENTSampleForm>
- If this REDCap is not completed prior to tubing the samples, please email biosend@iu.edu and iugbstat@iu.edu. Please include "Noyes" in the subject line.

### 8.2 Tube Station

All samples should be sent to Tube Station 260 (Noyes Pavilion). Samples may be tubed Monday-Friday from 8:30AM-4PM only. PBMC tubes should be delivered no later than noon on Fridays. Samples cannot be hand delivered to this location; samples must go through the tube station. Utilization of a courier service is also acceptable.

## 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 F: Whole Blood Collection for RNA
- Appendix G: Cerebrospinal Fluid Collection
- Appendix I: Sample Collection and Processing Form

## 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.

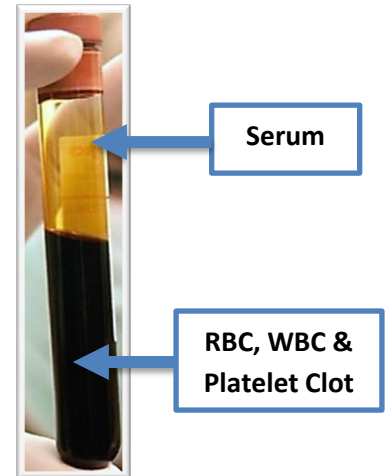


*The following techniques shall be used to prevent possible backflow:*

- 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.

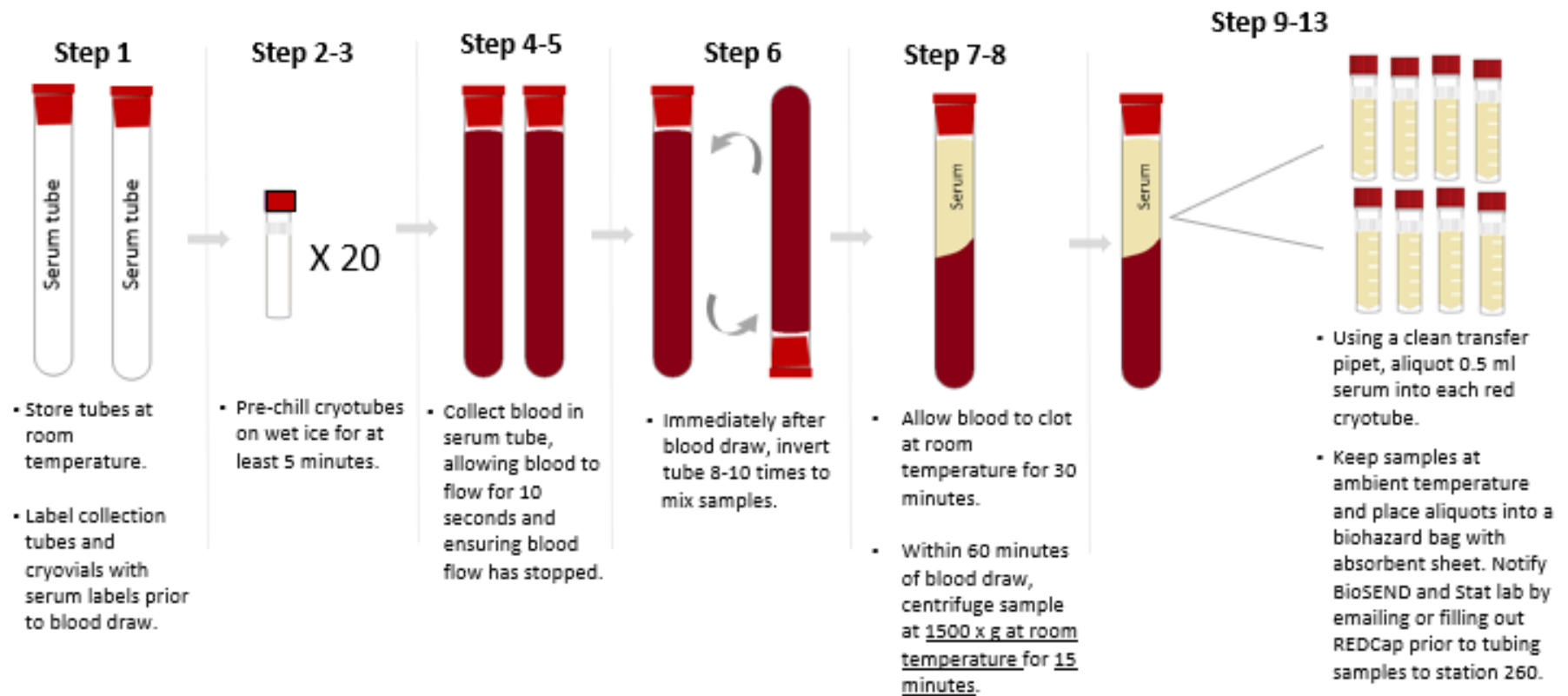
9. Remove the serum by tilting the tube and placing the pipette tip along the lower side of the tube wall. Use caution to pipet only the serum layer and not the red blood cell layer. Using a disposable tipped micropipette, transfer serum into the pre-labeled cryovials. Aliquot 0.5 ml per cryovial. Each 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 tubes into provided biohazard bag with absorbent sheet and seal.
12. Notify BioSEND and the IUGB lab of incoming samples prior to tubing. Notification should be in the form of the completed REDCap BioSEND Sample Form or an email to biosend@iu.edu and iugbstat@iu.edu. Please include "Noyes" in the subject line.
13. Tube samples to station #260 (Noyes Pavilion). Samples may be tubed Monday-Friday from 8:30AM-4PM only. Samples must be delivered via the tube station; hand deliveries to the Noyes Pavilion lab are not possible. Samples should be delivered to the lab within 2 hours of collection.
14. Ensure the sample form (Appendix I) is completed if it was not completed prior to the samples being tubed. Please note any issues that may have occurred during collection and processing.

