NINDS BIOSEND

Dissecting Oligogenic Biomarkers in Ashkenazi Jews with Parkinson's Disease (AJPD)

BIOSPECIMEN COLLECTION & PROCESSING

Overview

1. Specimen uniformity and quality
2. Site Equipment
3. Procedures
    - Kit Ordering
    - Sample Labels
    - Sample Collection and Processing
    - Shipping Closures
4. Contact Information

Specimen Uniformity and Quality

GENERAL REMINDERS

Specimen Uniformity and Quality

Most biomarkers are sensitive to ***time*** and ***temperature***

- Standardization of processing across sites is key
- Reference the *BioSEND 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 Equipment

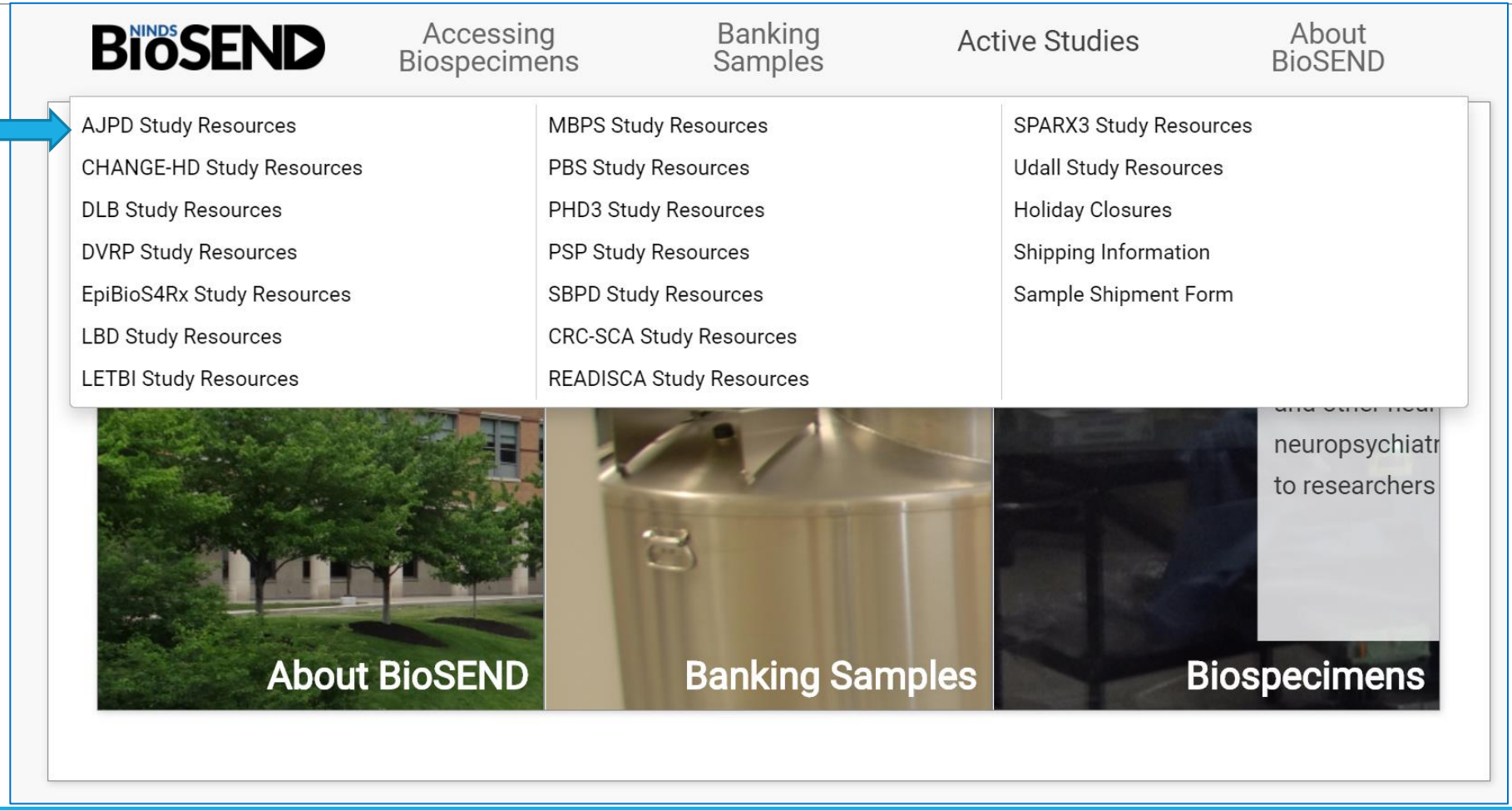
Sites will need to supply the following items:

- 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

Biospecimen Collection Protocol

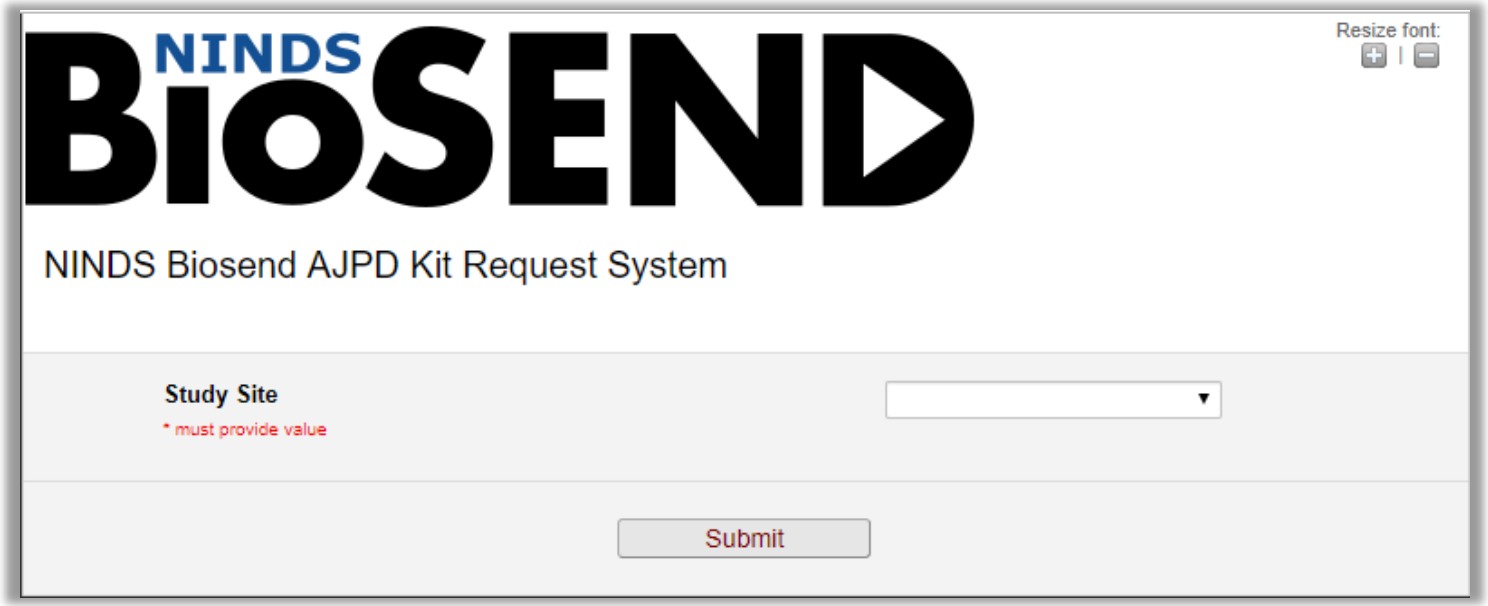
	BL	12M	24M	36M	48M
Whole Blood (6ml)	X	X	X	X	X
Plasma (6 x 1ml)	X	X	X	X	X
Serum (6 x 1ml)	X	X	X	X	X
Buffy Coat (2 aliquots)	X	X	X	X	X
RNA (2 x 2.5ml)	X	X	X	X	X
CSF (10 x 1ml)	X	X	X	X	X
Urine (2 x 10ml)	X	X	X	X	X

