BioSEND

Biospecimen Exchange for Neurological Disorders

Longitudinal Imaging Biomarkers of Disease Progression in DLB

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
Whole Blood (2x3ml)	X	X	X	X	X	X	X	X	X	X
Plasma (6 x 1ml)	X	X	X	X	X	X	X	X	X	X
Buffy coat (2 aliquots)	X	X	X	X	X	X	X	X	X	X
Serum (6 x 1ml)	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
CSF (10 x 1ml)	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 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

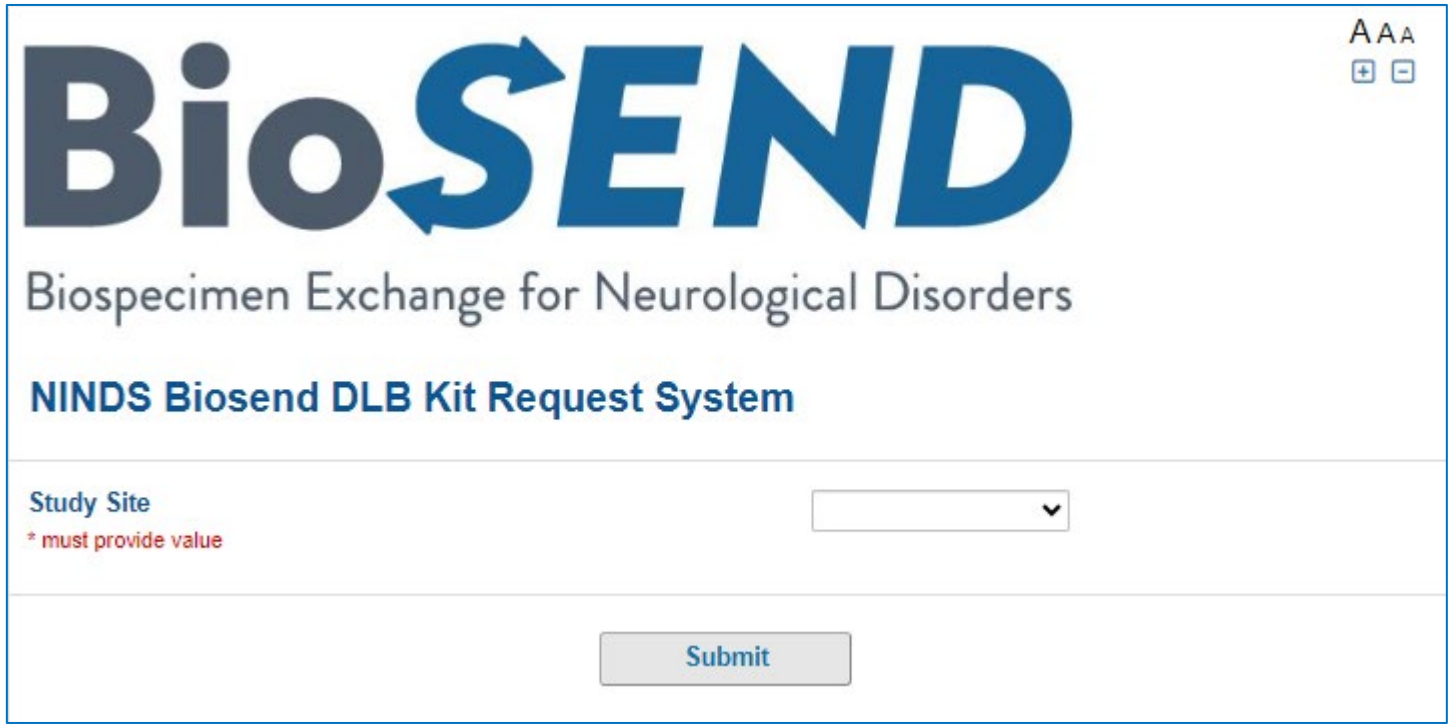
Kit Contents and Ordering – REDCap Survey

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

Order kits online through the Kit Request Module for:

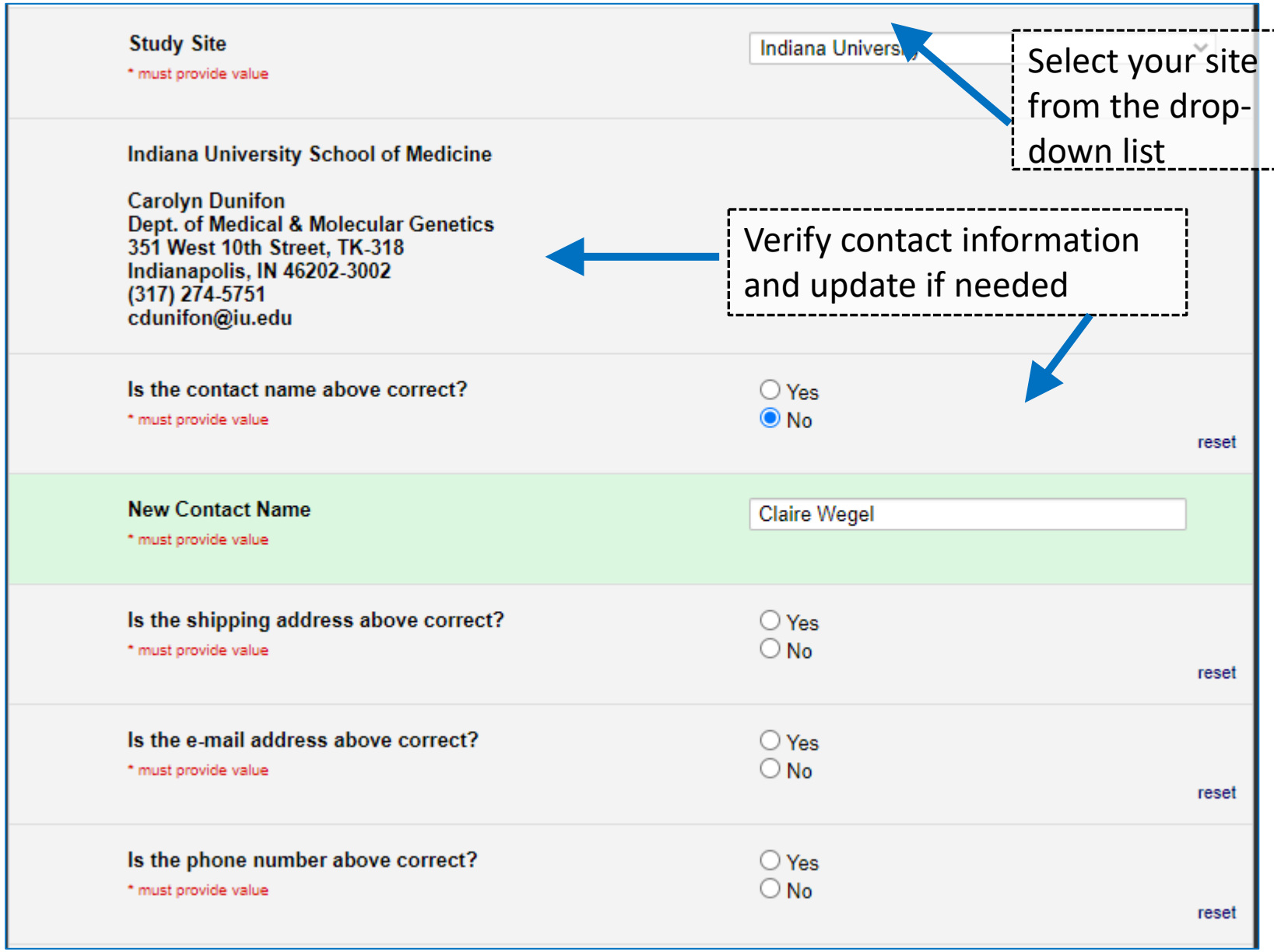
- Blood kits
- Supplemental Kit
- Extra Supplies

