BioSEND

Biospecimen Exchange for Neurological Disorders

NINDS Udall Centers of Excellence for Parkinson's Disease Research: University of Minnesota

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
Buffy coat (2 aliquots)	X
Plasma (6 x 1.5ml)	X
RNA (2 x 2.5ml)	X

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

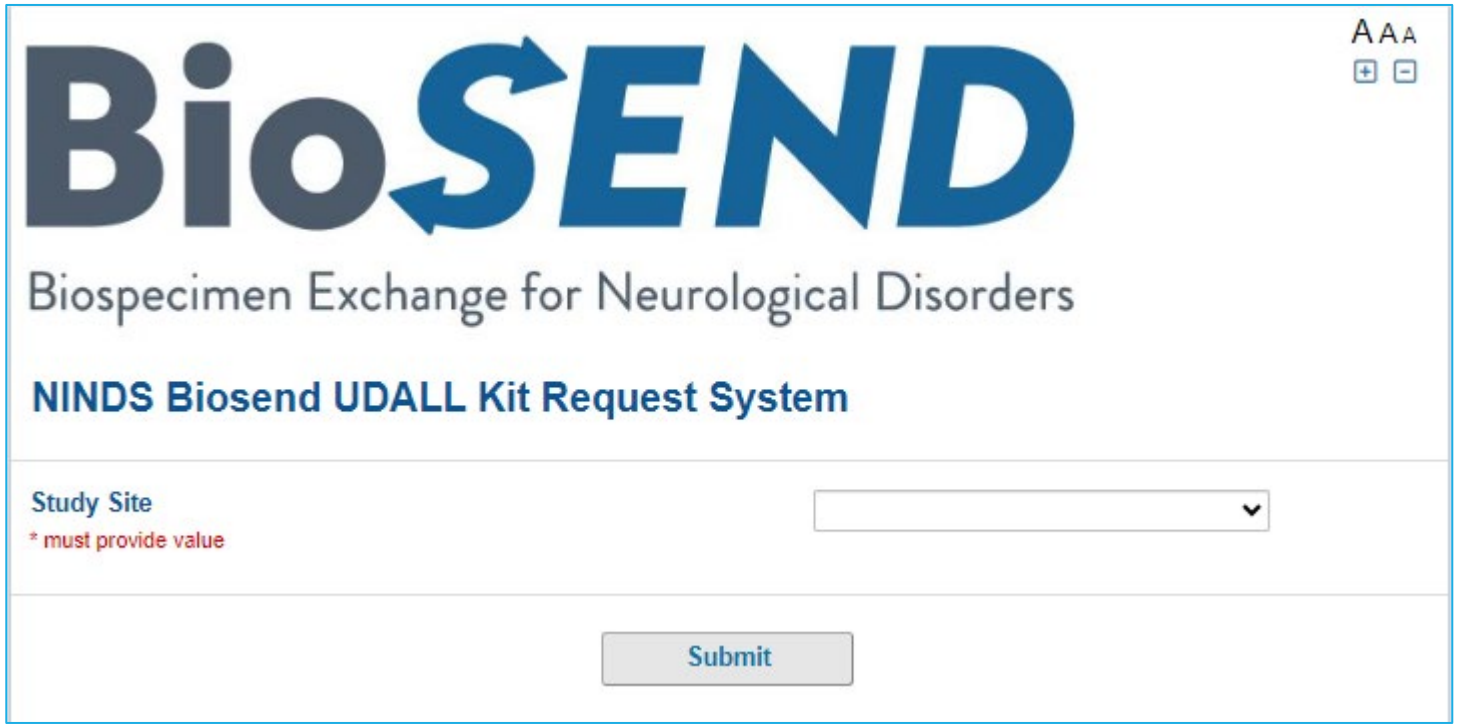
Kit Contents and Ordering – REDCap Survey

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

Order kits online through the Kit Request Module for:

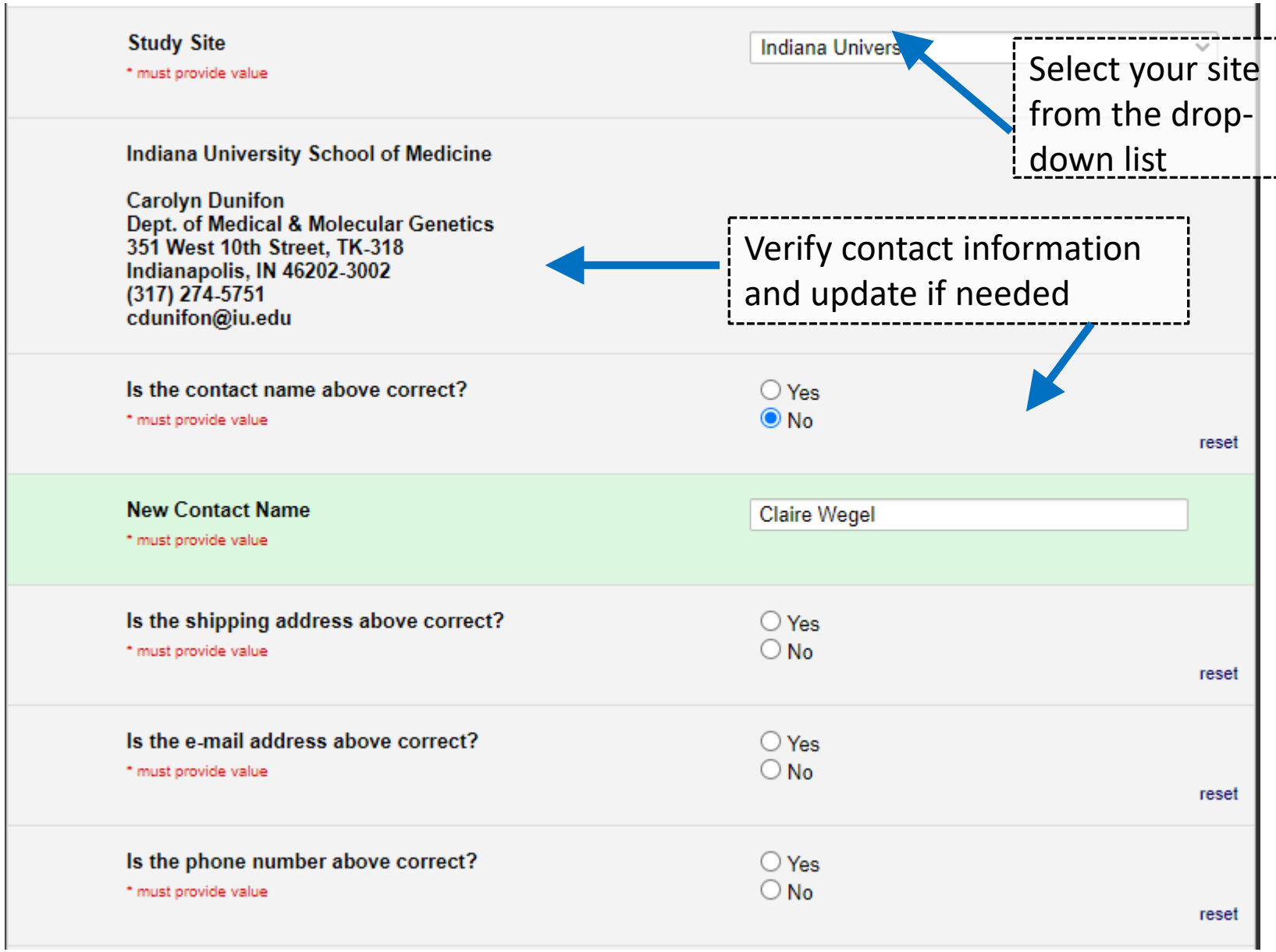
- Blood Kits
- Extra Supplies

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



Kit Contents and Ordering: Confirm Site Info

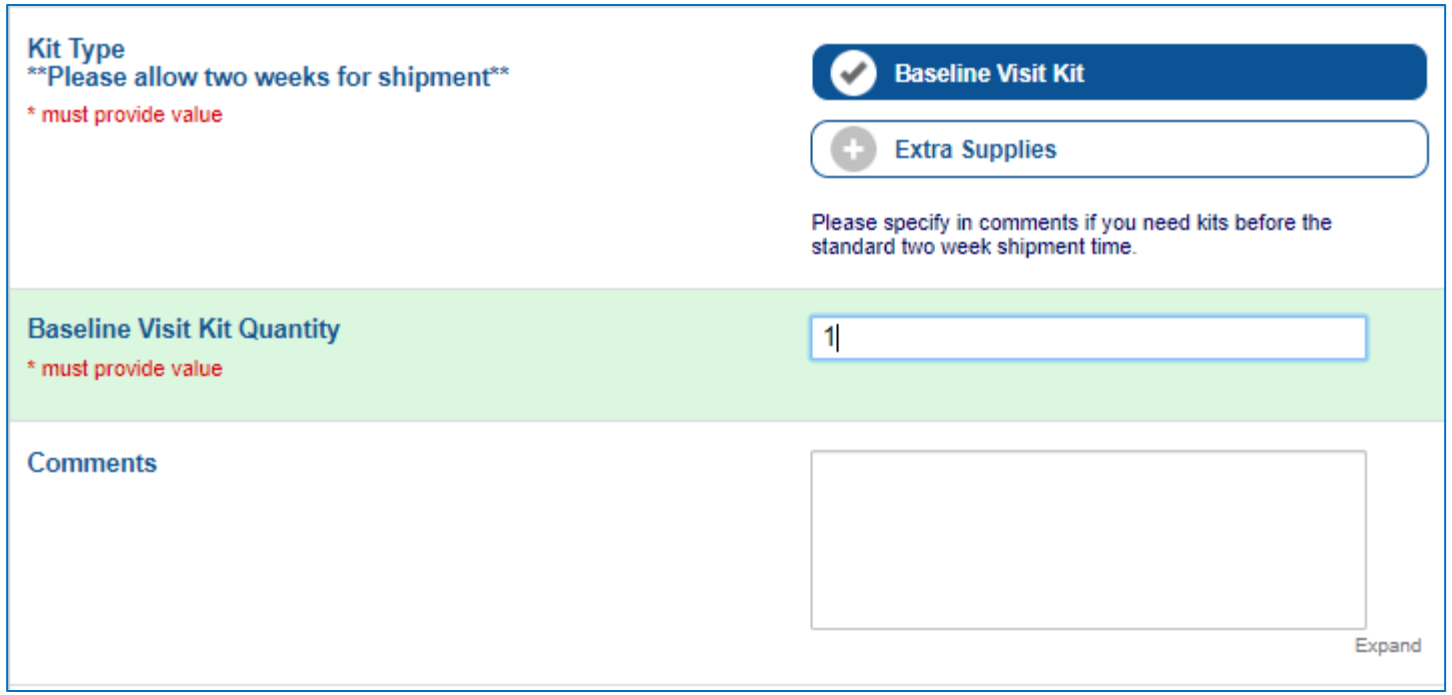
UDALL Kit Request Module



Kit Contents and Ordering: Kit Types

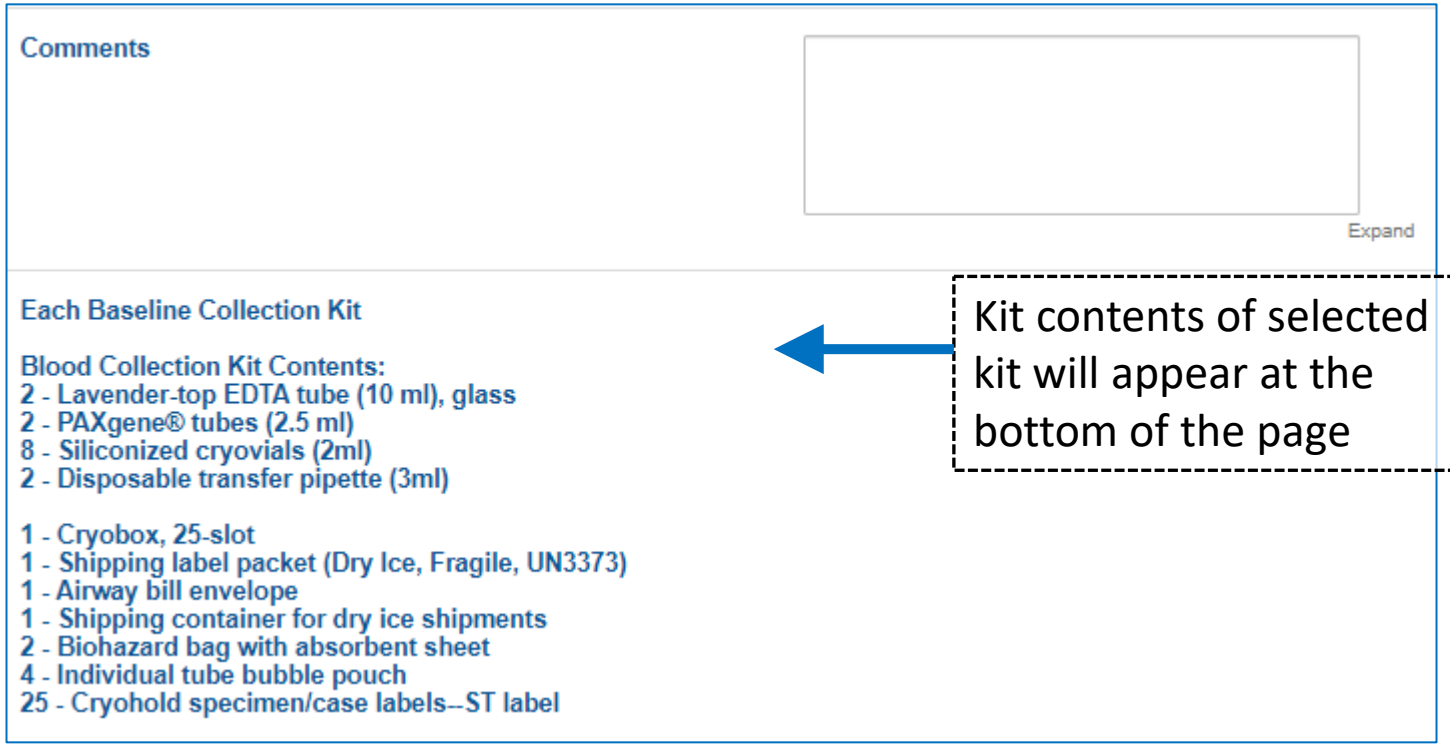
UDALL Kit Request Module

- 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 Contents and Ordering: Kit Breakdown

UDALL Kit Request Module



Kit Contents and Ordering: Blood Kit

Collection Volumes

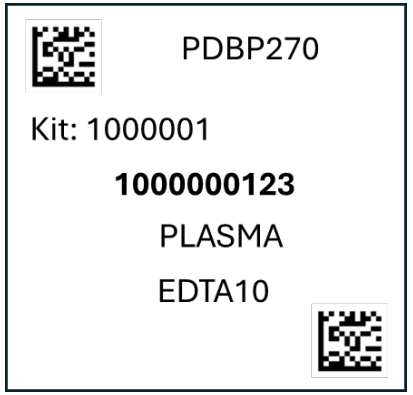