Kit Ordering – Biosend.org



BioSEND Kit Request Module

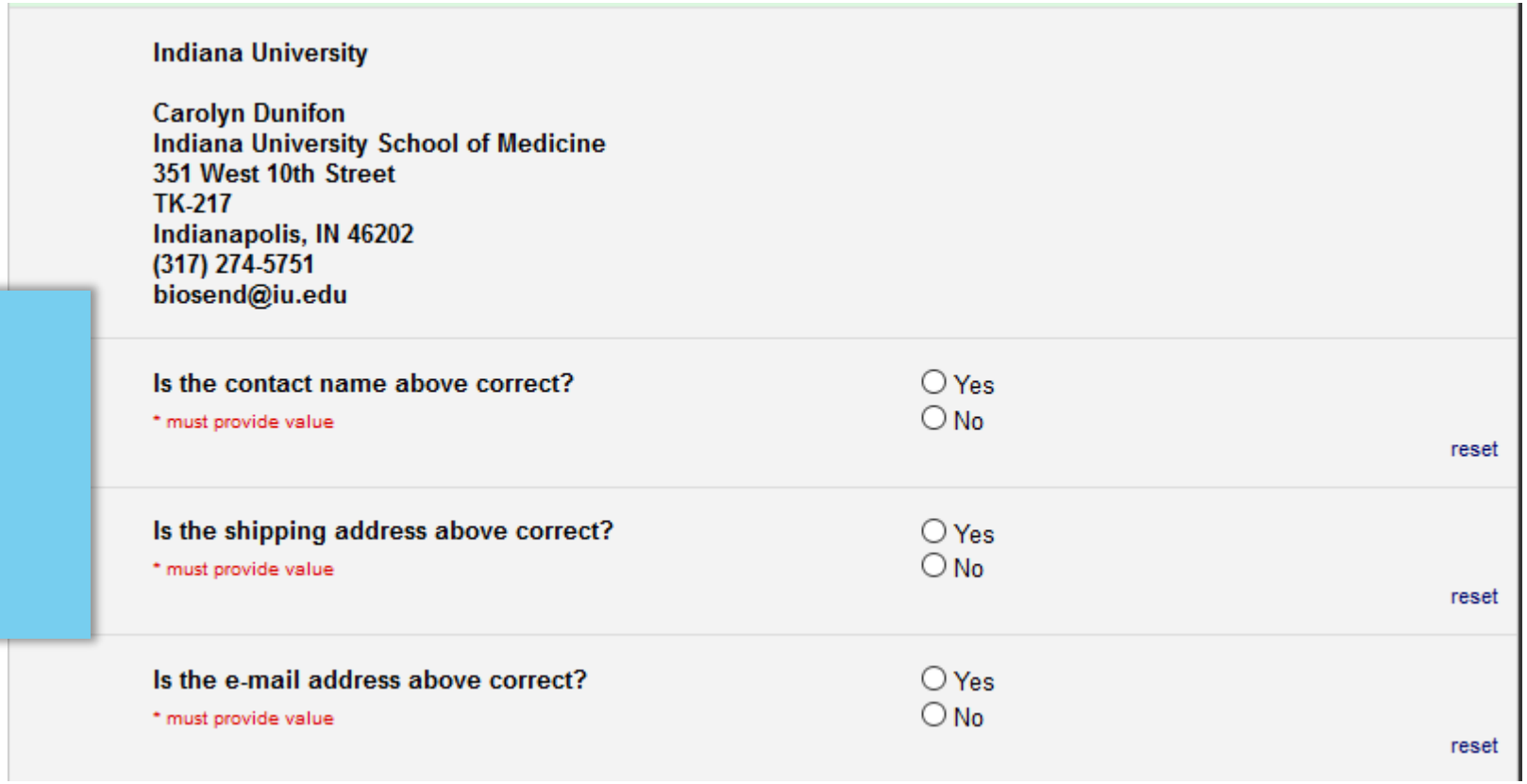
- http://kits.iu.edu/biosend/ajpd
- **Choose your site from the drop-down list.**