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



Kit Contents and Ordering: Confirm Site Info

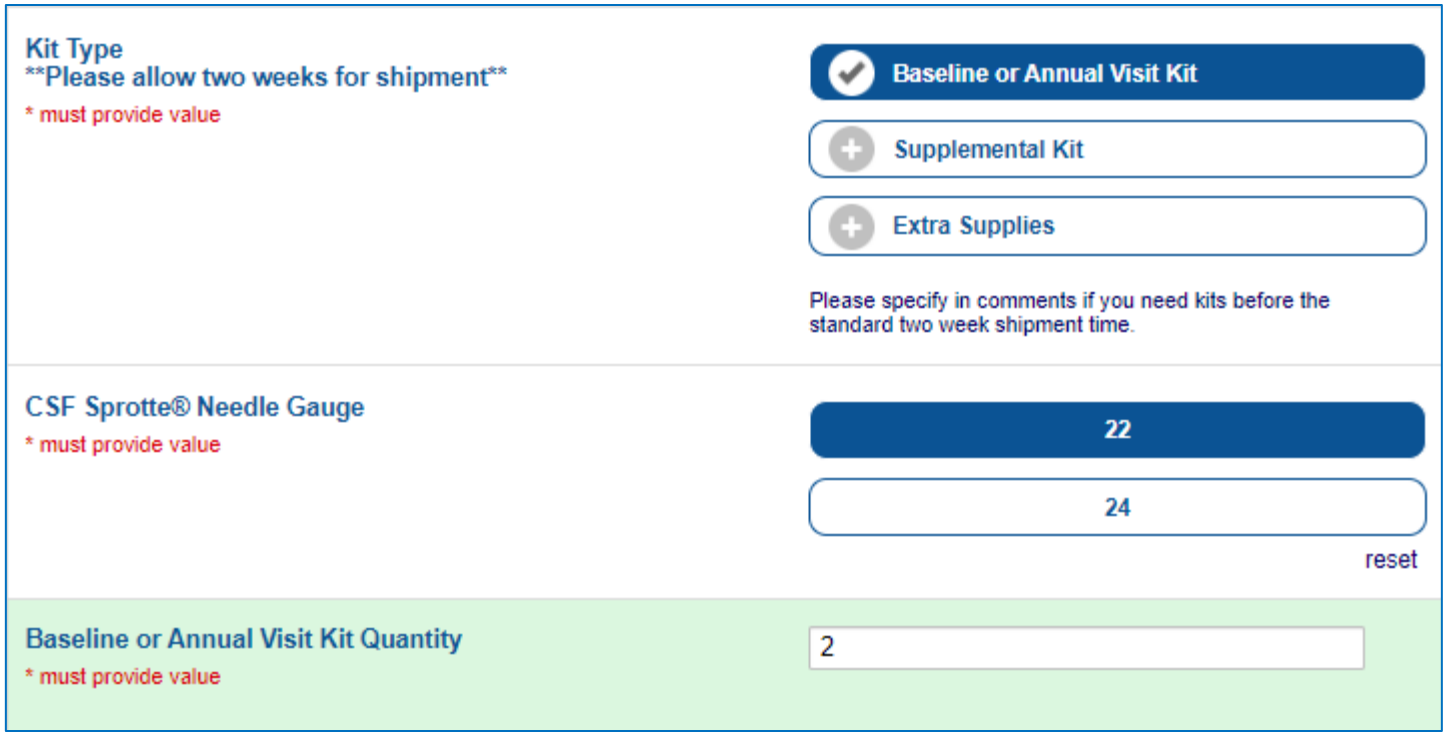
DLB Kit Request Module



Kit Contents and Ordering: Kit Types

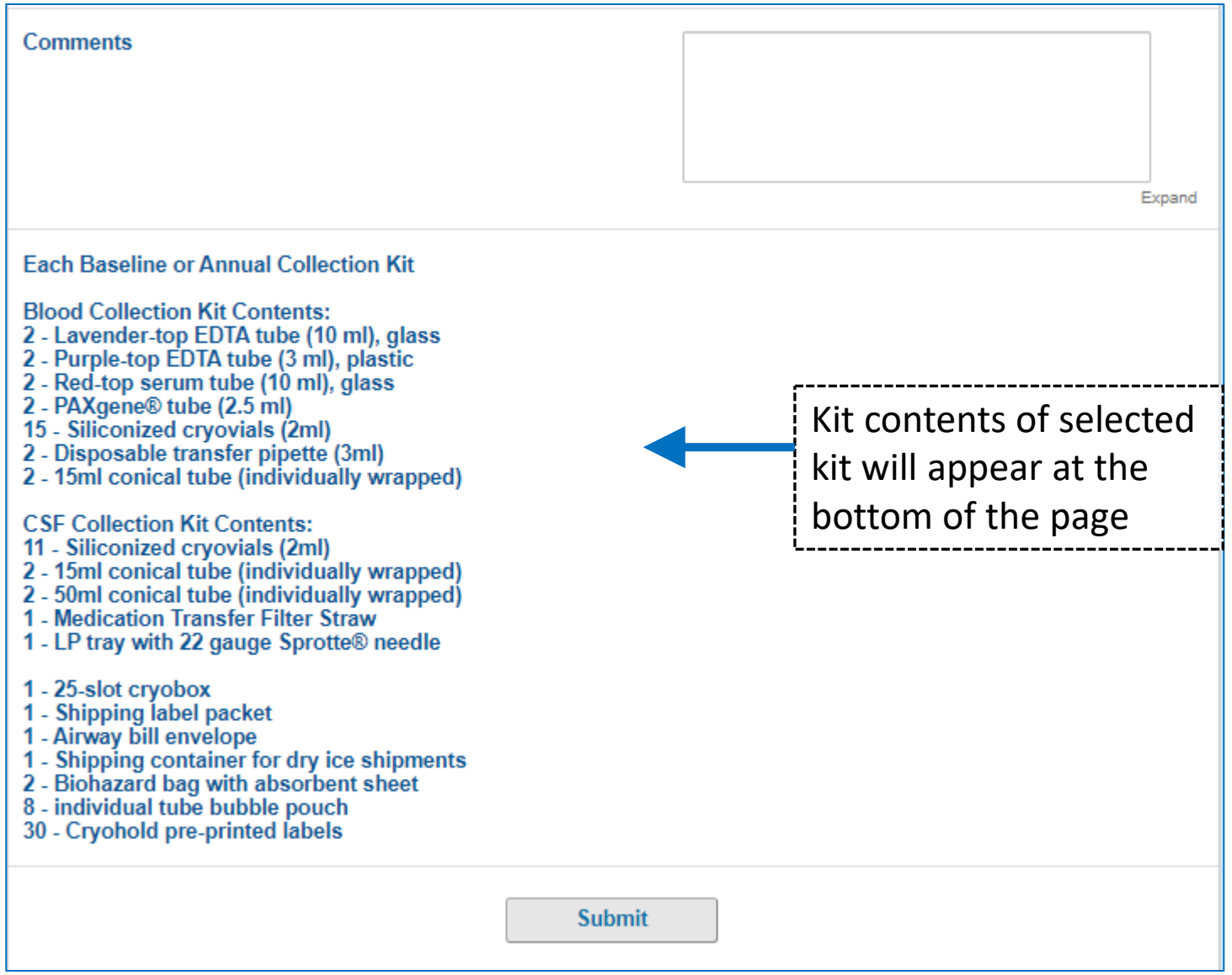
DLB Kit Request Module

- 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

