BioSEND

Biospecimen Exchange for Neurological Disorders

Study in Parkinson Disease of Exercise Phase 3 Clinical Trial: SPARX3

BIOSPECIMEN COLLECTION & PROCESSING

Overview

1. Specimen uniformity and quality
2. Site Equipment
3. Procedures
    - Kit Contents and Ordering
    - Sample Labeling
    - 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

Visit & Sample Type	BL	6M	12M	18M	24M
Serum aliquots, 1.5ml	6	6	6	6	6
Plasma aliquots, 1.5 ml	6	6	6	6	6
Buffy Coat	2	2	2	2	2
Whole Blood, 3ml	2	2	2	2	2

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

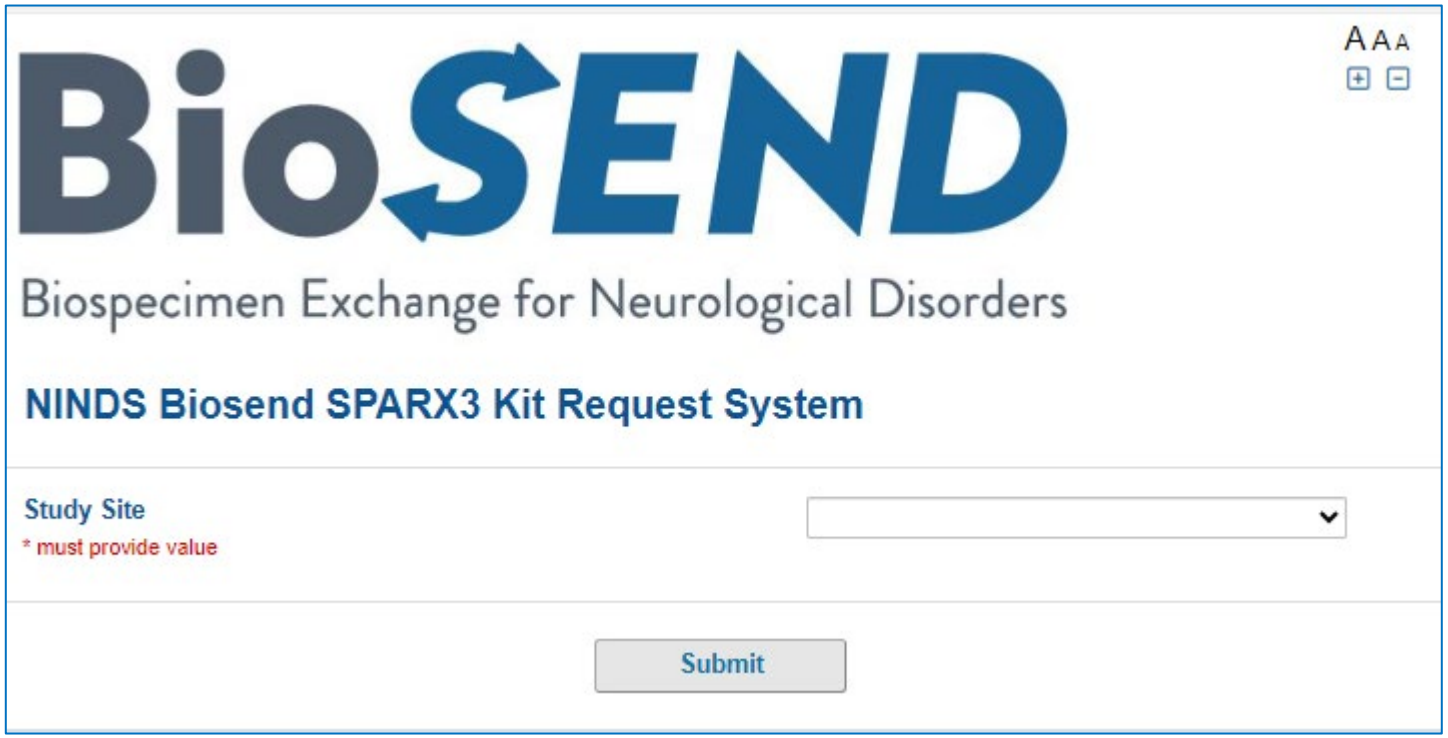
Kit Contents and Ordering – REDCap Survey

http://kits.iu.edu/biosend/sparx3/

Order kits online through the Kit Request Module for:

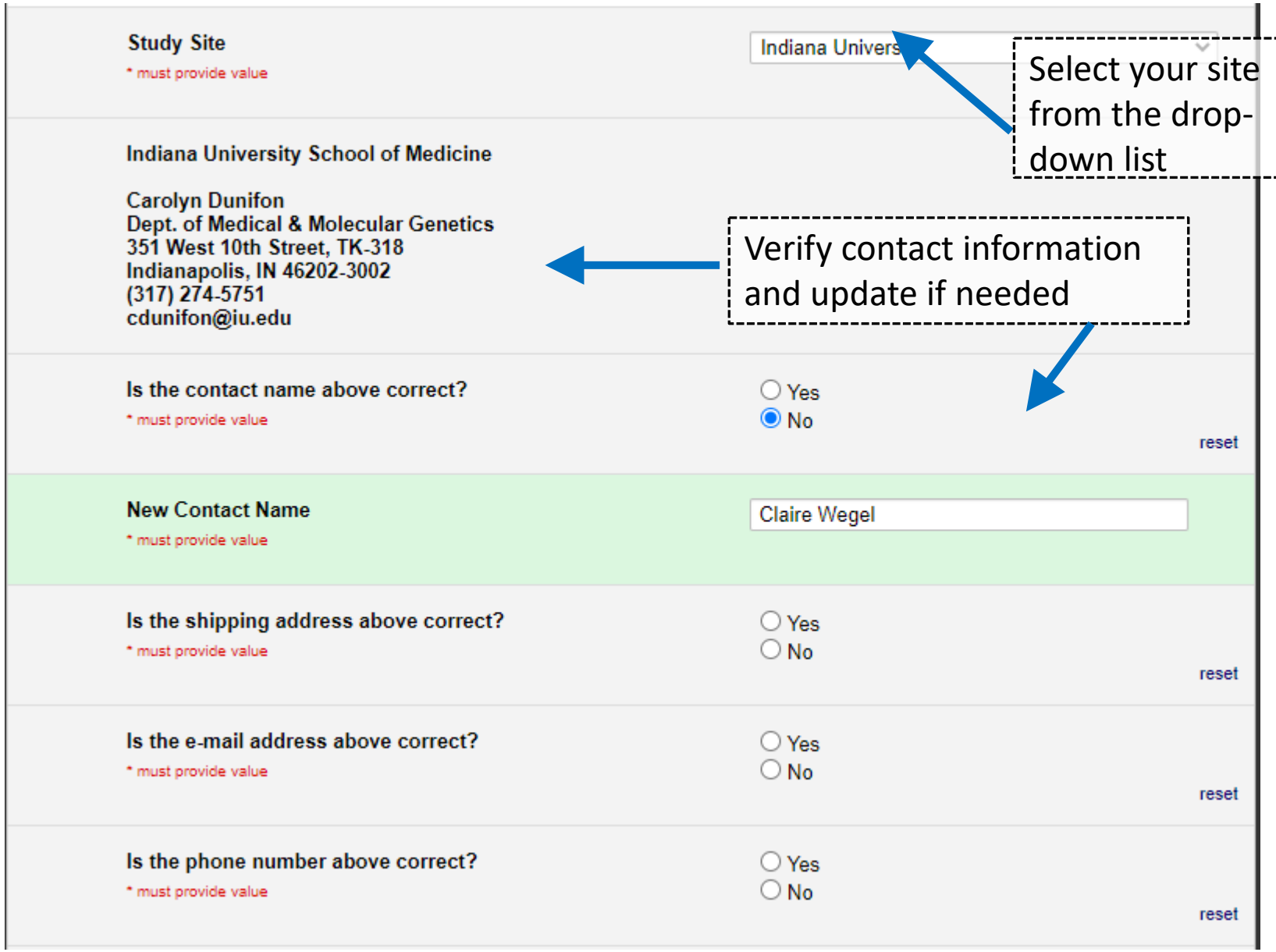
- Blood Kits
- Supplemental Kit
- Extra Supplies