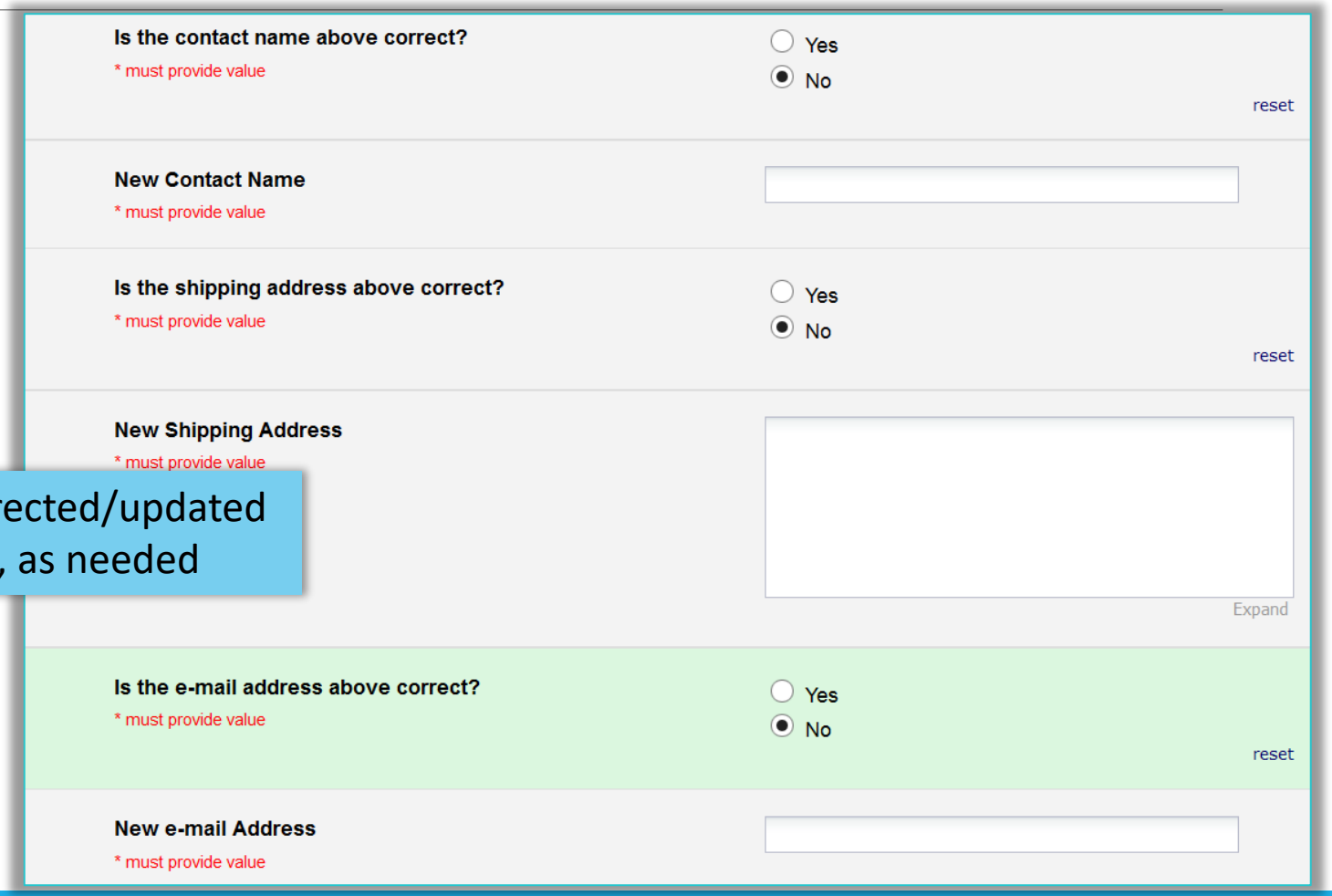
Confirm Shipping Info

Confirm site information:

- Study site
- Shipping address
- Contact name
- Email
- Phone Number



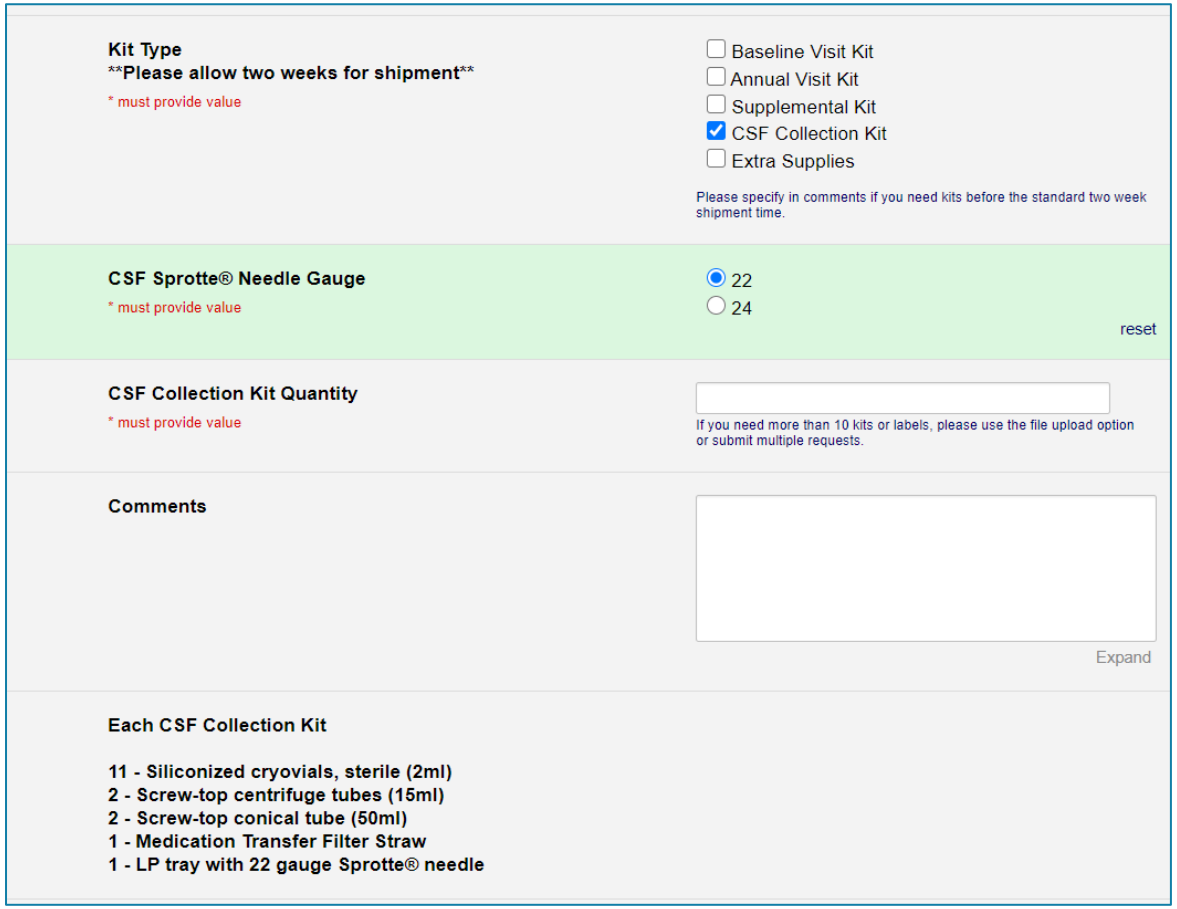
Update Information



Provide corrected/updated information, as needed

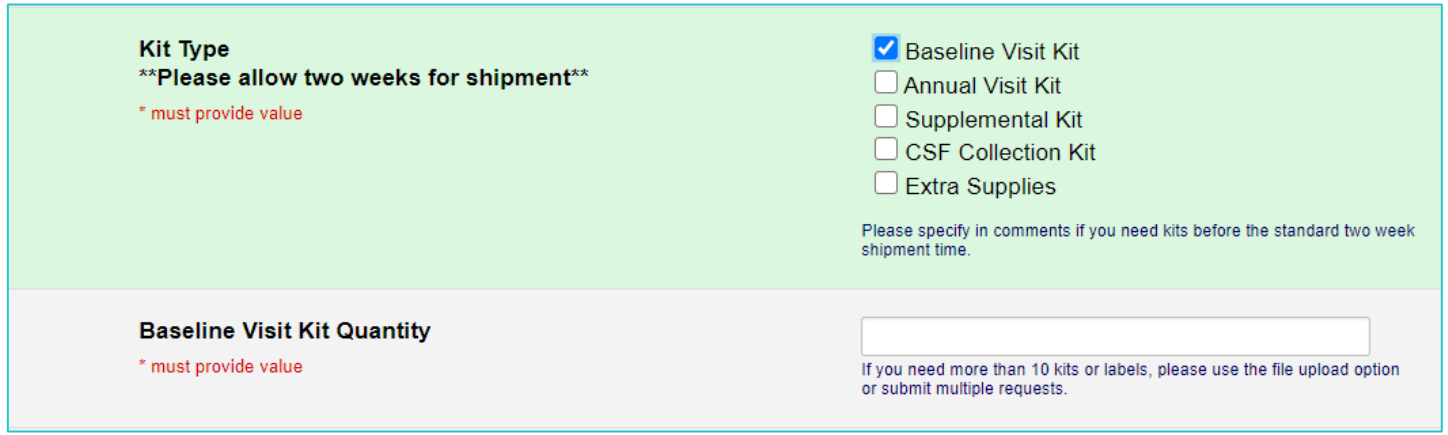
Kit Type

Multiple kit types available. Selecting a kit will populate the contents in that kit.



