BioSEND

Biospecimen Exchange for Neurological Disorders

# The Dementia With Lewy Bodies Consortium (DLBC) Study
# PDBP Study ID 233

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 within 2 hours of 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 maintaining 4°C
- -80°C Freezer
- Dry ice

# Procedures

MAINTAINING SPECIMEN UNIFORMITY AND QUALITY

# Biospecimen Collection Protocol

| | BL | 12M | 24M | 36M | 48M | 60M | 72M | 84M | 96M | 108M | 120M |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Buffy coat** (2 aliquots) | X | X | X | X | X | X | X | X | X | X | X |
| **Plasma** (6 x 1ml) | X | X | X | X | X | X | X | X | X | X | X |
| **Serum** (6 x 1ml) | X | X | X | X | X | X | X | X | X | X | X |
| **RNA** (2 x 2.5ml) | X | X | X | X | X | X | X | X | X | X | X |
| **Whole Blood** (1x3ml) | X | X | X | X | X | X | X | X | X | X | X |
| **CSF** (10 x 1ml) | X | X | X | X | X | X | X | X | X | X | X |

# 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

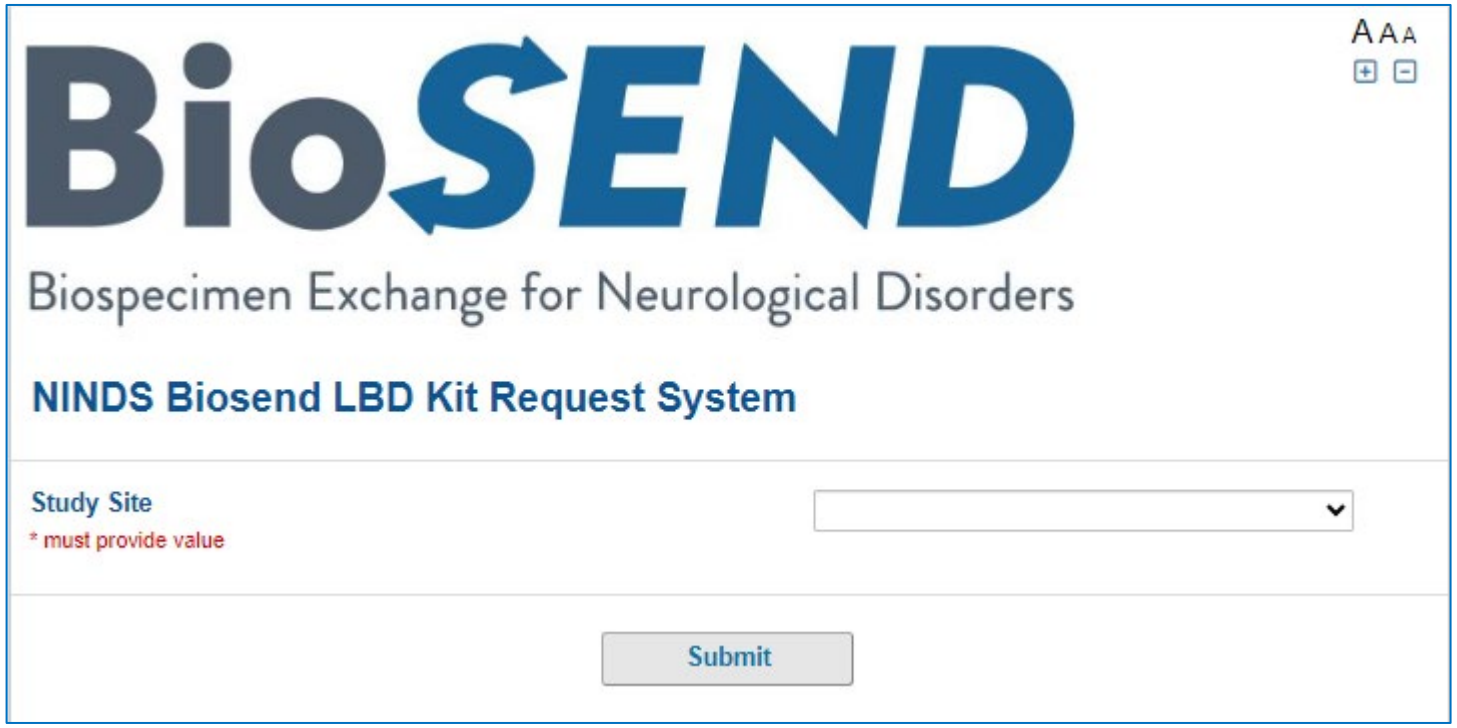
# Kit Contents and Ordering – REDCap Survey

- http://kits.iu.edu/biosend/LBD

Order kits online through the Kit Request Module for:

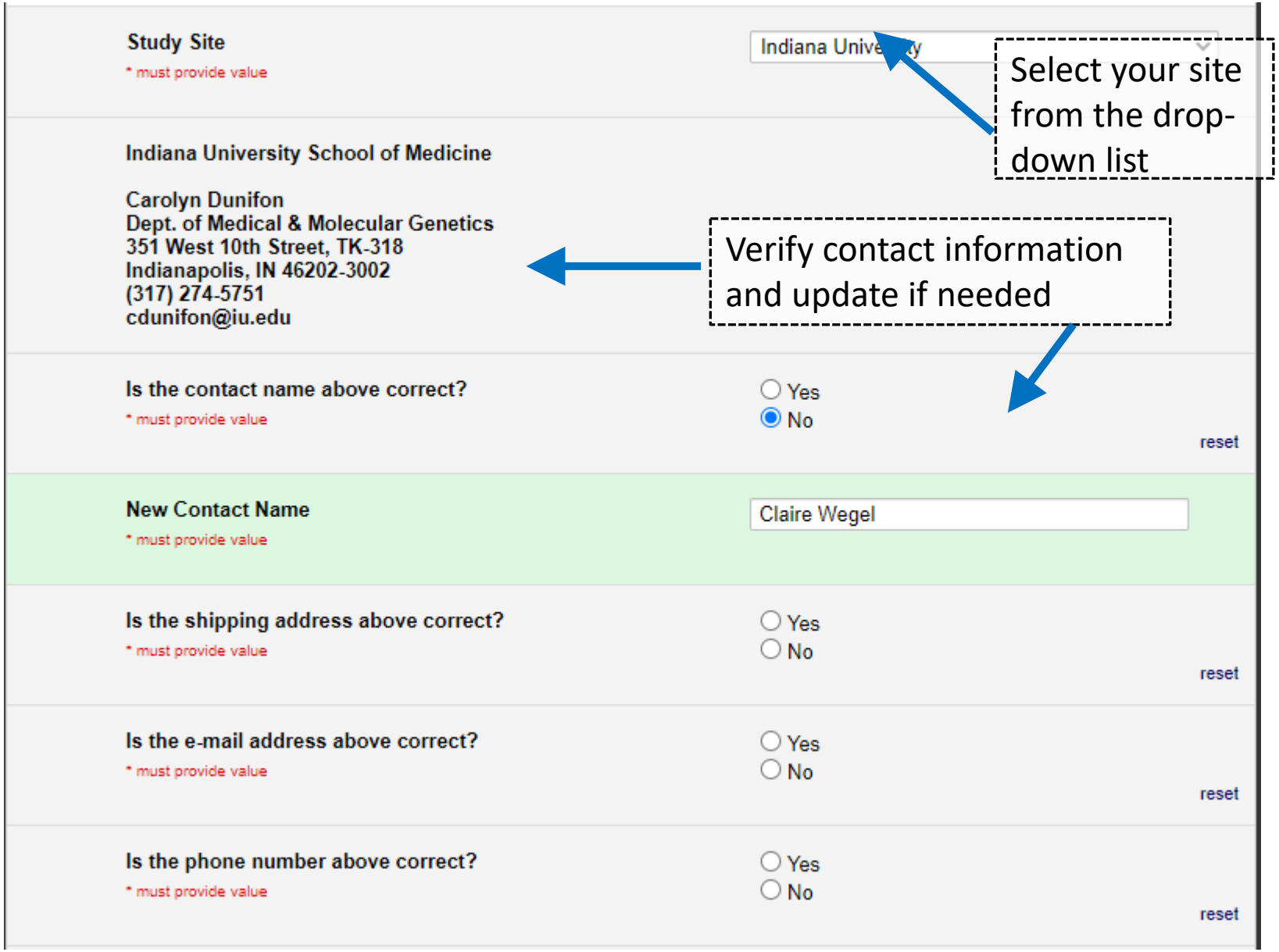
- Blood & CSF kits
- Extra Supplies

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



# Kit Contents and Ordering: Confirm Site Info

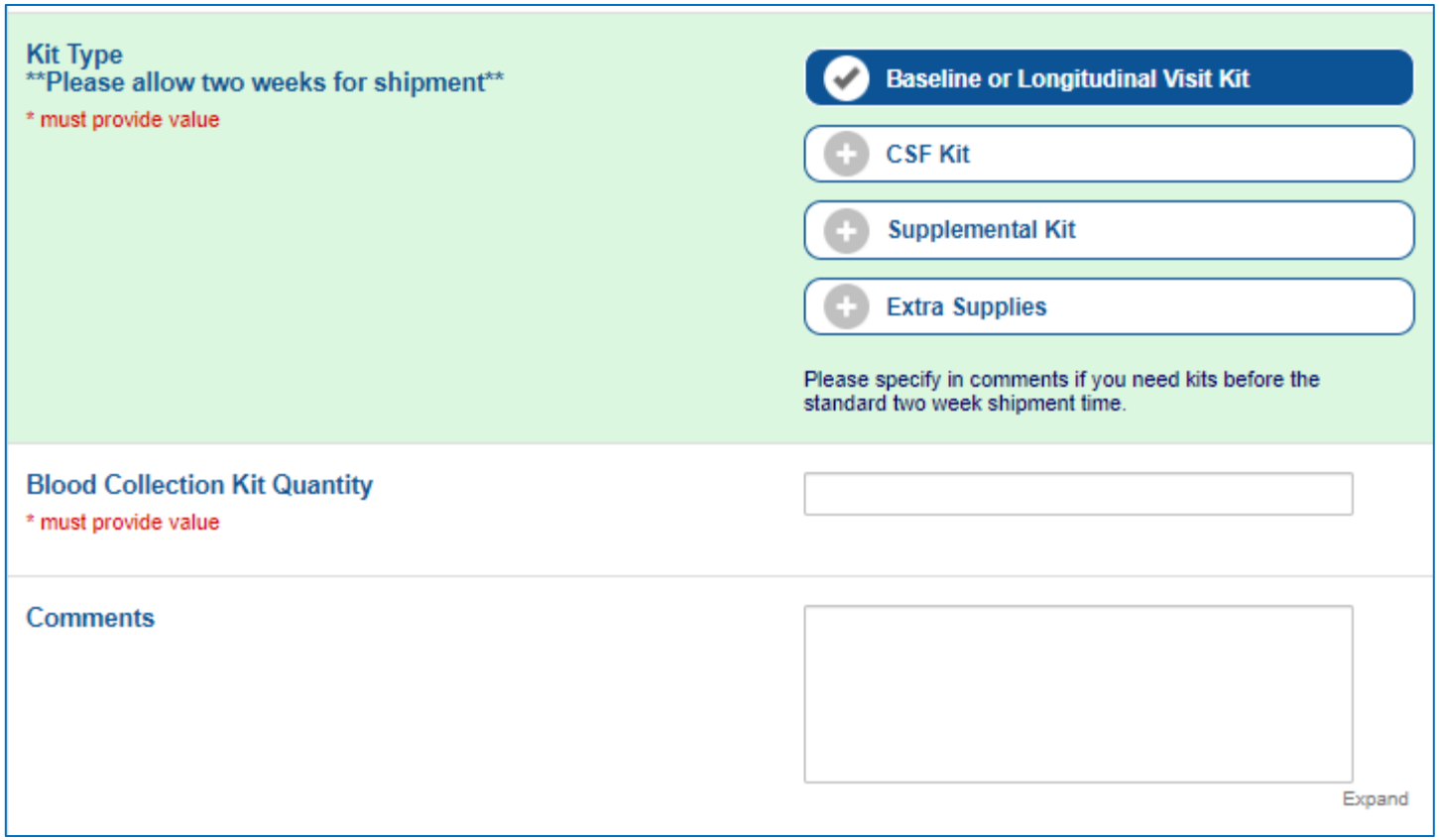
LBD Kit Request Module



# Kit Contents and Ordering: Kit Types

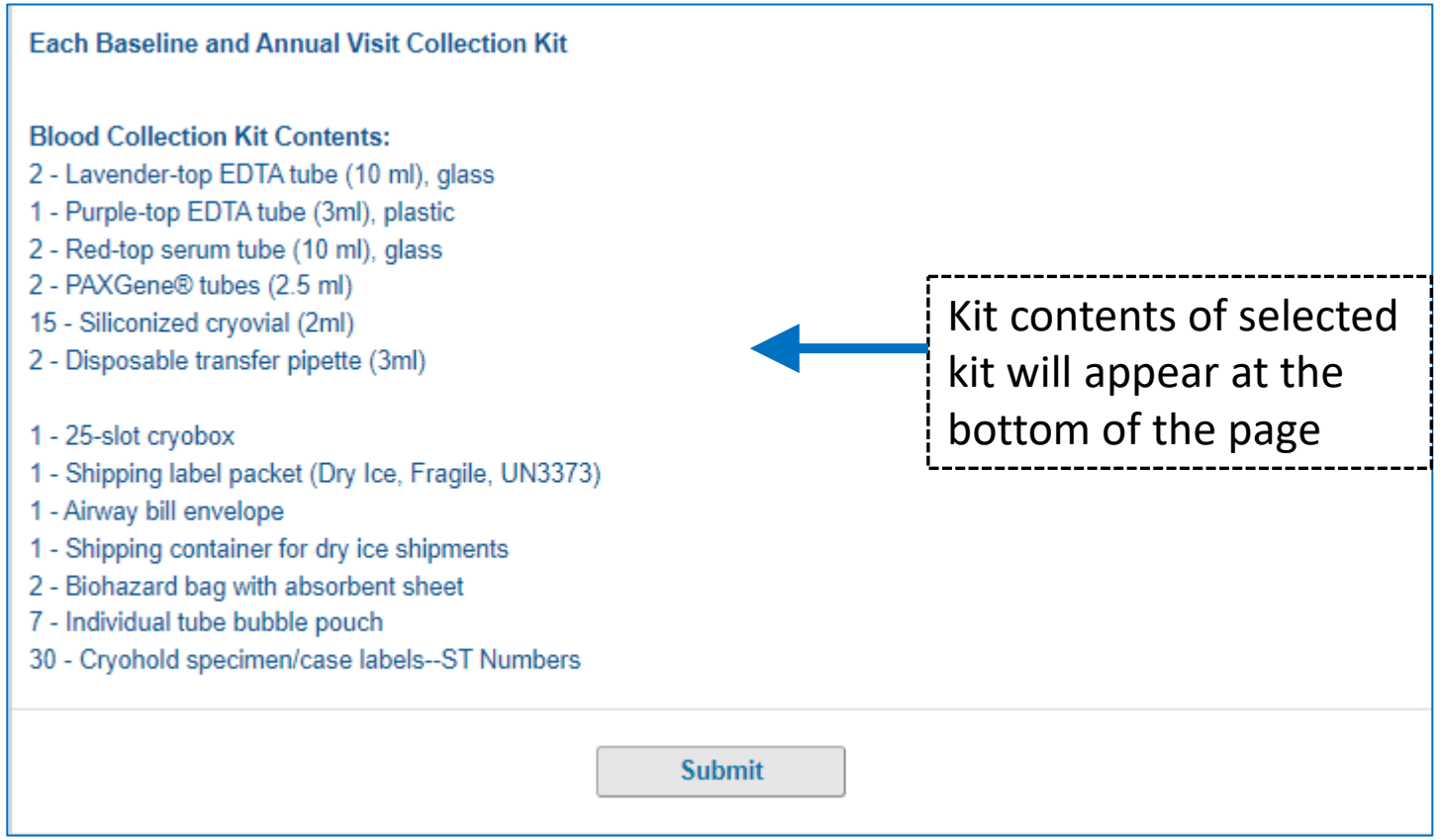
LBD Kit Request Module

- 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, as CSF is not required
- 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 Contents and Ordering: Kit Breakdown