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



Kit Contents and Ordering: Confirm Site Info

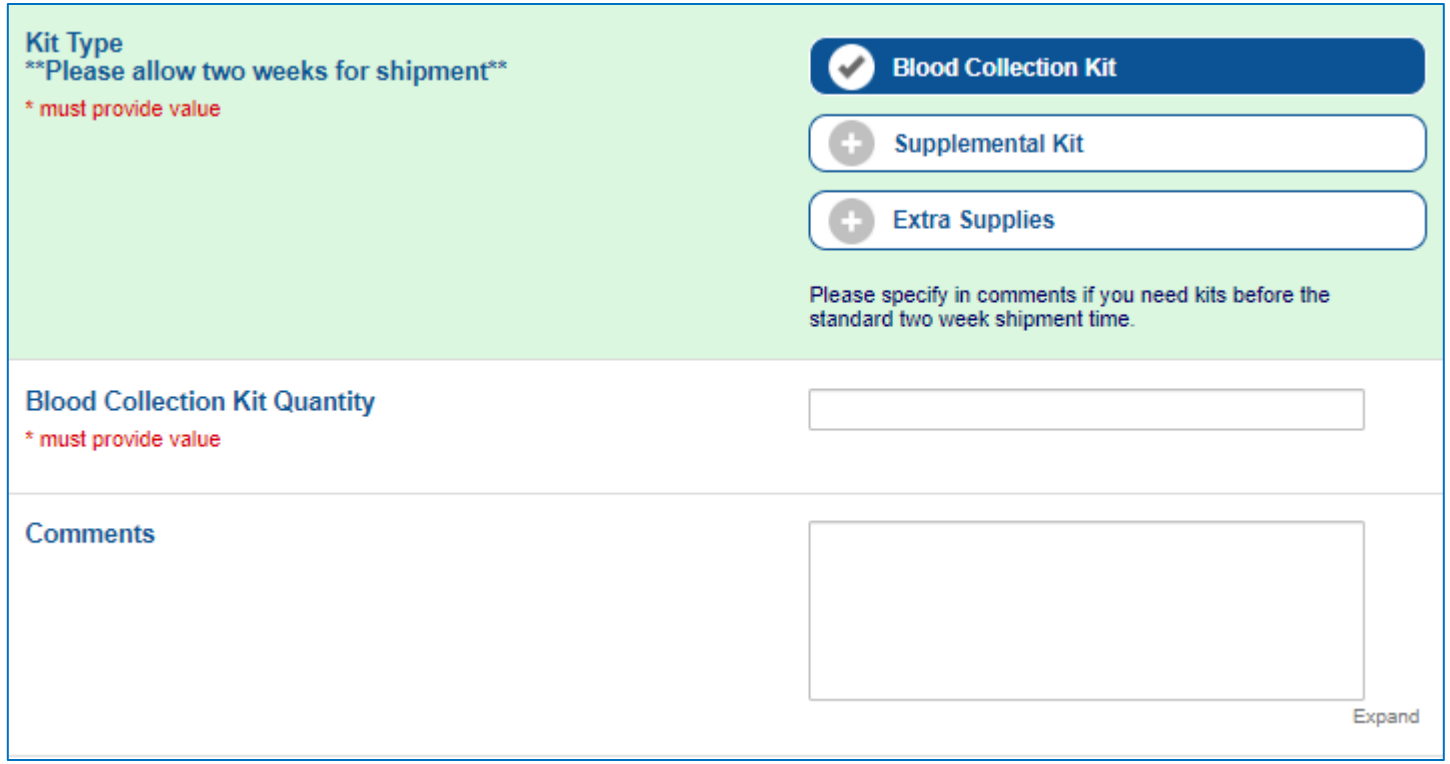
SPARX3 Kit Request Module



Kit Contents and Ordering: Kit Types

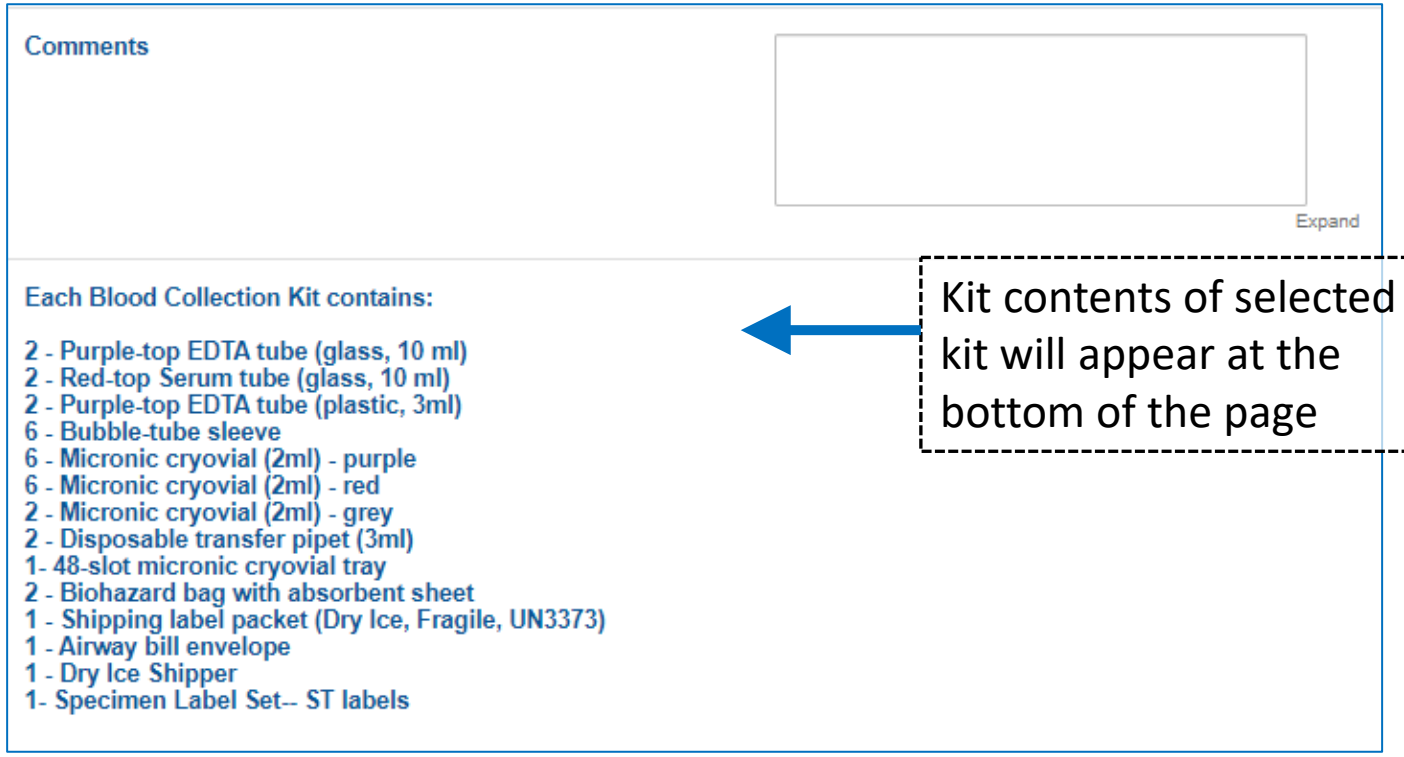
SPARX3 Kit Request Module

- 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

SPARX3 Kit Request Module



Kit Contents and Ordering: Blood Kit

Kit and Supply Ordering

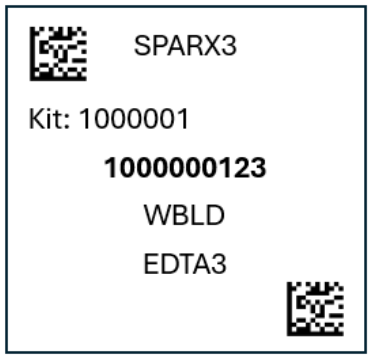
- Click “Submit” to send order to BioSEND; staff will confirm receipt of your order
- Please allow two week turn-around time for kit shipments
- If urgent request needed, please note date needed by in comments and email BioSEND. We cannot guarantee urgent orders, but we will do our best to accommodate.
- BioSEND will send confirmation of shipment and tracking number when supplies ship