Baseline Kits

- BioSEND creates ST numbers for baseline kits
- ST#s serve as the biorepository subject identifier
- Enter kit quantity



Important Note: ST-Numbers

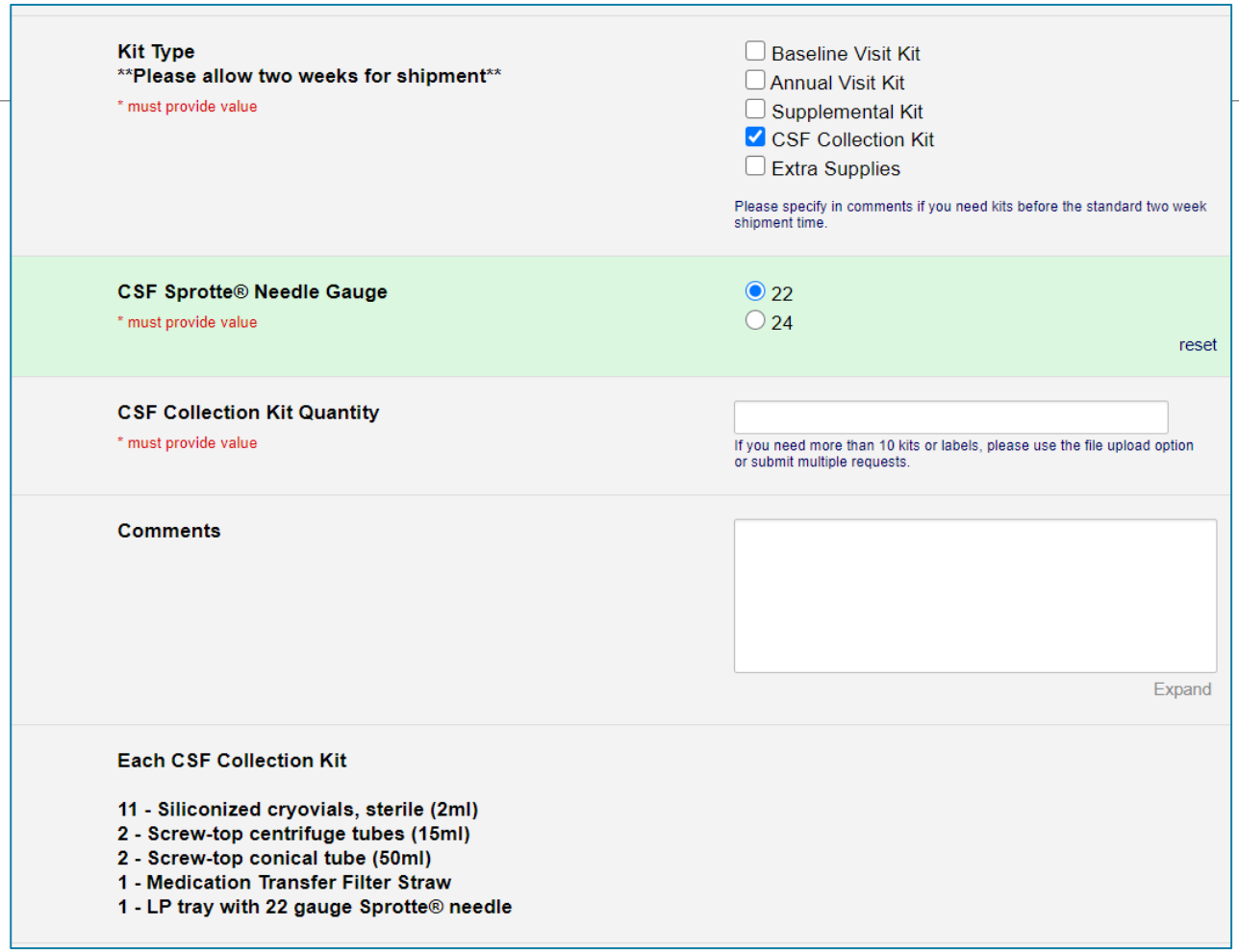
Please note: the ST-Number provided in a BL kit can be used for any subject's BL visit. **This ST number is a subject identifier** and will need to stay linked to the patient through the entirety of the study.

Annual Visits

Please provide ST-Number and time point. We are unable to complete your request without this information

CSF Kits

Labels for CSF included with every BL and Annual visit, so that CSF kit can be paired with any subject/time point.



Kit Type
****Please allow two weeks for shipment****
* must provide value

- [ ] Baseline Visit Kit
- [ ] Annual Visit Kit
- [ ] Supplemental Kit
- [x] CSF Collection Kit
- [ ] Extra Supplies

Please specify in comments if you need kits before the standard two week shipment time.

CSF Sprotte® Needle Gauge
* must provide value

- (•) 22
- ( ) 24

reset

CSF Collection Kit Quantity
* must provide value

If you need more than 10 kits or labels, please use the file upload option or submit multiple requests.

