



Biospecimen Exchange for Neurological Disorders

An Intermediate Expanded Access Protocol with CNM-Au8
for Amyotrophic Lateral Sclerosis for Protocol
CNMAU8.EAPo₄ (CNMAu8.EAPo₄)

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
- Specimens must be processed and frozen within specified windows (one hour for whole blood, two hours for all other sample types)
- 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 (for in-clinic storage)
- Dry ice

Procedures

MAINTAINING SPECIMEN UNIFORMITY AND QUALITY



Biospecimen Collection Protocol

Samples collected at visit weeks 1 (Screening/Baseline), 12, 24, 48, 72, 96, 120, and 144 or ET					
Draw Order	Collection Tube	Specimen Type	Aliquot Volume	Total Number of Aliquots	Cryovial Cap Color
1	1 PST LiHep (Green-top) Tube (4.5ml)	Plasma for creatinine testing	N/A	N/A (Entire collection tube to be shipped without aliquoting)	N/A
2	2 EDTA (purple-top) Tubes (10ml)	Plasma for Quanterix Nfl and UCHL1 analysis	1.5ml	6	Purple
		Buffy Coat for DNA extraction	~750ul	2	Clear
3	1 EDTA (purple-top) Tube (3ml)	Whole Blood for NadMED analysis	0.5ml	6	Green

*For females of child-bearing potential only: A urine pregnancy test should take place at the Screening/Baseline visit and as clinically indicated thereafter. Pregnancy tests and urine should **not** be sent to BioSEND.

Kit Contents and Ordering

- All sites will be sent a Supplemental Kit with their first kit shipment
 - Contains extra blood collection tubes and processing supplies
 - 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

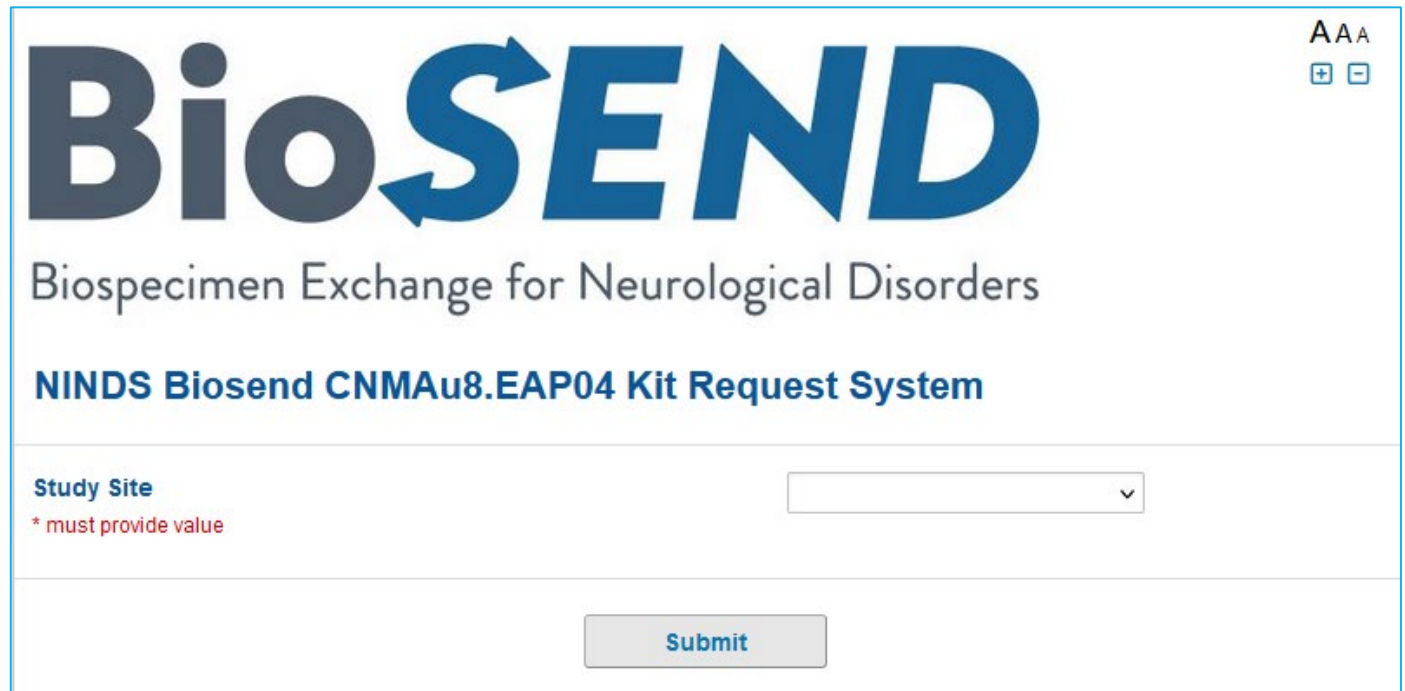
Kit Contents and Ordering – REDCap Survey

