



Biospecimen Exchange for Neurological Disorders

PREVENT ALL ALS Study

BIOSPECIMEN COLLECTION & PROCESSING

Overview

1. Specimen uniformity and quality
2. Site Equipment
3. Procedures
 - Kit Contents and Ordering
 - Sample Labelling
 - Sample Collection and Processing
 - Shipping Samples
 - Non-Conformance
4. Contact Information

Specimen Uniformity and Quality

GENERAL REMINDERS

Specimen Standardization and Quality

Most biomarkers are sensitive to *time* and *temperature*

- Standardization of processing across sites is key
- All frozen specimens must be processed and frozen within two hours
- Ambient PBMC must be shipped to BioSEND same day as collection
- Reference the *BioSEND Biomarker Specimen Collection, Processing, and Shipment Manual* as needed
- Do not replace or supplement any kit components without first receiving approval from BioSEND/NINDS

Questions? Email biosend@iu.edu

Site Consumables and Equipment

Sites will need to supply the following items:

- Gloves
- Alcohol wipes
- Butterfly needles
- Tourniquet
- Gauze pads
- Bandages
- Microcentrifuge tube rack
- Sharps bin and lid
- Crushed ice
- Pipettes and pipette tips
- Centrifuge capable of 1500 RCF (x g)
- -80°C Freezer
- Dry ice

Procedures

MAINTAINING SPECIMEN UNIFORMITY AND QUALITY

Biospecimen Collection Protocol- Symptomatic Participants

Sample Type & Volume	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 5 360 ± 30 days (Remote)	Visit 6 480 ± 30 days (Remote)	Visit 7 730 ± 60 days (Clinic)	Visit 8 600 ± 30 days (Remote)	Visit 9 720 ± 30 days (Remote)	Visit 10 1095 ± 60 days (Clinic)
Plasma aliquots, 0.5ml	20	20	20	20	20	20	20	20	20	20
Serum 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, 3ml	2	2	2	2	2	2	2	2	2	2
RNA Paxgene	1	-	-	-	-	-	-	-	-	-
PBMC, 10ml	1	-	-	-	-	-	-	-	-	-
CSF aliquots, 0.5ml (optional, on-site visits only)	30	-	-	30	-	-	30	-	-	30

Kit Contents and Ordering

- All sites will be sent a Supplemental Kit with their first kit shipment
 - Contains extra blood collection tubes, processing supplies, and LP needles
 - May be used to replace items in study visit kits
- Study Visit Kits should be ordered as soon as visits are planned
 - Contains collection, processing, and shipping supplies specific to each visit
 - Includes barcoded labels
 - The supplies/labels in each study visit kit are intended for that visit only

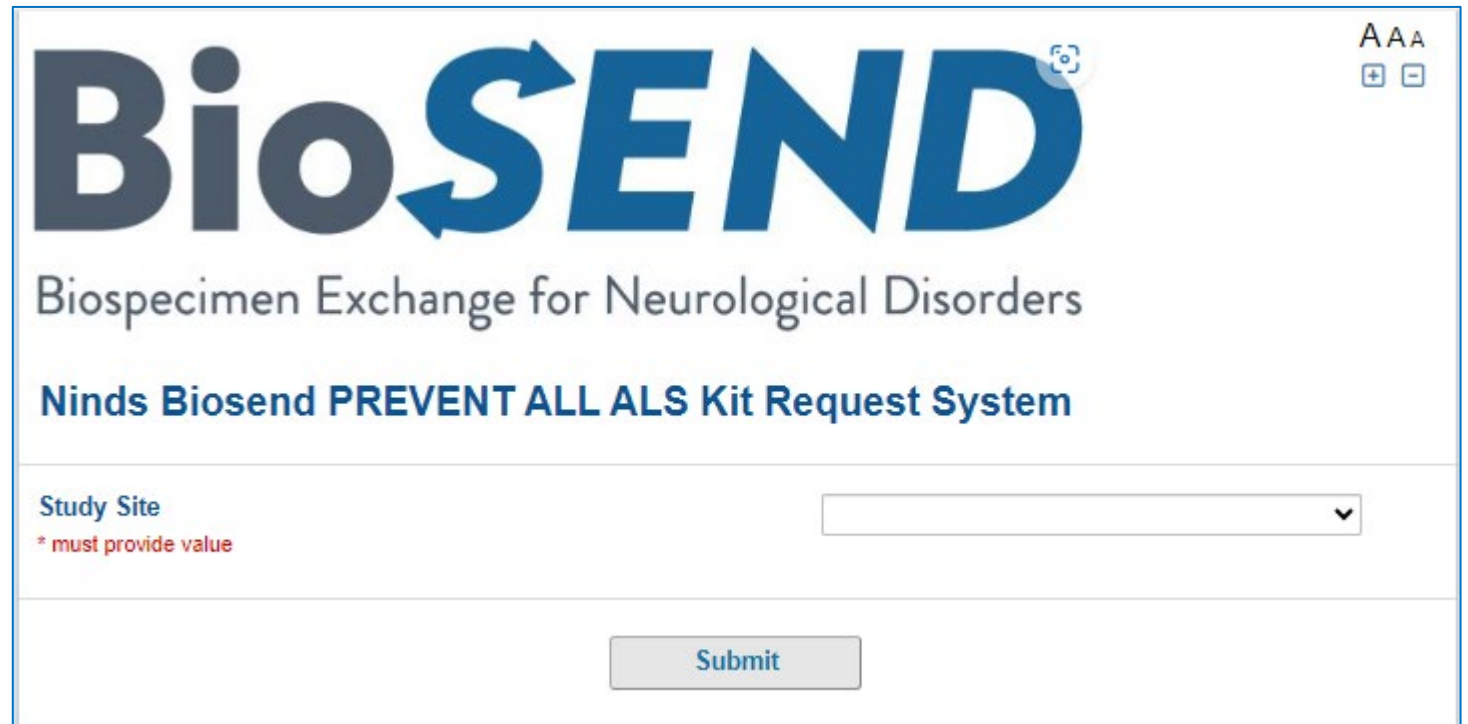
Kit Contents and Ordering – REDCap Survey

<https://redcap.link/preventalls>

Order kits online through the Kit Request Module for:

- Blood Kits
- CSF Kits
- Extra Supplies

Please provide as much notice as possible when ordering kits and/or supplies.



The screenshot shows a web form titled "BioSEND" with the subtitle "Biospecimen Exchange for Neurological Disorders". Below this is the heading "Ninds Biosend PREVENT ALL ALS Kit Request System". The form includes a "Study Site" dropdown menu with a red asterisk and the text "* must provide value" below it. A "Submit" button is located at the bottom right of the form. In the top right corner of the form, there are icons for font size adjustment (AAA) and a small camera icon.

