BioSEND

Biospecimen Exchange for Neurological Disorders

Concussion Assessment, Research, and Education for Kids **CARE4Kids**

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

Sample type	Visit	Tube Provided	Blood Volume Collected	Sample Volume	Samples shipped to BioSEND	Cryovial Color
Plasma	T1, T3	EDTA tube (purple-top)	12ml	500ul per cryovial	Up to 12 cryovials	Purple Cap
Buffy Coat	T1, T3			750ul	2 cryovials	Clear Cap
Plasma	T1, T3	P100	2ml	200ul per cryovial	Up to 5 cryovials	Lavender Sticker
Plasma	T1, T3	Tasso™ HemoLink w/ EDTA microtainer	500ul	200ul per cryovial	1 cryovial	White Sticker
RNA	T1, T3	PAXgene™ tube	2.5ml	N/A	1 tube	n/a

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
  - Tasso+ Hemolink™ devices will be provided with the Supplemental Kit

- 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

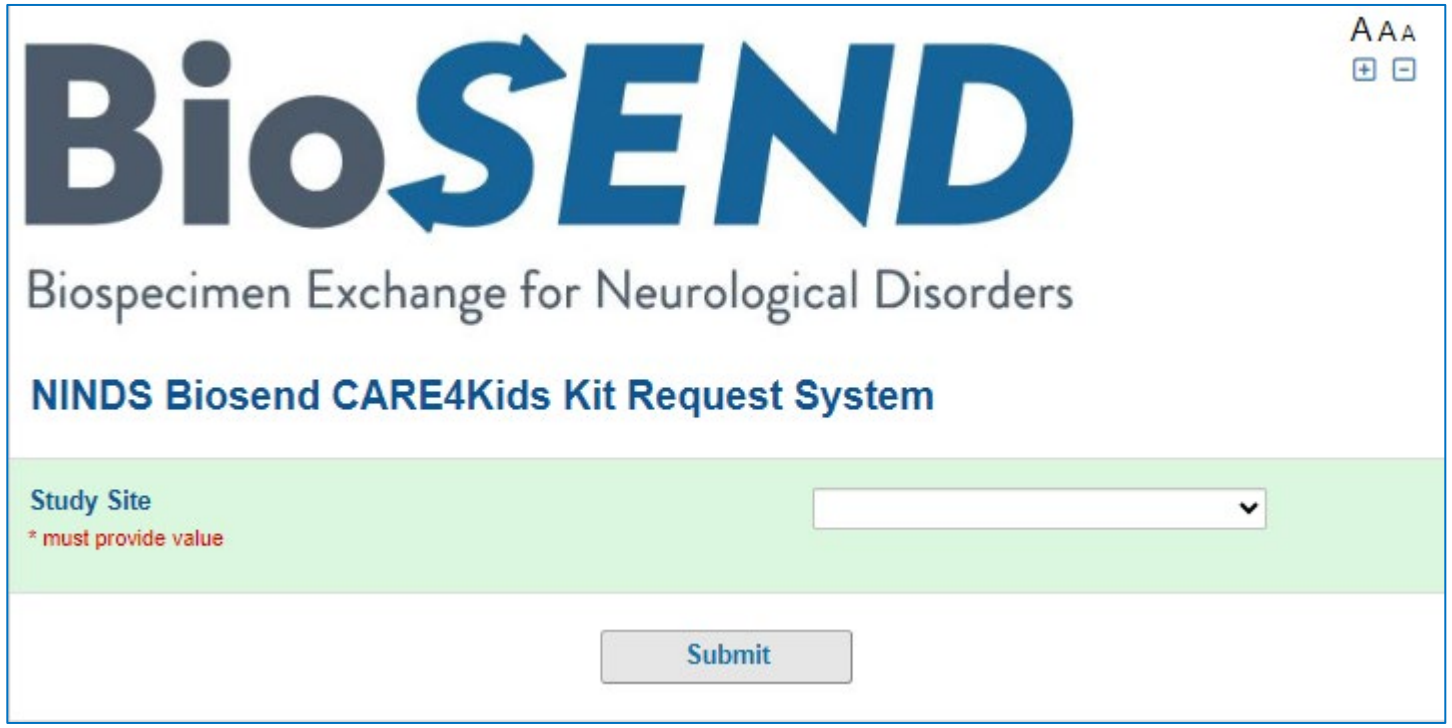
Kit Contents and Ordering – REDCap Survey

http://kits.iu.edu/biosend/care4kids

Order kits online through the Kit Request Module for:

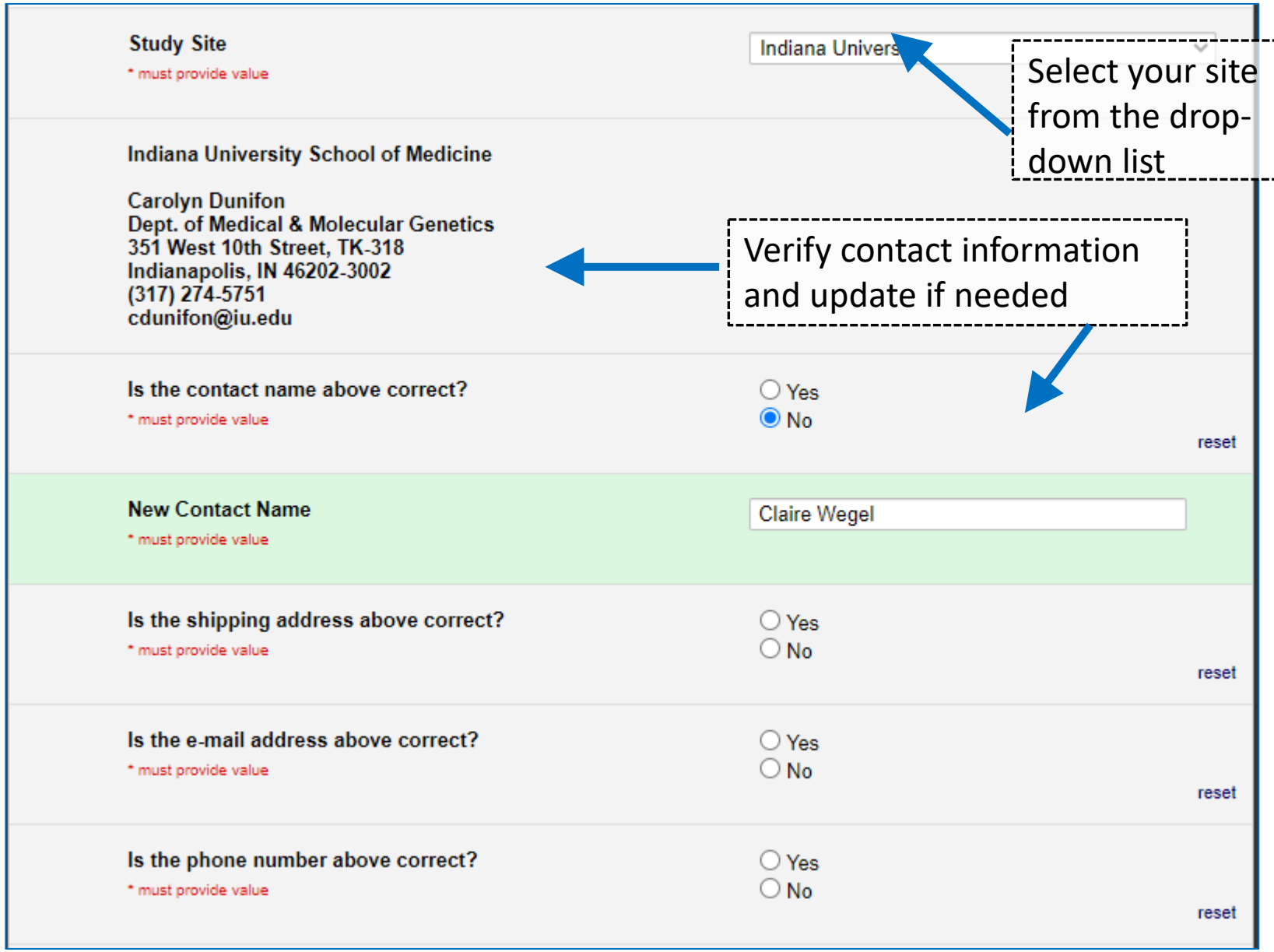
- T1/T3 Collection kits
- Supplemental Kit
- Extra Supplies