<http://kits.iu.edu/biosend/CNMAu8EAP04>

Order kits online through the Kit Request Module for:

- Blood Kits
- Supplemental Kit
- Extra Supplies

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



The screenshot shows the BioSEND web interface. At the top, the logo "BioSEND" is displayed in large blue letters, with the "S" containing a circular arrow icon. Below the logo, the text "Biospecimen Exchange for Neurological Disorders" is shown. Underneath, the title "NINDS Biosend CNMAu8.EAP04 Kit Request System" is displayed in blue. In the top right corner, there are accessibility icons for "AAA" and a plus/minus sign. A form field labeled "Study Site" is present, with a dropdown arrow and a red asterisk indicating it is required. Below the form field, a "Submit" button is visible.

BioSEND
Biospecimen Exchange for Neurological Disorders
NINDS Biosend CNMAu8.EAP04 Kit Request System

Study Site AAA
* must provide value

Submit

Kit Contents and Ordering: Confirm Site Info

Confirm where kits/supplies should be shipped

Study Site <small>* must provide value</small>	Indiana University
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	
Is the contact name above correct? <small>* must provide value</small>	<input type="radio"/> Yes <input checked="" type="radio"/> No reset
New Contact Name <small>* must provide value</small>	Claire Wegel
Is the shipping address above correct? <small>* must provide value</small>	<input type="radio"/> Yes <input type="radio"/> No reset
Is the e-mail address above correct? <small>* must provide value</small>	<input type="radio"/> Yes <input type="radio"/> No reset
Is the phone number above correct? <small>* must provide value</small>	<input type="radio"/> Yes <input type="radio"/> No reset

Select your site from the drop-down list

Verify contact information and update if needed

Kit Contents and Ordering: Kit Types

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.

Kit Type

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

* must provide value

+ Remote Collection & Shipping Kit

+ In Clinic Collection Kit

+ In Clinic Shipping Kit - Standard Size

+ In Clinic Shipping Kit - Bulk Size

+ Extra Supplies

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

Kit Contents and Ordering: Kit Breakdown

Select a kit type to view contents

Kit Type
****Please allow two weeks for shipment****
** must provide value*

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

In Clinic Collection Kit Quantity

** must provide value*

Comments

Expand

Each In Clinic Collection Kit contains:

Kit contains the supplies to collect samples for one study visit.

Supply	Quantity
Cryovial (Sarstedt®) with green cap, 2ml	6
Cryovial (Sarstedt®) with purple cap, 2ml	6
Cryovial (Sarstedt®) with clear cap, 2ml	2
LiHep (plastic) tube, 4.5ml	1
EDTA (plastic) tube, 10ml	2
EDTA (plastic) tube, 4ml	1
Disposable pipet, 3ml	3
Cryobox, 25 cell	1
Hcg pregnancy dipstick test	1
Urine cup	1
Label set (kit & specimen labels)	1