Kit Contents and Ordering: Confirm Site Info

PREVENT ALL ALS Kit Request Module

Study Site * must provide value	Indiana University	Select your site from the drop-down list
Indiana University School of Medicine Carolyn Dunifon Dept. of Medical & Molecular Genetics 351 West 10th Street, TK-318 Indianapolis, IN 46202-3002 (317) 274-5751 cdunifon@iu.edu		
Verify contact information and update if needed		
Is the contact name above correct? * must provide value	☐ Yes ☒ No	reset
New Contact Name * must provide value	Claire Wegel	
Is the shipping address above correct? * must provide value	☐ Yes ☐ No	reset
Is the e-mail address above correct? * must provide value	☐ Yes ☐ No	reset
Is the phone number above correct? * must provide value	☐ Yes ☐ No	reset

Kit Contents and Ordering: Kit Types

PREVENT ALL ALS Kit Request Module

- Kits are not specific to a subject or time point. After collection, sites will indicate the subject and time point to which BioSEND should link the samples.
- CSF processing kits are ordered independently of blood kits
- All specimen labels (including CSF) will be included in the Blood Collection Kit. If CSF is not collected at a visit, you may discard these extra labels

Kit Type

****Please allow two weeks for shipment****

* must provide value

☒ Blood Collection Kit

☐ Shipping Kit

☐ YourBio Kit

☐ CSF Kit

☐ Extra Supplies

Please specify in comments if you need kits before the standard two week shipment time.

Blood Collection Kit Quantity

* must provide value

1|

Comments

Expand

Kit Contents and Ordering: Kit Breakdown

PREVENT ALL ALS Kit Request Module

Comments

Expand

Blood Collection Kit

Blood Collection Kit Contents:

2 - EDTA tube (10ml), plastic

1- RNA Paxgene tube (10ml)

1- NaHep PBMC tube (10ml)

2- Red-top Serum tube (10ml) plastic

2 - EDTA tube for whole blood (3ml)

6 - Purple-capped cryovial (Sarstedt), 2ml

2 - Clear-capped cryovial (Sarstedt), 2ml

6 - Red-capped cryovial (Sarstedt), 2ml

2 - Disposable transfer pipette (3ml)

1 - 25-Slot Cryobox

3 - Bubble sleeves for tubes

30 - Cryohold specimen labels-- Kit numbers

Submit

Kit contents of selected kit will appear at the bottom of the page

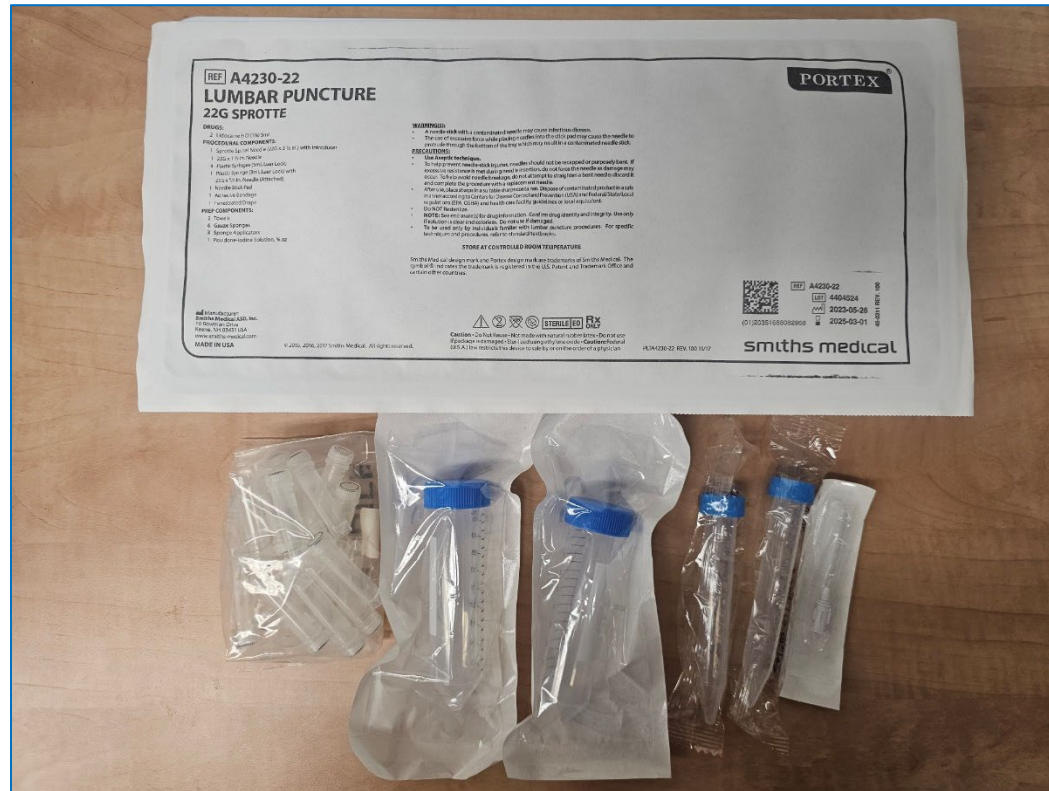
Kit Contents and Ordering: Blood Kits



Yellow kit bags will be used for PREVENT kits to help distinguish PREVENT kits from ASSESS kits

Kit Contents and Ordering: CSF Kits

CSF:



LP Tray:



Collection Volumes

Total blood and CSF volumes

Sample Type	Amount
Whole Blood for Serum	20 ml
Whole Blood for PBMC	10 ml
Whole Blood for Plasma and Buffy Coat	20 ml
Whole Blood for Banking	6 ml
Whole Blood for RNA	2.5 ml
Cerebrospinal Fluid	15 ml

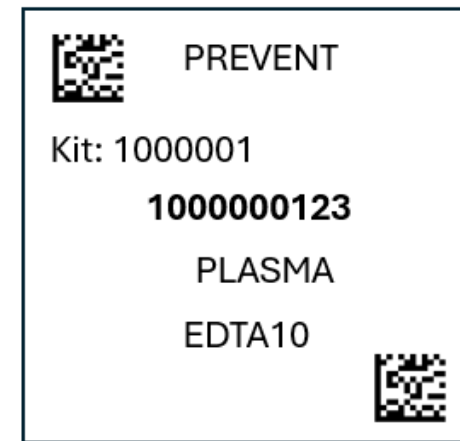
Kit and Supply Ordering

- Click “Submit” to send order to BioSEND; staff will confirm receipt of your order
- Please allow two week turn-around time for kit shipments
- If urgent request needed, please note date needed by in comments and email BioSEND. We cannot guarantee urgent orders, but we will do our best to accommodate.
- BioSEND will send confirmation of shipment and tracking number when supplies ship