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



Kit Contents and Ordering: Confirm Site Info

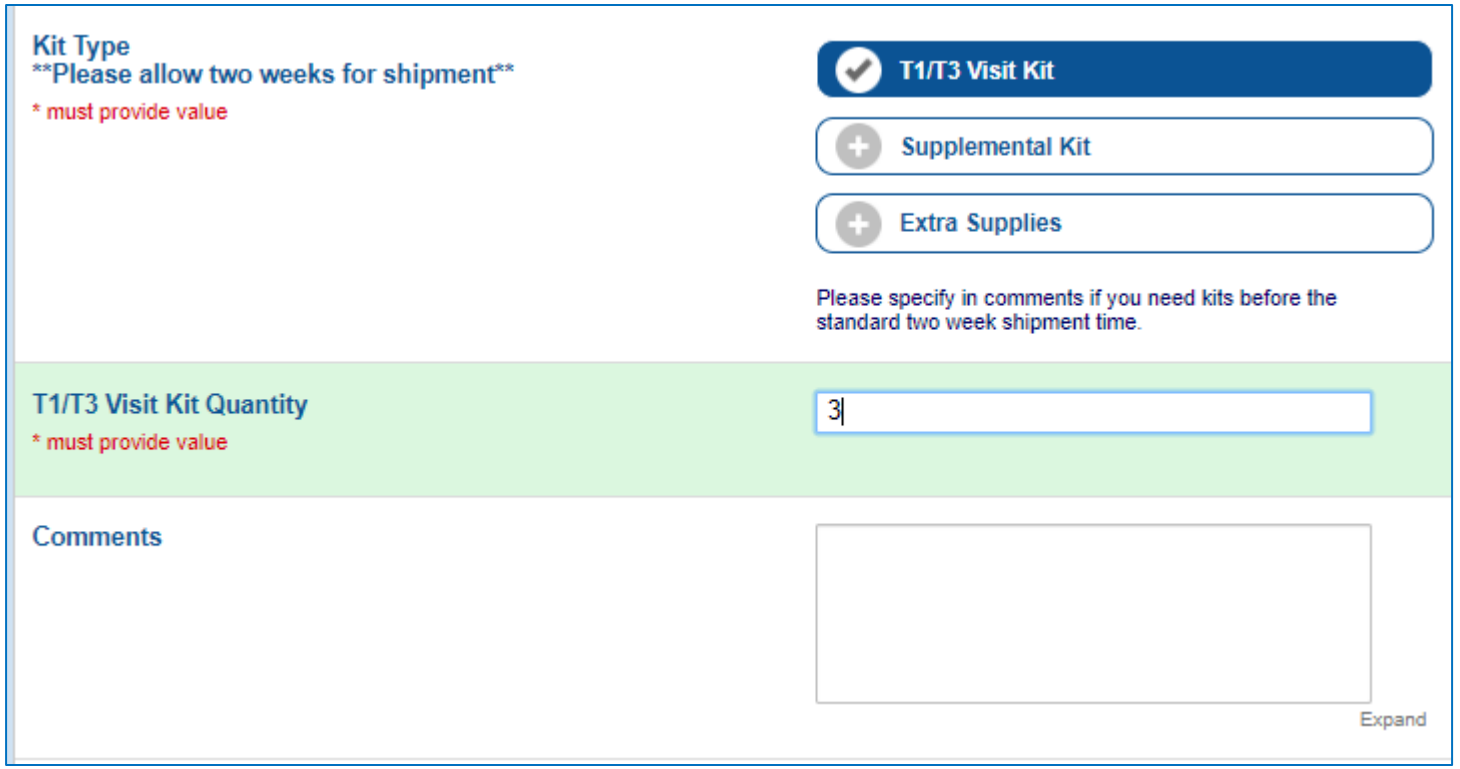
CARE4Kids Kit Request Module



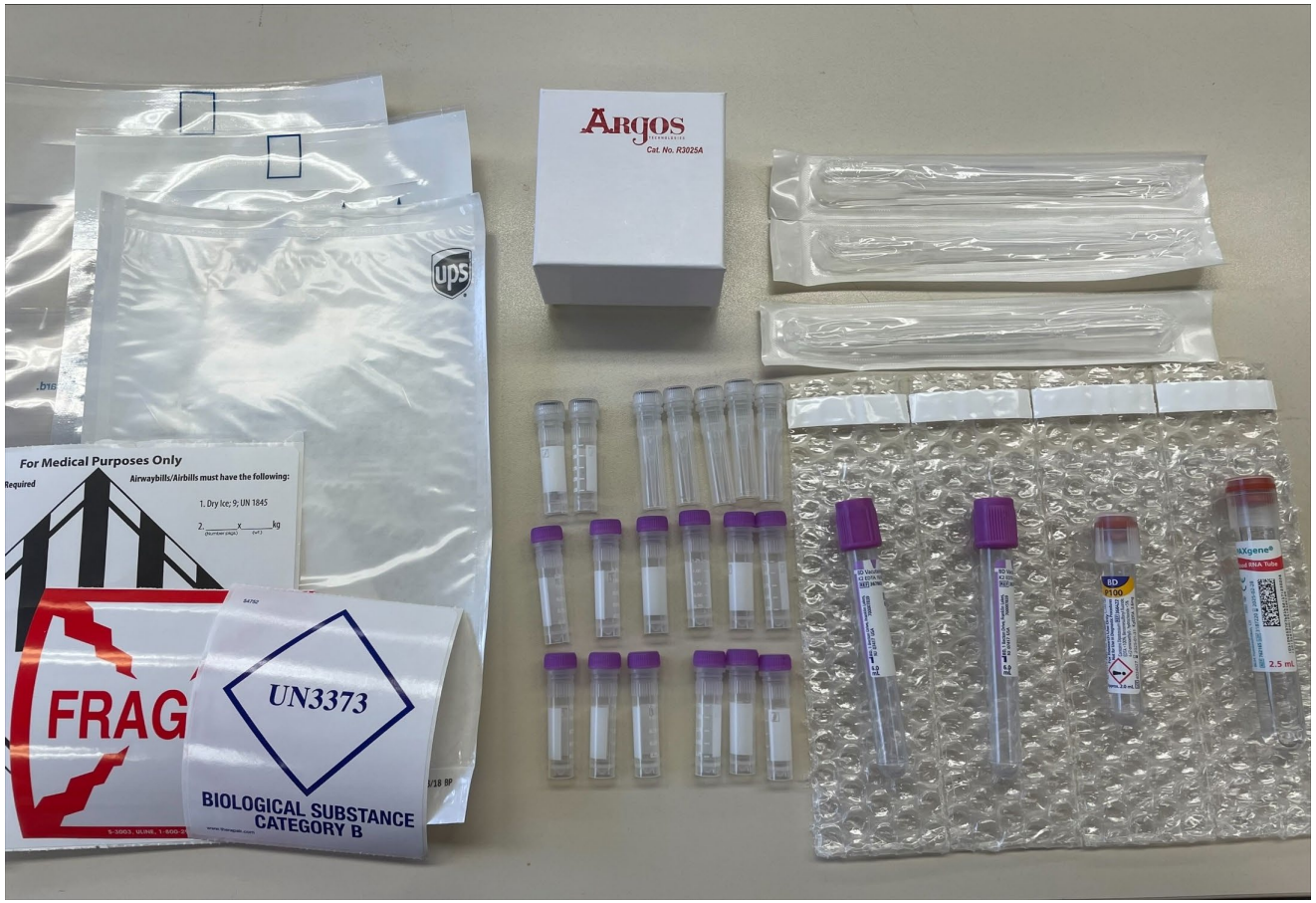
Kit Contents and Ordering: Kit Types

CARE4Kids 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: Blood Kit



Kit Contents and Ordering: Tasso Hemolink™

Collection Volumes

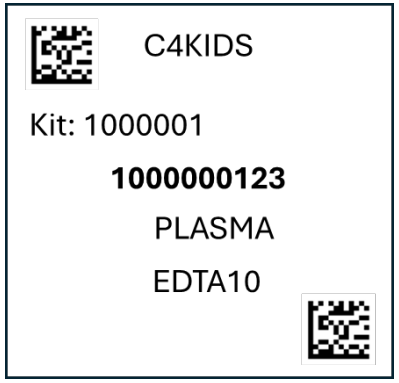
Total blood volumes

Sample Type	Amount
Whole Blood for RNA	2.5 ml
Whole Blood for Plasma and Buffy Coat	12 ml
Whole Blood for Plasma (P100 protease inhibitor tube)	2 ml
Whole Blood for Plasma (Tasso Hemolink™ device with EDTA microtainer)	0.5 ml

Sample Labelling: Example Labels

Labels are provided by Indiana University