## Serum Preparation –10 ml Serum (Red Top) Tube



## Appendix B – Whole Blood Collection for PBMC

**(BASELINE VISIT ONLY however if this is not collected at Visit 1, it can be collected at one other timepoint)**

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

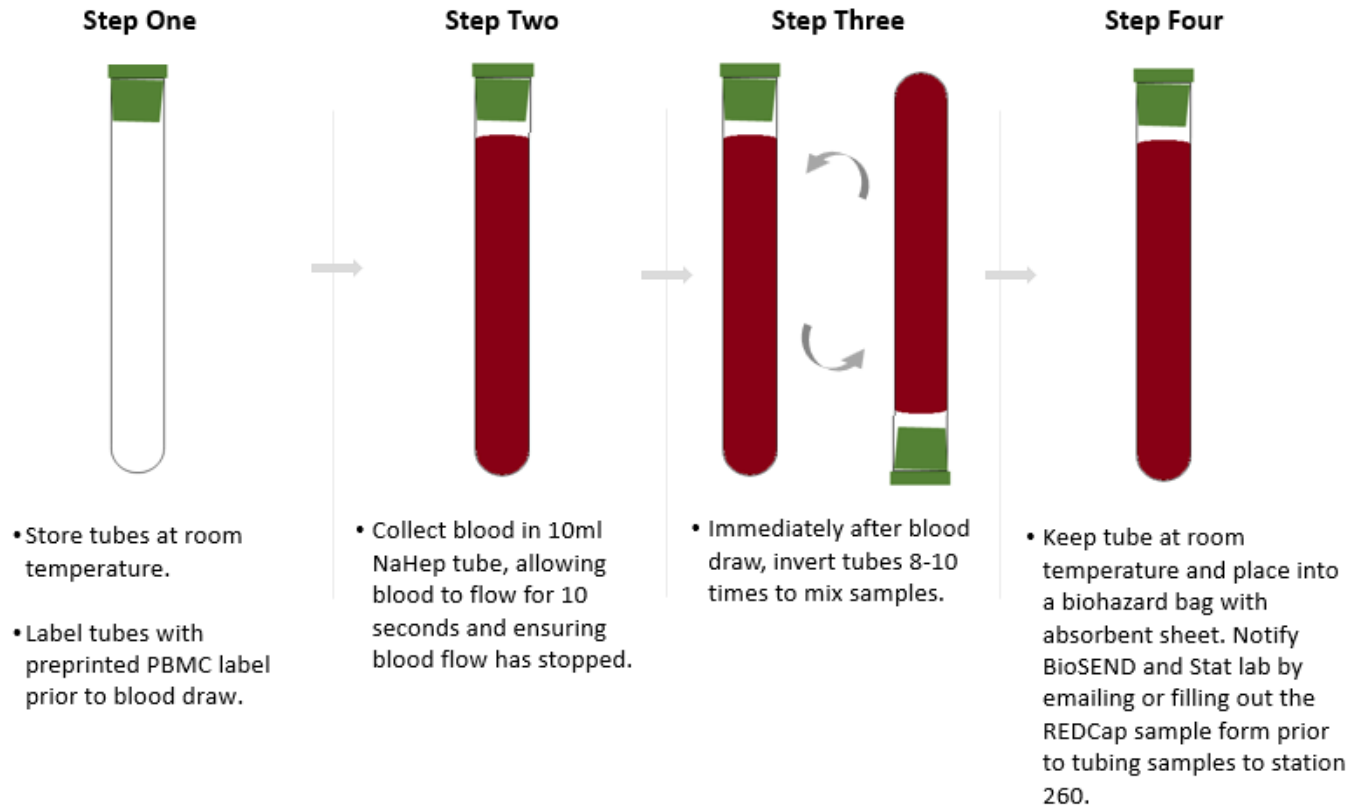


The following techniques shall be used to prevent possible backflow:

- 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.
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. Place the Sodium Heparin tube in the provided biohazard bag. Do NOT draw or send this sample past 12pm on a Friday.
  9. Notify BioSEND and the IUGB lab of incoming samples prior to tubing. Notification should be in the form of the completed REDCap BioSEND Sample Form or an email to biosend@iu.edu and iugbstat@iu.edu. Please include "Noyes" in the subject line.