Sample Labelling: Example Labels

Labels are provided by Indiana University

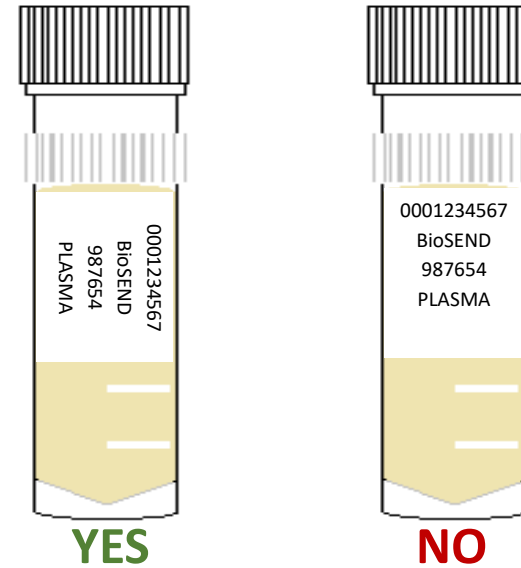
- Please check that all samples are properly labelled to ensure correct identification by IU
- If do not have enough labels to complete a visit, please contact IU *immediately*
- Note that you will have extra labels. If you do not need the extra labels, please dispose of them.
- Labelling the tubes during processing prevents sample mix-ups



Sample Labelling: Label Placement

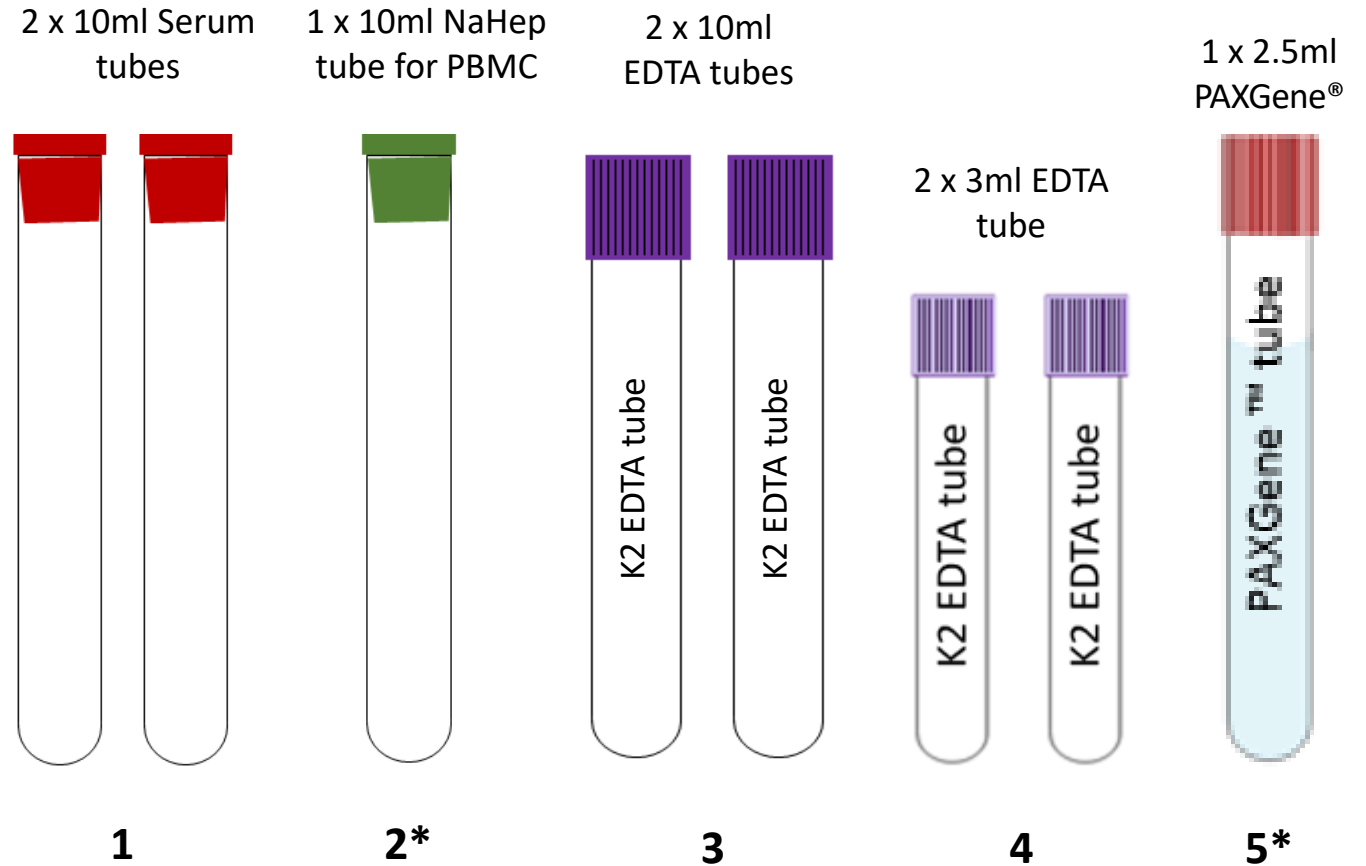
Please...

- Label all collection and aliquot tubes before cooling, collecting, processing, or freezing samples
- Label only 1 subject's tubes at a time to avoid mix-ups
- Wrap the label around the tube horizontally - label position is important for all tube types
- Make sure the label is completely adhered by rolling between your fingers