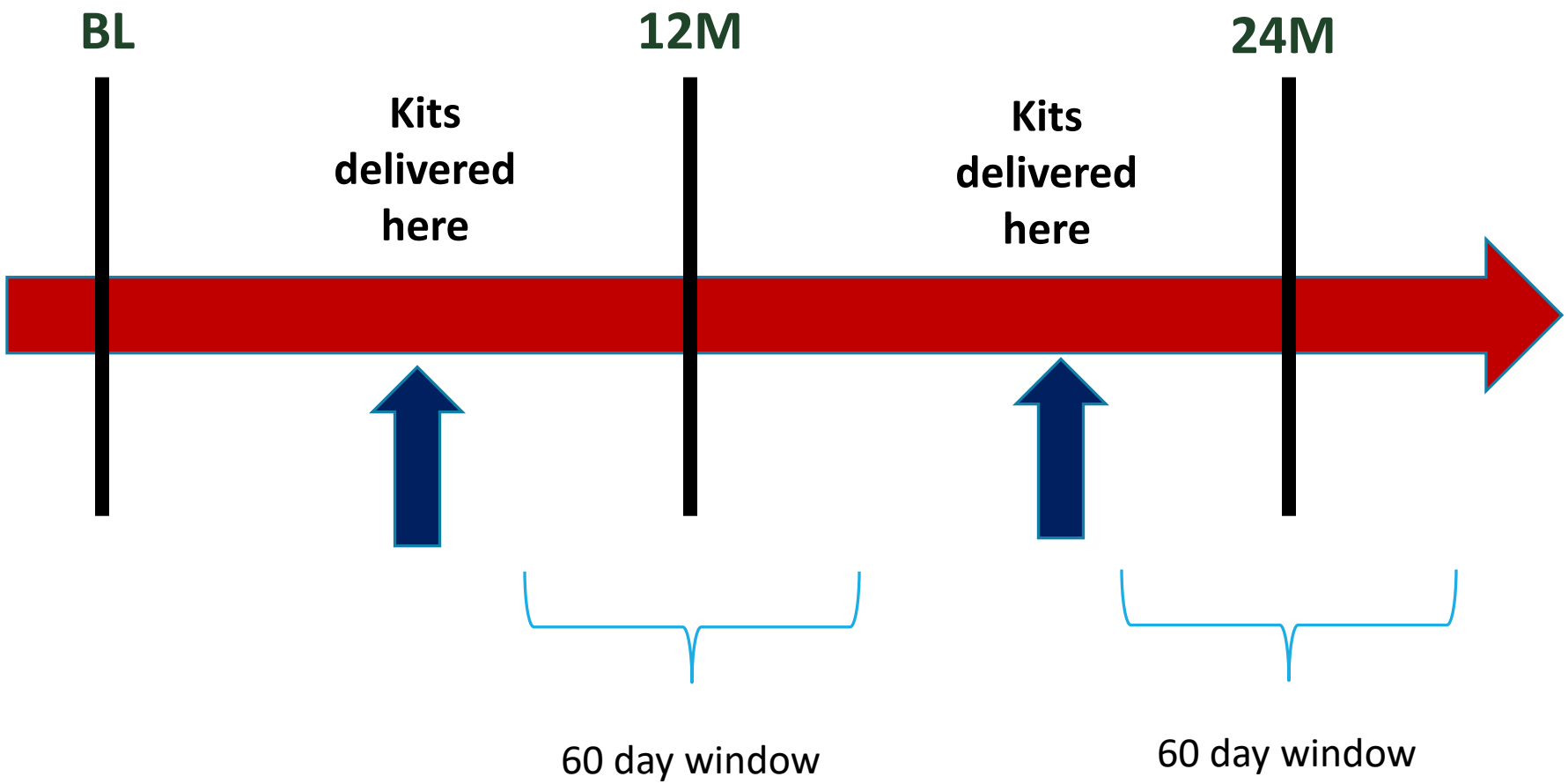
LBD Kit Request Module



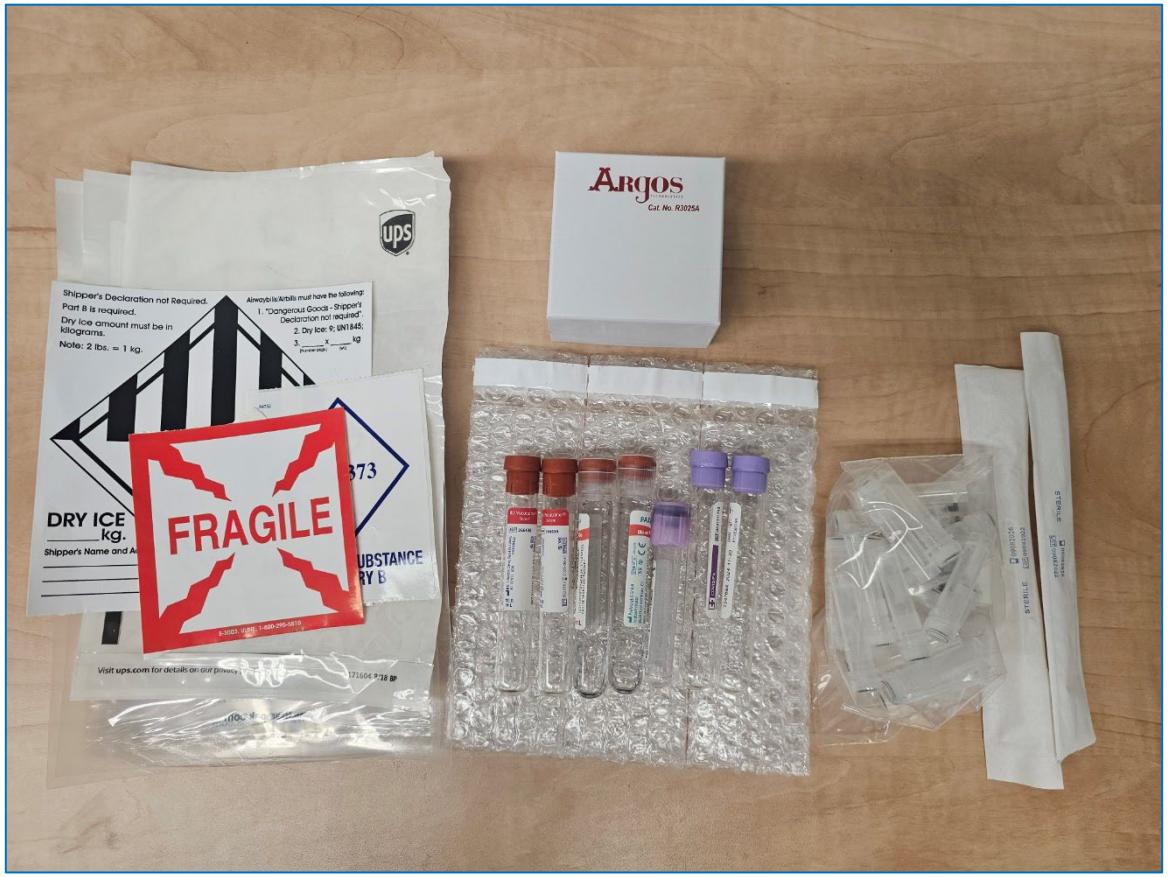
# Automatic Kit Shipments

- After subject completes baseline visit and BioSEND receives BL samples, BioSEND sets up automated kit sending schedule for subject's subsequent visits
- Schedule gives 2 month window around the longitudinal study visit target (1 month on either side)
- BioSEND will send kits prior to start of study window
  - Reduces effort for study coordinators
  - Sites only need to order kits if visit will occur AHEAD of the study visit window
- All study visit target dates are determined from Baseline Visit (not from last study visit date)

# Automatic Kit Shipments

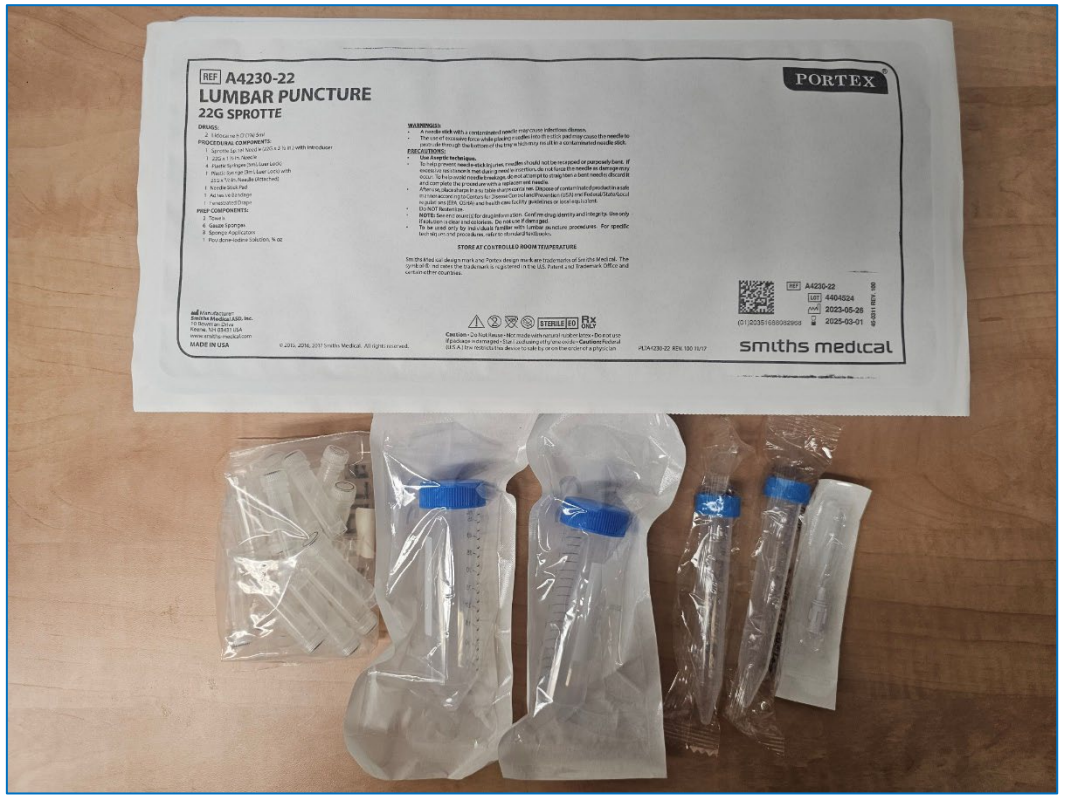


# Kit Contents and Ordering: Blood Kits



# Kit Contents and Ordering: CSF Kits

CSF:




LP Tray:

# Collection Volumes

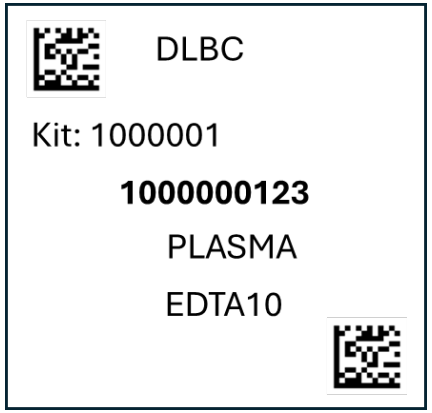
Total blood and CSF volumes

| Sample Type | Amount |
|---|---|
| Whole Blood for RNA | 5 ml |
| Whole Blood for Plasma and Buffy Coat | 20 ml |
| Whole Blood for Serum | 20 ml |
| Whole Blood for Banking | 3 ml |
| Cerebrospinal Fluid | 10 ml |

# Sample Labelling: Example Labels

**Labels are provided by Indiana University**

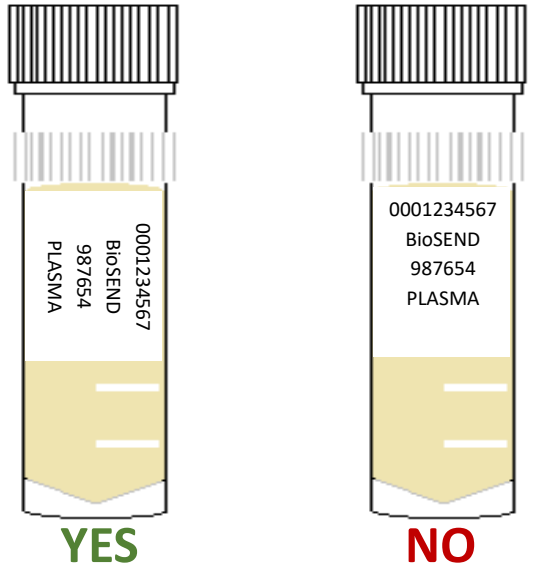
- Please check that all samples are properly labelled to ensure correct identification by IU
- If do not have enough labels to complete a visit, please contact IU *immediately*
- Labelling the tubes during processing prevents sample mix-ups

# Sample Labelling: Label Placement

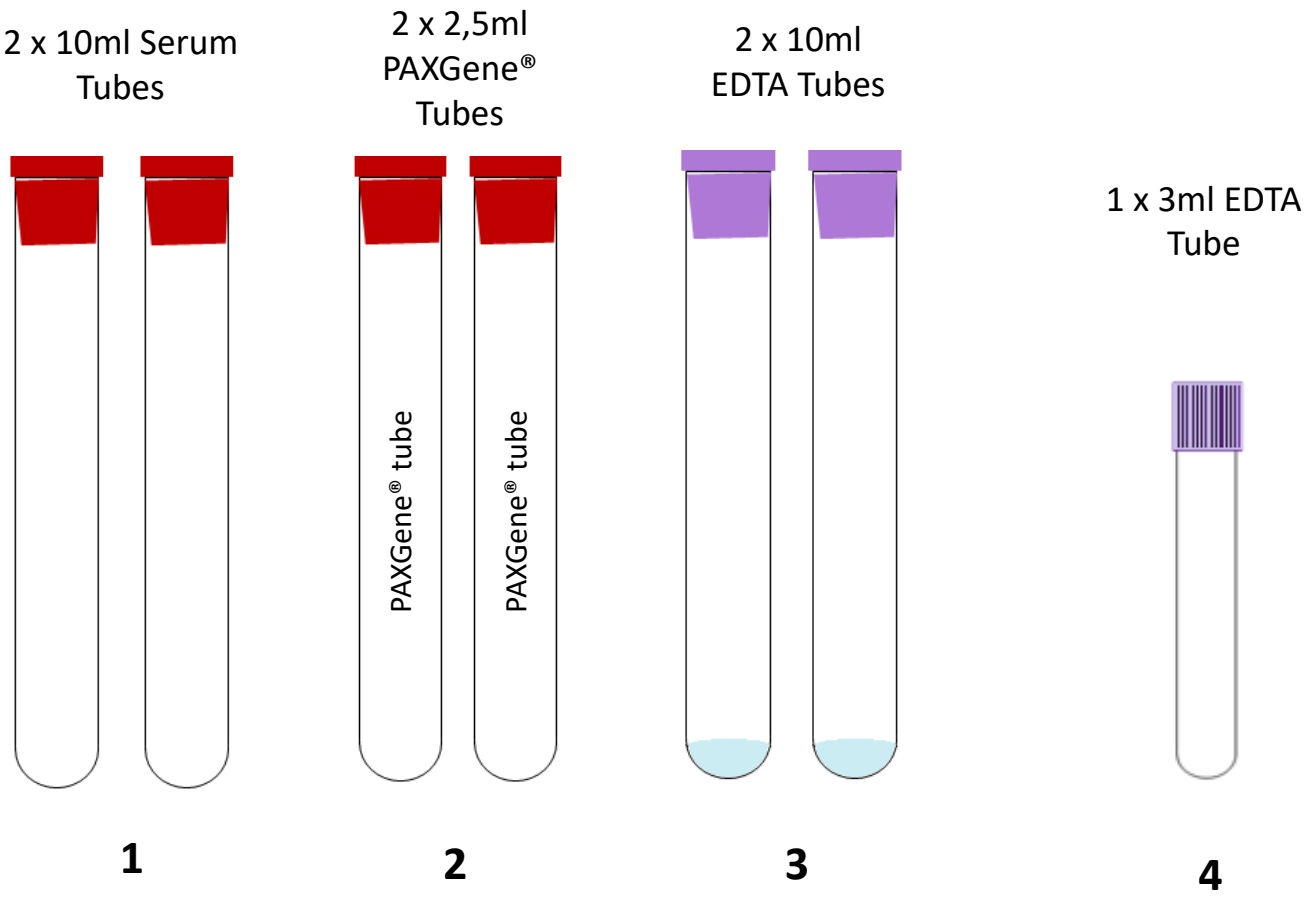
Please...

- 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



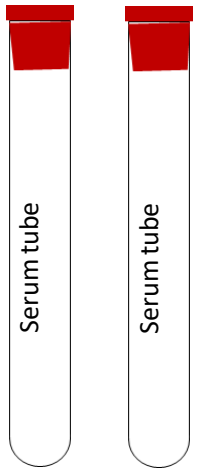
# Sample Collection and Processing

Blood Tube Draw Order



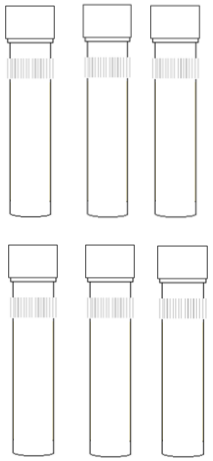
# Sample Collection and Processing: Serum

**Step One**



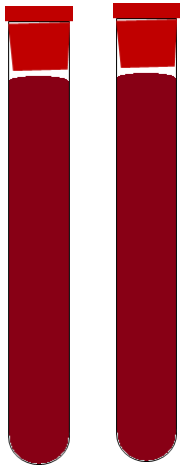

- Store tubes at room temperature.
- Label tubes with preprinted serum labels prior to blood draw.