  10. Tube samples to station #260 (Noyes Pavilion). Samples may be tubed Monday-Friday from 8:30AM-4PM only. Samples must be delivered via the tube station; hand deliveries to the Noyes Pavilion lab are not possible. Samples should be delivered to the lab within 2 hours of collection.
  11. Ensure the sample form (Appendix I) is completed if it was not completed prior to the samples being tubed. Please note any issues that may have occurred during collection and processing.



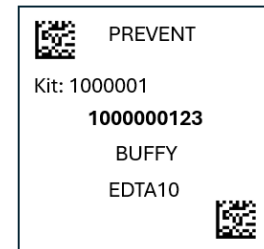
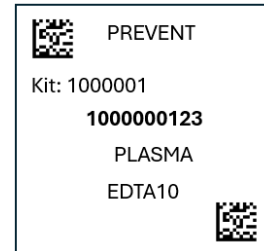
## Whole Blood for PBMC – 10ml Sodium Heparin (green top) Tube



## 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” 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.

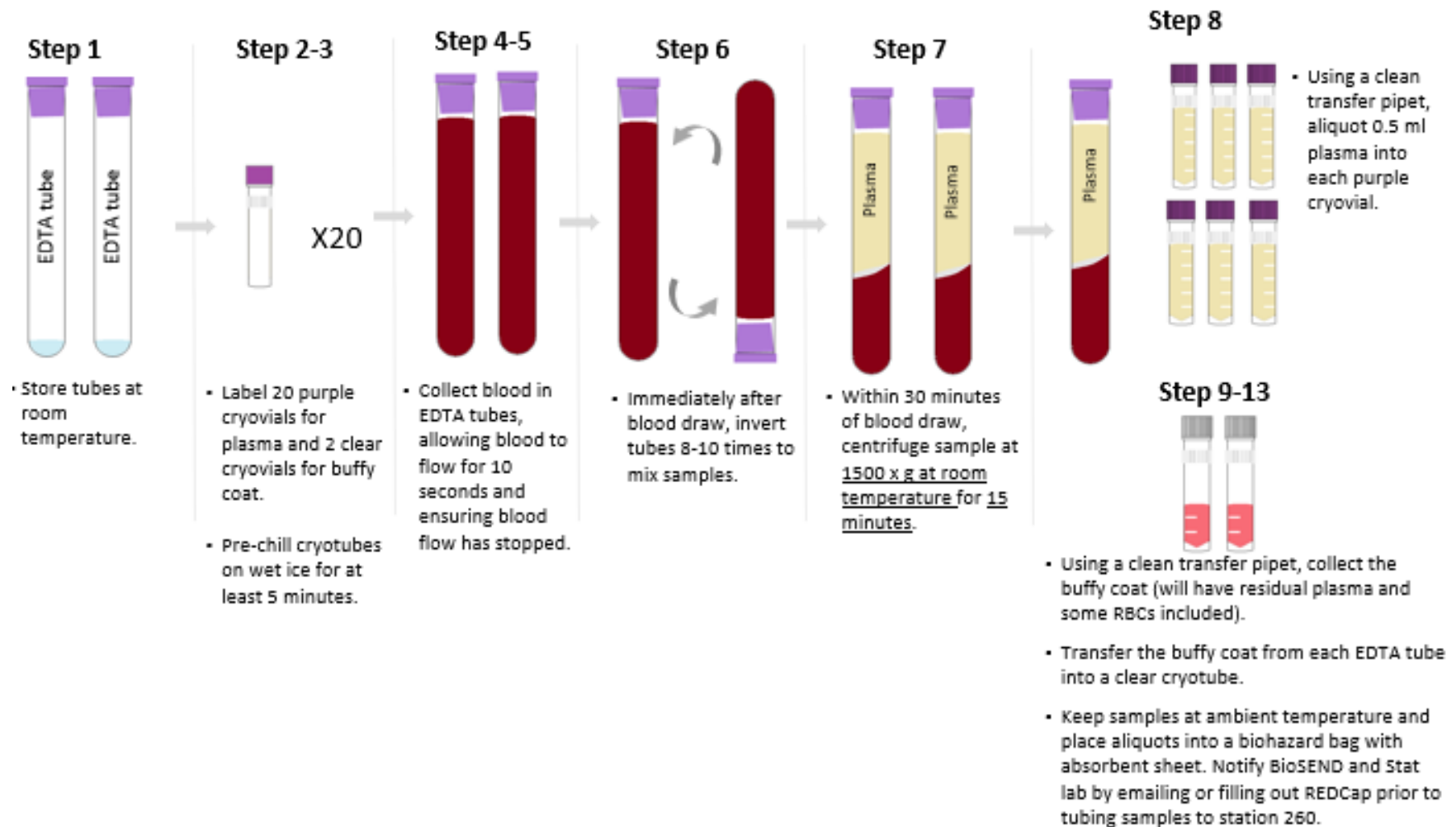
**Note:** If a low volume draw occurs, please generate as many 0.5ml aliquots as possible. Fewer standard size aliquots are preferred over 20 aliquots of non-standard size.