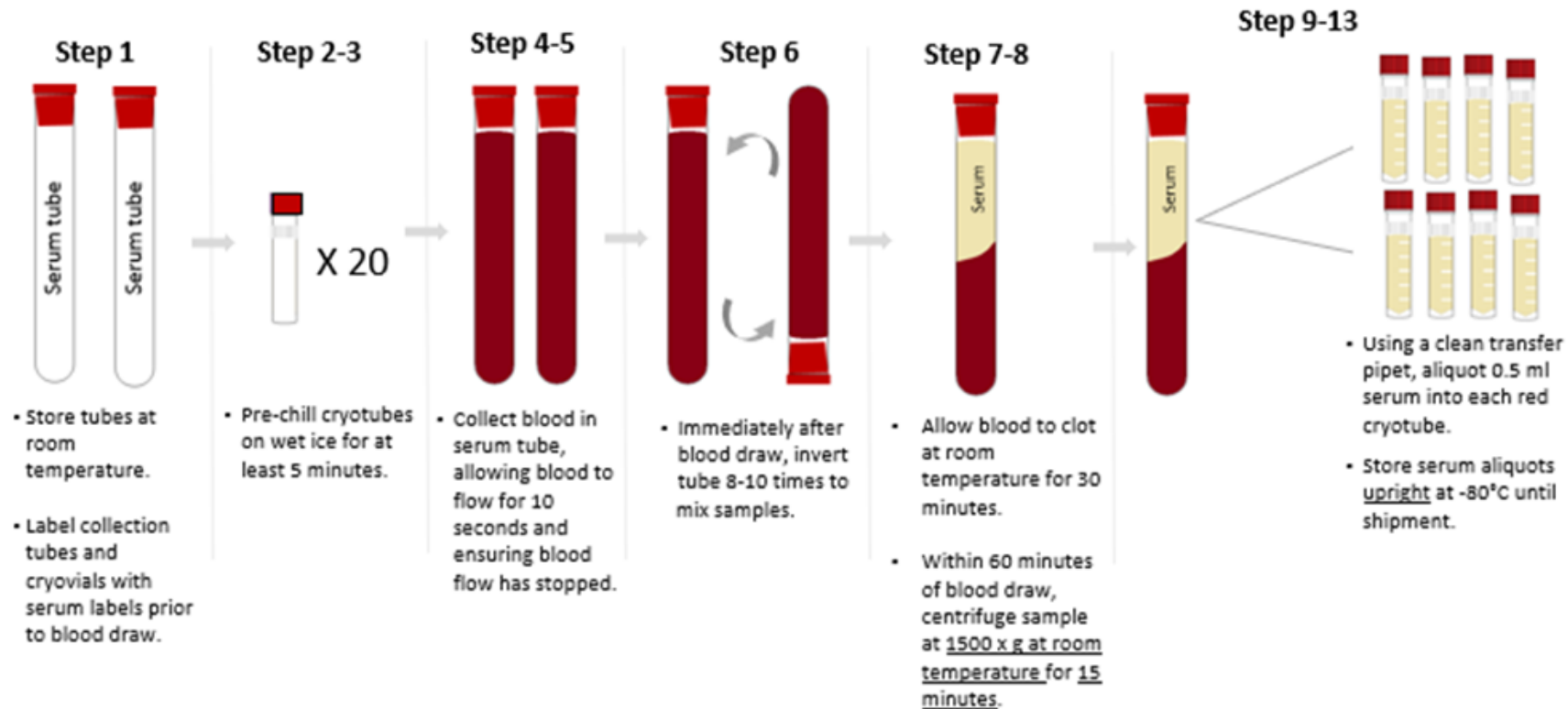
Sample Collection and Processing

Blood Tube Draw Order



**Collected at Screening Visit only*

Sample Collection and Processing: Serum



Sample Collection and Processing: PBMCs

Step One



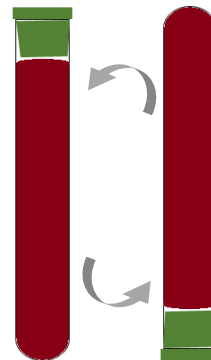
- Store tubes at room temperature.
- Label tubes with preprinted PBMC label prior to blood draw.

Step Two



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

Step Three



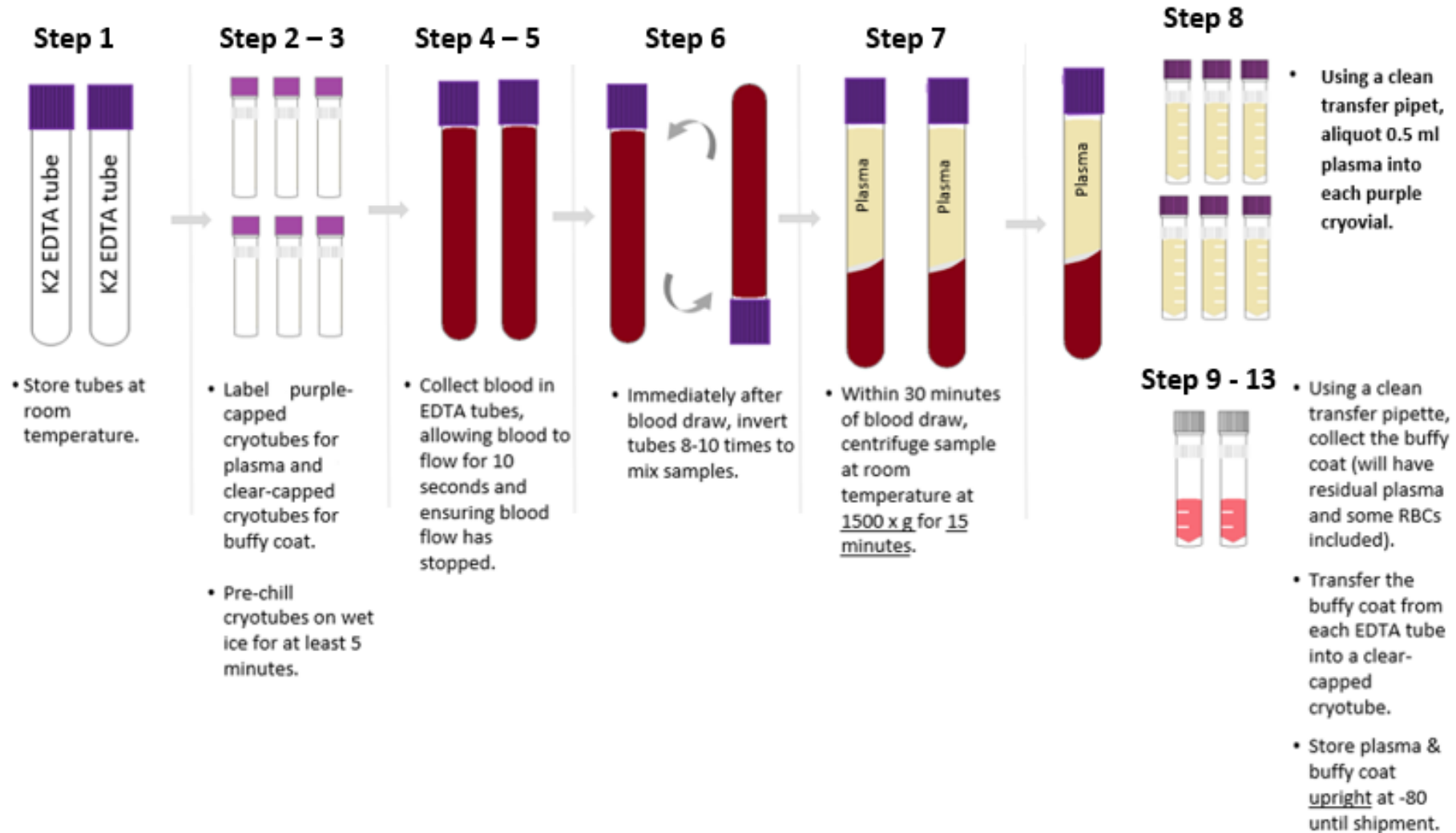
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Four