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



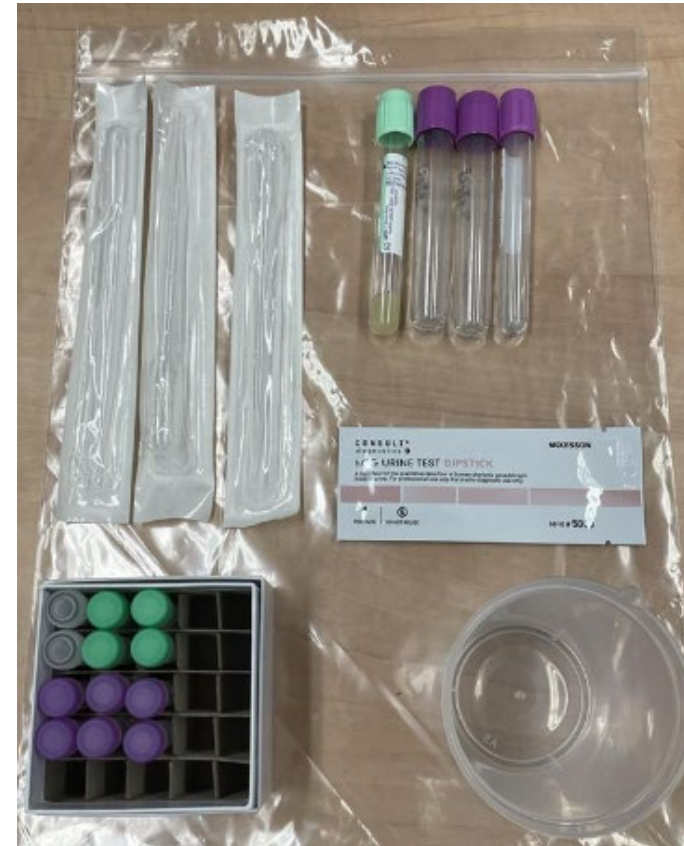
Kit Contents and Ordering: Remote Collection & Shipping Kit

- These kits will contain the supplies to collect and ship the samples from one participant-visit, as samples will be shipped as they are collected.



Kit Contents and Ordering: In-Clinic Collection Kit

- Because in-clinic samples may be stored and batch shipped on a quarterly basis, In-Clinic Collection Kits will contain only the supplies to collect and process samples. Shipping kits are ordered separately (see next slide).



Kit Contents and Ordering: In-Clinic Shipping Kit Options

STANDARD SIZE SHIPPING KIT

- This kit contains the supplies to ship the samples from up to two study visits (that is, up to 4 biohazard bags may be shipped in a single shipper).

BULK SIZE SHIPPING KIT

This kit contains the supplies to ship the samples from up to four study visits (that is, up to 8 biohazard bags may be shipped in a single shipper).

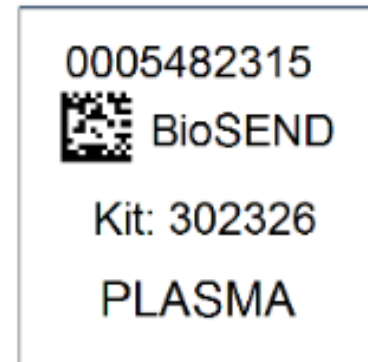
Kit and Supply Ordering

- 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 Labeling: Example Labels

Labels are provided by Indiana University

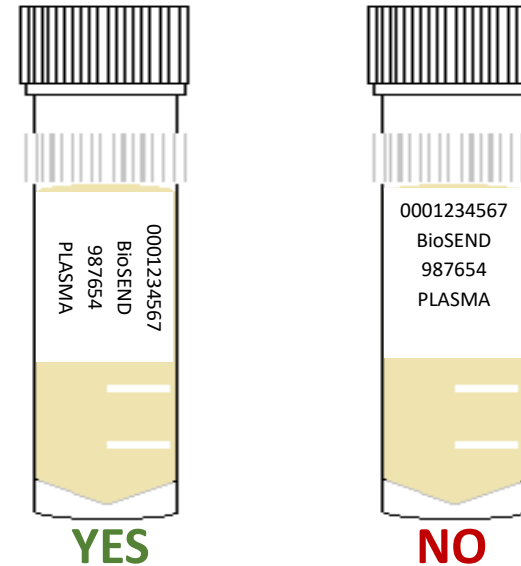
- Please check that all samples are properly labelled to ensure correct identification by IU
- If do not have enough labels to complete a visit, please contact IU *immediately*
- Note that you will have extra labels. If you do not need the extra labels, please dispose of them.
- Labelling the tubes during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