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 tubes into provided biohazard bag with absorbent sheet and seal.
12. Notify BioSEND and the IUGB lab of incoming samples prior to tubing. Notification should be in the form of the completed REDCap BioSEND Sample Form or an email to biosend@iu.edu and iugbstat@iu.edu. Please include "Noyes" in the subject line.
13. Tube samples to station #260 (Noyes Pavilion). Samples may be tubed Monday-Friday from 8:30AM-4PM only. Samples must be delivered via the tube station; hand deliveries to the Noyes Pavilion lab are not possible. Samples should be delivered to the lab within 2 hours of collection.
14. Ensure the sample form (Appendix I) is completed if it was not completed prior to the samples being tubed. Please note any issues that may have occurred during collection and processing.

## Plasma Preparation 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.

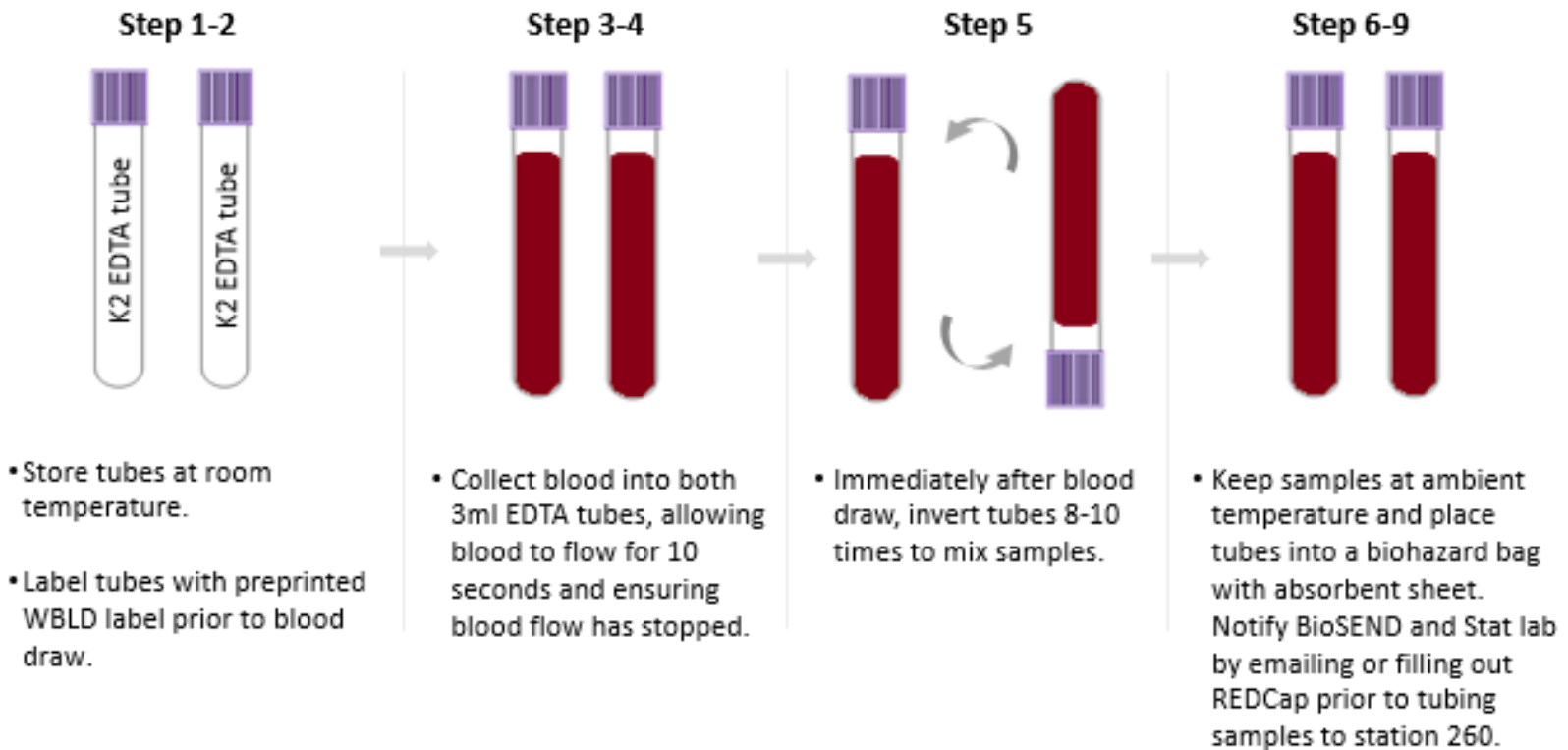


3. Using a blood collection set and a holder, collect whole blood into the tubes using your institution's recommended procedure for standard venipuncture technique.

*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. Place tubes into provided biohazard bag with absorbent sheet and seal.
  7. Notify BioSEND and the IUGB lab of incoming samples prior to tubing. Notification should be in the form of the completed REDCap BioSEND Sample Form or an email to biosend@iu.edu and iugbstat@iu.edu. Please include "Noyes" in the subject line.
  8. Tube samples to station #260 (Noyes Pavilion). Samples may be tubed Monday-Friday from 8:30AM-4PM only. Samples must be delivered via the tube station; hand deliveries to the Noyes Pavilion lab are not possible. Samples should be delivered to the lab within 2 hours of collection.
  9. Ensure the sample form (Appendix I) is completed if it was not completed prior to the samples being tubed. Please note any issues that may have occurred during collection and processing.

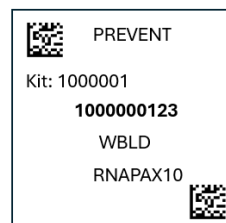
## WBLD Preparation – 2 x 3 ml K2 EDTA (Purple Top) Tube