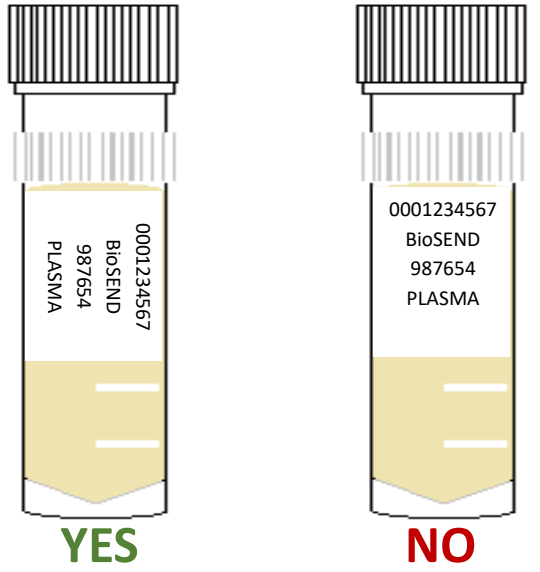
- Please check that all samples are properly labelled to ensure correct identification by IU
- If do not have enough labels to complete a visit, please contact IU *immediately*
- Labelling the tubes during processing prevents sample mix-ups



Sample Labelling: Label Placement

Please...

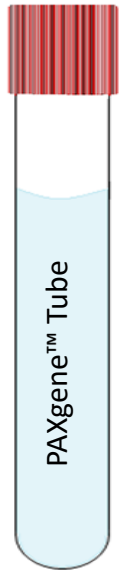
- Label all collection and aliquot tubes before cooling, collecting, processing, or freezing samples
- Label only 1 subject's tubes at a time to avoid mix-ups
- Wrap the label around the tube horizontally - label position is important for all tube types
- Make sure the label is completely adhered by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