**Step Two**



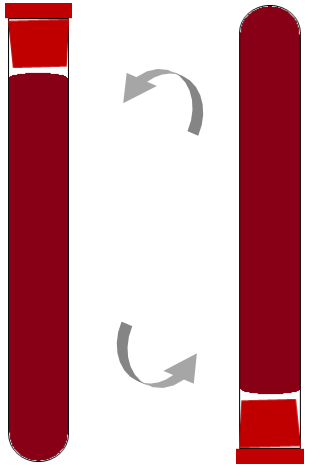
- Label 6 cryotubes with serum aliquot labels.
- Pre-chill cryotubes on wet ice for at least 5 minutes

**Step Three**



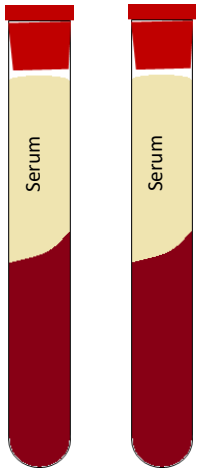
- Collect blood in serum tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

**Step Four**



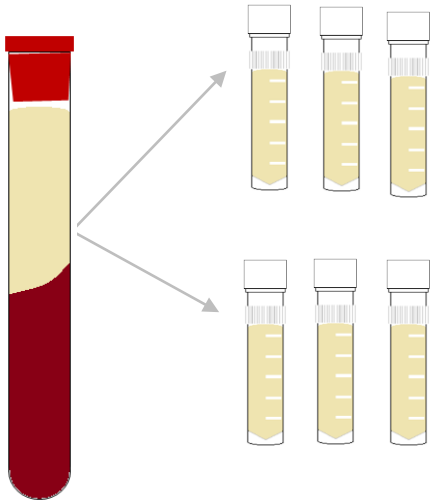
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

**Step Five**




- **Allow blood to clot for 30 minutes**
- Within 60 minutes of blood draw, centrifuge sample at 4°C at 1500 x g for 15 minutes.

**Step Six**



- Using a clean transfer pipet, aliquot 1.0 ml serum into each cryotube.
- Store serum aliquots upright at -80°C until shipment.

# Sample Collection and Processing: Whole blood RNA

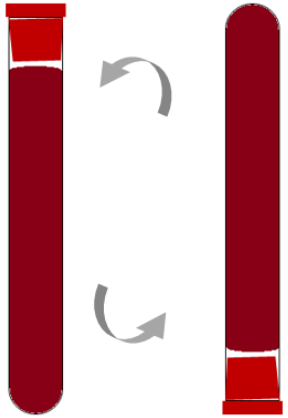
**Step One**


- Store tubes at room temperature.
- Label tubes with preprinted RNA labels prior to draw.

**Step Two**

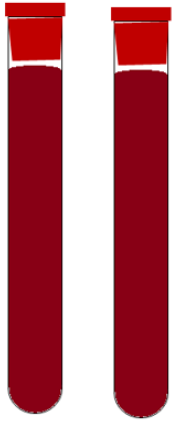
- Collect blood into PAXGene tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

**Step Three**



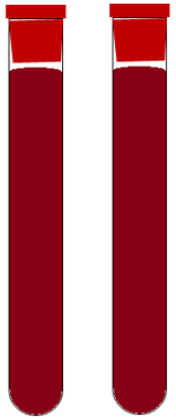
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

**Step Four**



- Incubate tubes upright at room temperature for 24 hours.

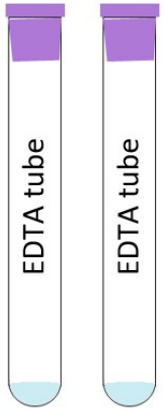
**Step Five**



- After incubation period, freeze tubes upright in -80 in a **wire** rack. Keep frozen until shipment.

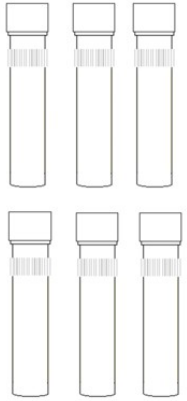
# Sample Collection and Processing: Plasma & Buffy Coat

## Step One



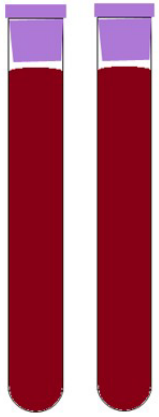

- Store tubes at room temperature.
- Label tubes with preprinted plasma labels prior to blood draw.

## Step Two



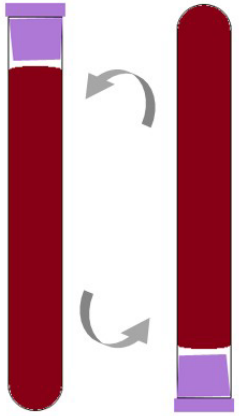
- Label 6 cryotubes with plasma labels and 2 with buffy coat labels.
- Pre-chill cryotubes on wet ice for at least 5 minutes

## Step Three



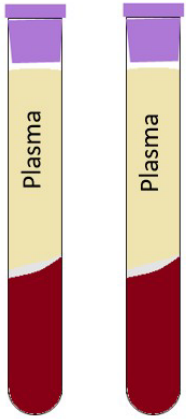
- Collect blood in EDTA tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

## Step Four

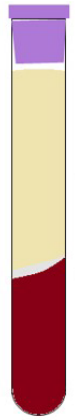


- Immediately after blood draw, invert tubes 8-10 times to mix samples.

## Step Five




- Within 30 minutes of blood draw, centrifuge sample at 4°C at 1500 x g for 15 minutes.



## Step Six

- Using a clean transfer pipet, aliquot 1.0 ml plasma into each cryotube.

## Step Seven

- Using a clean transfer pipet, collect the buffy coats (will include residual plasma and some red blood cells).
- Transfer each buffy coat to separate cryotubes.
- Store plasma and buffy coat aliquots upright at -80°C until shipment.

# Sample Collection and Processing: Whole Blood

**Step One**


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

**Step Two**

- Collect blood into 3ml EDTA tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

**Step Three**

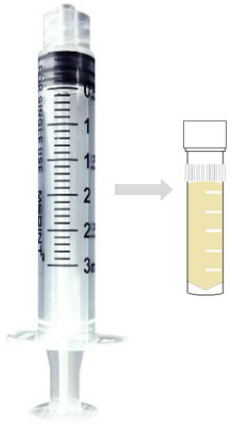
- Immediately after blood draw, invert tube 8-10 times to mix sample.

**Step Four**

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

# Sample Collection and Processing: CSF

## Step One

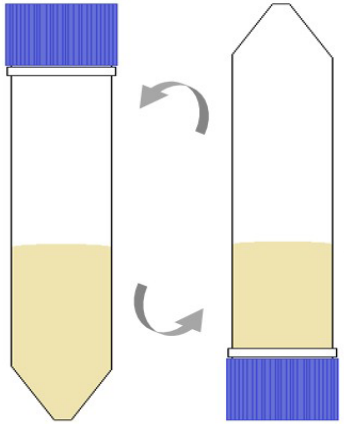


- Collect CSF into the 3ml luer lock syringe or by gravitational pull.
- Dispense 1-2 ml into cryovial.
- Send to local lab for testing, if applicable.

## Step Two

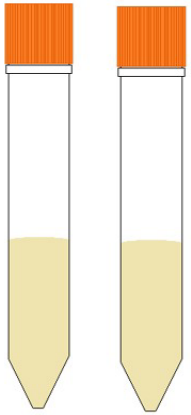

- Collect CSF into the 5ml luer lock syringe or by gravitational pull
- Transfer sample into 50 ml conical tube.

## Step Three



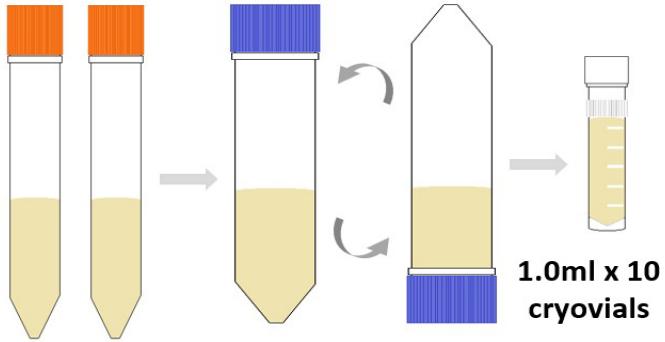
- Immediately after collection, gently invert the 50 ml conical tube 3-4 times to mix the sample.

## Step Four



- Transfer CSF into <u>two</u> 15 ml conical tubes.
- Within 15 minutes of collection, centrifuge samples at room temperature at 2000 x g for 10 minutes.