## Appendix F – Whole Blood Collection for of RNA

**One 2.5 ml PAXgene® tube is provided by BioSEND for the collection of blood for RNA  
(BASELINE VISIT ONLY)**

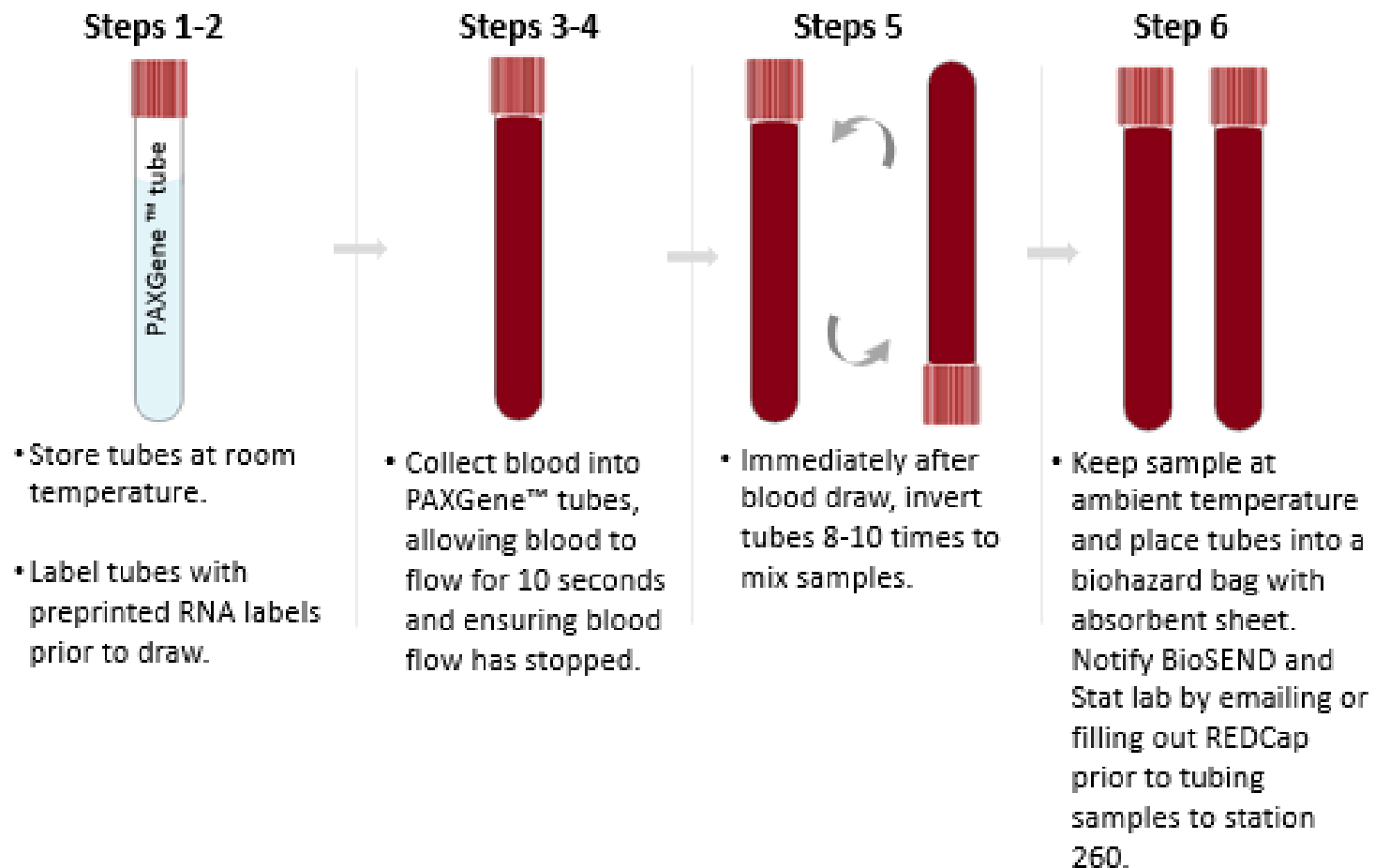
1. Store unused kits and supplies at room temperature 64<sup>0</sup>F - 77<sup>0</sup>F (18°C to 25°C) before use.
2. Place specimen label on the PAXgene® tube prior to blood draw
3. Using a blood collection set and a holder, collect blood into the PAXgene® tube using your institution's recommended procedure for standard venipuncture technique.



The following techniques shall be used to prevent possible backflow:

- a. Place donor's arm in a downward position.
  - b. Hold tube in a vertical position, below the donor's arm during blood collection.
  - c. Release tourniquet as soon as blood starts to flow into tube.
  - d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.
4. Allow at least 10 seconds for a complete blood draw to take place in each tube. Ensure that the blood has stopped flowing into the tube before removing the tube from the holder. The PAXgene® tube with its vacuum is designed to draw 2.5 ml of blood into the tube.
  5. Immediately after blood collection, gently invert/mix (180 degree turns) the PAXgene® tube 8 – 10 times. **Do not shake the tube!**
  6. Place tubes into provided biohazard bag with absorbent sheet and seal.
  7. Notify BioSEND and the IUGB lab of incoming samples prior to tubing. Notification should be in the form of the completed REDCap BioSEND Sample Form or an email to biosend@iu.edu and iugbstat@iu.edu. Please include "Noyes" in the subject line.
  8. Tube samples to station #260 (Noyes Pavilion). Samples may be tubed Monday-Friday from 8:30AM-4PM only. Samples must be delivered via the tube station; hand deliveries to the Noyes Pavilion lab are not possible. Samples should be delivered to the lab within 2 hours of collection.
  9. Ensure the sample form (Appendix I) is completed if it was not completed prior to the samples being tubed. Please note any issues that may have occurred during collection and processing.

## RNA Collection and Preparation – 2.5 ml PAXgene® Tube





## 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 <b>OR</b> 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 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, sexual activity, or any other strenuous activities) for 24 hours after the LP.
- Advise the subject to continue with increased fluid intake.