Sample Collection and Processing

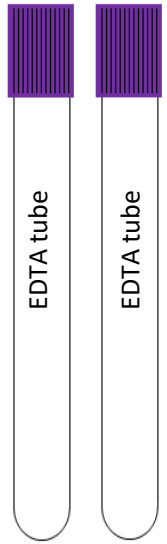
Blood Tube Draw Order

2.5ml PAXGene™ tube



1

Two 6ml EDTA tubes



2

2ml P100 EDTA + protease inhibitor cocktail tube

3

**Plasma from Tasso™ HemoLink Device collected through non-venipuncture method*

Sample Collection and Processing: Whole blood RNA

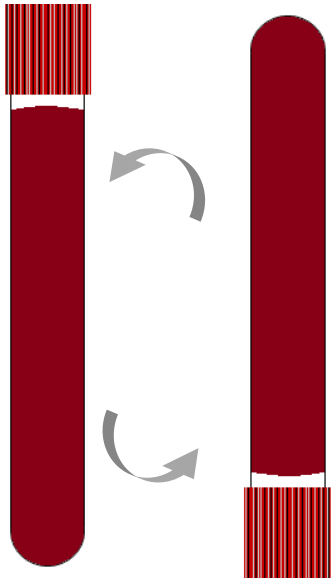
Steps 1-2

- 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

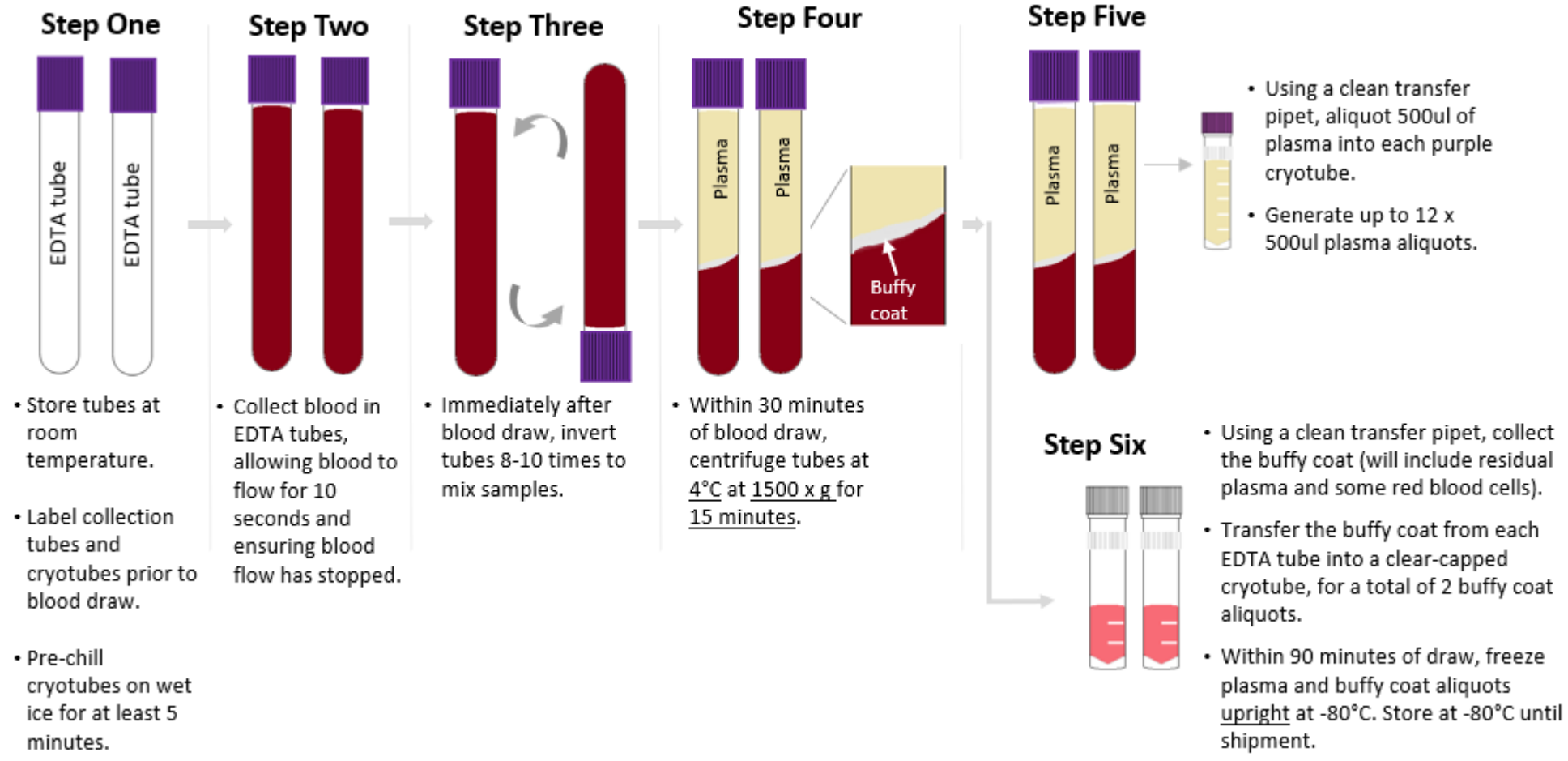


- Immediately after blood draw, invert tubes 8-10 times to mix samples.

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



Sample Collection and Processing: Plasma (P100)

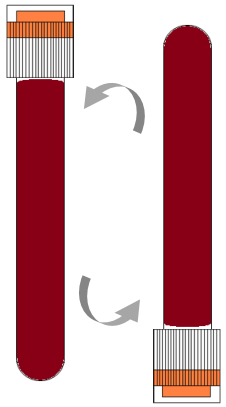
Step One

- Pre-chill cryotubes on wet ice for at least 5 minutes.

Step Two

- Collect blood in P100 tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three

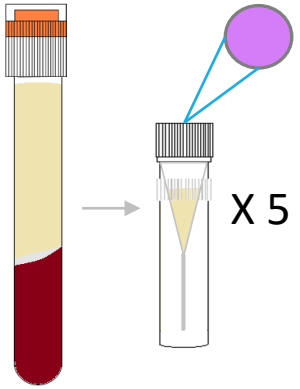


- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Four

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

Step Five



- Using a clean transfer pipet, aliquot 200ul of plasma into each 0.5ml cryotube (lavender dot on top).
- Generate up to 5 x 200ul plasma aliquots.
- Within 90 minutes of draw, freeze plasma and buffy coat aliquots <u>upright</u> at -80°C. Store at -80°C until shipment.

Tasso+ HemoLink™ Device

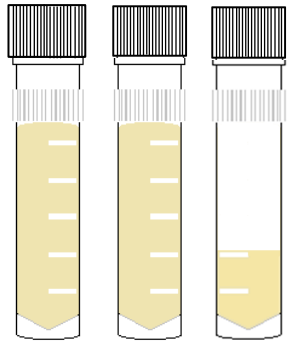
Collection instructions – please follow manufacturer's guide

- Within 30 minutes of blood collection, centrifuge tube for 15 minutes at 1500 RCF (x g) at 4°C.
- Remove the plasma by tilting the tube and placing the pipette tip along the lower side of the wall.
- Using a disposable tipped micropipette, transfer plasma into the 0.5ml (white dot) cryotube.
- Aliquot 200 ul into cryotube.