Sample Labeling: 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

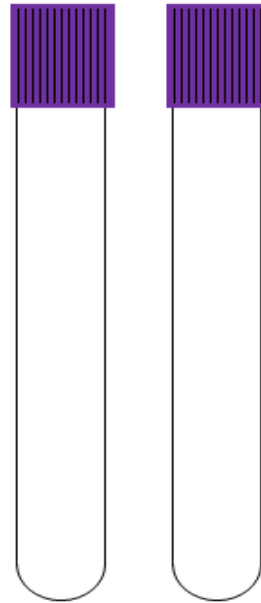
Blood Tube Draw Order

1 x 4.5ml PST
LiHep for
creatinine testing



1

2 x 10ml EDTA
for plasma and
buffy coat



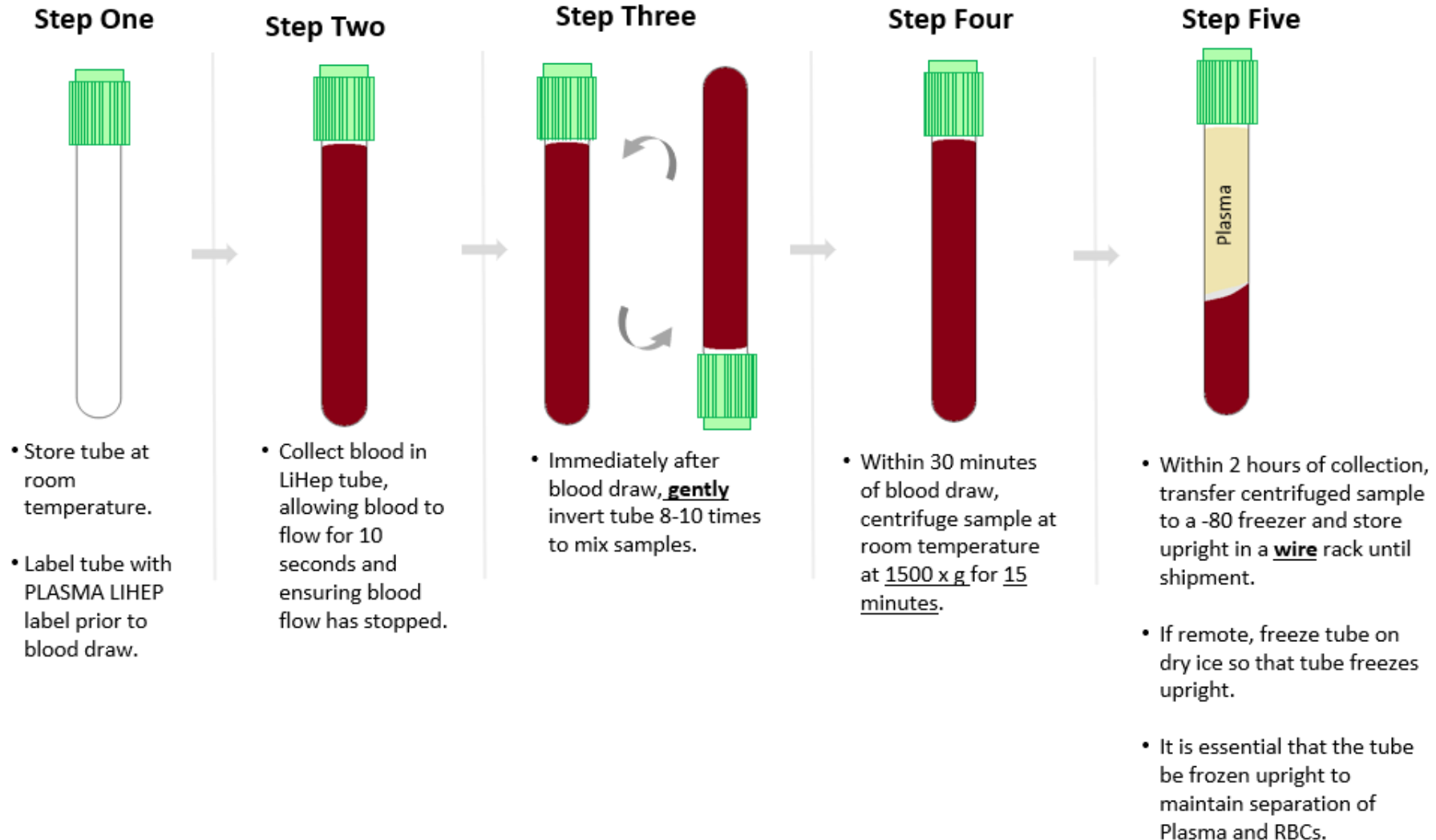
2

1 x 3ml EDTA
for whole
blood

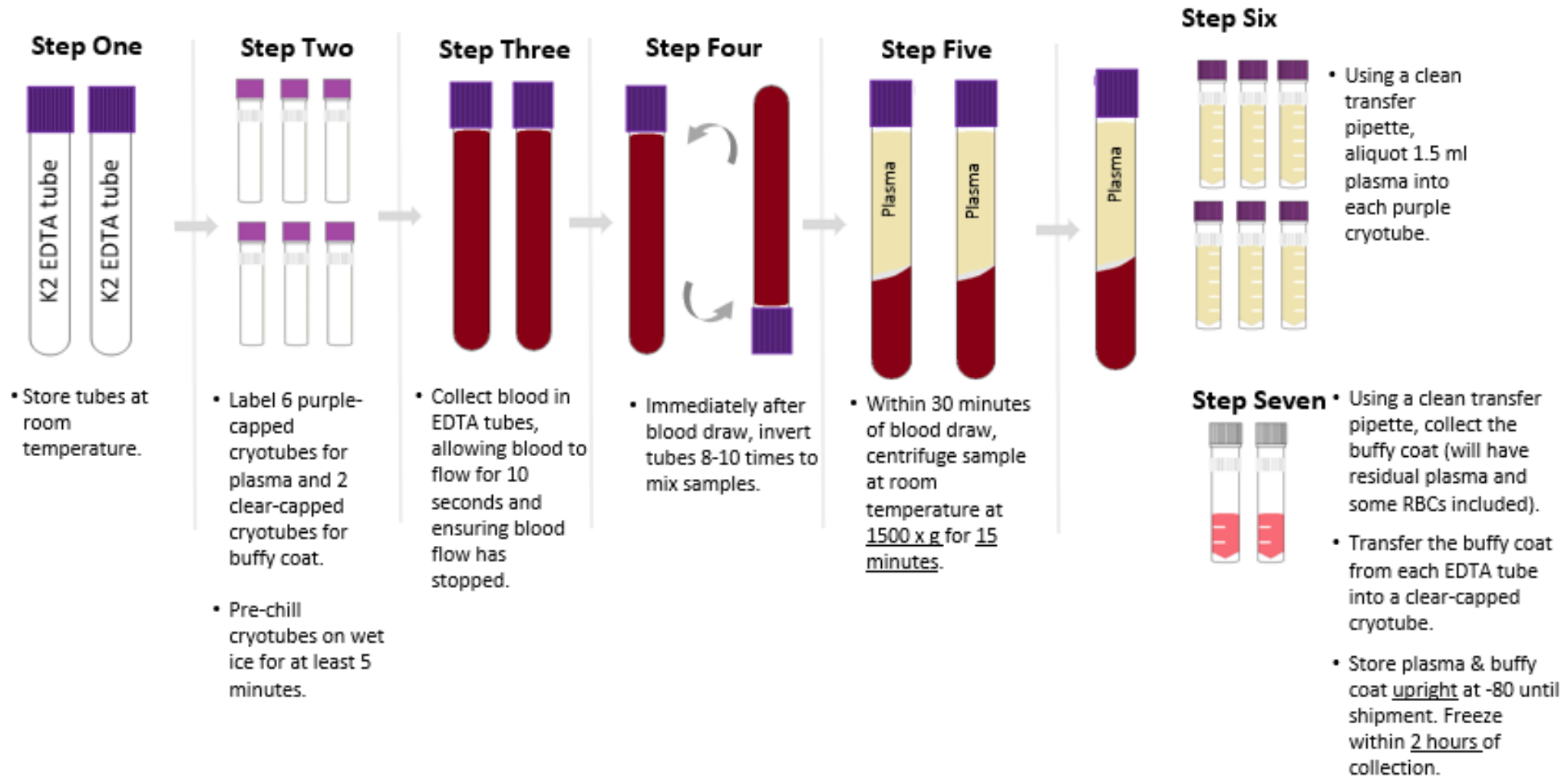


3

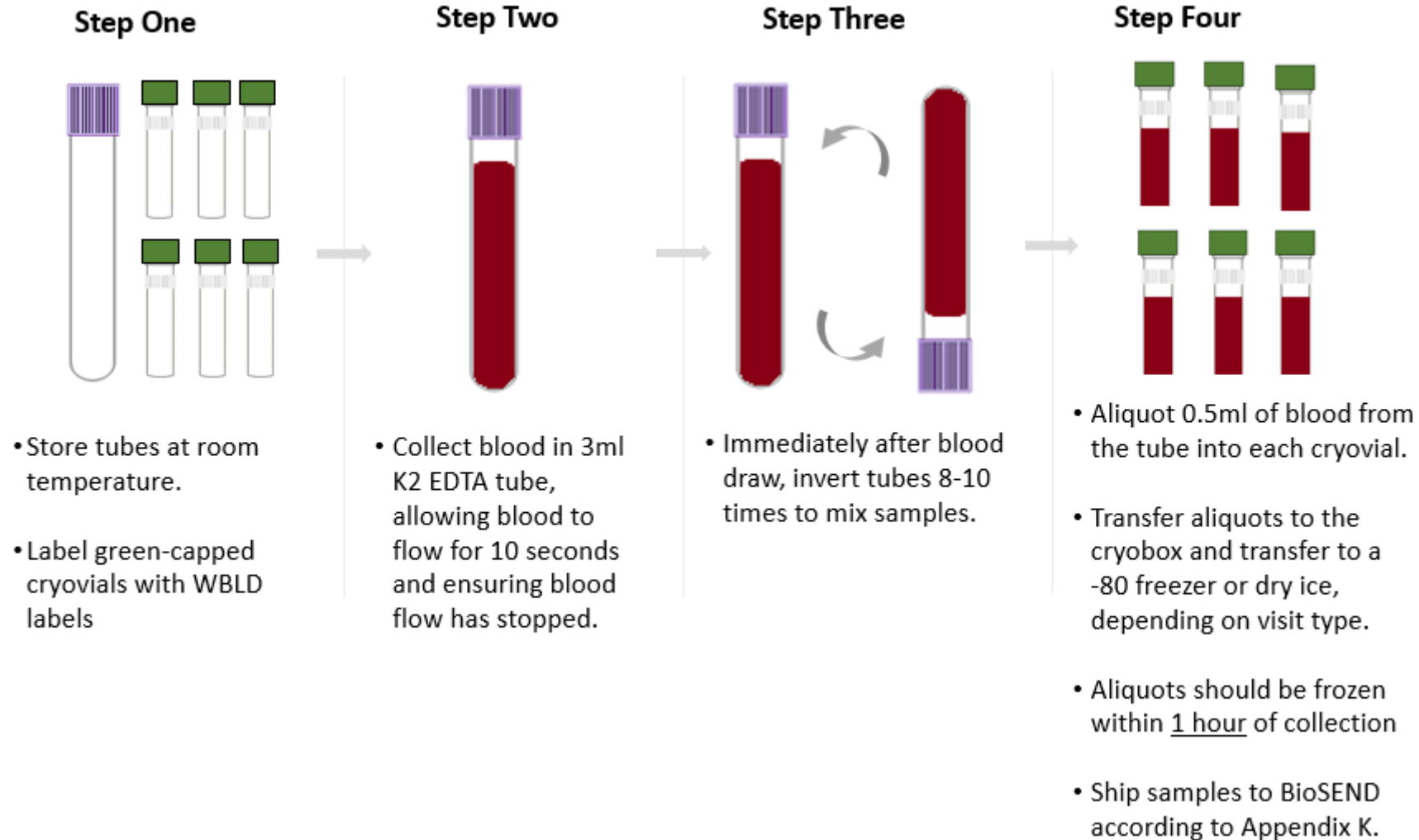
Sample Collection and Processing: 4.5ml PST LiHep



Sample Collection and Processing: Plasma & Buffy Coat



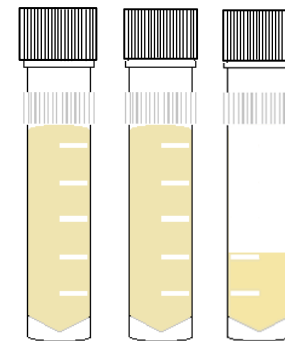
Sample Collection and Processing: Whole Blood