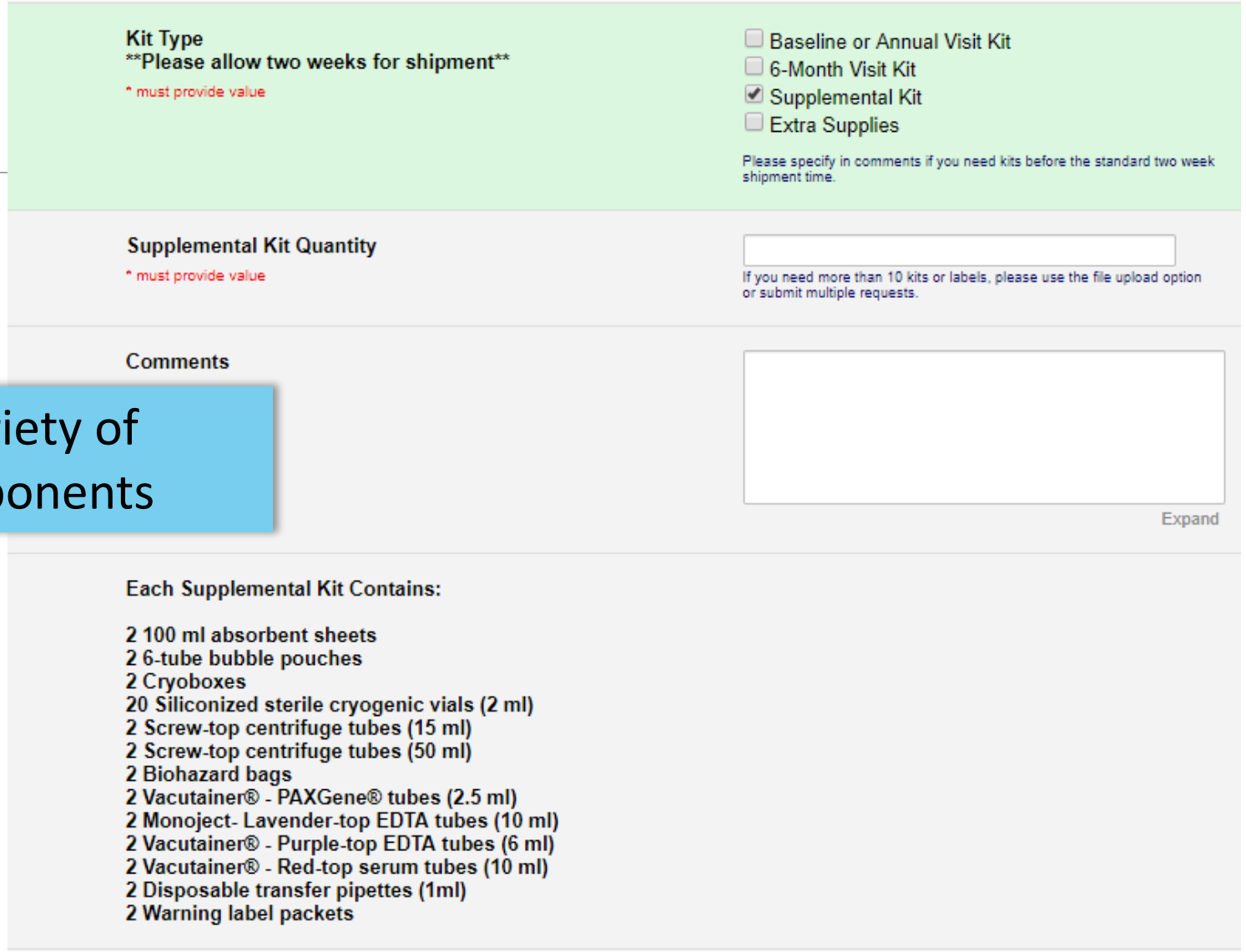
Comments

Expand

Each CSF Collection Kit

11 - Siliconized cryovials, sterile (2ml)
2 - Screw-top centrifuge tubes (15ml)
2 - Screw-top conical tube (50ml)
1 - Medication Transfer Filter Straw
1 - LP tray with 22 gauge Sprotte® needle