### a. 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.

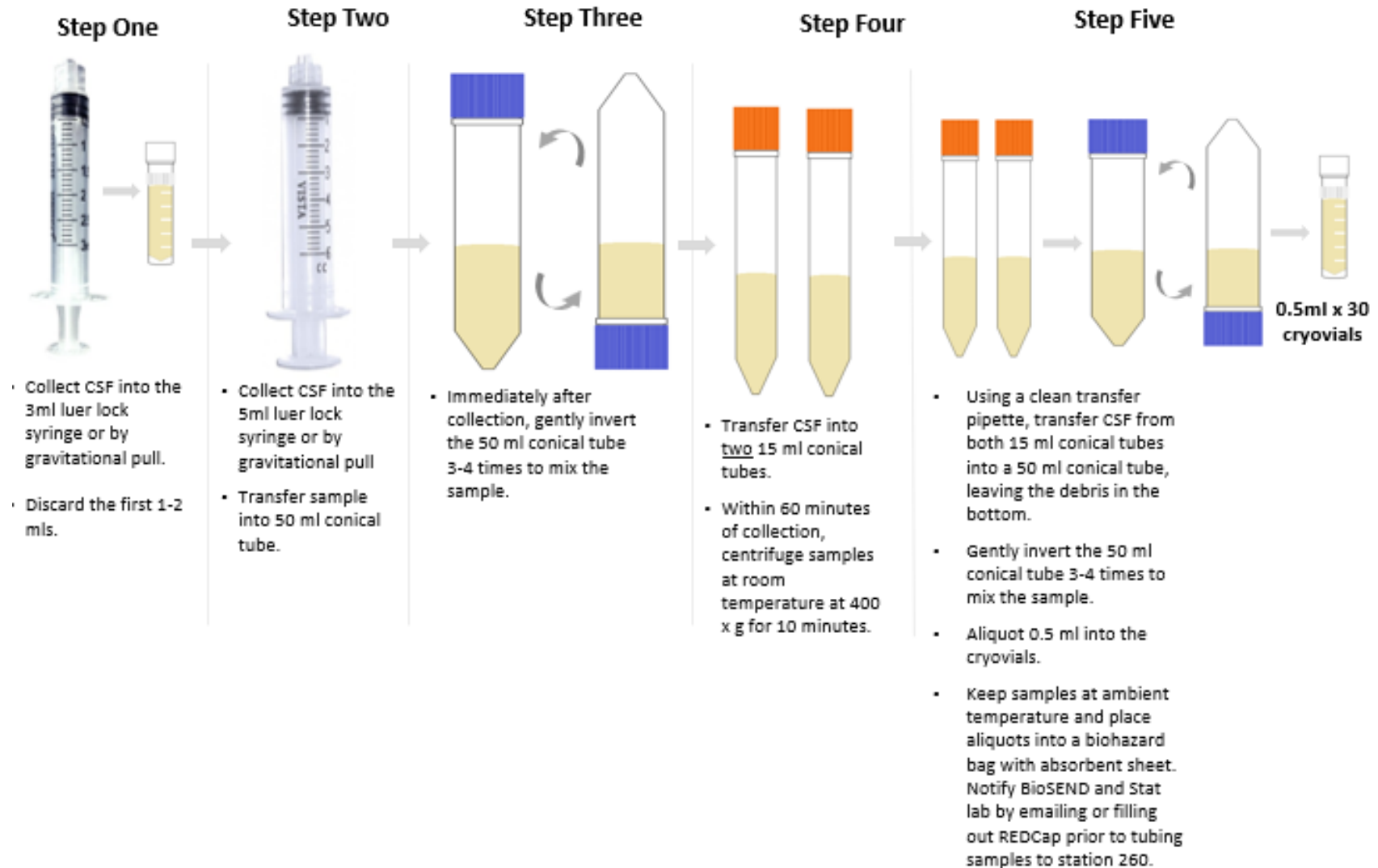
### b. Severe headache after a lumbar puncture

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 tubes into provided biohazard bag with absorbent sheet and seal.
- j. Notify BioSEND and the IUGB lab of incoming samples prior to tubing. Notification should be in the form of the completed REDCap BioSEND Sample Form or an email to biosend@iu.edu and iugbstat@iu.edu. Please include "Noyes" in the subject line.
- k. Tube samples to station #260 (Noyes Pavilion). Samples may be tubed Monday-Friday from 8:30AM-4PM only. Samples must be delivered via the tube station; hand deliveries to the Noyes Pavilion lab are not possible. Samples should be delivered to the lab within 2 hours of collection.
- l. Ensure the sample form (Appendix I) is completed if it was not completed prior to the samples being tubed. Please note any issues that may have occurred during collection and processing.