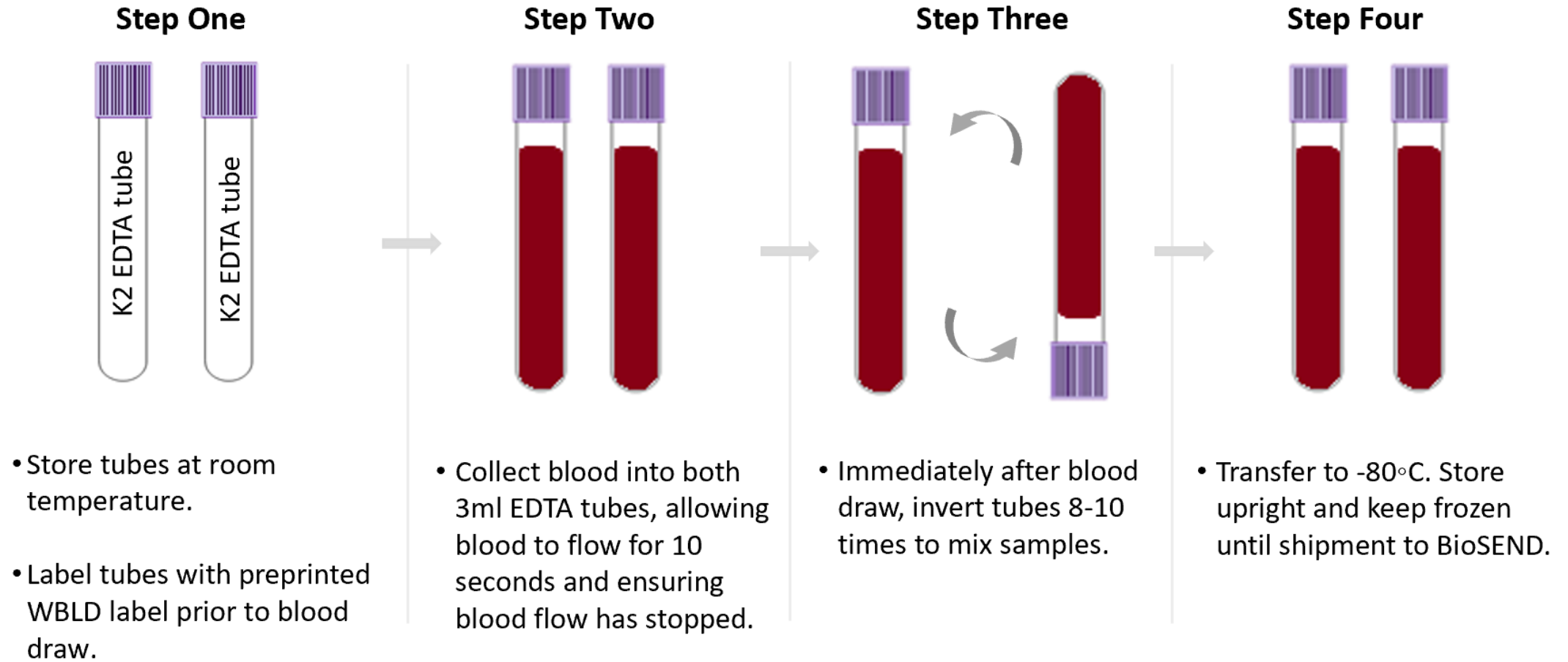


- Store tubes at room temperature until shipment.
- ***Must be shipped ambient to IU on day of blood draw!***

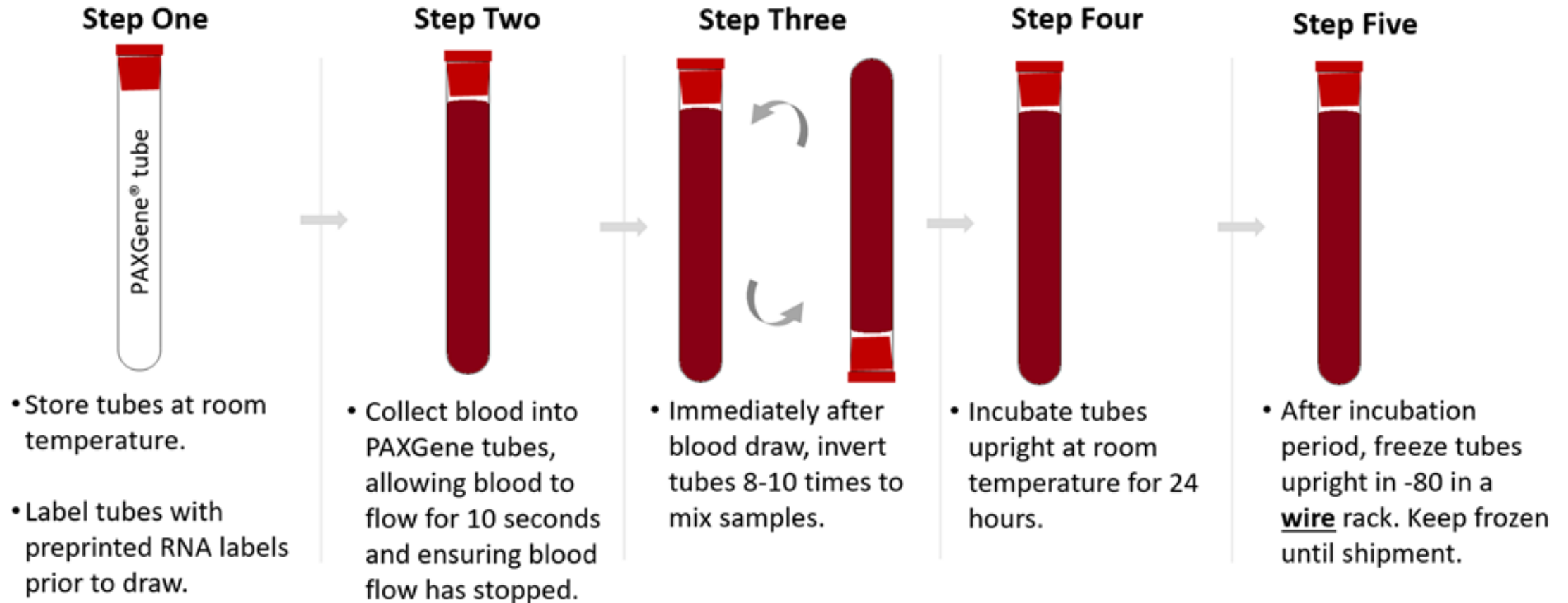
Sample Collection and Processing: Plasma & Buffy Coat