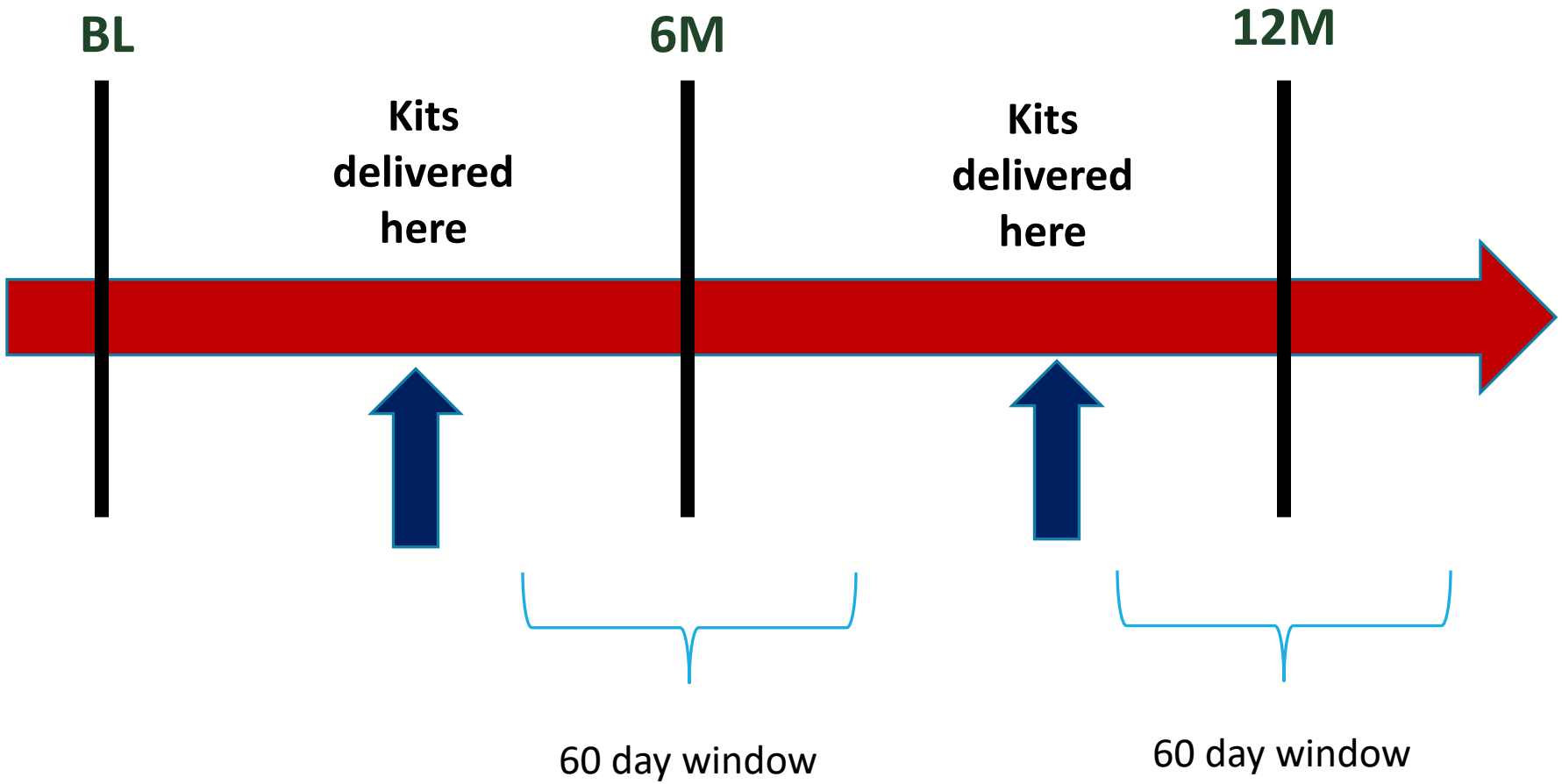
DLB Kit Request Module



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)

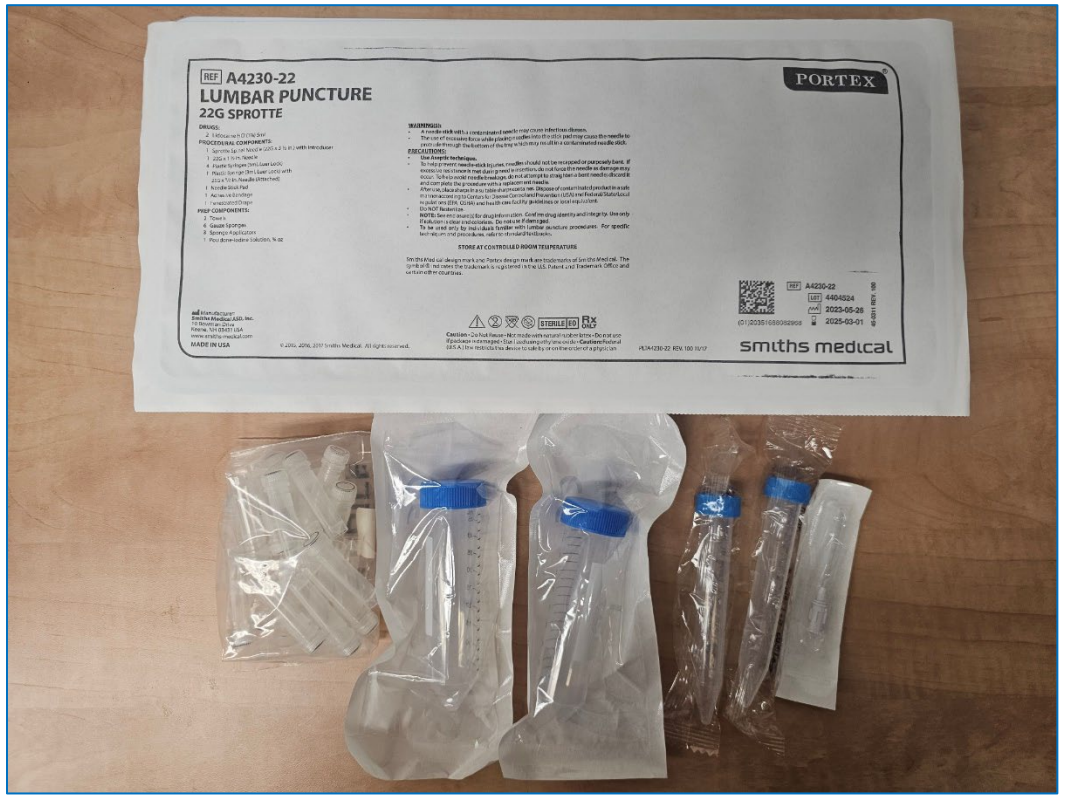
Automatic Kit Shipments



Kit Contents and Ordering: Blood Kit

Kit Contents and Ordering: CSF Supplies

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	6 ml
Cerebrospinal Fluid	10 ml