## Step Five

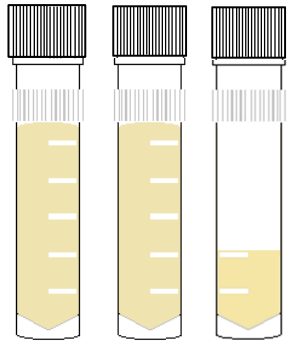



- Using a clean transfer pipette, transfer CSF from both 15 ml conical tubes into a 50 ml conical tube, leaving the debris in the bottom.
- Gently invert the 50 ml conical tube 3-4 times to mix the sample.
- Aliquot 1.0 ml into the cryovials.
- Store CSF aliquots <u>upright</u> at -80$^{o}$C until shipment to BioSEND.

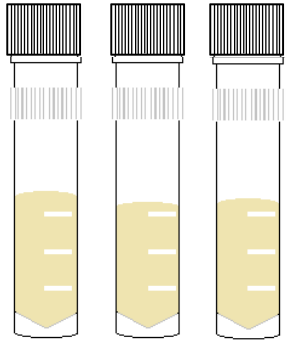
# Sample Collection and Processing: Aliquots

Filling biomarker serum, plasma, and CSF aliquots:

- Fill as many cryovials as possible to 1.0 ml (plasma, serum & CSF)
- Over-filled vials may burst in freezer!
- Ship ALL material to IU, even if final vial is less than standard volume



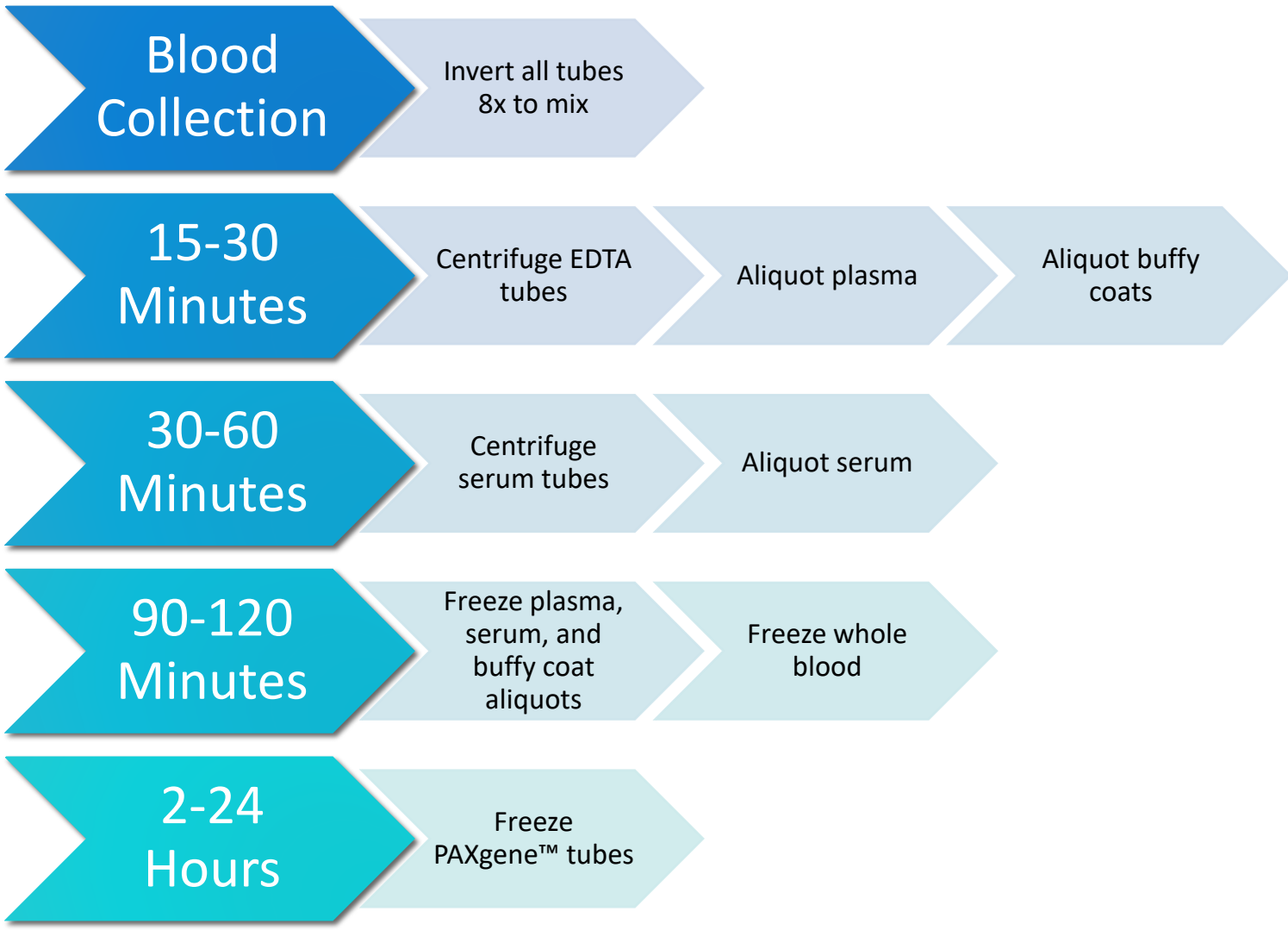
YES



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 serum and/or plasma caused by incorrect collection

| Cause: Blood Collection Methods | Corrective Action |
|---|---|
| Improper venipuncture site | Draw from median cubital, basalic, 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

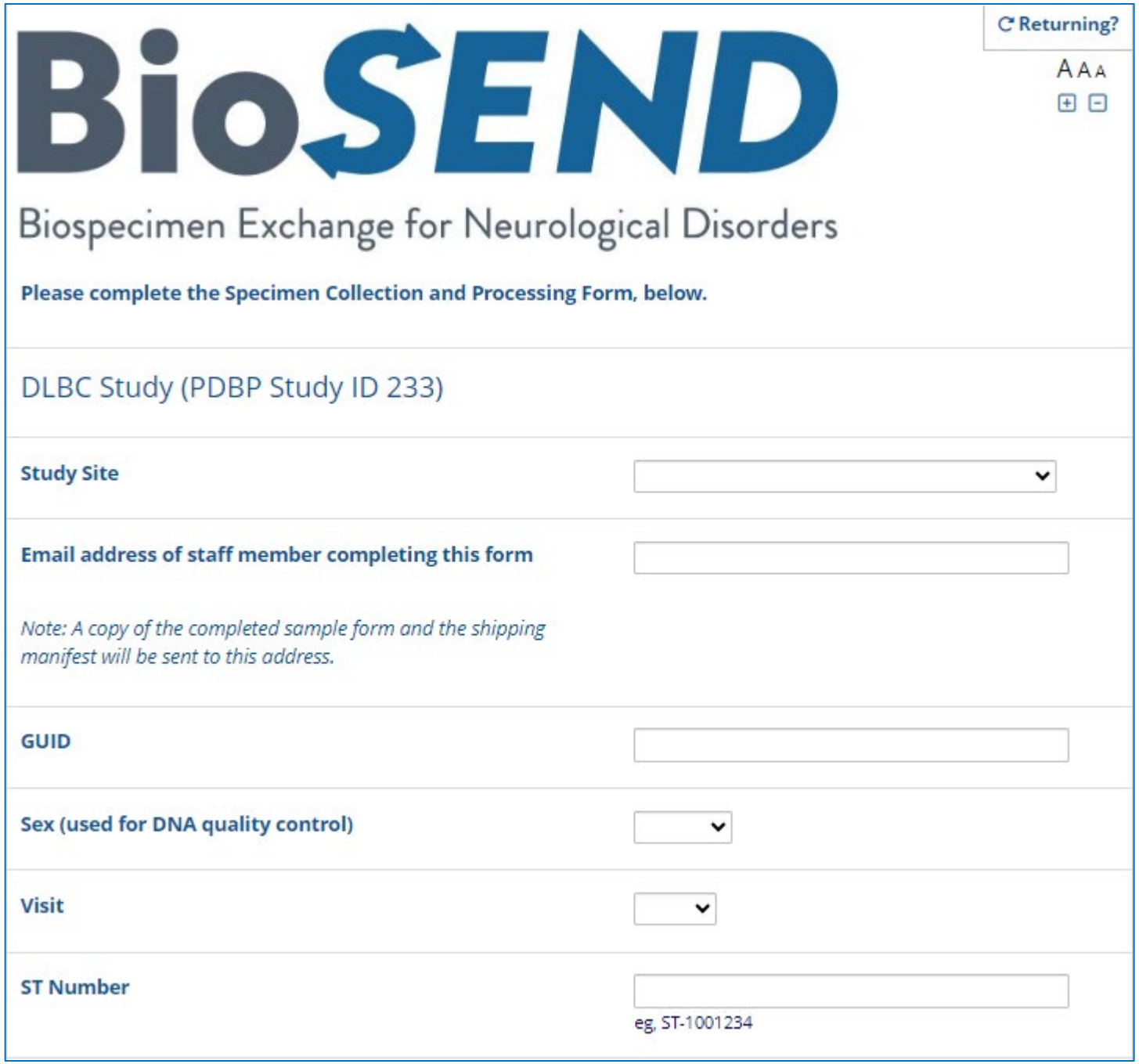
| Cause: Sample Processing Methods | Corrective Actions |
|---|---|
| Vigorous mixing/shaking | Gently invert blood collection tube when mixing additive with specimen, follow guidelines in Biologics Manual regarding number of times to invert each type of tube |
| Not allowing serum to clot for recommended 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:
http://kits.iu.edu/biosend/DLBCSampleForm