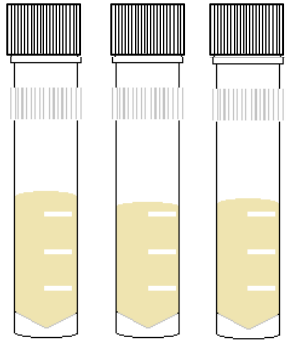
Sample Collection and Processing: Aliquots

Filling biomarker plasma aliquots:

- Fill as many cryovials as possible to 0.5 ml (Plasma) or 0.2ml (P100 or Tasso)
- Over-filled vials may burst in freezer!
- Ship ALL material to IU, even if final vial is less than standard volume



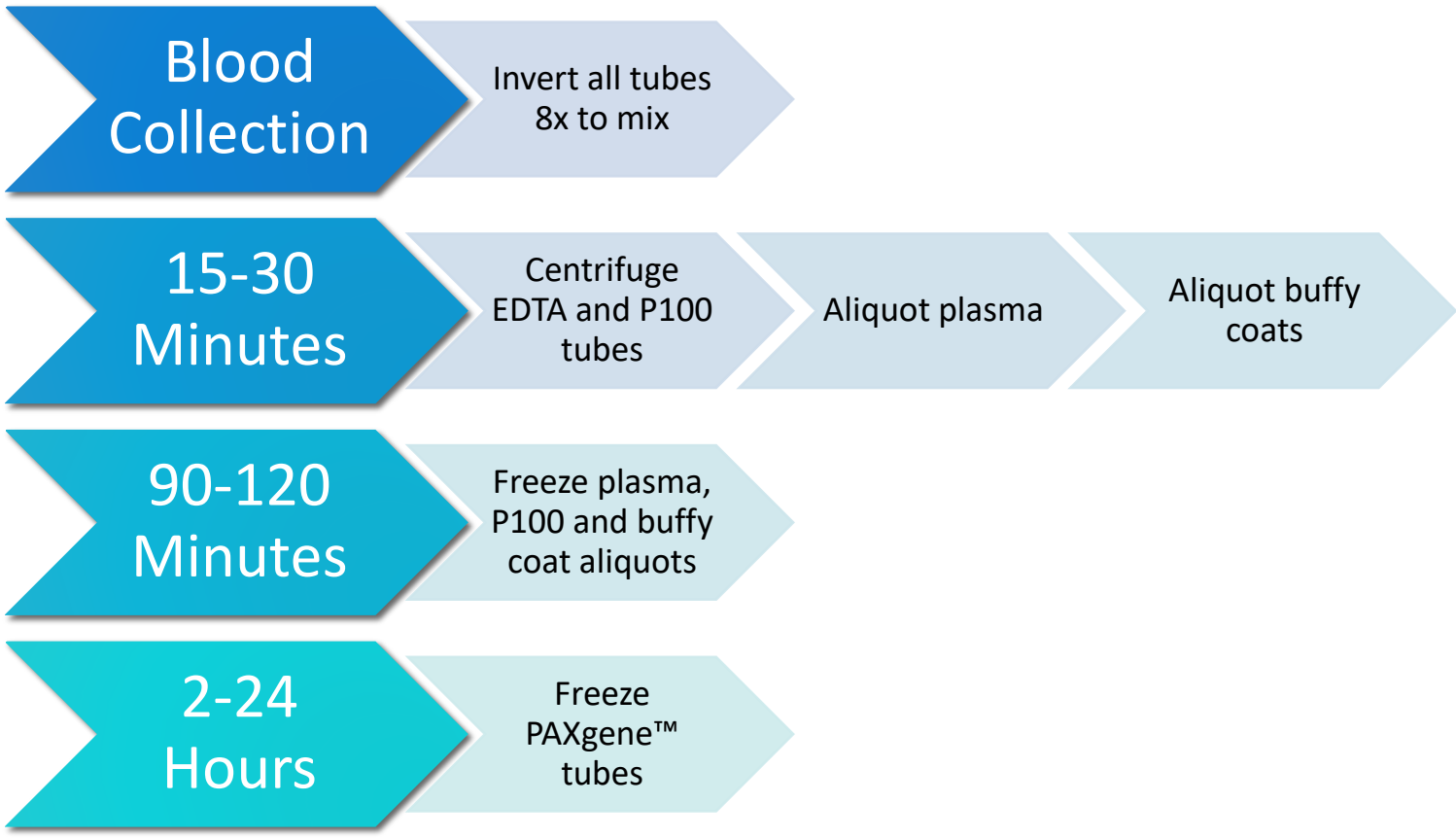
YES



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, 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 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

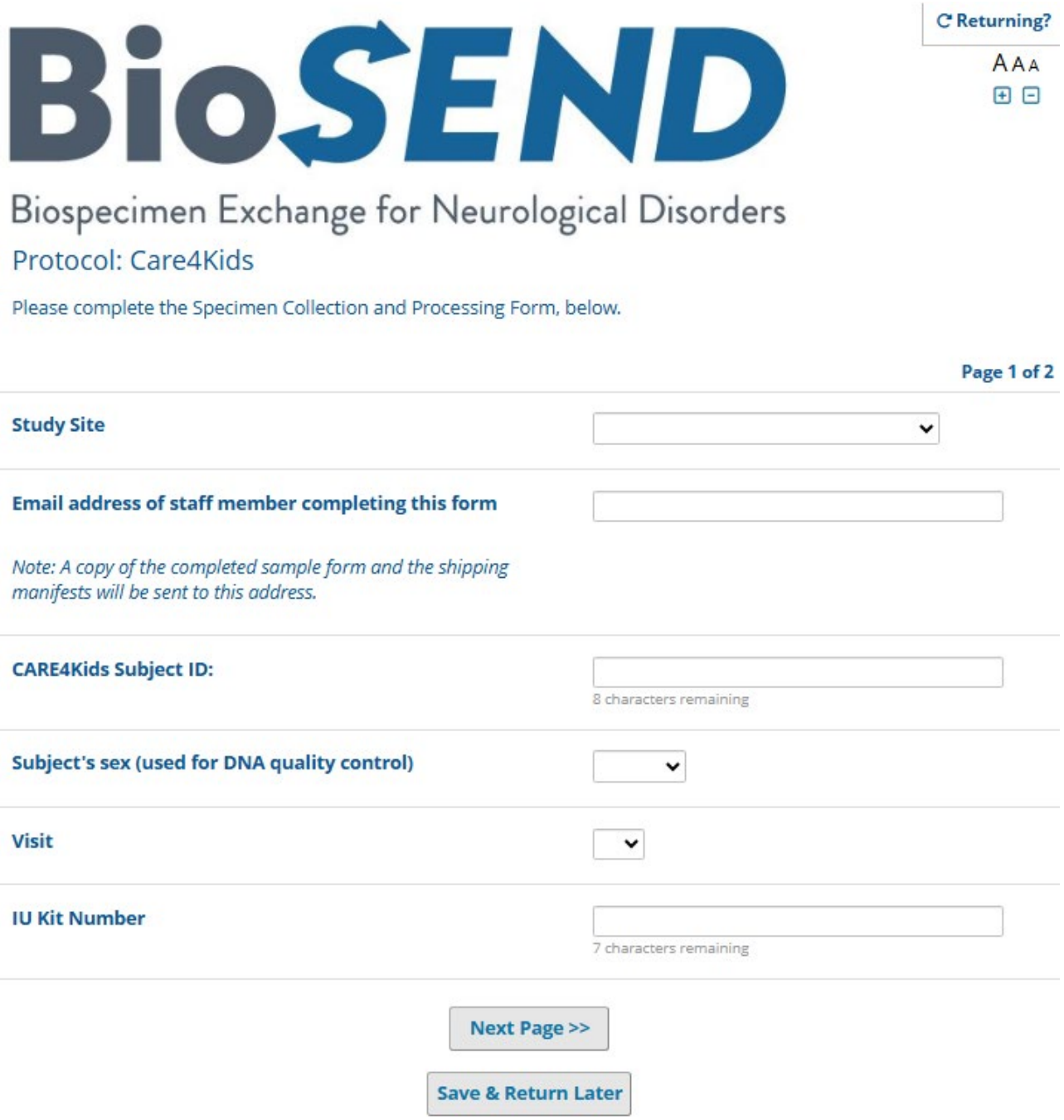
Sample Collection and Processing: Online Form