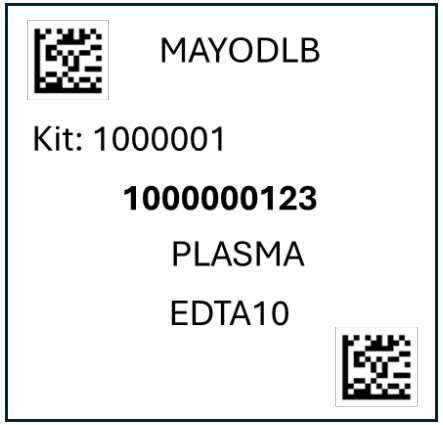
Kit and Supply Ordering

- 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

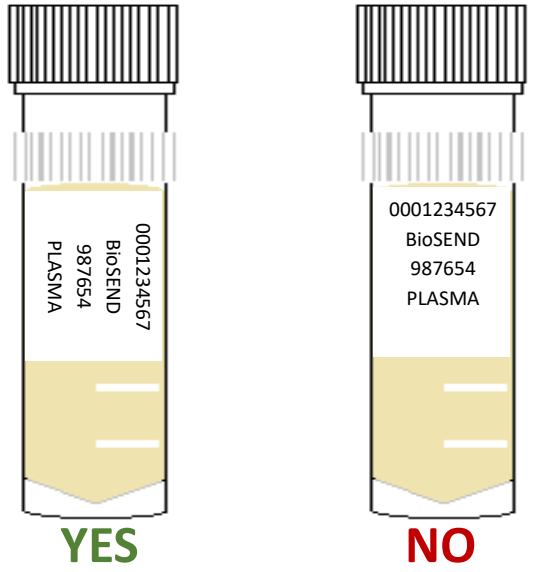
- Please check that all samples are properly labelled to ensure correct identification by IU
- If do not have enough labels to complete a visit, please contact IU *immediately*
- Labelling the tubes during processing prevents sample mix-ups



Sample Labelling: Label Placement

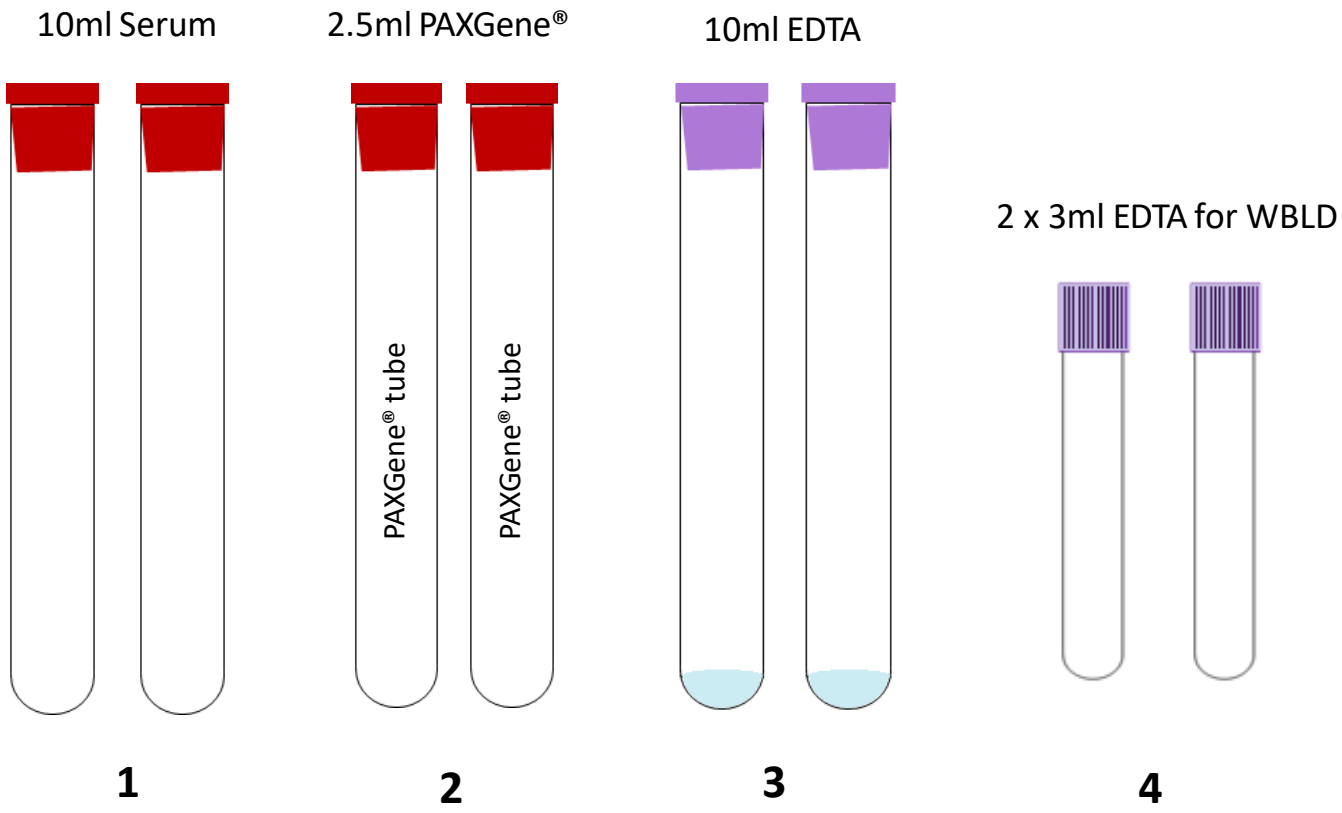
Please...

- 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



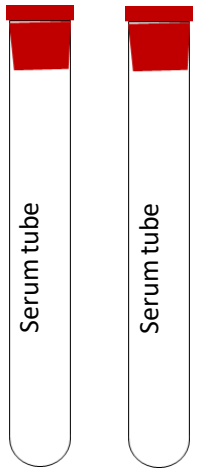
Sample Collection and Processing

Blood Tube Draw Order



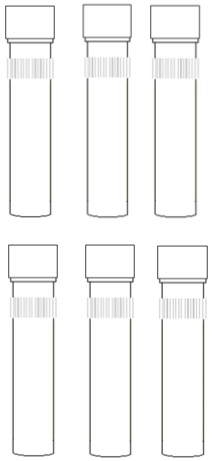
Sample Collection and Processing: Serum

Step One



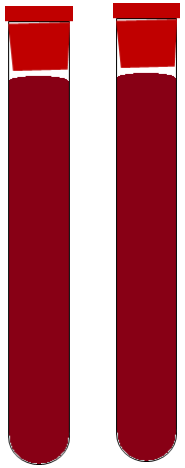
- Store tubes at room temperature.
- Label tubes with preprinted serum labels prior to blood draw.