Total blood volumes

Sample Type	Amount
Whole Blood for Plasma and Buffy Coat	10 ml
Whole Blood for RNA	5 ml

Sample Labelling: Example Labels

Labels are provided by Indiana University

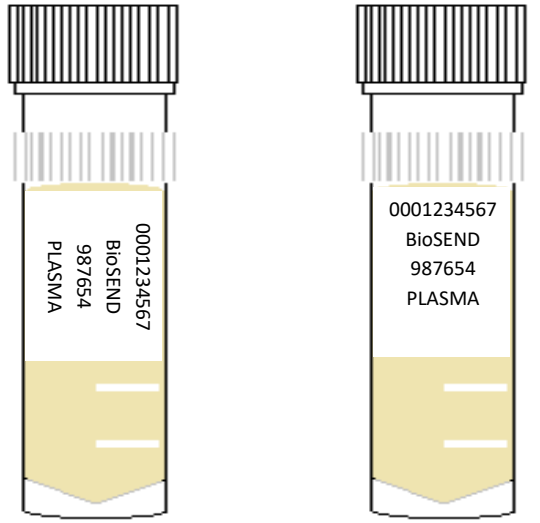
- Please check that all samples are properly labelled to ensure correct identification by IU
- If do not have enough labels to complete a visit, please contact IU *immediately*
- Labelling the tubes during processing prevents sample mix-ups



Sample Labelling: Label Placement

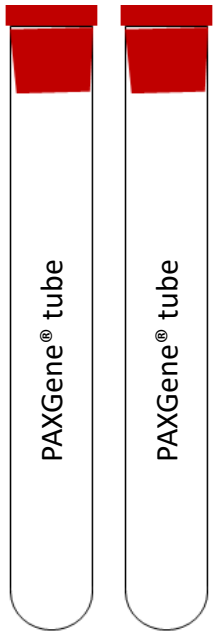
Please...