Sample Collection and Processing: Aliquots

Filling biomarker serum and plasma aliquots:

- In the event of a low volume draw, please generate as many standard-sized (1.5ml) plasma and (0.5ml) whole blood aliquots as possible. 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



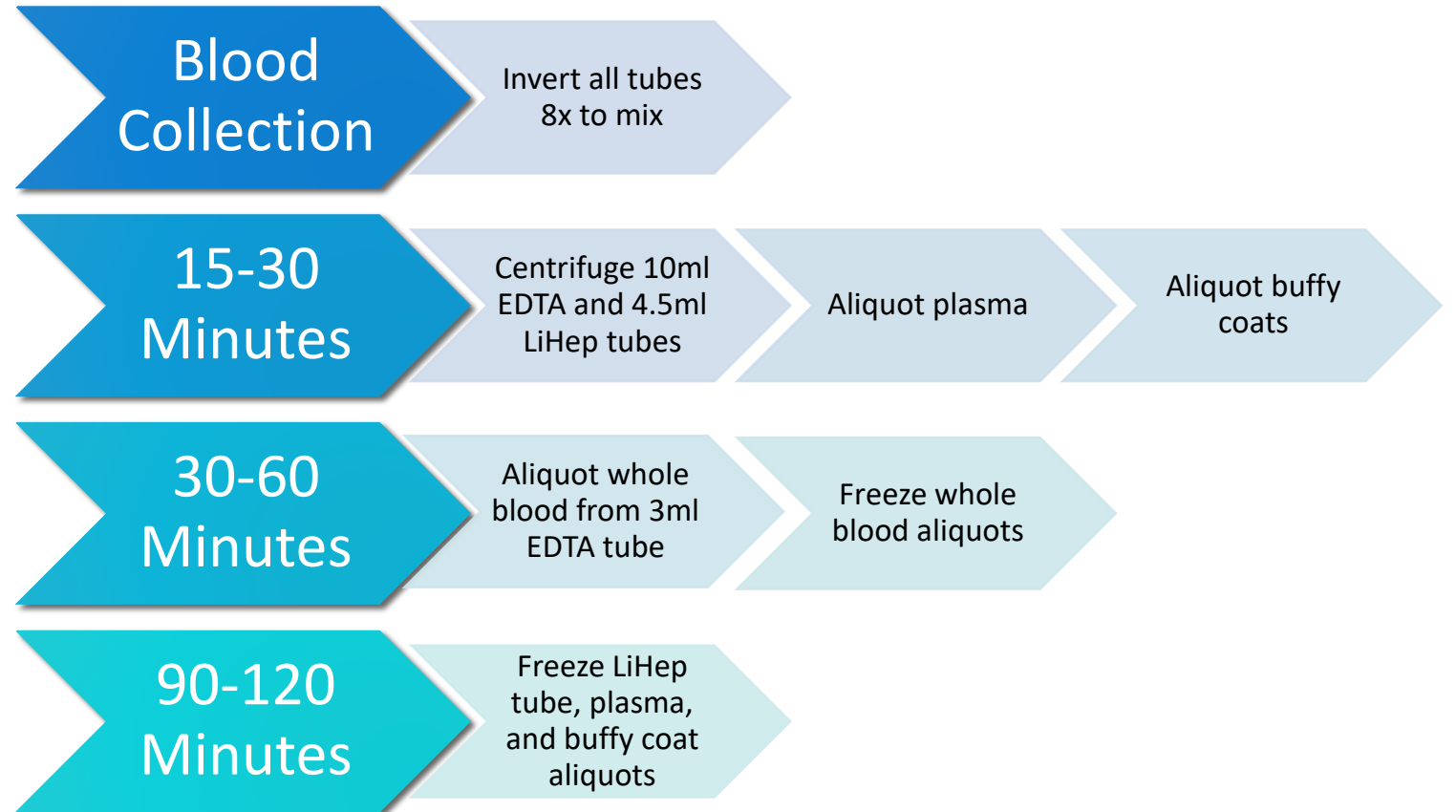
YES



NO

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 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 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:

<http://kits.iu.edu/biosend/CNMAu8EAP04SampleForm>

First page captures basic participant and visit information

BioSEND
Biospecimen Exchange for Neurological Disorders

[Returning?](#) AAA
+ -

Please complete the Specimen Collection and Processing Form, below.