Supplemental Kit

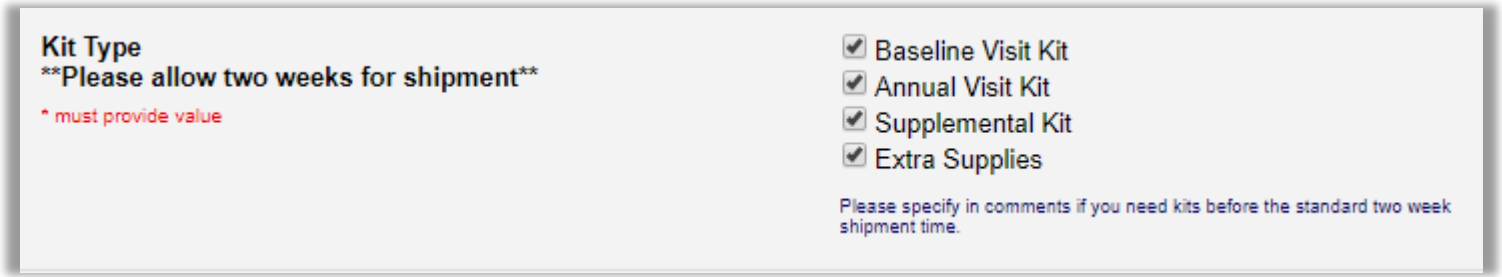


Contains a variety of extra kit components

Extra Supplies

Allows you to choose specific supplies and particular quantities

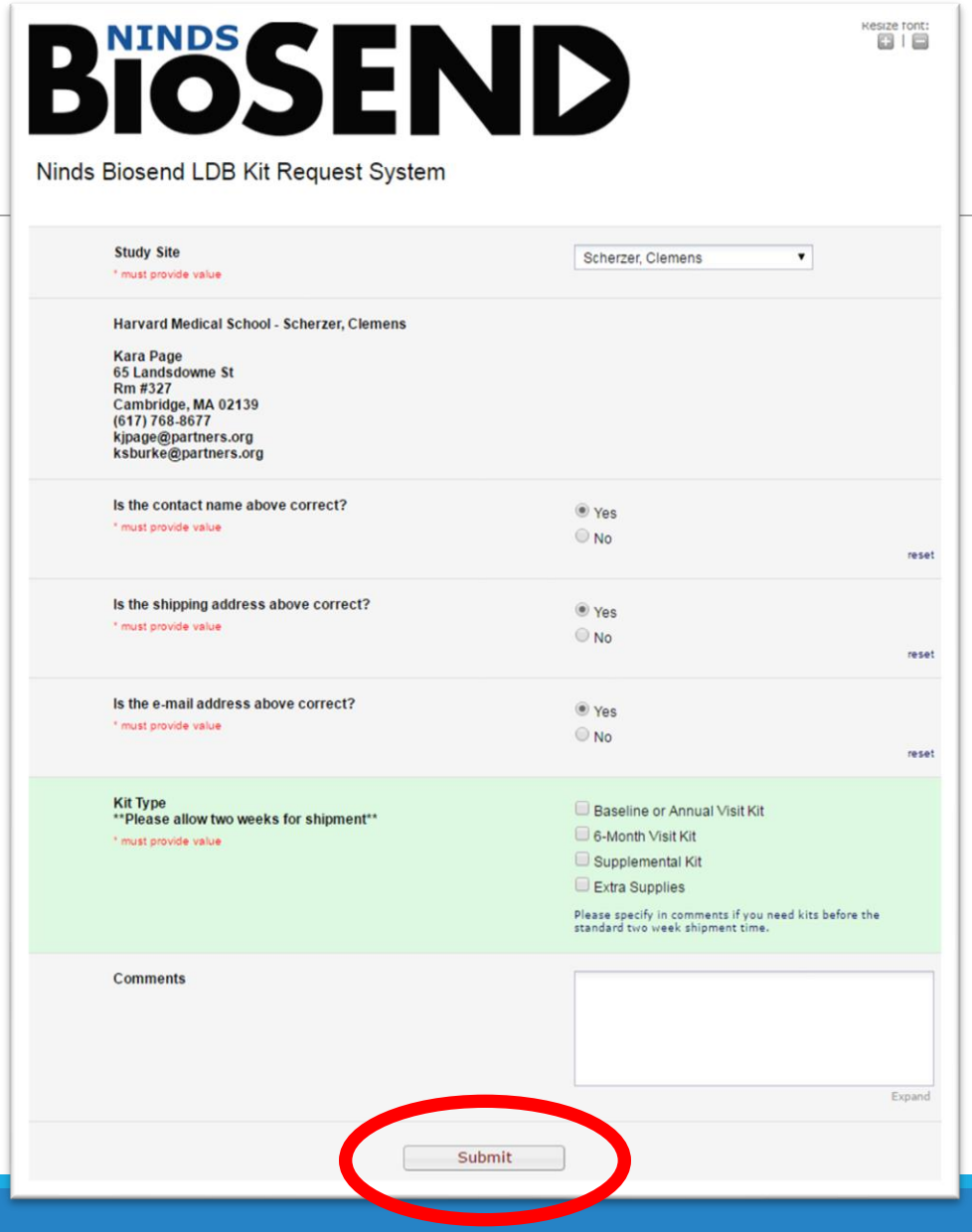
Multiple Orders



You can order more than one type of kit in a single kit request

Submit Request

- 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

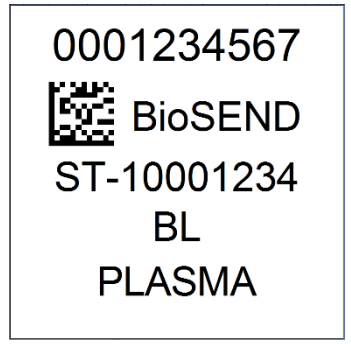


Sample Labelling

Labels are provided by Indiana University

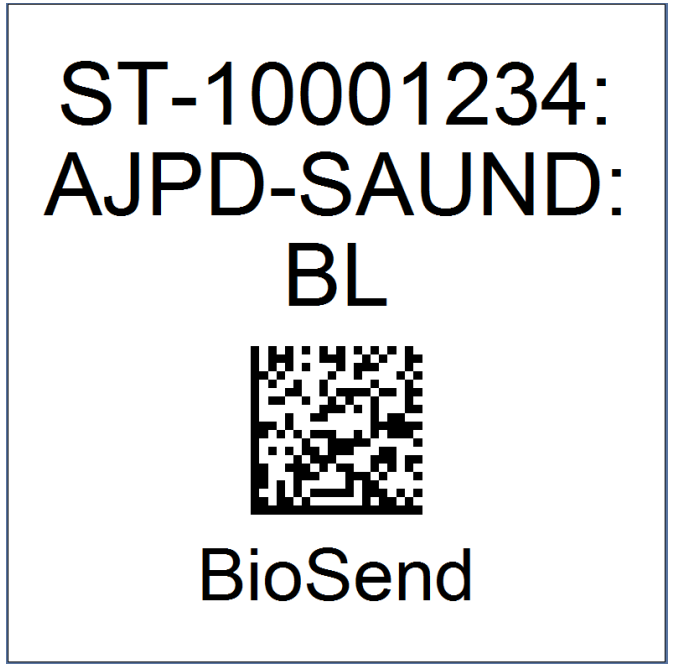
- Please check that all samples are properly labelled with correct specimen type and visit

Case Labels



Specimen Labels

Case Label



Subject Number

Study – PI & Visit

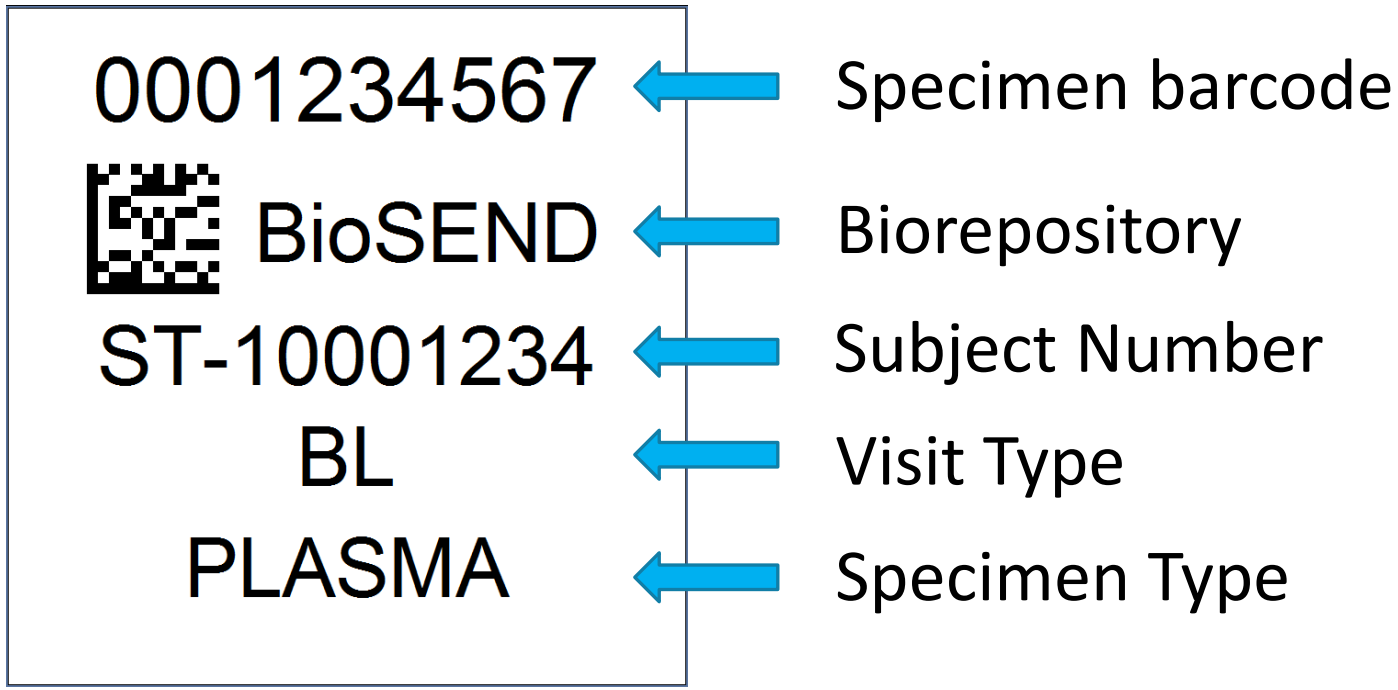
Biorepository Name

Case Labels

Case labels are placed:

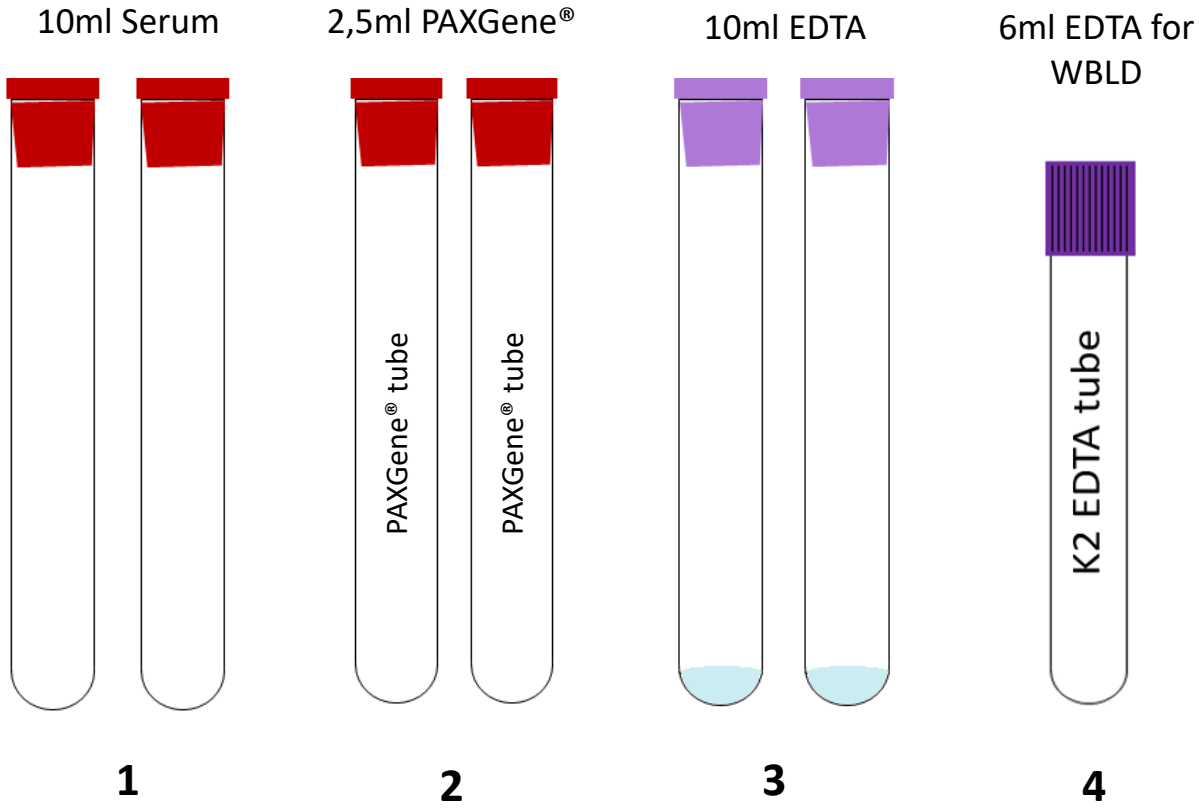
- On the plastic biohazard bags
- On the lid of frozen shippers