- 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



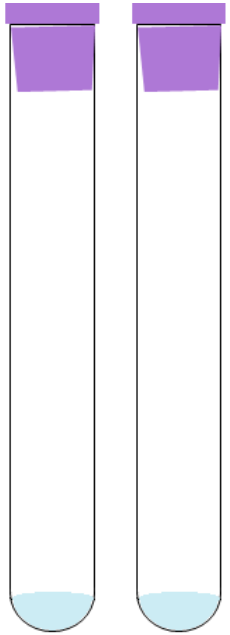
Sample Collection and Processing

2,5ml PAXGene®



1

10ml EDTA



2

Sample Collection and Processing: RNA

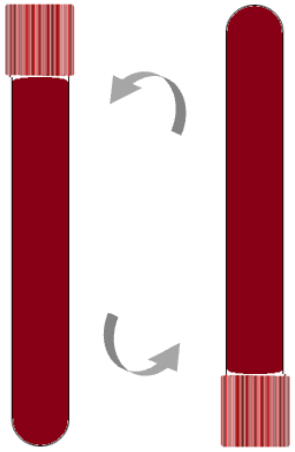
Step One

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

Step Two

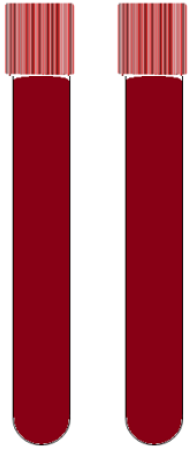
- Collect blood into PAXGene™ tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



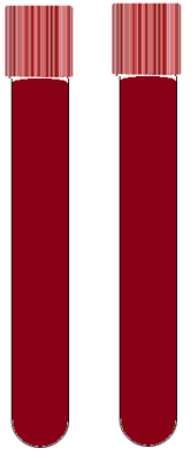
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Four



- Incubate tubes upright at room temperature for 24 hours.

Step Five



- After incubation period, freeze tubes upright at -80°C 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.

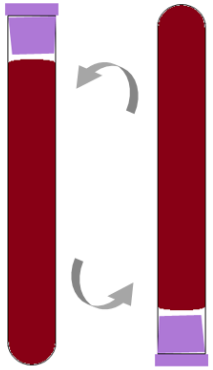
Step Two

- 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

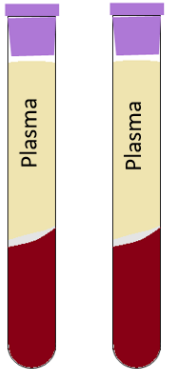
- Collect blood in EDTA tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Four



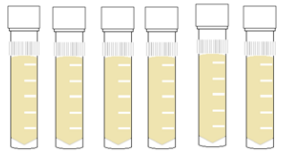
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

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 1.5 ml plasma into each cryotube.

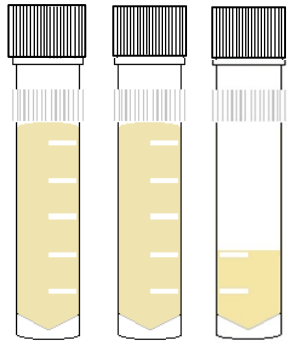
Step Seven

- 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.

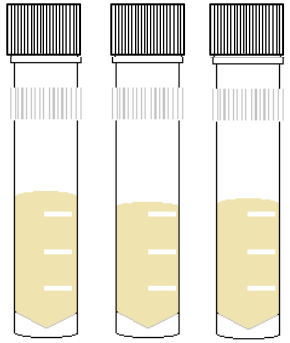
Sample Collection and Processing: Aliquots

Filling biomarker plasma aliquots:

- Fill as many cryovials as possible to 1.5 ml (plasma)
- Over-filled vials may burst in freezer!
- Ship ALL material to IU, even if final vial is less than standard volume