Specimen Collection and Processing Form

Direct Link:

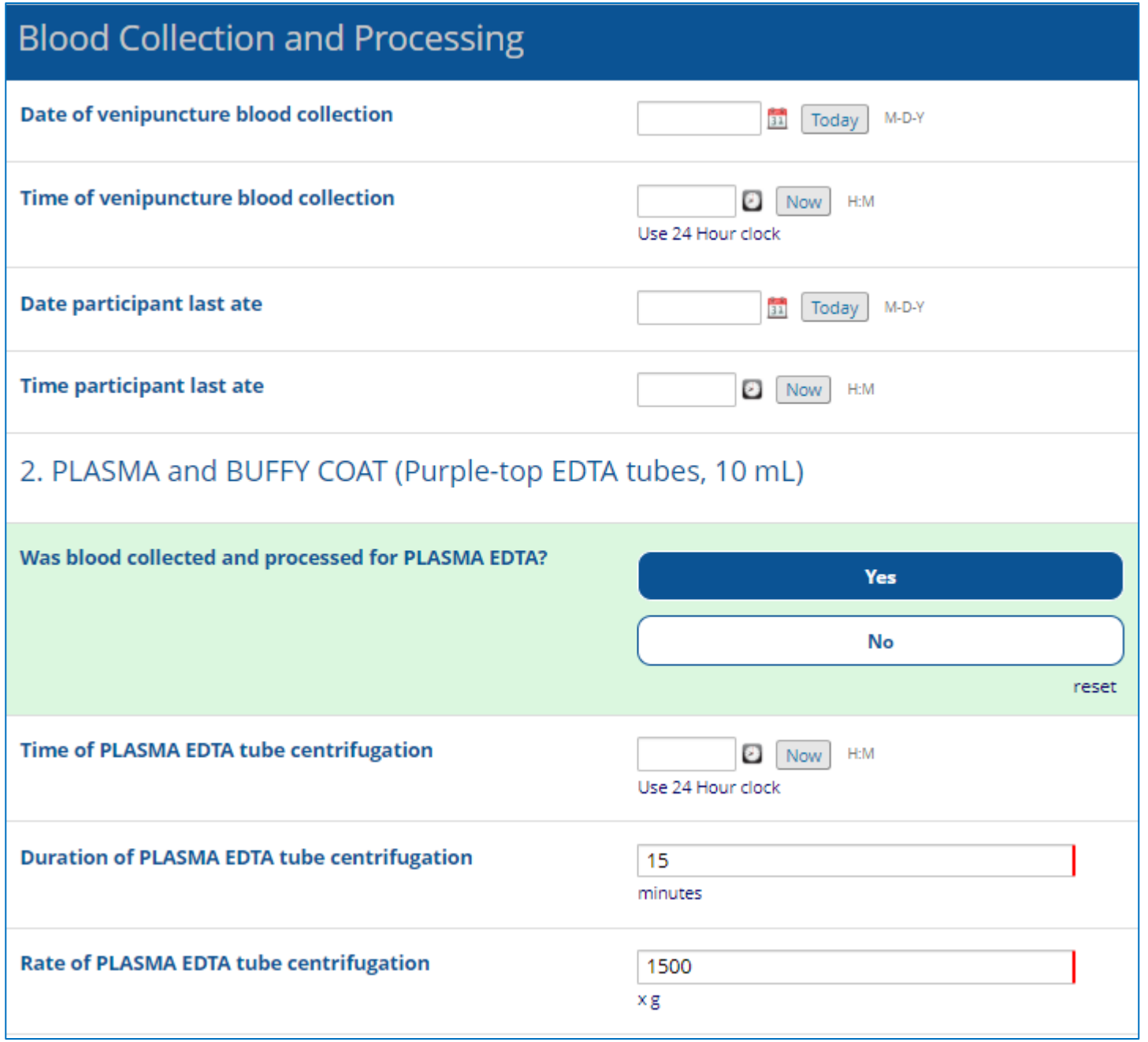
https://redcap.link/Care4KIDSSampleForm

First page captures basic subject and visit information



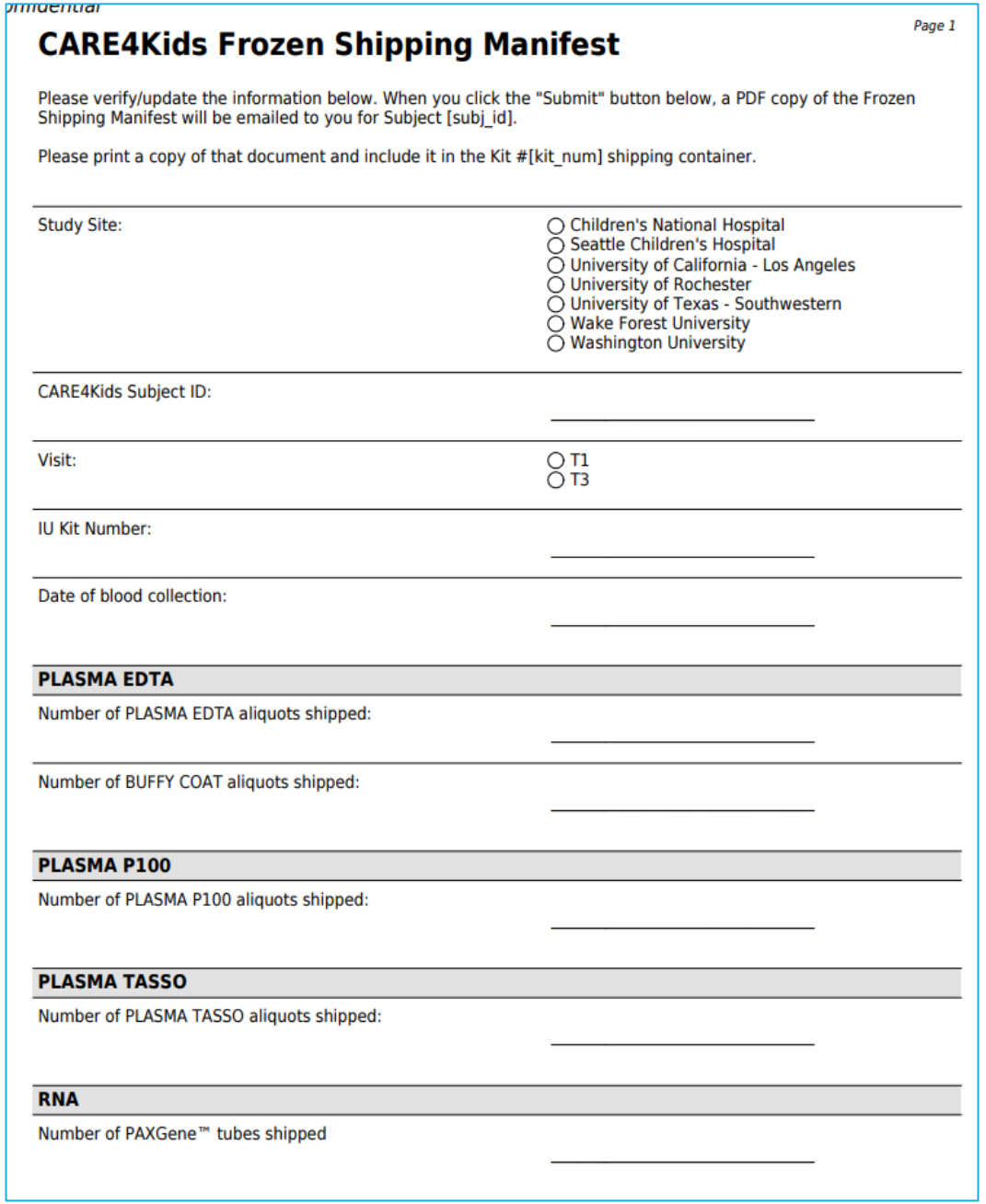
Sample Collection and Processing Form

Second page captures processing information



Sample Collection and Processing Form

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



pnfidential

Page 1

CARE4Kids 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 Kit #[kit_num] shipping container.

Study Site:

- ◯ Children's National Hospital
- ◯ Seattle Children's Hospital
- ◯ University of California - Los Angeles
- ◯ University of Rochester