Specimen Label



Sample Collection and Processing

Blood Tube Draw Order



Sample Collection and Processing: RNA

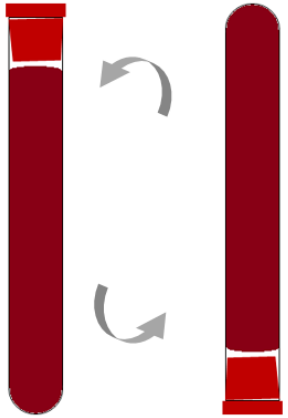
Step One

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

Step Two

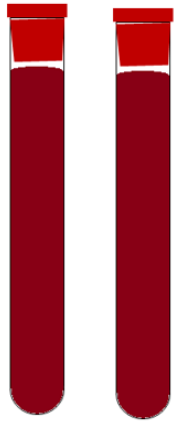
- Collect blood into PAXGene tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



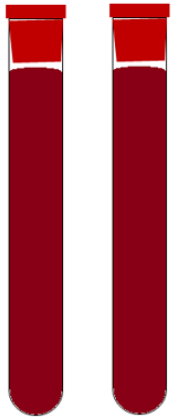
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Four



- Incubate tubes upright at room temperature for 24 hours.

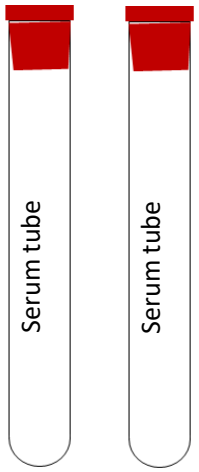
Step Five



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

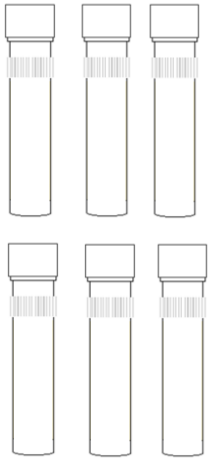
Sample Collection and Processing: Serum

Step One



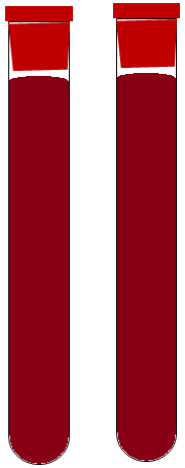
- Store tubes at room temperature.
- Label tubes with preprinted serum labels prior to blood draw.

Step Two



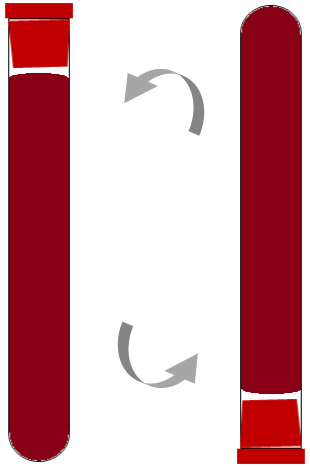
- Label 6 cryotubes with serum aliquot labels.
- Pre-chill cryotubes on wet ice for at least 5 minutes

Step Three



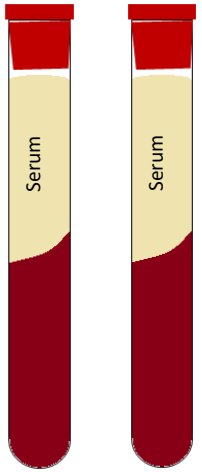
- Collect blood in serum tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Four



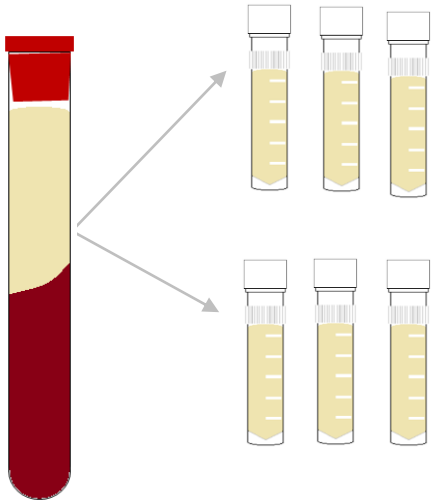
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

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.

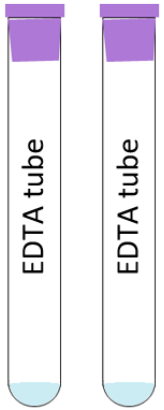
Step Six



- 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: Plasma & Buffy Coat

Step One

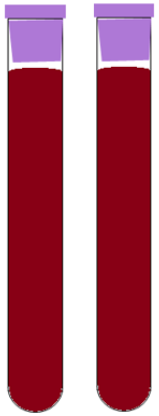


- Store tubes at room temperature.
- Label tubes with preprinted plasma labels prior to blood draw.

Step Two

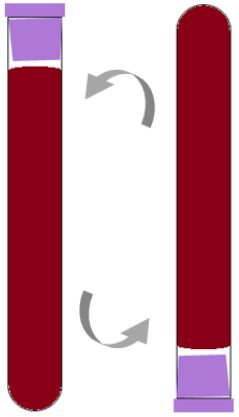
- Label 6 cryotubes with plasma labels and 2 with buffy coat labels.
- Pre-chill cryotubes on wet ice for at least 5 minutes

Step Three



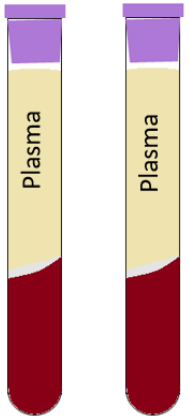
- Collect blood in EDTA tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Four

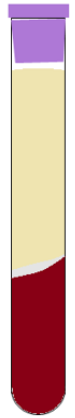


- Immediately after blood draw, invert tubes 8-10 times to mix samples.

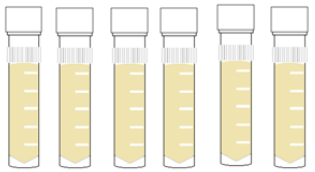
Step Five



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



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 <u>upright</u> at -80°C until shipment.

Sample Collection and Processing: Whole Blood

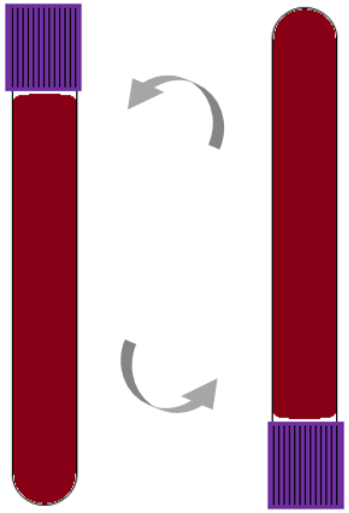
Step One

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

Step Two

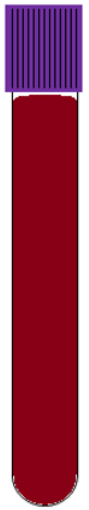
- Collect blood in 6ml EDTA tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



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

Step Four



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

Blood Collection: Troubleshooting

Issue #1: Collection tube with little/no vacuum

Always check expiration dates before beginning blood draw and discard expired tubes

- Tubes expire on last day of month printed on tube

Store tubes at ambient temperature

- Extreme temperatures can affect vacuum

Keep extra collection tubes from supplemental kit nearby during blood draw to replace “bad” tubes. These can also be requested through the Kit Request Module.