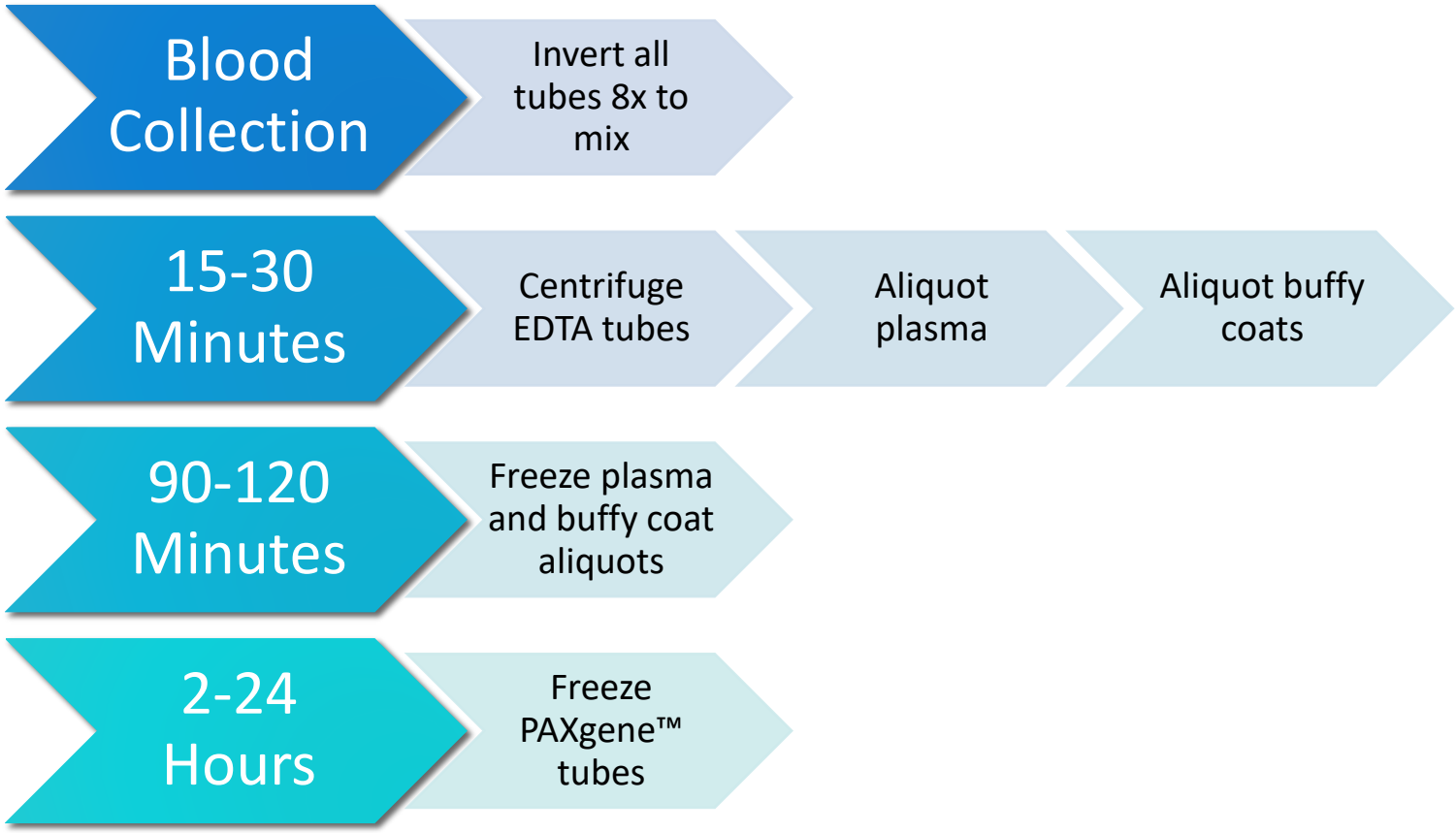
YES



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

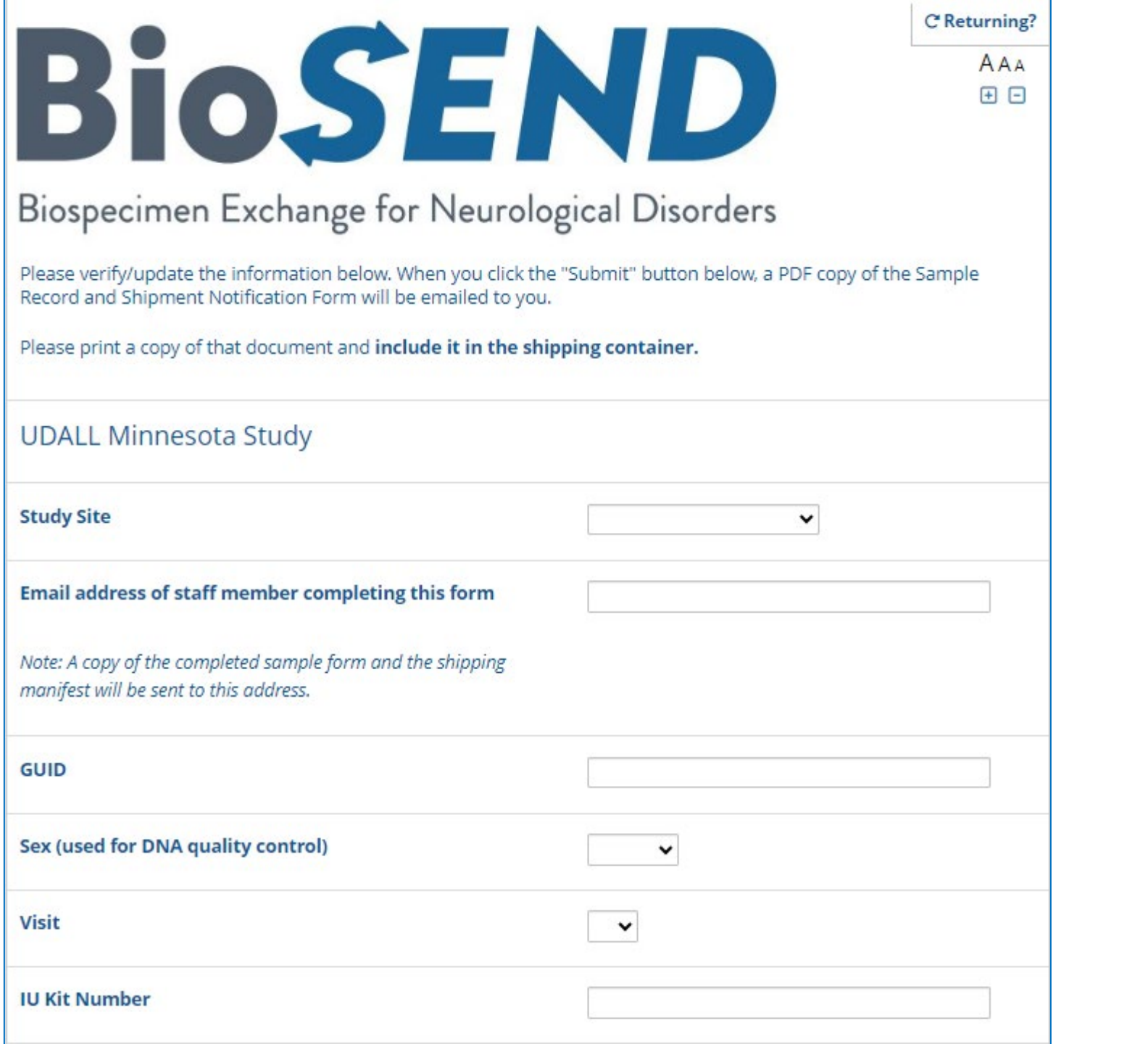
Cause: Sample Processing Methods	Corrective Actions
Vigorous mixing/shaking	Gently invert blood collection tube when mixing additive with specimen, follow guidelines in Biologics Manual regarding number of times to invert each type of tube
Not allowing serum to clot for recommended time	Serum tubes without 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: https://redcap.link/UDALLMinnesotaSampleForm