Sample Labelling

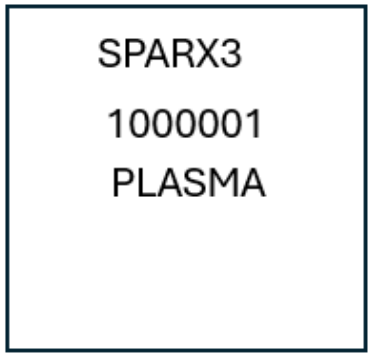
Labels are provided by Indiana University for BioSEND samples only

- Please check that all samples are properly labelled with correct specimen type

Kit Labels



Specimen (Collection tube) Labels



Cryotube Labels

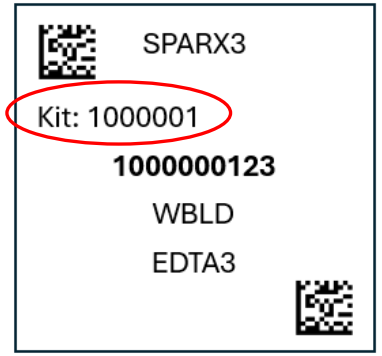
Sample Labelling

Kit Number

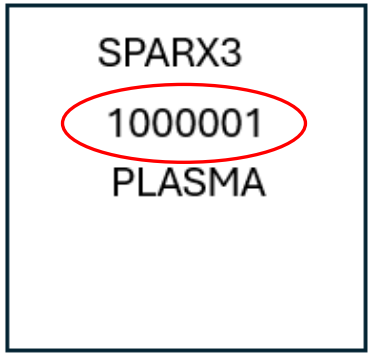
- Any kit number can be used for any subject or visit—which is why it is very important to document which kit number was used for which subject and visit

Each Blood Collection Kit will have a unique "Kit Number" used to track participant samples and provide quality assurance

Kit Labels



Specimen (Collection tube) Labels



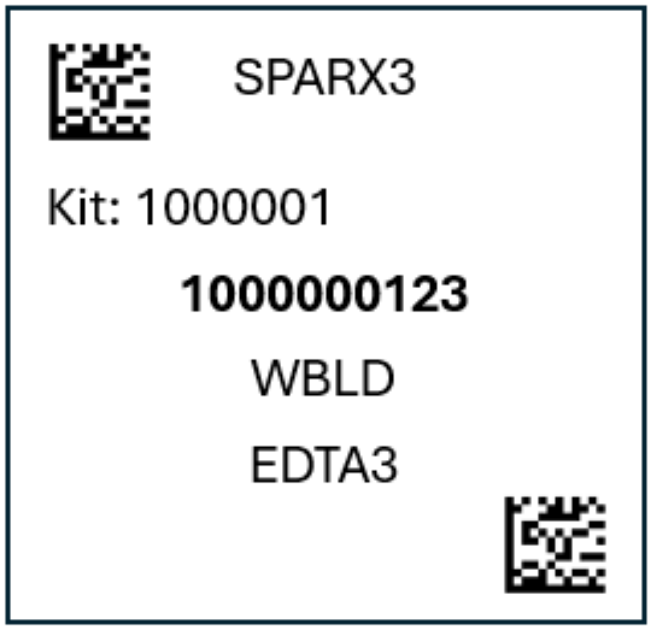
Cryotube Labels

Sample Labelling – Kit Labels

Kit Number labels are placed:

- On the biohazard bag of the cryovial transport box and biohazard bag with EDTA.
- On the lid of frozen shippers

Sample Labelling – Collection Tube Labels



Study

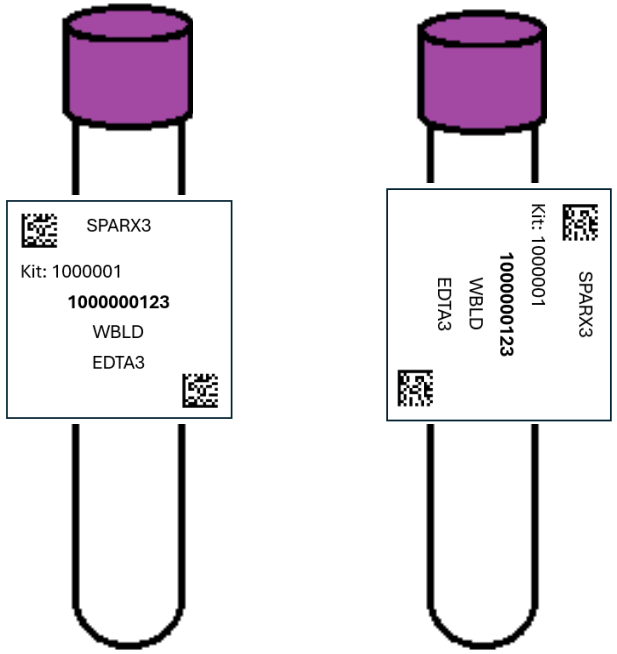
Kit Number

Unique Barcode

Sample Type

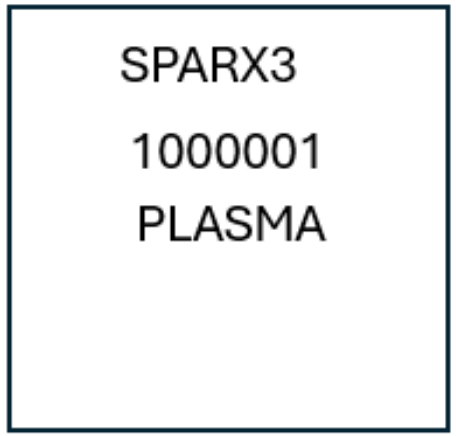
Collection Tube

Sample Labelling – Collection Tube Labels



Incorrect **Correct**

Sample Labelling – Cryotube Labels



Biorepository/Study

Kit Number

Sample Type

Sample Labelling – Cryotube Labels

Note: cryotube barcodes are printed on tube, not on the label. Barcodes are tied to the specific kit they arrive in (ie, a specific subject and visit). **Please do not mix the labels from one kit with the cryotubes from another.**