Page 1 of 2

Intermediate Expanded Access Protocol CNMAu8.EAP04

Study Site	<input type="text"/>
Email address of staff member completing this form	<input type="text"/>
<i>Note: A copy of the completed sample form and the shipping manifest will be sent to this address.</i>	
Participant ID	<input type="text"/>
Sex (used for DNA quality control)	<input type="text"/>
Weight	<input type="text"/> In pounds
Height	<input type="text"/> In inches
Visit	<input type="text"/>
Kit Number	<input type="text"/> 6-digit number on specimen labels

Sample Collection and Processing Form

Direct link:

<http://kits.iu.edu/biosend/CNMAu8EAP04SampleForm>

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

Date participant last ate  Today M-D-Y

Time participant last ate  Now H:M

1. PLASMA for Creatinine Testing (one green-top LiHep tube, 4.5 mL)

Was blood collected and processed for PLASMA LIHEP?
 Yes
 No
reset

Time of LiHep tube centrifugation  Now H:M
Use 24 Hour clock

Duration of LiHep tube centrifugation
minutes

Rate of LiHep tube centrifugation
x g

Was LiHep tube centrifuged at room temperature?
 Yes
 No
reset

Time LiHep was placed in freezer  Now H:M
24 Hr clock

Sample Collection and Processing Form

Direct link:

<http://kits.iu.edu/biosend/CNMAu8EAP04SampleForm>

PDF form of responses will be emailed to you. Print a copy of the Frozen Shipping Manifest and include with shipment.

CNMAu8 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 123.

Please print a copy of that document and include it in the shipping container.

Response was added on 05/02/2024 11:37am.

Study Site Duke University

Participant ID 123

Visit: 12 Weeks

Kit Number: 123456

Date of blood collection: 2024-05-02

LiHep TUBE

Number of LiHep tubes shipped: 1

PLASMA EDTA

Number of PLASMA EDTA aliquots shipped: 7

Number of BUFFY COAT aliquots shipped: 3

WHOLE BLOOD EDTA

Number of WHOLE BLOOD aliquots shipped: 5

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 Frozen Samples: Tips

Packing and Shipping Frozen Samples

- All 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



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

Shipping Samples

Packing and Shipping Frozen Samples

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

9

06426 1/01 RRD

Shipping Samples: Frozen

Packing and Shipping Frozen Samples



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

The screenshot shows the shipping interface for Indiana University School of Medicine. The header includes the IU logo and the text "INDIANA UNIVERSITY SCHOOL OF MEDICINE". Below the header, the text "and Affiliated Biorepositories" is displayed. The interface is divided into two main sections: "Ship From" and "Shipment Information".

Ship From Section:

- Search for address: A search bar with a magnifying glass icon and a "Clear" button.
- Code: Input field.
- Company: Icahn School of Medicine - Mt. Sinai
- Contact: Kenny Persaud
- Address 1: 1425 Madison (Icahn Building)
- Address 2: Icahn L4 39
- Address 3: Input field.
- City: New York
- State/Province: NY
- Postal Code: 10029
- Country/Territory: United States

Shipment Information Section:

- Study Group: SSBC (dropdown menu)
- Weight: 20 LB (input field and dropdown menu)
- Dry Ice Weight: 10 LB (input field and dropdown menu)
- Description of Return: Biologic Specimens
- Pickup Request: Button

Annotations:

- "Search for address" points to the search bar.
- "Choose Study" points to the Study Group dropdown.
- "Enter weight" points to the Weight and Dry Ice Weight input fields.
- "Schedule Pickup" points to the Pickup Request button.
- "Click 'Ship'" points to the Ship button at the bottom right.

Buttons: Clear, Pickup Request, Reset, 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



BILLING: P/P
DESC: Biological Specimens
RETURN SERVICE

Reference No. 1: 4087277

XOL 20.03.09 NV45 83.0A 12/2019



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:

- Processing samples outside of processing window allows for breakdown of certain proteins and small molecules. Whole blood aliquots should be frozen within 1 hour of collection, while plasma/buffy coat/LiHep should be processed and frozen within 2 hours of collection.
- Over/under centrifuging changes plasma 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:

<http://kits.iu.edu/biosend/CNMAu8EAP04>