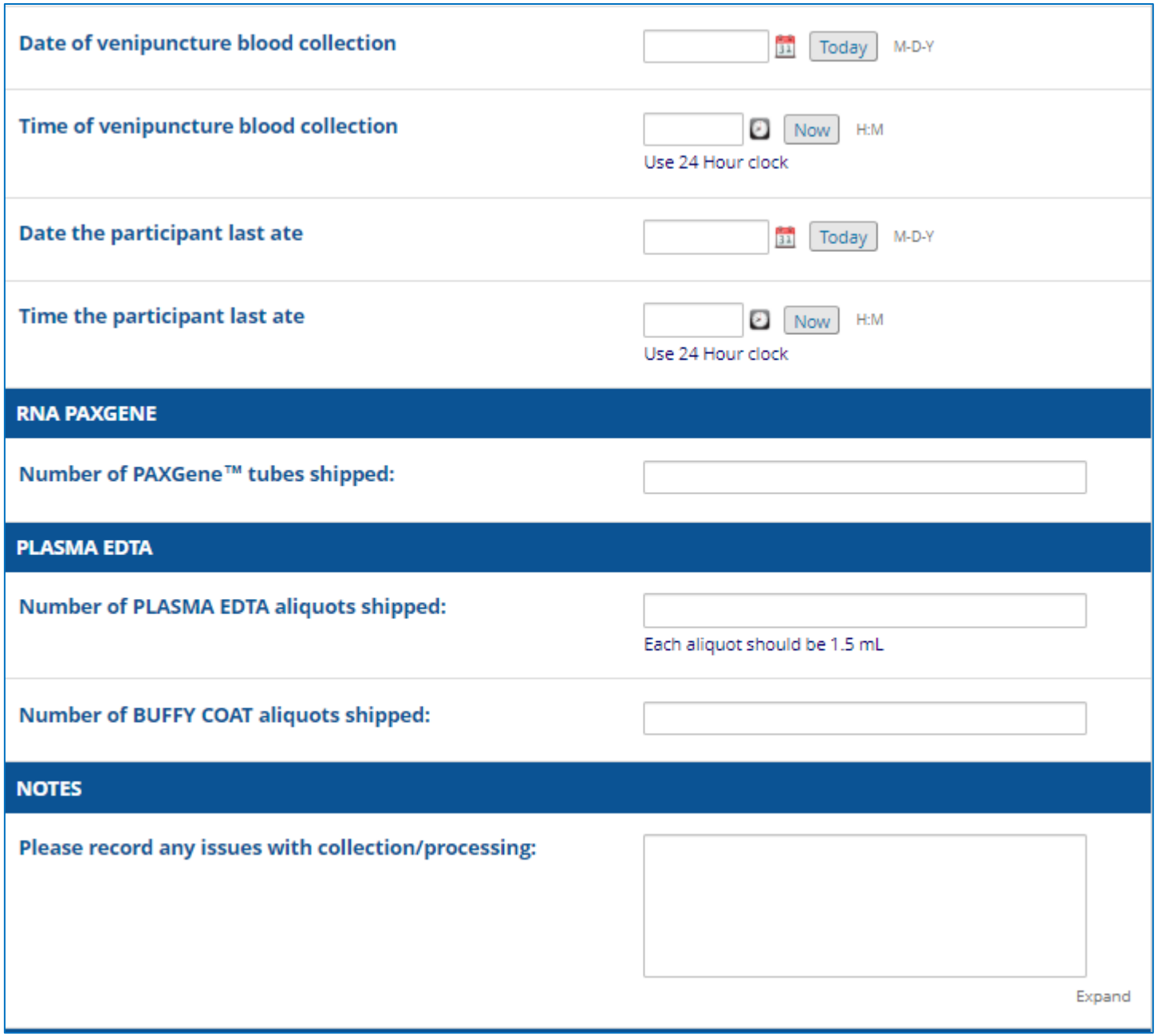
First part captures basic subject and visit information



Sample Collection and Processing Form

Direct link: https://redcap.link/UDALLMinnesotaSampleForm

Second part captures collection information



Sample Collection and Processing Form

Direct link: **https://redcap.link/UDALLMinnesotaSampleForm**

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

UDALL Minnesota 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:	◯ University of Minnesota
GUID:	_____
Visit:	◯ BL
IU Kit Number:	_____
Date of blood collection:	_____

RNA

Number of PAXGene™ tubes shipped:	_____

PLASMA EDTA

Number of PLASMA EDTA aliquots shipped:	_____
Number of BUFFY COAT 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 are unsure whether or not it is safe to ship.