## CSF Collection and Preparation – ALL ALS



## 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 a Frozen Shipping Manifest that should be completed in advance of tubing samples to BioSEND. A copy of the form will be emailed to you upon completion. For links to your sample processing form, to download the most recent version of of this manual, and access recordings of BioSEND trainings, please visit <https://biosend.org/coordinate-studies/active-studies> and choose your study from the drop-down list.

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

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 Frozen Shipping Manifest.

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

If unable to access REDCap during collection and processing, the form on the following may be printed and completed by hand. If your site has a separate processing lab from your coordinating team, you can share this form with them so that they may capture and return this data to you. This form is provided to aid with processing data collection. Data must be entered into REDCap when site staff is able to do so.

## Appendix I: PREVENT 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/PREVENTSampleForm>. If you have any questions about sample processing for this study, visit [biosend.org](http://biosend.org) or email [biosend@iu.edu](mailto:biosend@iu.edu).

ALL ALS Subject ID: \_\_\_\_\_

Visit:  V1  V2  V3  V4  V5  V6  V7

Kit Number (6-7 digits): \_\_\_\_\_

V8  V9  V10

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

### 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"/> <b>SOP – spin for 15 minutes</b>
Temperature of centrifuge (°C):		<input type="checkbox"/> <b>SOP – spin at room temperature</b>
Force of centrifugation (x g):		<input type="checkbox"/> <b>SOP – spin at 1500 x g</b>
# 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"/> <b>SOP – store at -80°C (±10°)</b> 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):		If the PBMC tube is unable to be collected at the V1, this tube can be collected at one other timepoint. 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"/>	<b>SOP – spin for 15 minutes</b>
Temperature of centrifuge (°C):	<input type="checkbox"/>	<b>SOP – spin at room temperature</b>
Force of centrifugation (x g):	<input type="checkbox"/>	<b>SOP – spin at 1500 x g</b>
# 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"/>	<b>SOP – store at -80°C (±10°)</b> 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"/>	<b>SOP – store at -80°C (±10°)</b> Samples <i>may</i> be stored at -20°C for 2-4 hours.

***(Form continues on next page)***



2.5ml PAXGene® tube		Standard Procedures (See BioSEND MOP for details)
Total volume collected for whole blood (mL):		Tube collected at V1 only
Duration of room temperature incubation:		Tube should incubate upright, at room temperature, for 24 hours. Minimum incubation time of 2 hours is required
Time tubes frozen (24 HR):		Tubes should be frozen upright within 24 hours of collection
Storage temperature (°C):		<input type="checkbox"/> <b>SOP – store at -80°C (±10°)</b> Samples <i>may</i> be stored at -20°C for 2-4 hours.

**Notes (including any deviations from protocol):**

## Appendix I: PREVENT 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/PREVENTSampleForm>. If you have any questions about sample processing for this study, visit [biosend.org](https://biosend.org) or email [biosend@iu.edu](mailto:biosend@iu.edu).

ALL ALS Subject ID: \_\_\_\_\_

Visit:  V1  V4  V7  V10

Kit Number (6-7 digits): \_\_\_\_\_

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

### Lumbar Puncture Details:

Date of CSF Collection:

Time of CSF Collection:

Date Participant last ate:

Time subject last ate:

[DD/MMM/YYYY]

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):		15ml expected
Time spin started (24 HR):		CSF should be spun within 60 minutes of collection
Duration of centrifugation (min):		<input type="checkbox"/> <b>SOP – spin for 10 minutes</b>
Temperature of centrifuge (°C):		<input type="checkbox"/> <b>SOP – spin at room temperature</b>
Force of centrifugation (x g):		<input type="checkbox"/> <b>SOP – spin at 400 x g</b>
# of CSF aliquots created: <i>Use clear-capped cryovials</i>		Thirty 0.5ml serum cryovials expected.
Time aliquots frozen (24 HR):		Cryovials should be frozen upright within 2 hours of collection
Storage temperature (°C):		<input type="checkbox"/> <b>SOP – store at -80°C (±10°)</b> Samples <i>may</i> be stored at -20°C for 2-4 hours.

**Notes (including any deviations from protocol):**

# PREVENT 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], Visit [visit].

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.

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

---

BioSEND Internal: Date Received

---

# PREVENT 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].

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:

\_\_\_\_\_

## RNA

Number of RNA PAXgene™ 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:

---

Days from collection to frozen shipping

---

Days since collection for samples not yet shipped  
(frozen)

---

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?

---

BioSEND Internal: Date Received

---