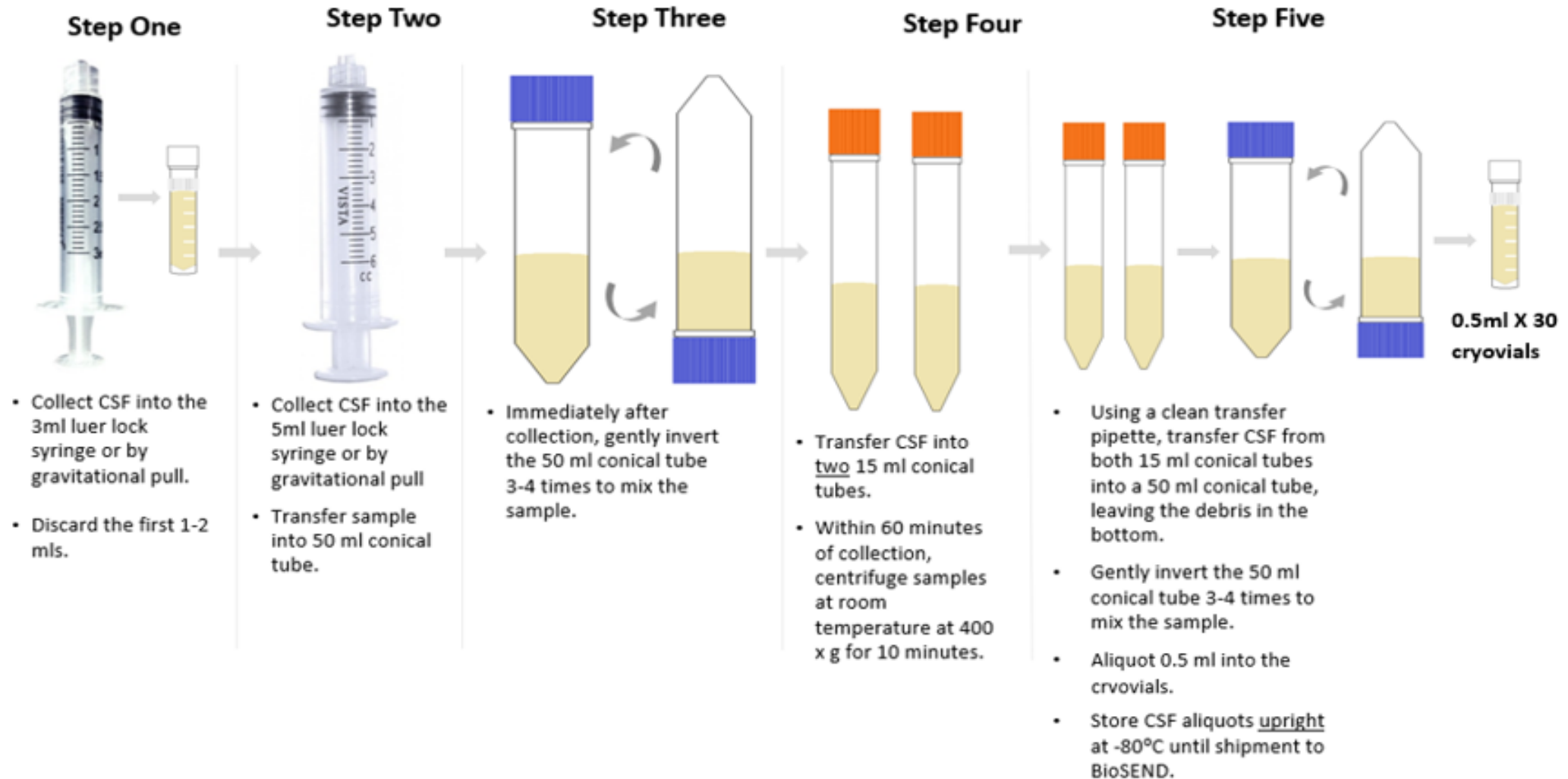
Sample Collection and Processing: Whole Blood