Step Two



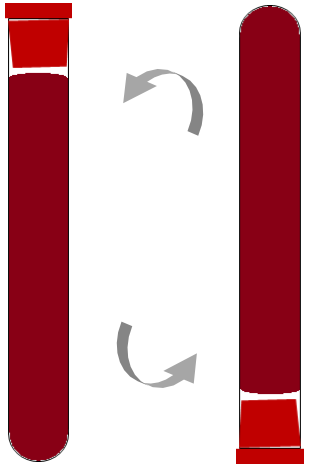
- Label 6 cryotubes with serum aliquot labels.
- Pre-chill cryotubes on wet ice for at least 5 minutes

Step Three



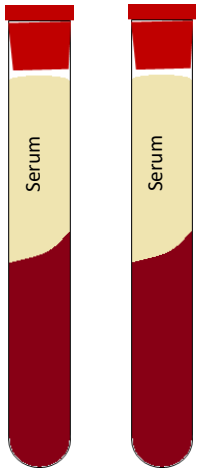
- Collect blood in serum tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Four



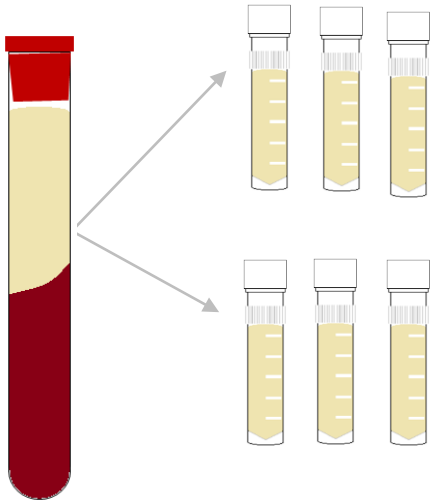
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

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

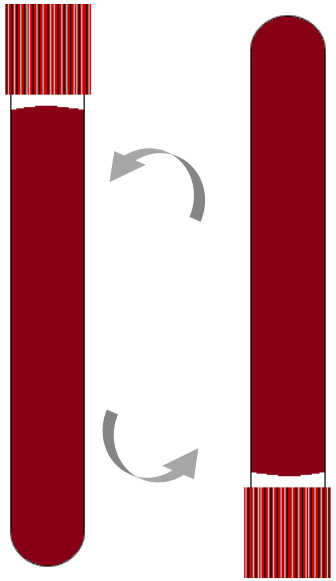
Steps 1-2

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

Steps 3-4

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

Steps 5



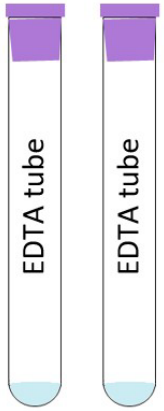
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Steps 7-8

- Incubate the tubes at room temperature for 2 to 24 hours.
- Freeze tubes upright at -80°C until shipment. (DO NOT freeze in solid Styrofoam tube racks)

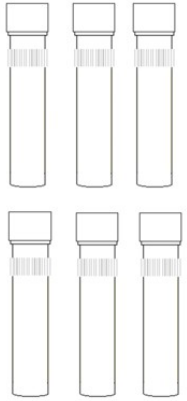
Sample Collection and Processing: Plasma & Buffy Coat

Step One



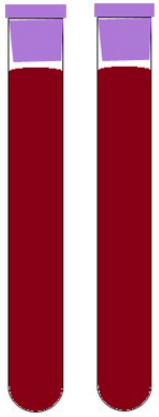
- Store tubes at room temperature.
- Label tubes with preprinted plasma labels prior to blood draw.

Step Two



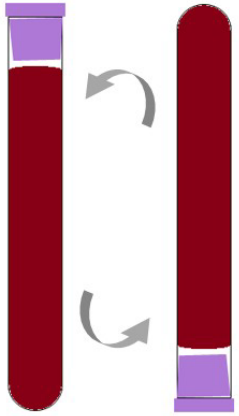
- Label 6 cryotubes with plasma labels and 2 with buffy coat labels.
- Pre-chill cryotubes on wet ice for at least 5 minutes

Step Three



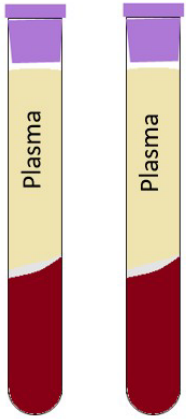
- Collect blood in EDTA tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Four

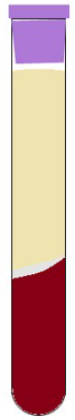


- Immediately after blood draw, invert tubes 8-10 times to mix samples.

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

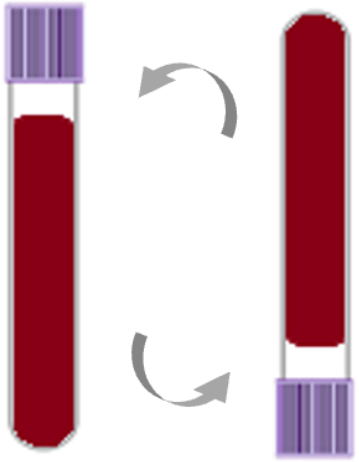
Step One

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

Step Two

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

Step Three



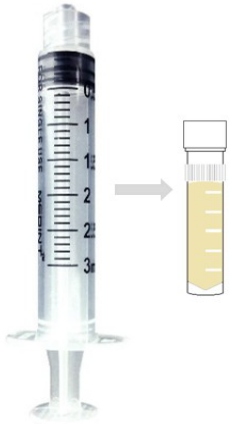
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Four

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

Sample Collection and Processing: CSF

Step One

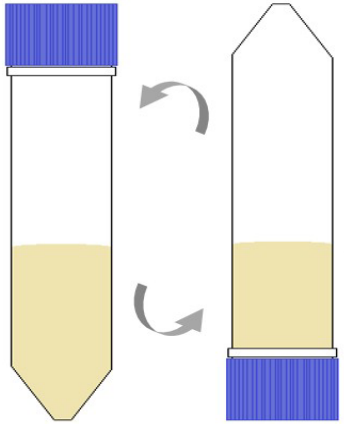


- Collect CSF into the 3ml luer lock syringe or by gravitational pull.
- Dispense 1-2 ml into cryovial.
- Send to local lab for testing, if applicable.

Step Two

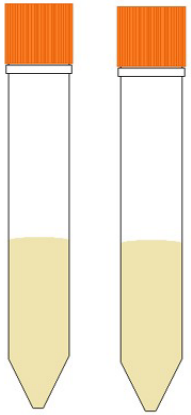
- Collect CSF into the 5ml luer lock syringe or by gravitational pull
- Transfer sample into 50 ml conical tube.

Step Three



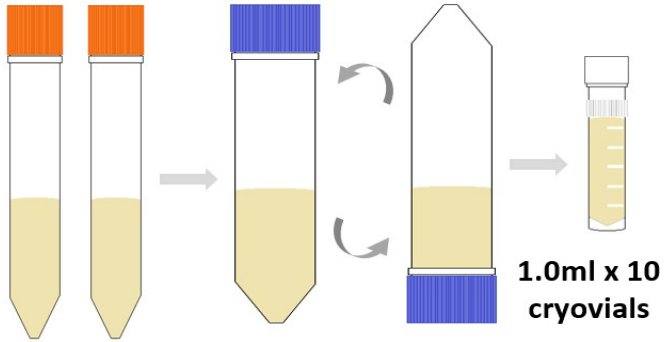
- Immediately after collection, gently invert the 50 ml conical tube 3-4 times to mix the sample.