Date of shipment:	_____
Did/will you use the IU UPS interface to generate the shipping label?	◯ Yes ◯ No
Which shipping service did you use?	◯ UPS ◯ FedEx ◯ World Courier ◯ Other

Shipping Frozen Samples: Tips

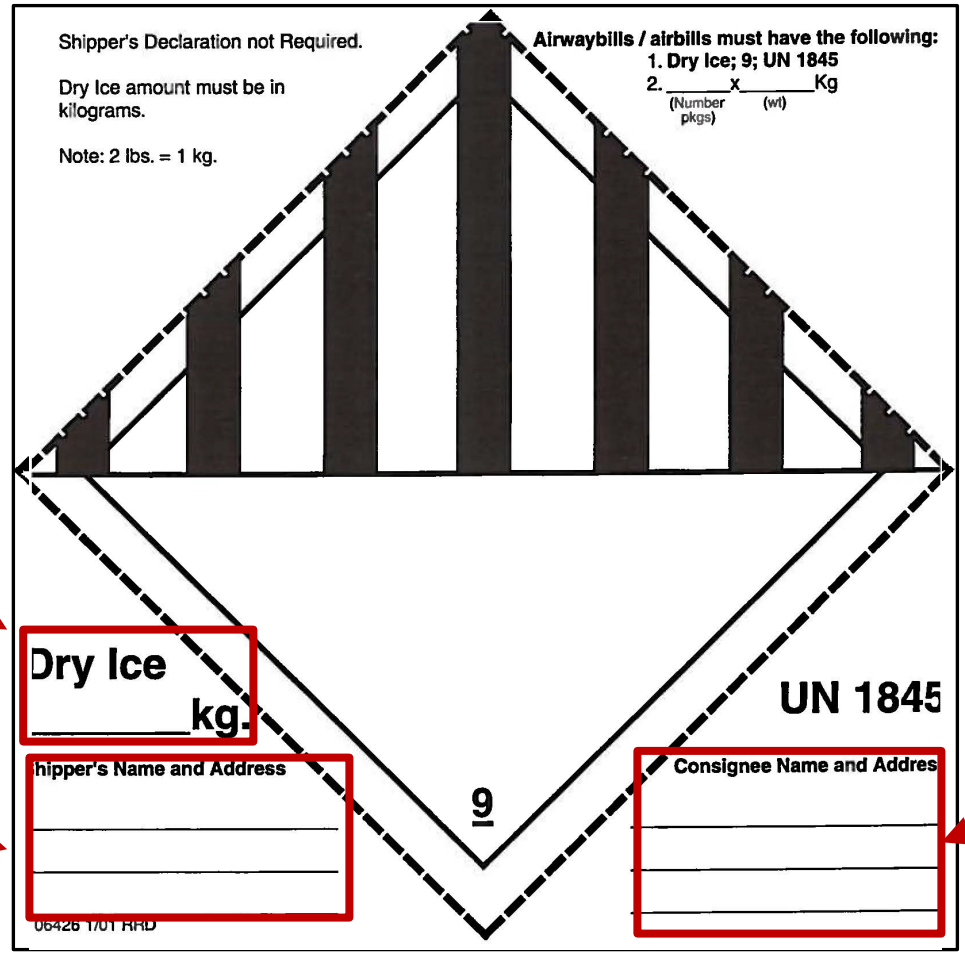
Packing and Shipping Frozen Samples

- Plasma, buffy coats 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

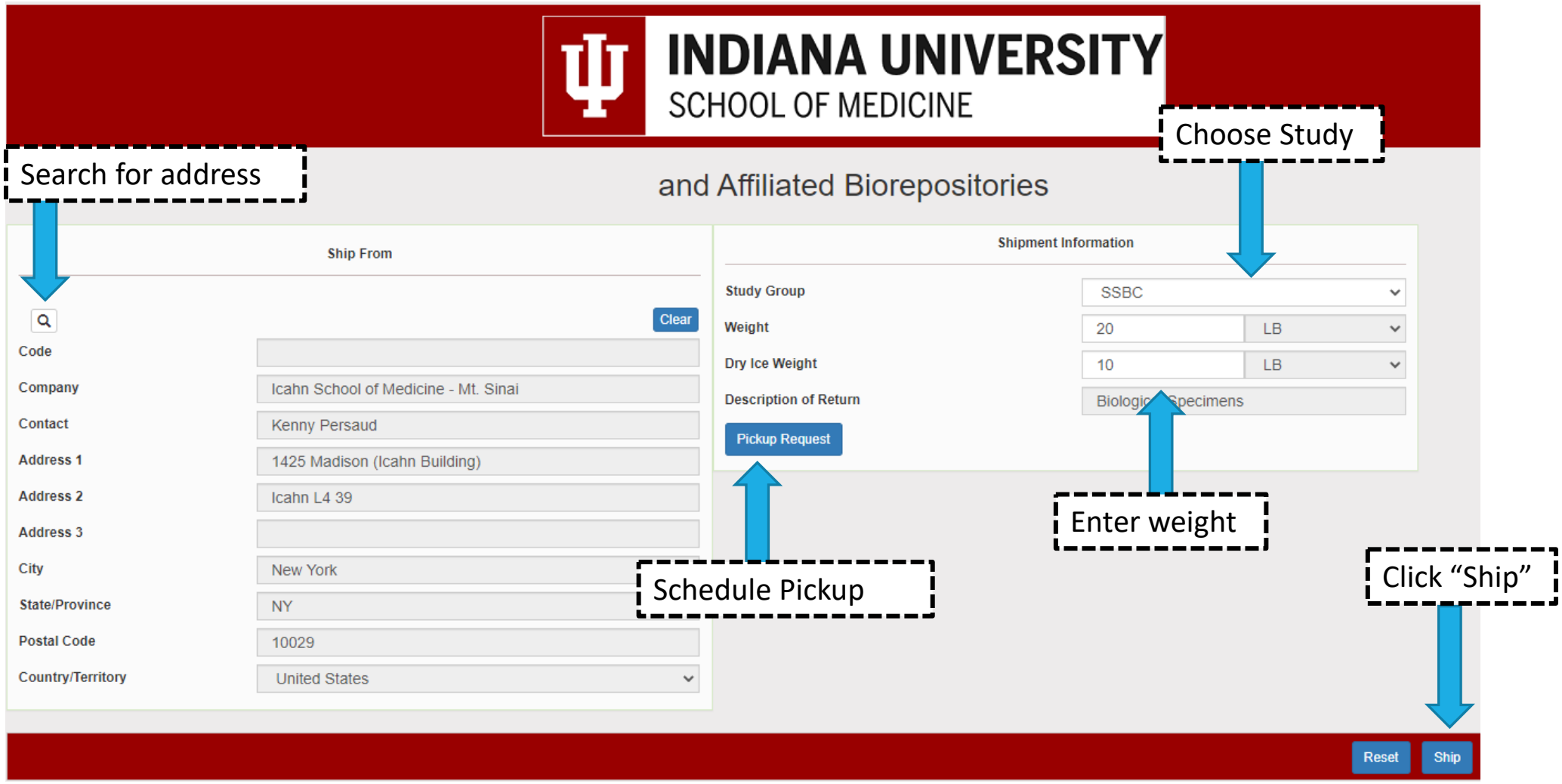
Class 9 Dry Ice Label should not be covered with other stickers and must be completed, or UPS will reject/return your package!