Sample Collection and Processing: Whole Blood RNA



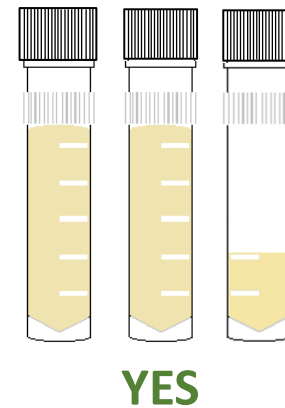
Sample Collection and Processing: CSF



Sample Collection and Processing: Aliquots

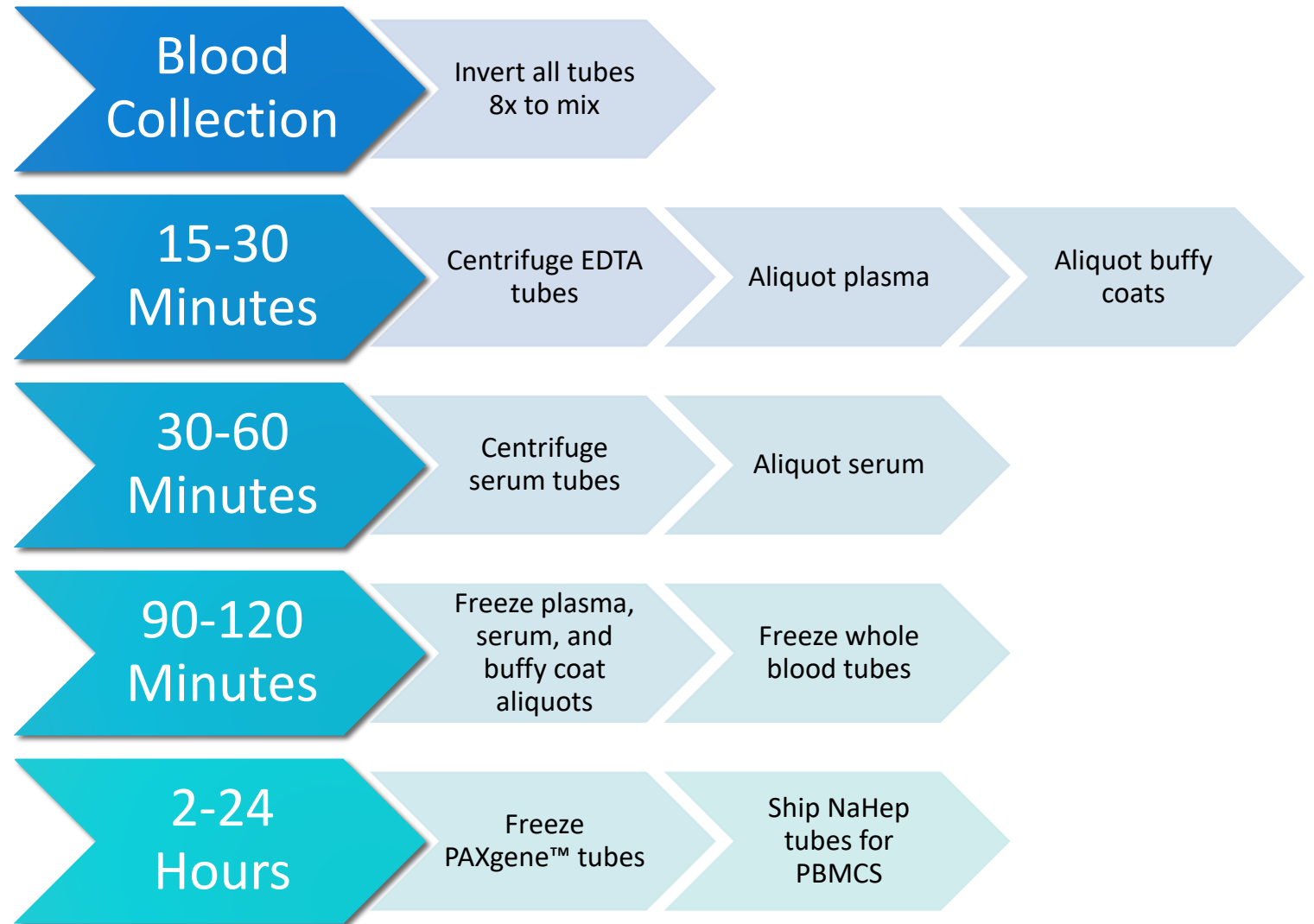
Filling biomarker serum, plasma, and CSF aliquots:

- In the event of a low volume draw, please generate as many standard-sized (0.5ml) plasma, serum and CSF. Fewer standard sized aliquots (with potentially one residual aliquot) is preferable to more aliquots with non-standard volume.
- Over-filled vials may burst in freezer!
- Ship ALL material to IU, even if final vial is less than standard volume



Sample Collection and Processing: Timeline

Timeline for blood processing



Sample Collection and Processing: Issue #1

Troubleshooting Blood Collection

Issue #1: Tube with little/no vacuum

- Always check expiration date on the tube before beginning blood draw and discard expired tubes
- Store tubes at “room temperature” – extreme temperature can affect vacuum
- Keep extra vacutainer tubes from supplemental kit nearby during blood draw to replace “bad” tubes
- If this is a frequent occurrence, report tube type and lot number to IU.

Sample Collection and Processing: Issue #2

Troubleshooting Blood Collection

Issue #2: Hemolyzed serum and/or plasma caused by incorrect collection

Cause: Blood Collection Methods	Corrective Action
Improper venipuncture site	Draw from median cubital, basilic, and cephalic veins from antecubital region of arm
Prolonged tourniquet use	Tourniquet should be released after no more than 1 min, excessive fist clenching should be avoided
Not allowing alcohol to dry on skin before venipuncture	Without touching, allow the venipuncture site to air dry
Use of too large/small bore needle resulting in excess force applied to blood	Avoid using too small/large needle. Needle size dependent on the subject's physical characteristics & amount of blood to be drawn. Most commonly used sizes are 19 – 23.
Pulling/pushing plunger too fast while drawing/transferring blood	Avoid drawing the syringe plunger too forcefully when collecting blood
Ensure all blood collection assemblies are fitted securely, to avoid frothing	

For more information, visit: http://www.bd.com/vacutainer/pdfs/techtalk/TechTalk_Jan2004_VS7167.pdf

Sample Collection and Processing: Issue #2 continued

Troubleshooting Blood Collection

Issue #2: Hemolyzed serum and/or plasma caused by incorrect processing

Cause: Sample Processing Methods	Corrective Actions
Vigorous mixing/shaking	Gently invert blood collection tube when mixing additive with specimen, follow guidelines in Biologics Manual regarding number of times to invert each type of tube
Not allowing serum to clot for recommended time	Serum tubes without clot activator should be allowed to clot for 60 min in a vertical position
Exposure to excessive heat or cold	Keep samples at ambient temperature until processing
Prolonged contact of serum/plasma with cells	Do not store uncentrifuged samples beyond recommended time

For more information, visit: http://www.bd.com/vacutainer/pdfs/techtalk/TechTalk_Jan2004_VS7167.pdf

Sample Collection and Processing Form

Direct Link:

<https://redcap.link/PREVENTSampleForm>

First page captures basic subject and visit information

BioSEND

Biospecimen Exchange for Neurological Disorders

Please complete the Specimen Collection and Processing Form, below.

[Returning?](#)

AAA



Page 1 of 2

Study Site



Email address of staff member completing this form

Note: A copy of the completed sample form and the shipping manifests will be sent to this address.

ALL ALS Subject ID:

8 characters remaining

Subject's biological sex (used for DNA quality control)



Visit



IU Kit Number

6 characters remaining

[Next Page >>](#)

[Save & Return Later](#)

Sample Collection and Processing Form

Second page captures processing information. Protocol instructions, such as centrifugation rate, are pre-populated.

Blood Collection and Processing	
Date of venipuncture blood collection	 Today M-D-Y
Time of venipuncture blood collection	 Now H:M Use 24 Hour clock
1. SERUM (red-top tubes, 10 mL)	
Was blood collected and processed for SERUM?	Yes No reset
Time of SERUM tube centrifugation	 Now H:M Use 24 Hour clock
Duration of SERUM tube centrifugation	15 minutes
Rate of SERUM tube centrifugation	1500 x g
Temperature of SERUM tube centrifugation	4 degrees Celsius
Total volume of SERUM collected	 mL
Number of SERUM aliquots created for BioSEND	 Each aliquot should be 1.5 mL
Time SERUM aliquots were placed in freezer	 Now H:M Use 24 Hour clock

Sample Collection and Processing Form

PDF form of responses will be emailed to you. **Print a copy and include with shipment.**

PREVENT ALL ALS Frozen Shipping Manifest

Page 1

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:

☐ V1
☐ V2
☐ V3
☐ V4
☐ V5
☐ V6
☐ V7

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:

Sample Collection and Processing Form

Submission of the Shipping Manifest portion of the form serves as shipment notification to BioSEND