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

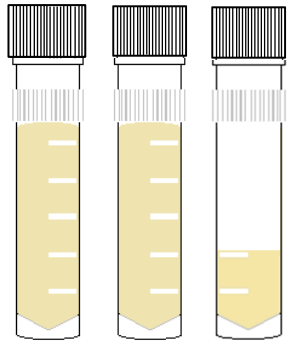


- Using a clean transfer pipette, transfer CSF from both 15 ml conical tubes into a 50 ml conical tube, leaving the debris in the bottom.
- Gently invert the 50 ml conical tube 3-4 times to mix the sample.
- Aliquot 1.0 ml into the cryovials.
- Store CSF aliquots <u>upright</u> at -80^{o}C until shipment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

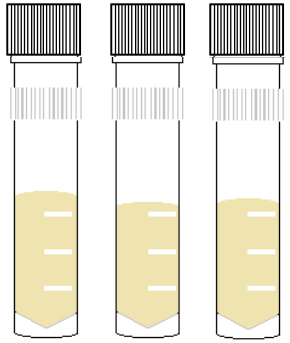
Sample Collection and Processing: Aliquots

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



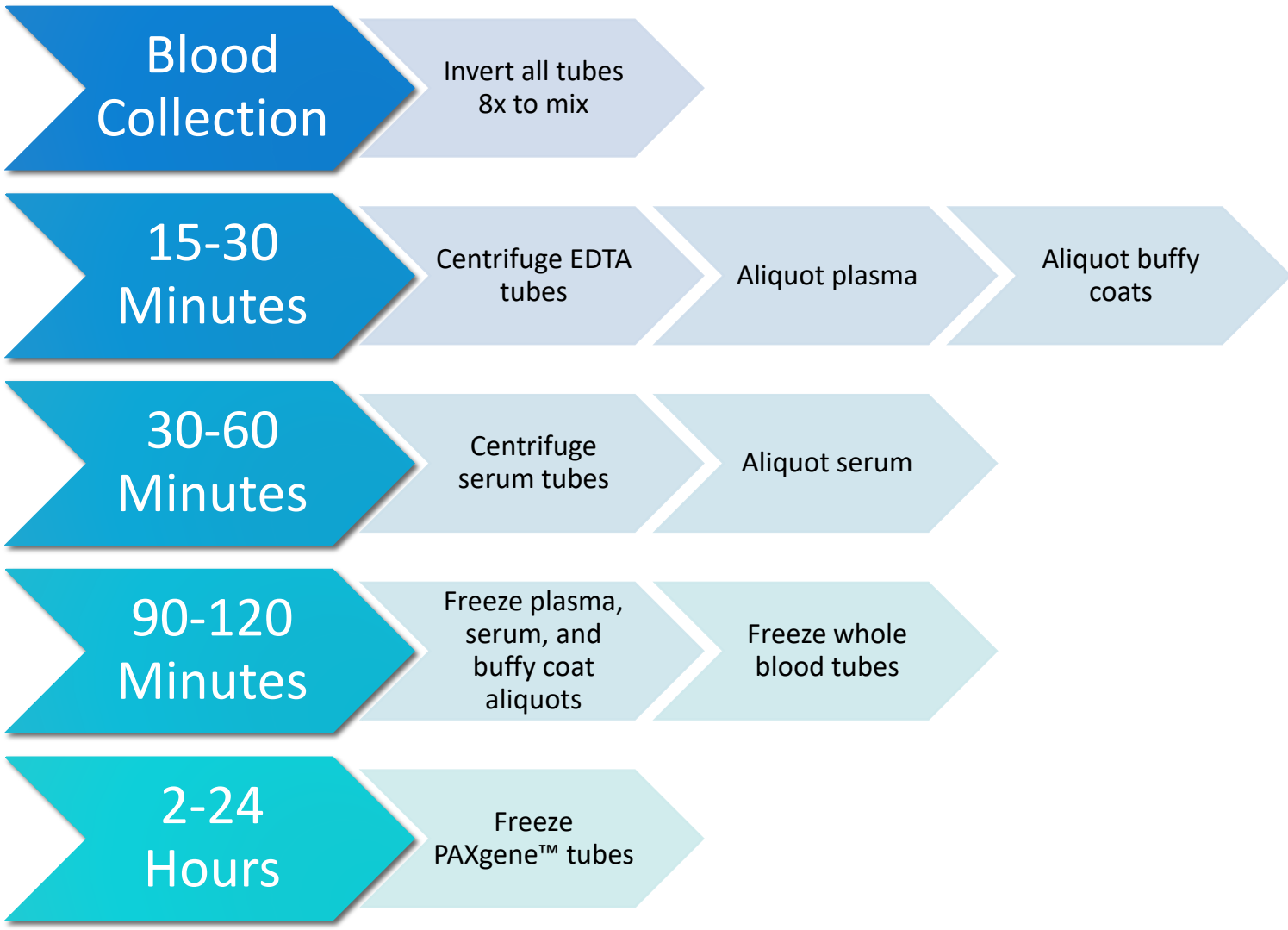
YES



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

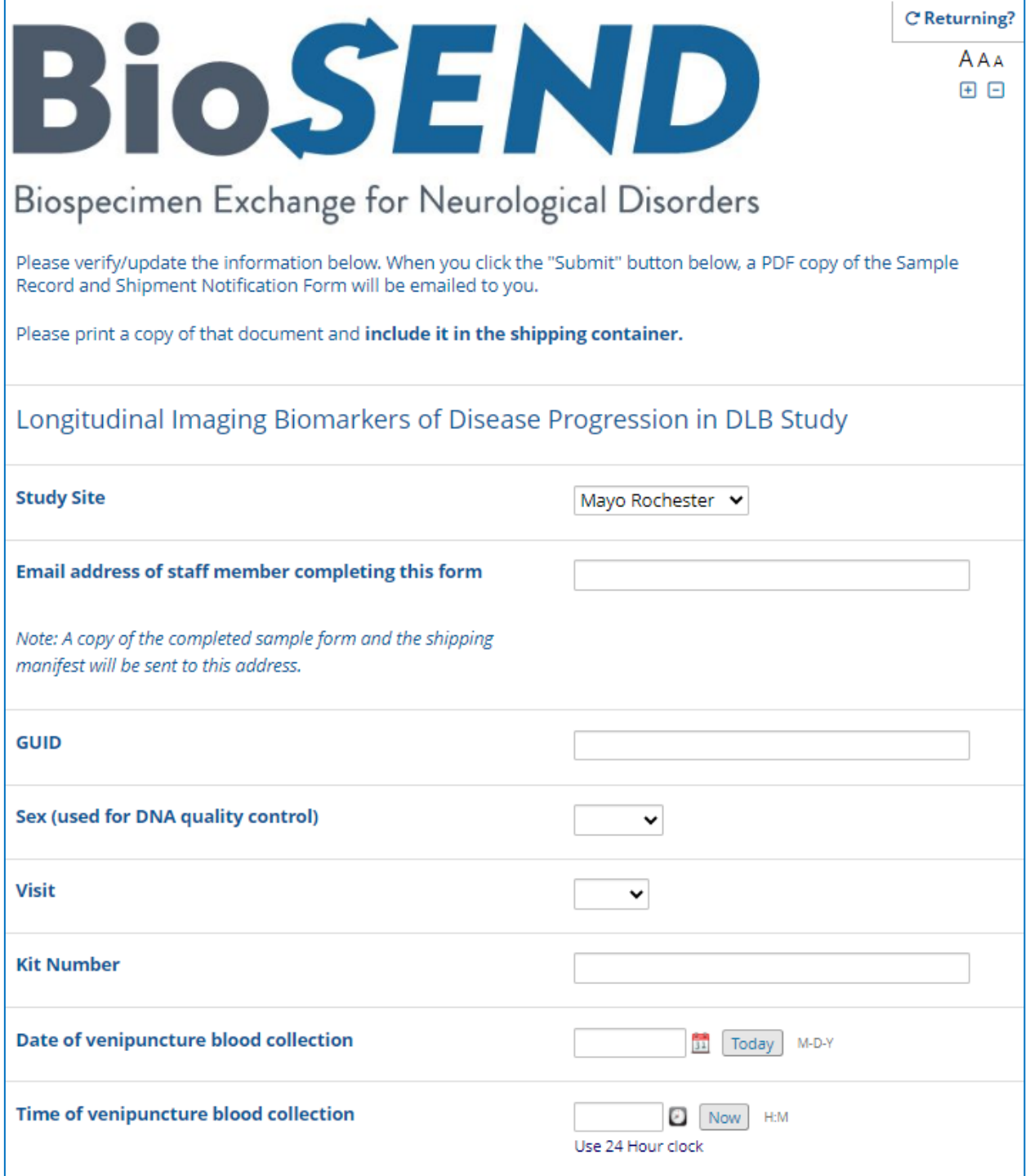
For more information, visit: http://www.bd.com/vacutainer/pdfs/techtalk/TechTalk_Jan2004_VS7167.pdf