Barcode is in human readable format on side of tube, 2D format on bottom of tube

Sample Labelling – Cryotube Labels

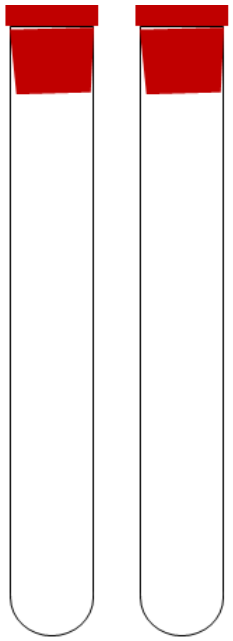
Please verify that you are using the correct cryotube for the correct specimen type

Cap Color	Specimen Type
Purple	Plasma
Grey	Buffy Coat
Red	Serum

Sample Collection and Processing

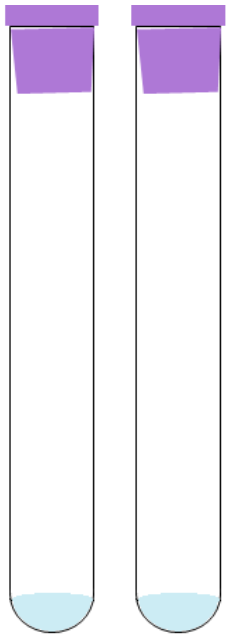
Blood Tube Draw Order

2 x 10ml Serum



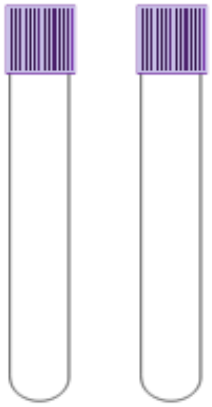
1

2 x 10ml EDTA for plasma and buffy coat



2

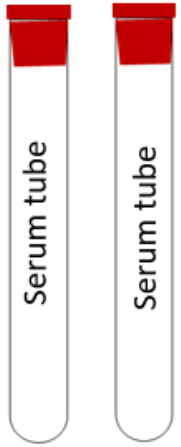
2 x 3ml EDTA for whole blood



3

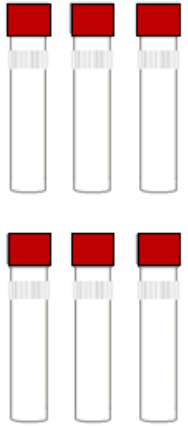
Sample Collection and Processing: Serum

Step One



- Store tubes at room temperature.
- Label collection tubes and red-capped cryovials with serum labels prior to blood draw.

Step Two

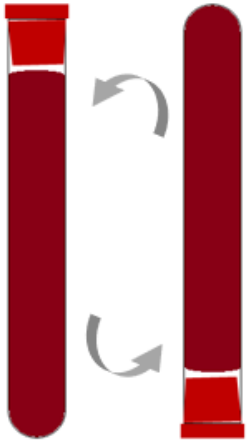


- Pre-chill cryovials on wet ice for at least 5 minutes.

Step Three

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

Step Four

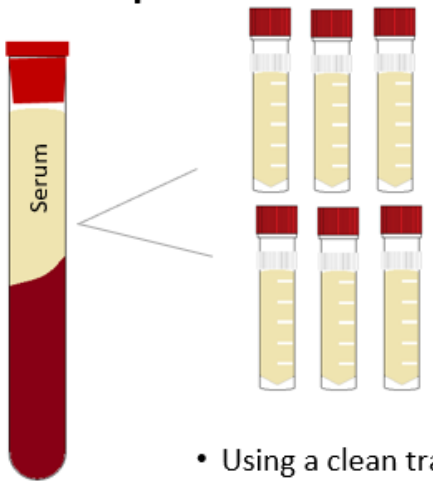


- Immediately after blood draw, gently invert tubes 8-10 times to mix samples.

Step Five

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

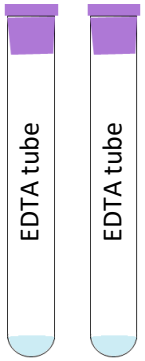
Step Six



- Using a clean transfer pipet, aliquot 1.5 ml serum into each red cryovial.
- Store serum aliquots upright at -80°C until shipment.

Sample Collection and Processing: Plasma & Buffy Coat

Step One

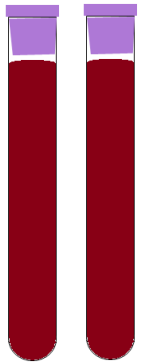


- Store tubes at room temperature.
- Label tubes with preprinted plasma labels prior to blood draw.

Step Two

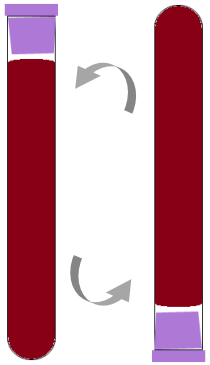
- Label 6 purple cryotubes for plasma and 2 grey for buffy coat
- Pre-chill cryotubes on wet ice for at least 5 minutes.