- ◯ University of Texas - Southwestern
- ◯ Wake Forest University
- ◯ Washington University

CARE4Kids Subject ID: _____

Visit:

- ◯ T1
- ◯ T3

IU Kit Number: _____

Date of blood collection: _____

PLASMA EDTA

Number of PLASMA EDTA aliquots shipped: _____

Number of BUFFY COAT aliquots shipped: _____

PLASMA P100

Number of PLASMA P100 aliquots shipped: _____

PLASMA TASSO

Number of PLASMA TASSO aliquots shipped: _____

RNA

Number of PAXGene™ 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 Frozen Samples: Tips

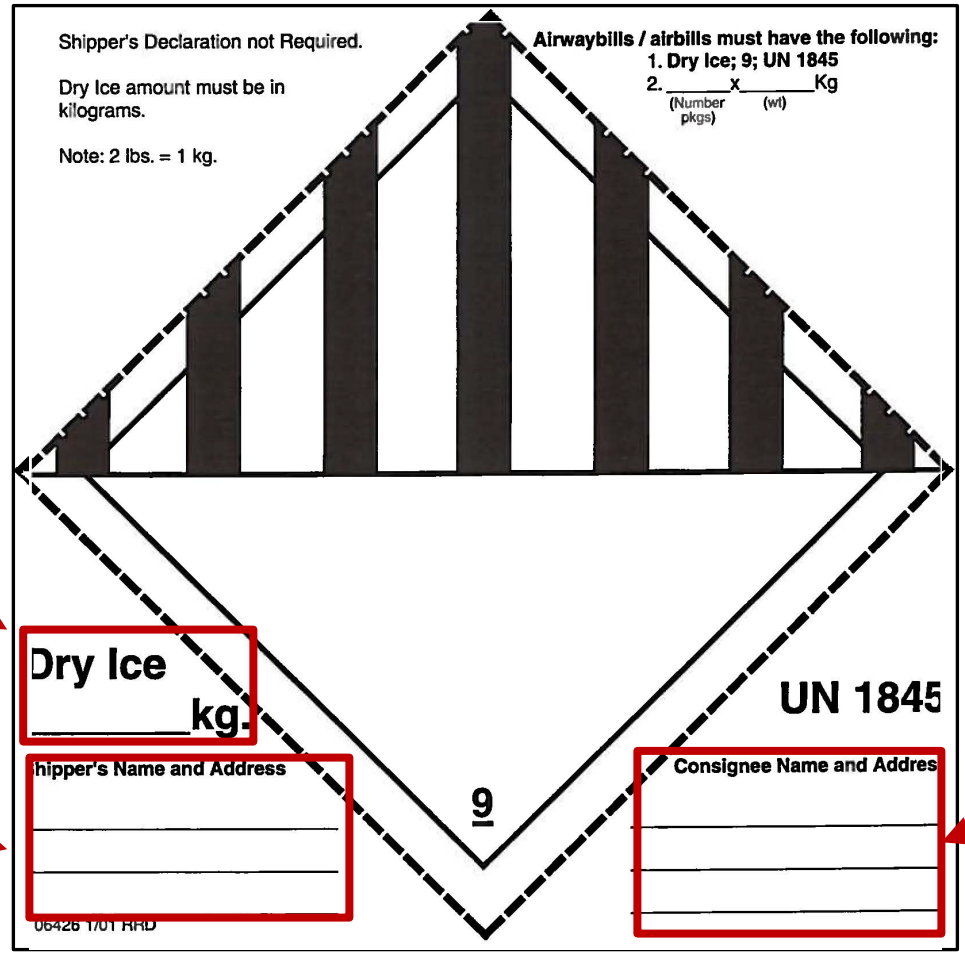
Packing and Shipping Frozen Samples

- 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

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!