Sample Collection and Processing Form

Direct link:

https://redcap.link/DLBSampleForm

First part captures basic subject and visit information



BioSEND

Biospecimen Exchange for Neurological Disorders

Returning?

A A A

Please verify/update the information below. When you click the "Submit" button below, a PDF copy of the Sample Record and Shipment Notification Form will be emailed to you.

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

Longitudinal Imaging Biomarkers of Disease Progression in DLB Study

Study Site Mayo Rochester

Email address of staff member completing this form

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

GUID

Sex (used for DNA quality control)

Visit

Kit Number

Date of venipuncture blood collection Today M-D-Y

Time of venipuncture blood collection Now H:M

Use 24 Hour clock

Sample Collection and Processing Form

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

Second part captures processing information

SERUM

Number of SERUM aliquots shipped:

Each aliquot should be 1 mL

RNA PAXGENE

Number of PAXGene™ tubes shipped:

WHOLE BLOOD EDTA

Number of WHOLE BLOOD tubes shipped:

PLASMA EDTA

Number of PLASMA EDTA aliquots shipped:

Each aliquot should be 1 mL

Number of BUFFY COAT aliquots shipped:

CSF

Was CSF collected at this visit?

Yes

No

reset

NOTES

Please record any issues with collection/processing:

Expand

Sample Collection and Processing Form

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

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

DLB Mayo Sample Record and Shipment Notification Form

Please verify/update the information below. When you click the "Submit" button below, a PDF copy of the Sample Record and Shipment Notification Form will be emailed to you.

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

Longitudinal Imaging Biomarkers of Disease Progression in DLB Study

Study Site
- ◯ Mayo Rochester
- ◯ Mayo Jacksonville

Email address of staff member completing this form

_____

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

GUID

_____

Sex (used for DNA quality control)
- ◯ Male
- ◯ Female
- ◯ Other

Visit
- ◯ BL
- ◯ 12M
- ◯ 24M
- ◯ 36M
- ◯ 48M
- ◯ 60M
- ◯ 72M
- ◯ 84M
- ◯ 96M
- ◯ 108M

Kit Number

_____

Date of venipuncture blood collection

_____

Time of venipuncture blood collection

_____
(Use 24 Hour clock)

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

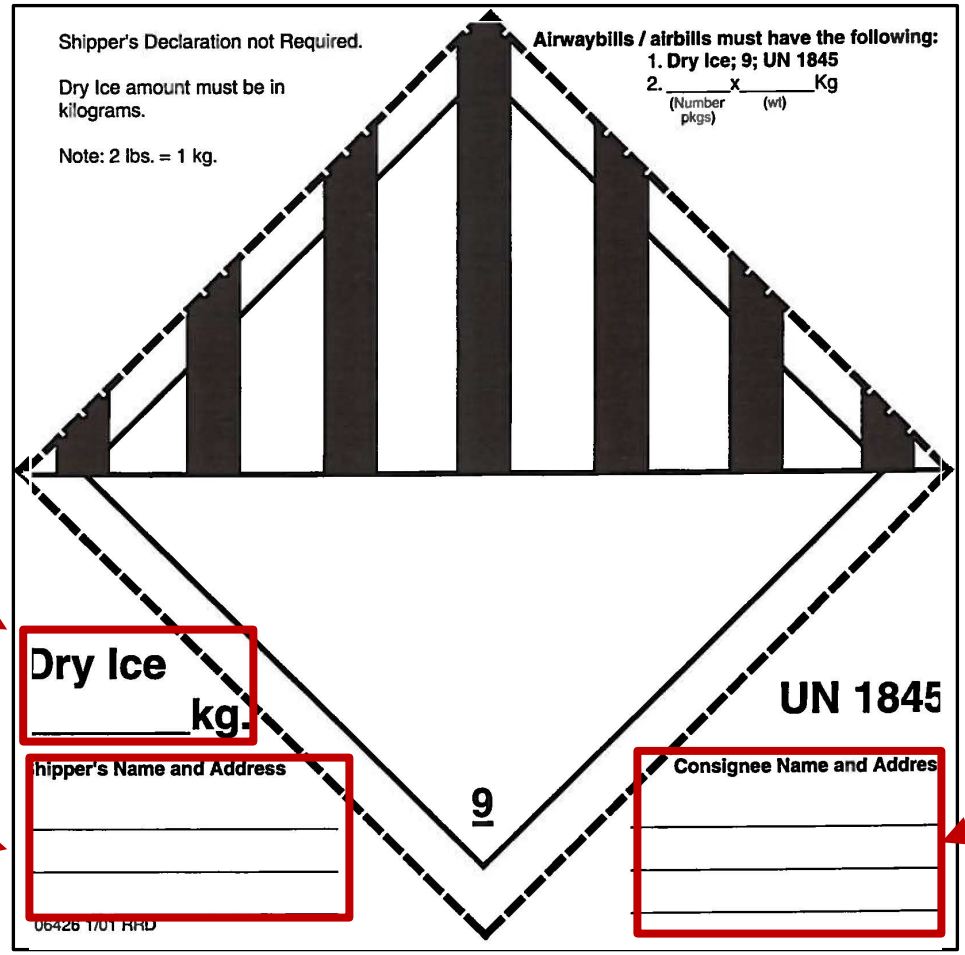
Packing and Shipping Frozen Samples

- 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!