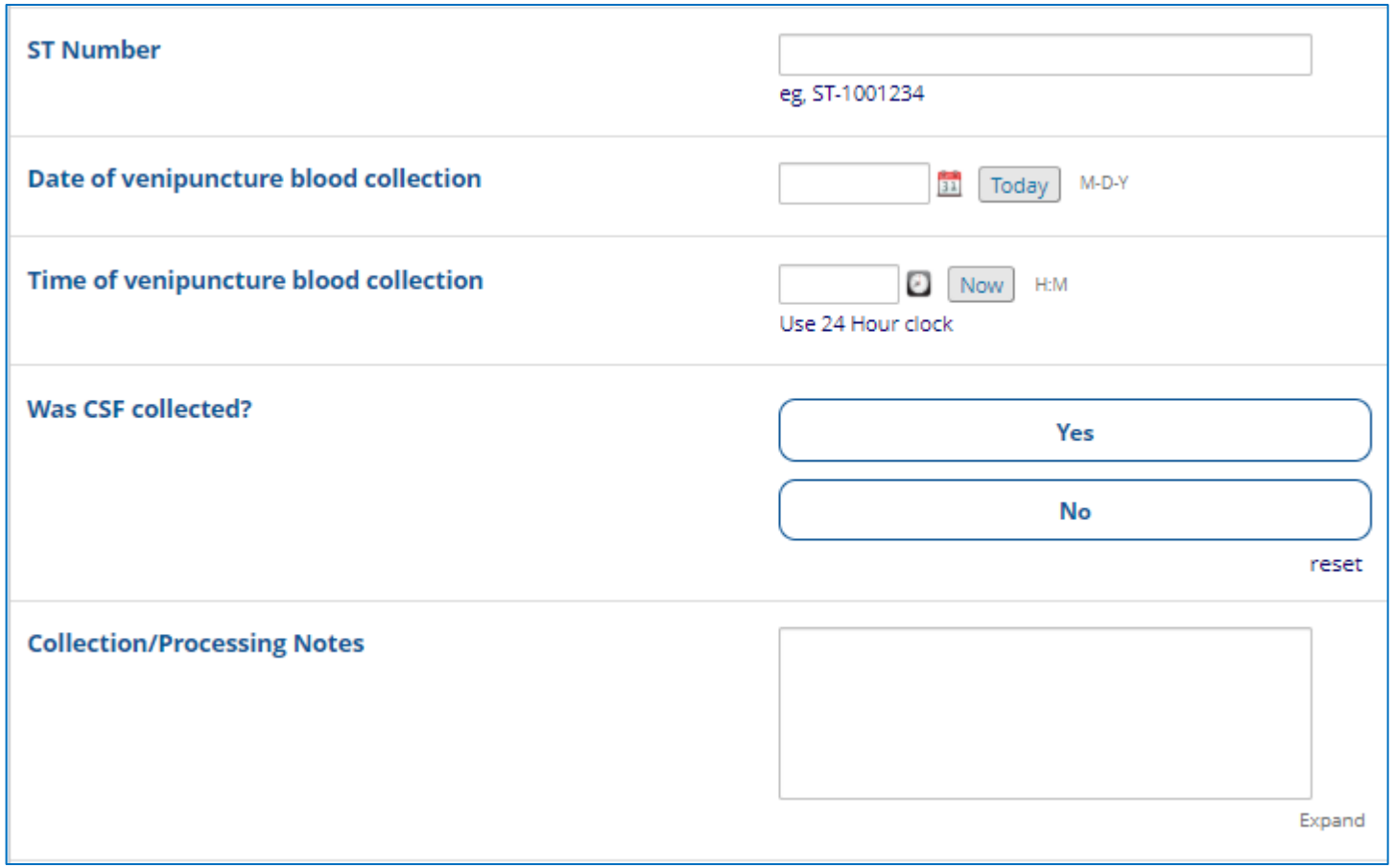
First part captures basic subject and visit information



# Sample Collection and Processing Form

Direct link:
http://kits.iu.edu/biosend/DLBCSampleForm

Second part captures collection information



# Sample Collection and Processing Form

Direct link:
http://kits.iu.edu/biosend/DLBCSampleForm

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



## DLBC 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 shipping container.

Study Site:

- ○ Cleveland Clinic
- ○ Cleveland Clinic- Las Vegas
- ○ Rush University
- ○ Thomas Jefferson University
- ○ University of California San Diego
- ○ University of North Carolina
- ○ University of Pennsylvania
- ○ University of Pittsburgh
- ○ VA-Puget Sound Health Care System/University of Washington

GUID: _____

Visit:

- ○ BL
- ○ 12M
- ○ 24M
- ○ 36M
- ○ 48M
- ○ 60M
- ○ 72M

Kit number: _____

Date of blood collection: _____

Date of CSF collection: _____

**SERUM**

Number of SERUM aliquots shipped: _____

**RNA**

Number of PAXGene™ tubes shipped: _____

# Shipping Frozen Samples: Tips

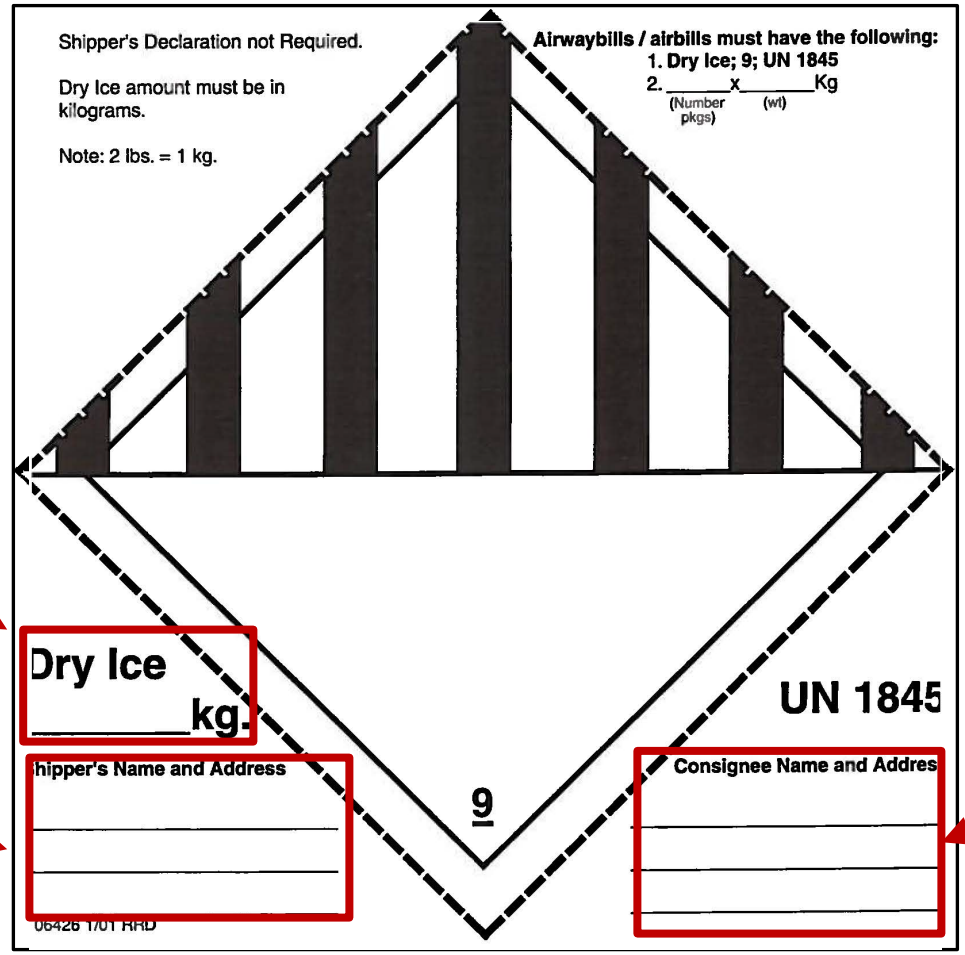
## Packing and Shipping Frozen Samples

- Serum, plasma, buffy coats, CSF, whole blood and RNA all ship frozen
- Ship frozen samples 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