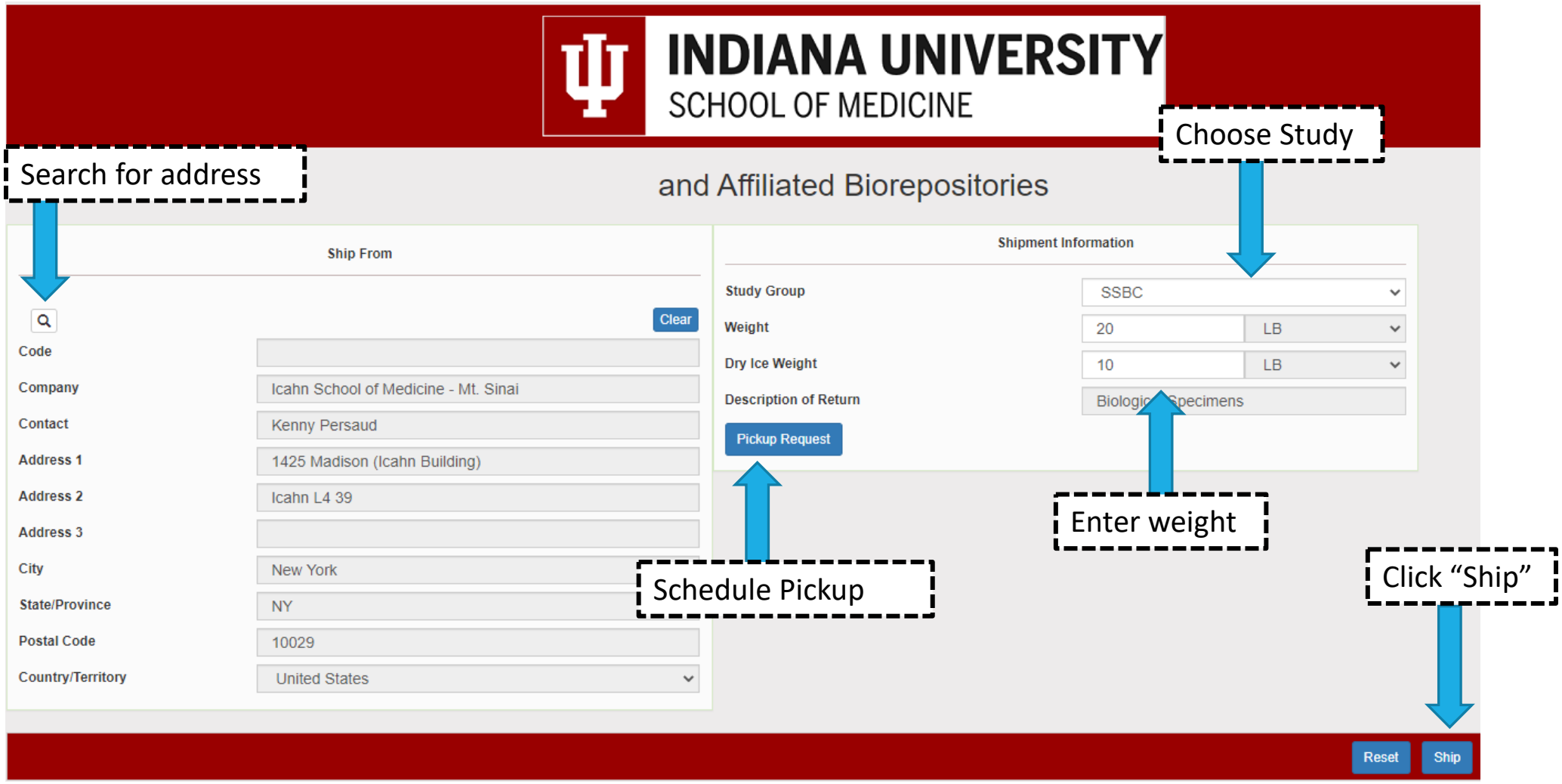


Shipping Samples: Frozen

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 plasma within 2 hours of collection allows for breakdown of certain proteins and small molecules
- Over/under centrifuging changes specimen 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

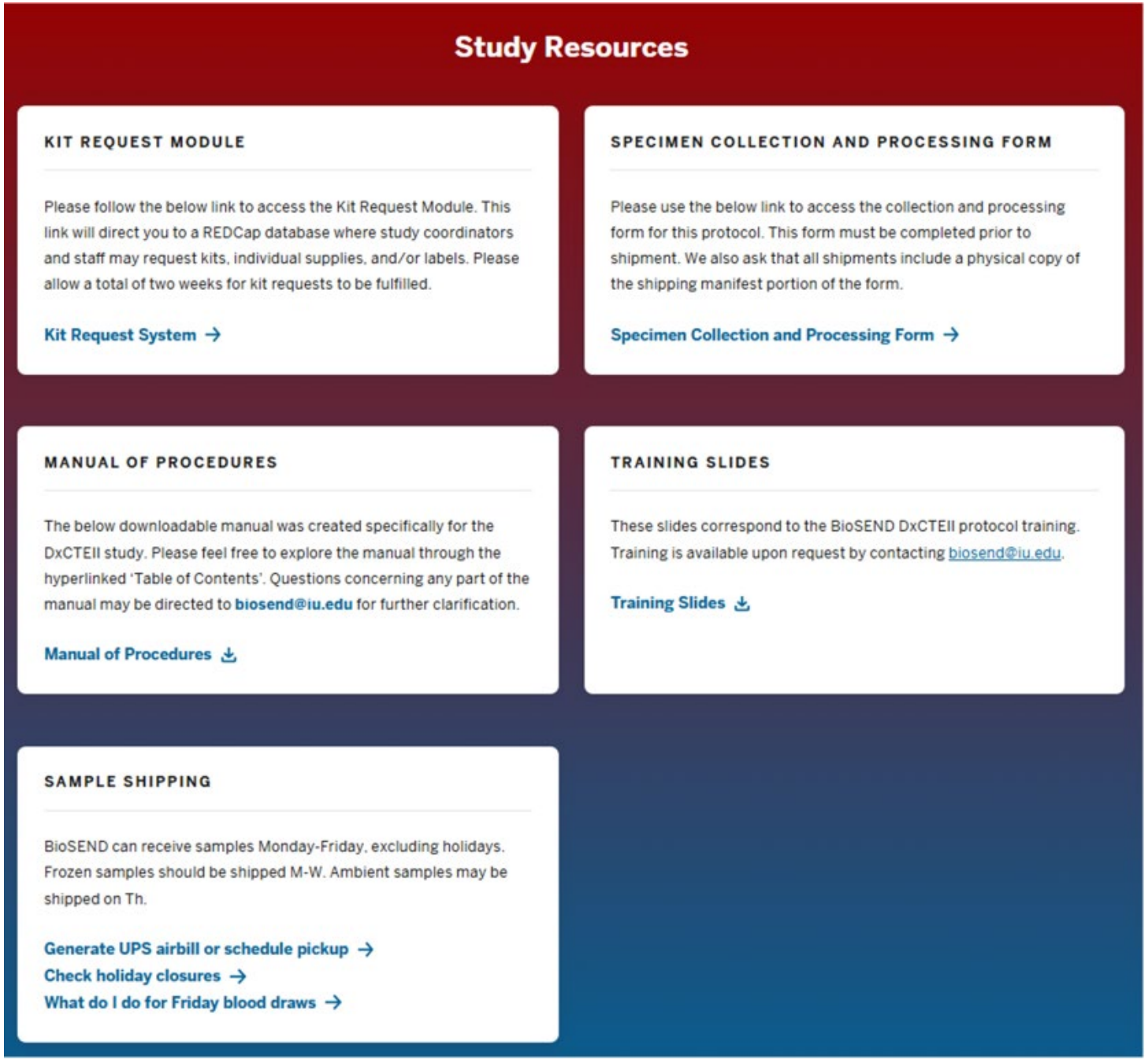
Most common non-conformance issues:

- 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/care4kids