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 Reporting

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

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

Non-Conformance Reporting con’t

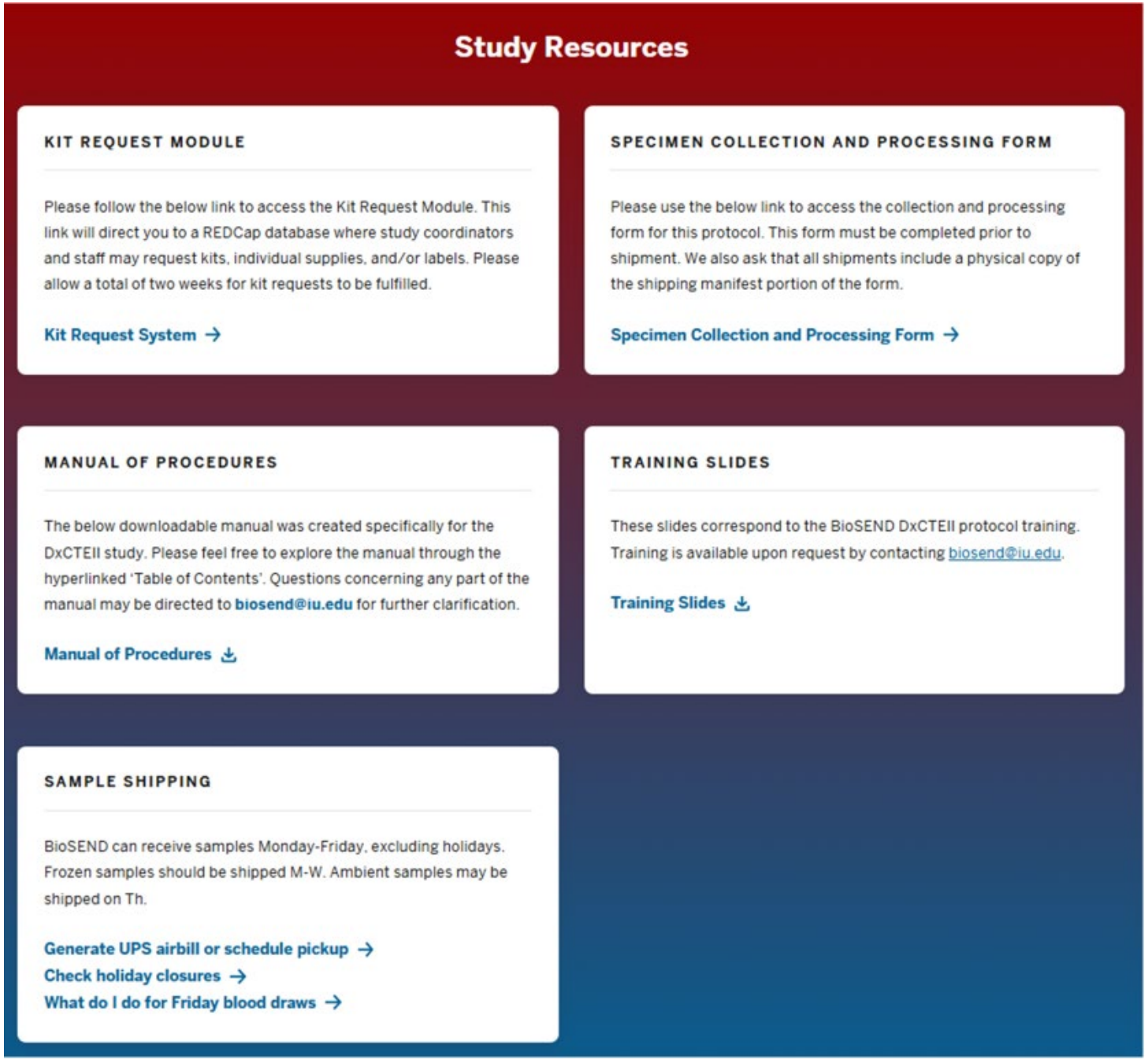
Most common non-conformance issues:

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

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/UDALL