Step Three



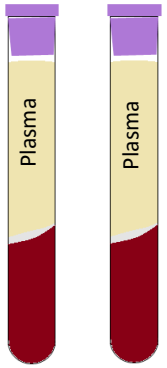
- Collect blood in EDTA tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Four

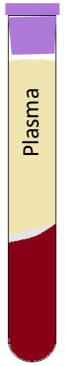


- Immediately after blood draw, invert tubes 8-10 times to mix samples.

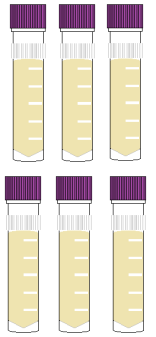
Step Five



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



Step Six



- Using a clean transfer pipet, aliquot 1.5 ml plasma into each purple cryotube.

Step Seven

- Using a clean transfer pipet, collect the buffy coat (will have residual plasma and some RBCs included).
- Transfer the buffy coat from each EDTA tube into a grey cryotube.
- Store plasma & buffy coat at -80 until shipment.

Sample Collection and Processing: Whole Blood

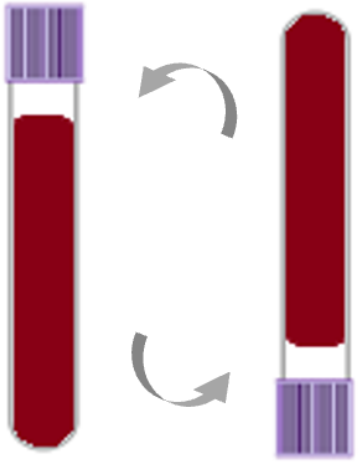
Step One

- 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.

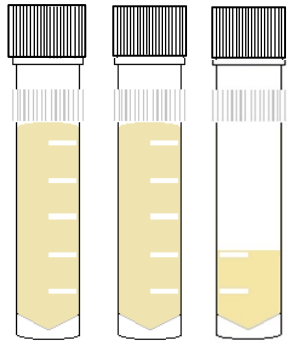
Step Four

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

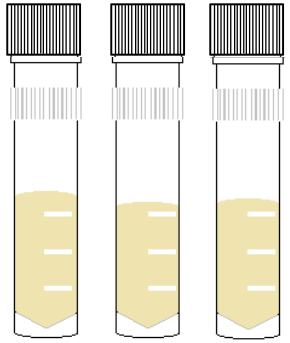
Sample Collection and Processing: Aliquots

Filling biomarker serum and plasma aliquots:

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



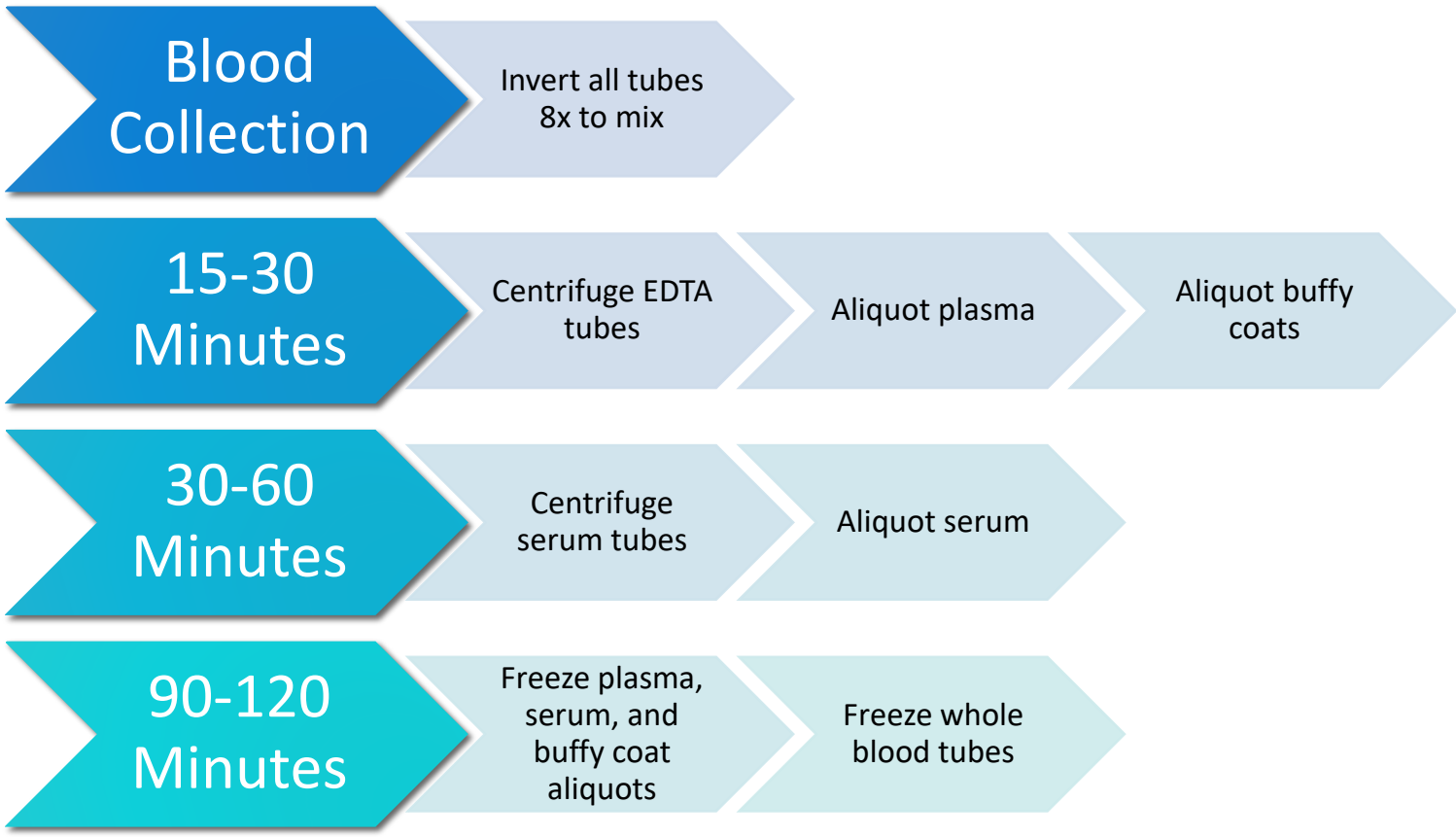
YES



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

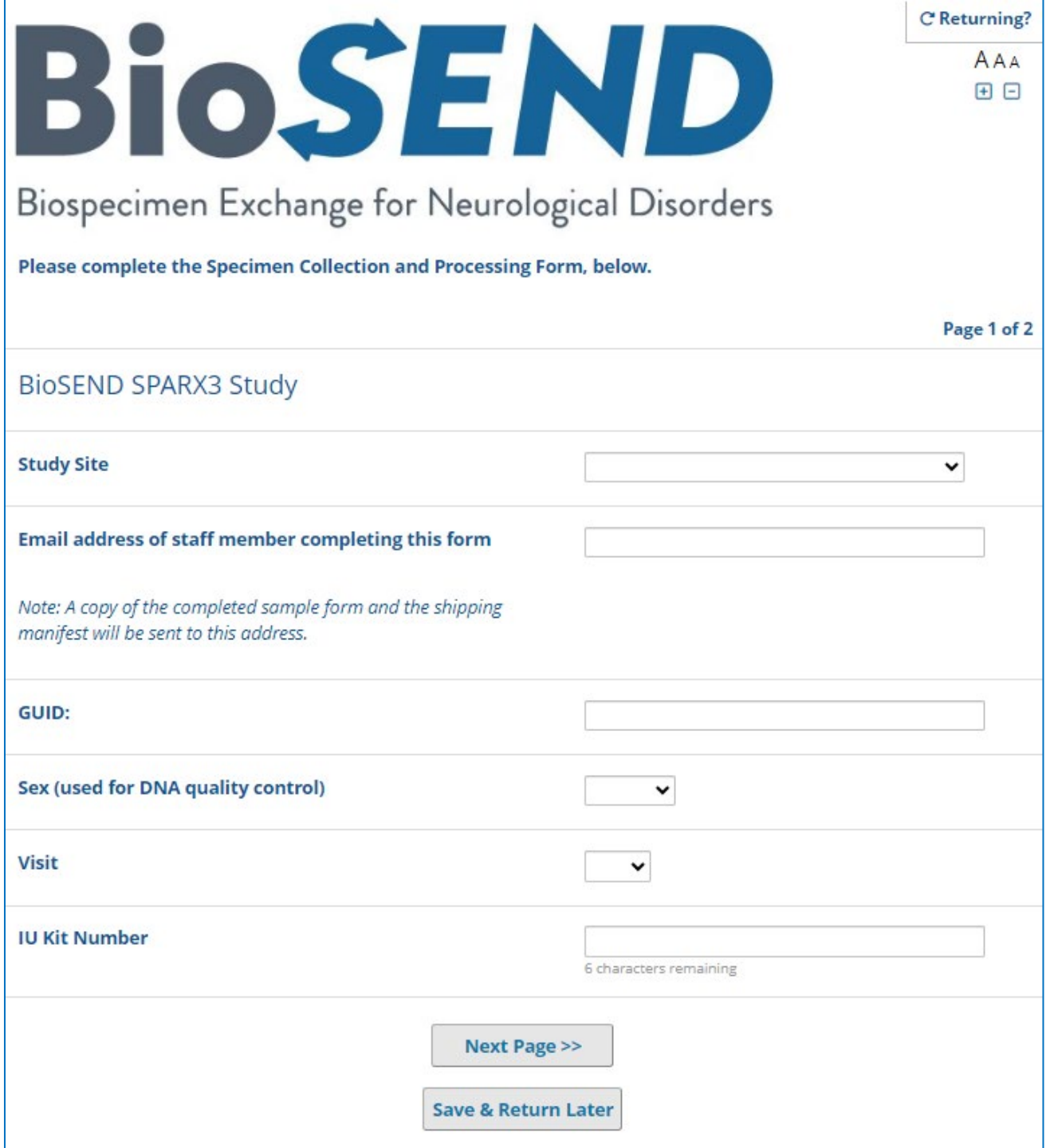
Cause: Sample Processing Methods	Corrective Actions
Vigorous mixing/shaking	Gently invert blood collection tube when mixing additive with specimen, follow guidelines in Biologics Manual regarding number of times to invert each type of tube
Not allowing serum to clot for recommended time	Serum tubes without 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/SPARX3SampleForm

First page captures basic subject and visit information



Sample Collection and Processing Form

Direct link:
https://redcap.link/SPARX3SampleForm

Second page captures processing information

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

SERUM (red-top tubes, 10 mL)

Was blood collected and processed for SERUM?
Yes
No
reset

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

Duration of SERUM tube centrifugation
15
minutes

Rate of SERUM tube centrifugation
1500
x g

Temperature of SERUM tube centrifugation
4
degrees Celsius

Total volume of SERUM collected
ml

Sample Collection and Processing Form

Direct link:
https://redcap.link/SPARX3SampleForm

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

SPARX3 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:

- ◯ Boston University (Charles River Campus)
- ◯ Cleveland Clinic
- ◯ Columbia University Medical Center
- ◯ Emory University
- ◯ Iowa State University
- ◯ Kent State University
- ◯ Louisiana State University
- ◯ Mayo Clinic, Rochester
- ◯ New York University Langone Health
- ◯ Northwestern University
- ◯ Ohio Health
- ◯ Oregon Health & Science University
- ◯ Rush University Medical Center
- ◯ University of Alabama at Birmingham
- ◯ University of Alberta
- ◯ University of California, San Francisco
- ◯ University of Cincinnati
- ◯ University of Colorado, Denver
- ◯ University of Florida
- ◯ University of Michigan
- ◯ University of Minnesota
- ◯ University of Pennsylvania
- ◯ University of Pittsburgh
- ◯ University of Southern California
- ◯ University of Texas Medical Branch
- ◯ University of Texas San Antonio
- ◯ University of Utah
- ◯ University of Virginia
- ◯ Washington University St. Louis
- ◯ Wildred Laurier University of Canada

GUID: _____

Visit:

- ◯ BL
- ◯ 6M
- ◯ 12M
- ◯ 18M
- ◯ 24M

IU Kit Number: _____

Date of blood collection: _____

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

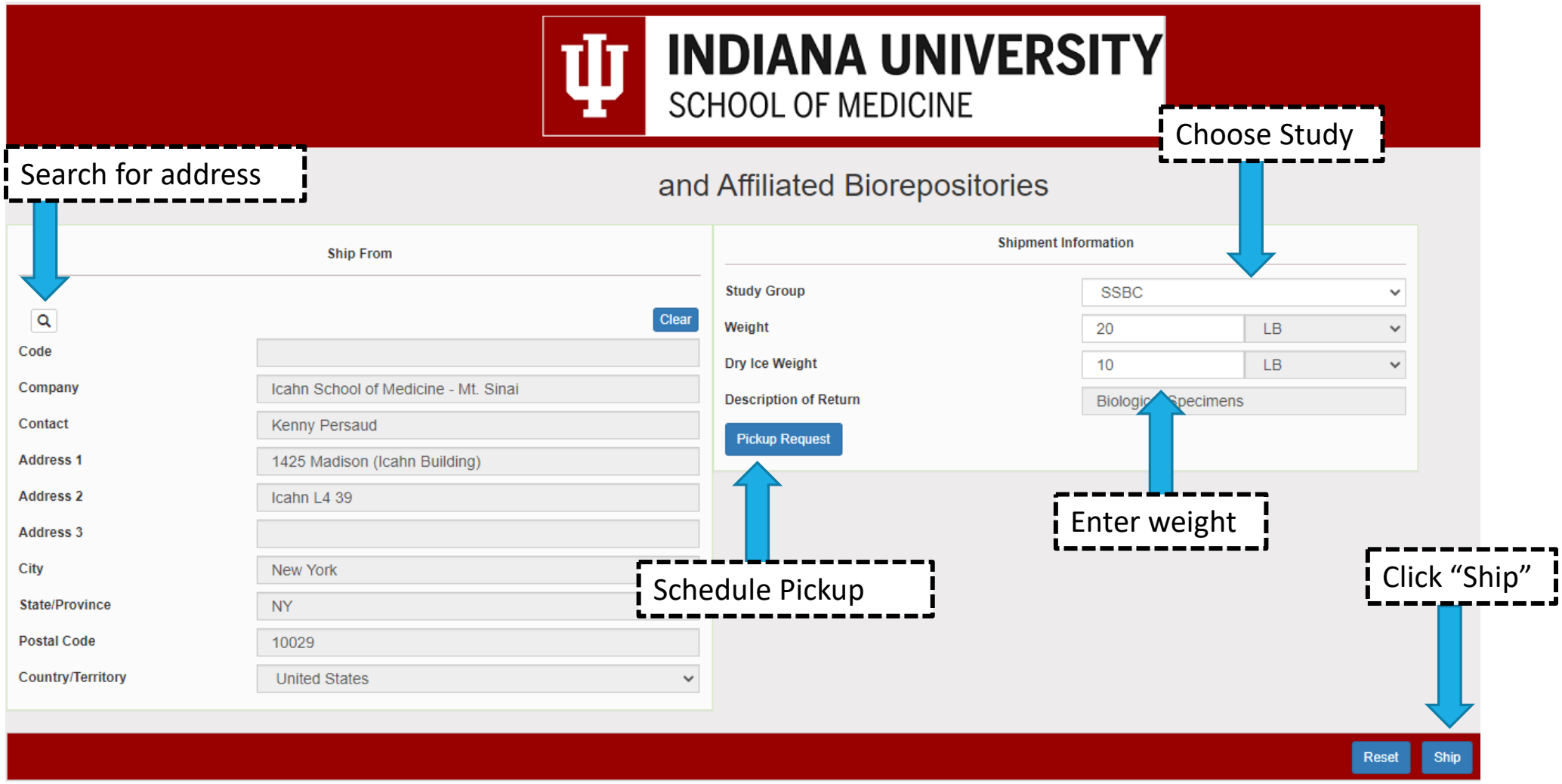
- SPARX3 samples all ship frozen on dry ice
- Frozen samples should be shipped ***only*** Monday through Wednesday
- Always fill carton to **top** with dry ice
- Do not pack shipment until the day of pickup
- Do not overlap the dry ice label with any other labels or the airway bill

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 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 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/sparx3/