- Must be completed prior to shipment
- If samples are shipped and not received, BioSEND will follow-up with courier. It is recommended that sites also track shipment to ensure safe delivery

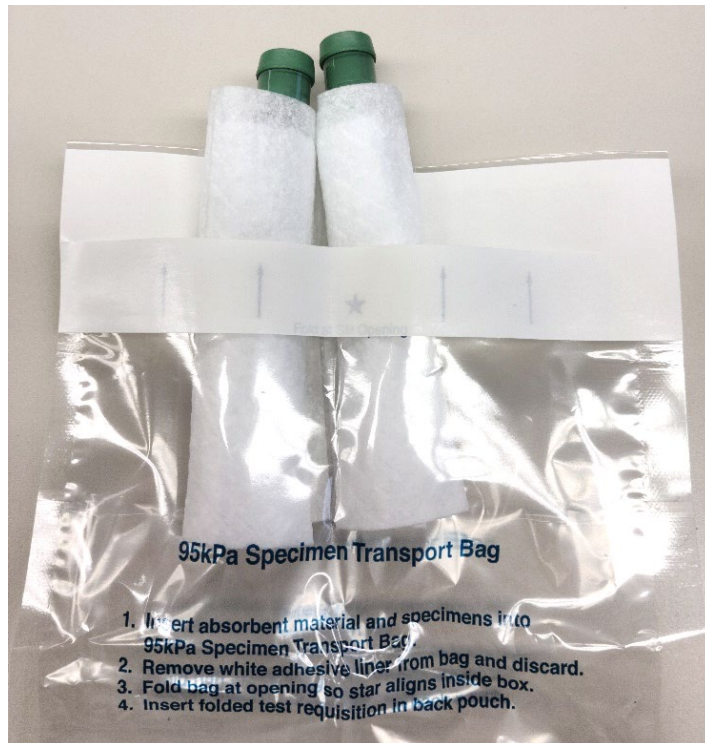
Shipping Samples: Ambient

Packing and Shipping Ambient Samples

- Whole blood for PBMCs ships at ambient temperature
- Ship ambient samples **only** Monday through Thursday
- Ship on **same day** of collection
- Only ship one subject's specimens in each shipping carton (unless otherwise instructed)

Shipping Ambient Samples

Packing and Shipping Ambient Samples



Shipping Frozen Samples: Tips

Packing and Shipping Frozen Samples

- All serum, plasma, buffy coat, RNA, whole blood and CSF samples ship frozen on dry ice
- Frozen samples should be shipped **only** Monday through Wednesday
- Always fill carton to **top** with dry ice
- Do not pack shipment until the day of pickup



Shipping Samples

Packing and Shipping Frozen Samples

Class 9 Dry Ice Label should not be covered with other stickers and must be completed, or UPS will reject/return your package!

The image shows a Class 9 Dry Ice Label template. It features a large triangle with vertical bars inside, and a large '9' at the bottom. The label contains the following text:

Shipper's Declaration not Required.

Dry Ice amount must be in kilograms.

Note: 2 lbs. = 1 kg.

Airwaybills / airbills must have the following:

1. Dry Ice; 9; UN 1845

2. $\frac{\text{Number}}{\text{(Number pkgs)}} \times \frac{\text{wt}}{\text{(wt)}} \text{ Kg}$

Net weight of dry ice in kg

Dry Ice _____ kg.

Your name & address

Shipper's Name and Address

UN 1845

Consignee Name and Address

IU information and address

06426 1/01 RRD

Red arrows point to the following fields:

- Net weight of dry ice in kg
- Dry Ice _____ kg.
- Your name & address
- Shipper's Name and Address
- UN 1845
- Consignee Name and Address
- IU information and address

Shipping Samples: Frozen

Do not ship more than 4 biohazard bags in a single shipper (equivalent to two subject-visits).

- Allows room for dry ice to keep samples frozen in transit
- Minimizes loss in the rare but unfortunate event of courier issues



Shipping Samples – UPS: <https://kits.iu.edu/UPS>

**INDIANA UNIVERSITY**
SCHOOL OF MEDICINE

and Affiliated Biorepositories

Search for address

Code

Company

Contact

Address 1

Address 2

Address 3

City

State/Province

Postal Code

Country/Territory

Icahn School of Medicine - Mt. Sinai

Kenny Persaud

1425 Madison (Icahn Building)

Icahn L4 39

New York

NY

10029

United States

Shipment Information

Study Group

Weight

Dry Ice Weight

Description of Return

SSBC

20

10

Biologic Specimens

LB

LB

Choose Study

Enter weight

Click "Ship"

Shipping Samples via UPS

IU UPS ShipExec Shipping Portal

- Print out UPS air waybill
- Ensure all elements (barcode, return address, etc.) printed clearly
- Fold and insert UPS air waybill into clear plastic sleeve on package

JOHN SMITH INDIANA UNIVERSITY 410 WEST 10TH STREET INDIANAPOLIS IN 46202	2 LBS	1 OF 1
RS		
SHIP TO: SCHOOL OF MEDICINE 317-278-2694 INDIANA UNIVERSITY TK 217 351 W 10TH ST INDIANAPOLIS IN 46202		
	IN 461 9-01	
		
UPS NEXT DAY AIR		1
TRACKING #: 1Z 976 R8W 84 3985 8595		
 SAMPLE		
BILLING: P/P DESC: Biological Specimens RETURN SERVICE		
Reference No.1: 4087277		
XOL 20.03.09 NV45 83.0A 12/2019		 TM

Shipping Samples: Closures

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

Please also consider weather when shipping. UPS will post service updates on their webpage. Reach out to BioSEND if you are unsure if it is safe to ship.

Non-Conformance

Non-conformance to standard procedures may reduce the utility of the biospecimens:

- Not processing serum/plasma within 2 hours of collection allows for breakdown of certain proteins and small molecules
- Over/under centrifuging changes plasma, serum, CSF composition



Non-Conformance Reporting con't

Most common non-conformance issues:

- Shipment notification not sent
- Samples shipped for weekend/holiday delivery
- Sample form incomplete/inaccurate
- Low volume
- Unlabeled or mislabeled tube(s)
- Sample hemolysis



Non-Conformance and Inventory Reporting

Most common non-conformance issues:

- BioSEND will notify sites directly of any issues upon receipt
- BioSEND will email sites a monthly inventory report of all samples received from that site to date
- ***If you are experiencing issues, please reach out to us for help! It is much easier to prevent an issue before sample collection & shipment than trying to fix it after the fact***

Contacts

Indiana University

General Questions/Shipment Notifications:

biosend@iu.edu

317-278-6158

Request kits:

<https://redcap.link/preventallals>