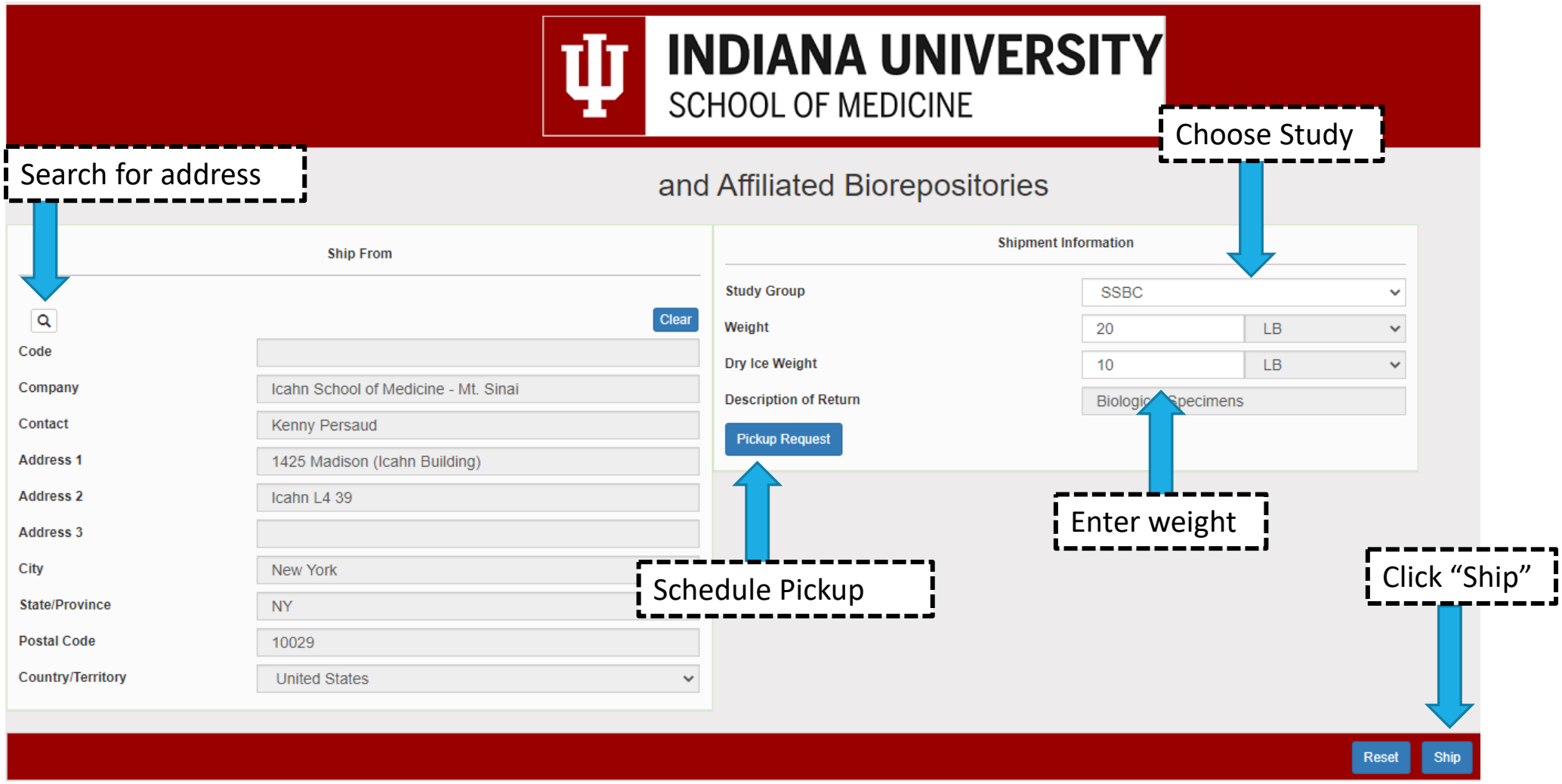
Class 9 Dry Ice Label should not be covered with other stickers and must be completed, or UPS will reject/return your package!



# Shipping Samples: Frozen

Packing and Shipping Frozen Samples


# Shipping Samples – UPS: https://kits.iu.edu/UPS



# 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

# 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

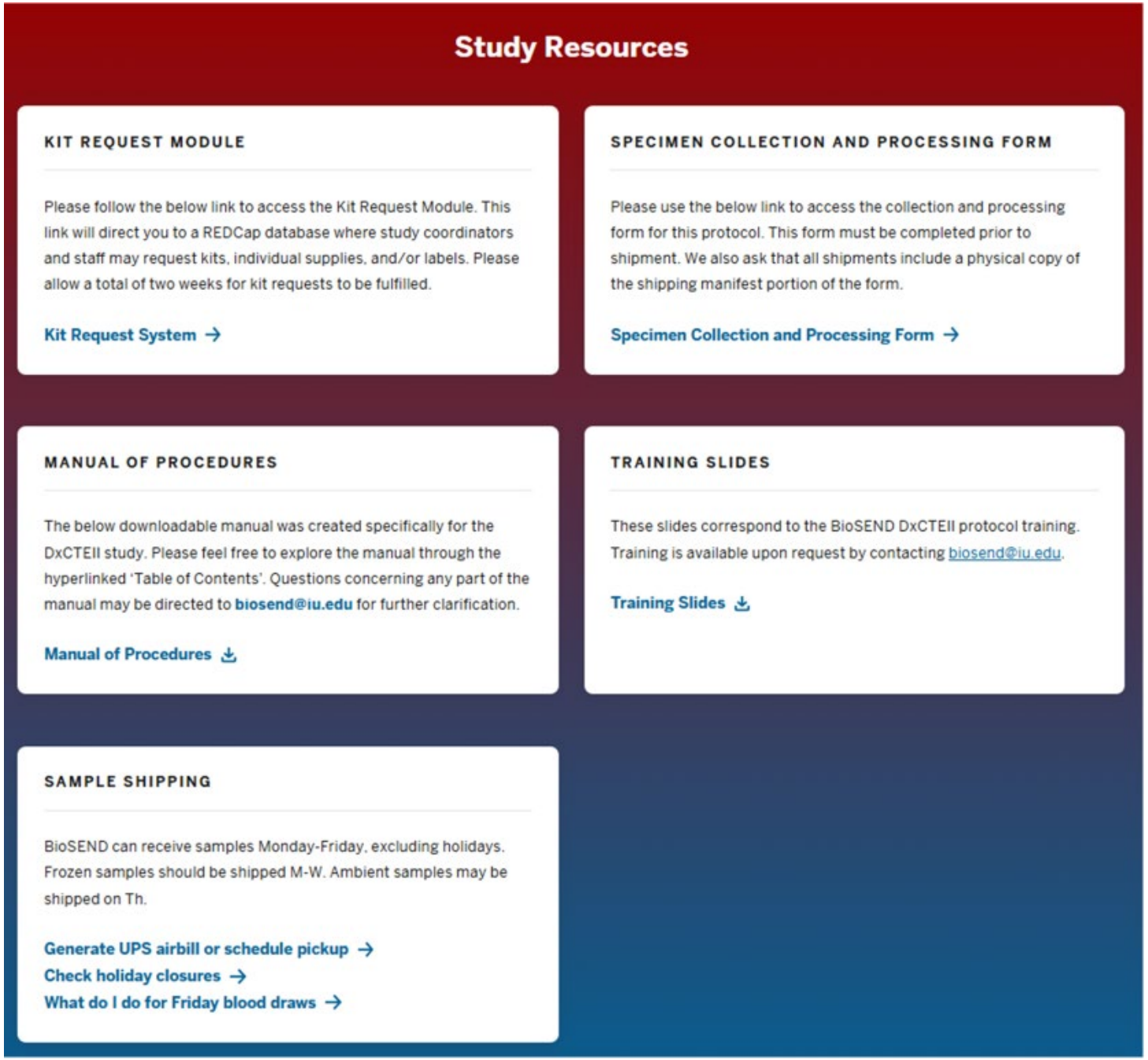
Most common non-conformance issues:

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

# BioSEND.org

On the website, you can:

- Access your study's kit request module and sample submission form
- Download the most recent version of the Manual of Procedures
- View a recording of this training
- Find information about holiday closures
- Access shipping resources



# Contacts

Indiana University

General Questions/Shipment Notifications:

biosend@iu.edu

317-278-6158

Request kits:

http://kits.iu.edu/biosend/LBD