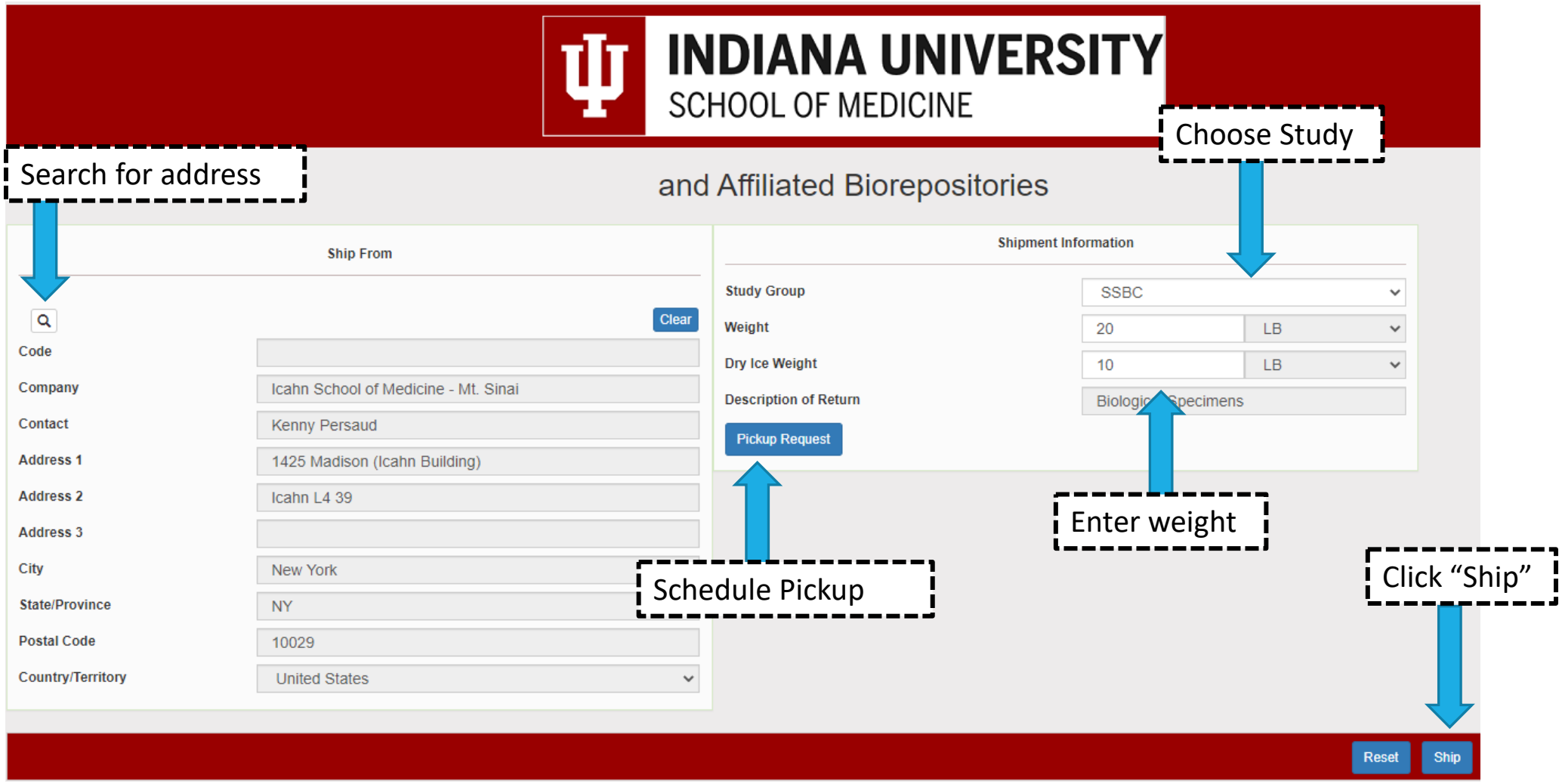


Shipping Samples: Frozen

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



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

Shipping Samples: Closures

Date	Holiday
January 1	New Year's Day
3rd Monday in January	Martin Luther King, Jr Day
4th Monday in May	Memorial Day
June 19	Juneteenth (observed)
July 4	Independence Day (observed)
1st Monday in September	Labor Day
4th Thursday in November	Thanksgiving
4th 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 an 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

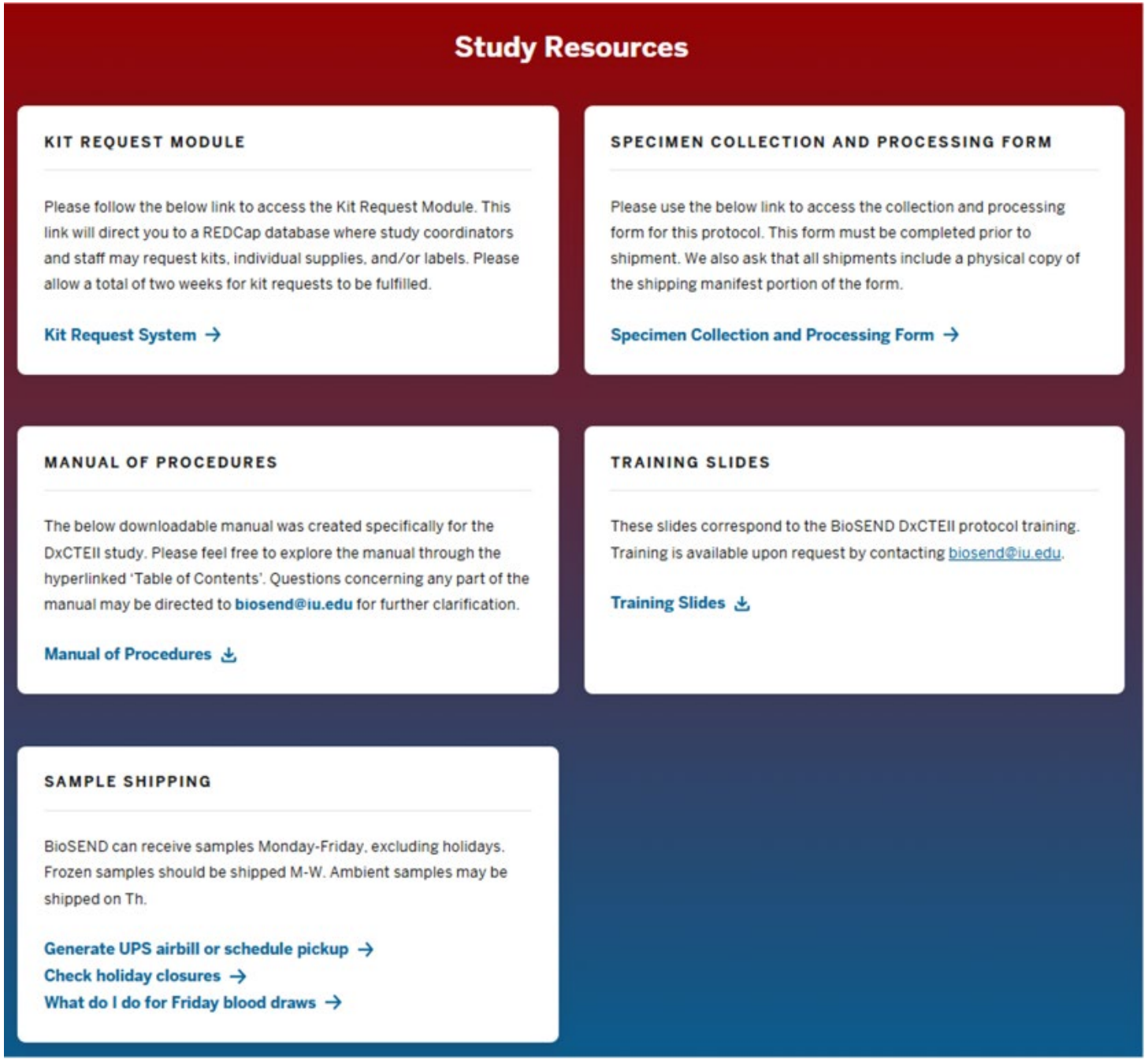
Most common non-conformance issues:

- 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***

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/dlb