If frequent occurrence, report tube type and lot numbers to Indiana University

Blood Collection: Troubleshooting

Issue #2: Hemolyzed (pink/red) plasma & serum

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
Lumen of needle too close to inner wall of vein (indicated by slow blood flow)	
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

Reference: BD's "Tech Talk" newsletter, Vol. 2, No. 2, October 2003 (http://www.bd.com/vacutainer/pdfs/techtalk/TechTalk_Jan2004_VS7167.pdf)

Blood Collection: Troubleshooting

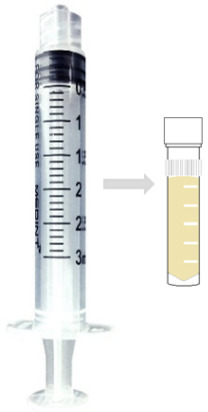
Issue #2: Hemolyzed (pink/red) plasma & serum

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 temp
Prolonged contact of serum/plasma with cells	Do not store uncentrifuged samples beyond recommended time

Reference: BD's "Tech Talk" newsletter, Vol. 2, No. 2, October 2003 (http://www.bd.com/vacutainer/pdfs/techtalk/TechTalk_Jan2004_VS7167.pdf)

Sample Collection and Processing: CSF

Step One

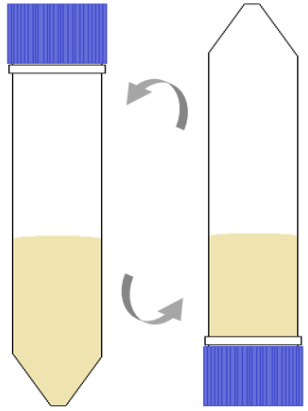


- Collect CSF into the 3ml luer lock syringe or by gravitational pull.
- Dispense 1-2 ml into cryovial.
- Send to local lab for testing, if applicable.

Step Two

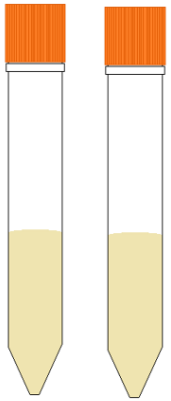
- Collect CSF into the 5ml luer lock syringe or by gravitational pull
- Transfer sample into 50 ml conical tube.

Step Three



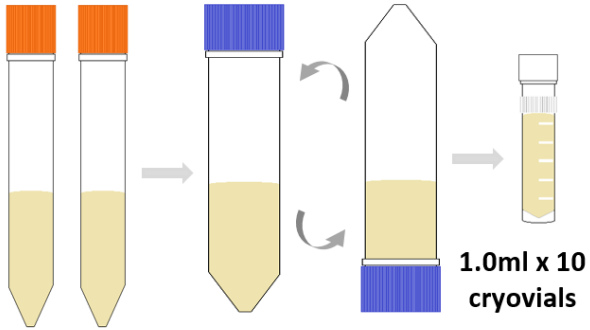
- Immediately after collection, gently invert the 50 ml conical tube 3-4 times to mix the sample.

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.

Urine Sample Collection

1. Label one urine collection cup prior to urine collection with a pre-printed “URINE” label.
2. Ask study subject to collect a urine specimen in the collection cup. Urine should be collected midstream and should remain as sterile as possible.
3. Label two 15 ml conical tubes with pre-printed “URINE” labels.
4. Transfer 10mL urine from the collection cup into each of the two 15 mL conical tubes.
5. Within 60 minutes of collection, freeze and store samples **upright** at -80°C until shipment.

Shipping Samples: Frozen

Packing and Shipping Frozen Samples

- All other samples 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: Frozen

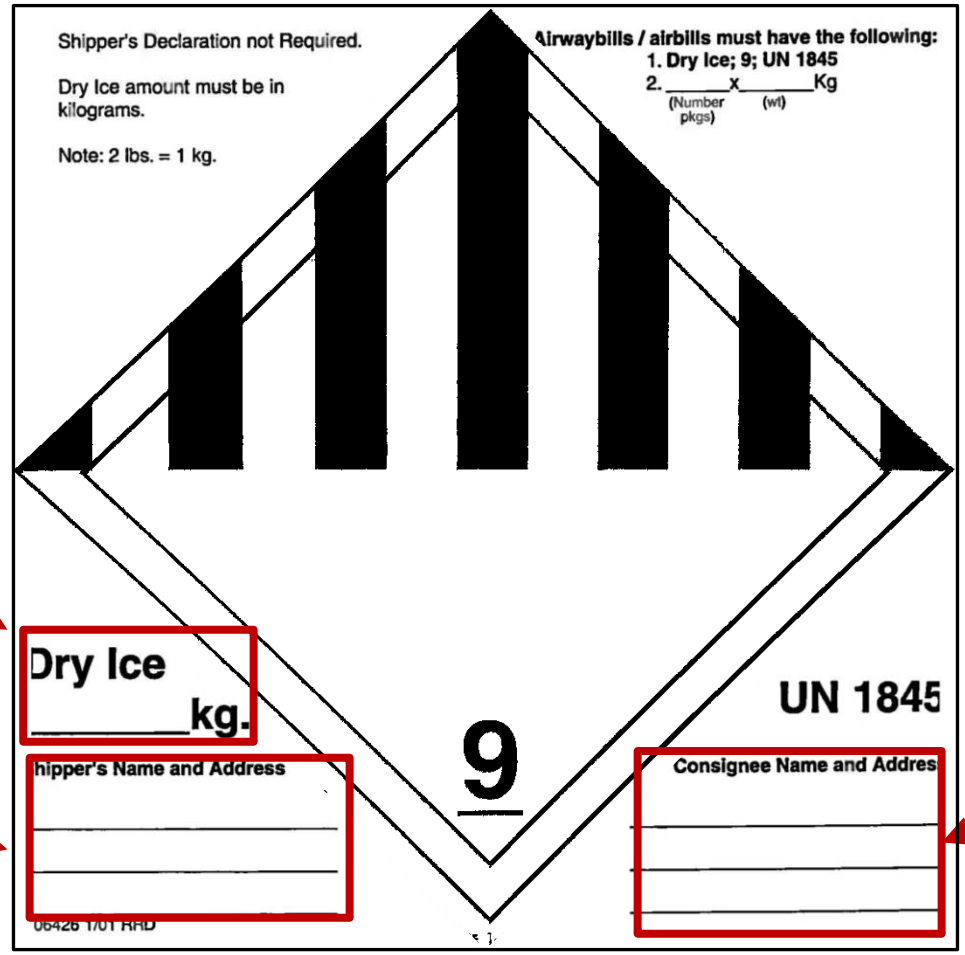
Packing and Shipping Frozen Samples

- Shippers use approx. 10lbs of dry ice
- Place layer of dry ice in between cryoboxes

Shipping Samples

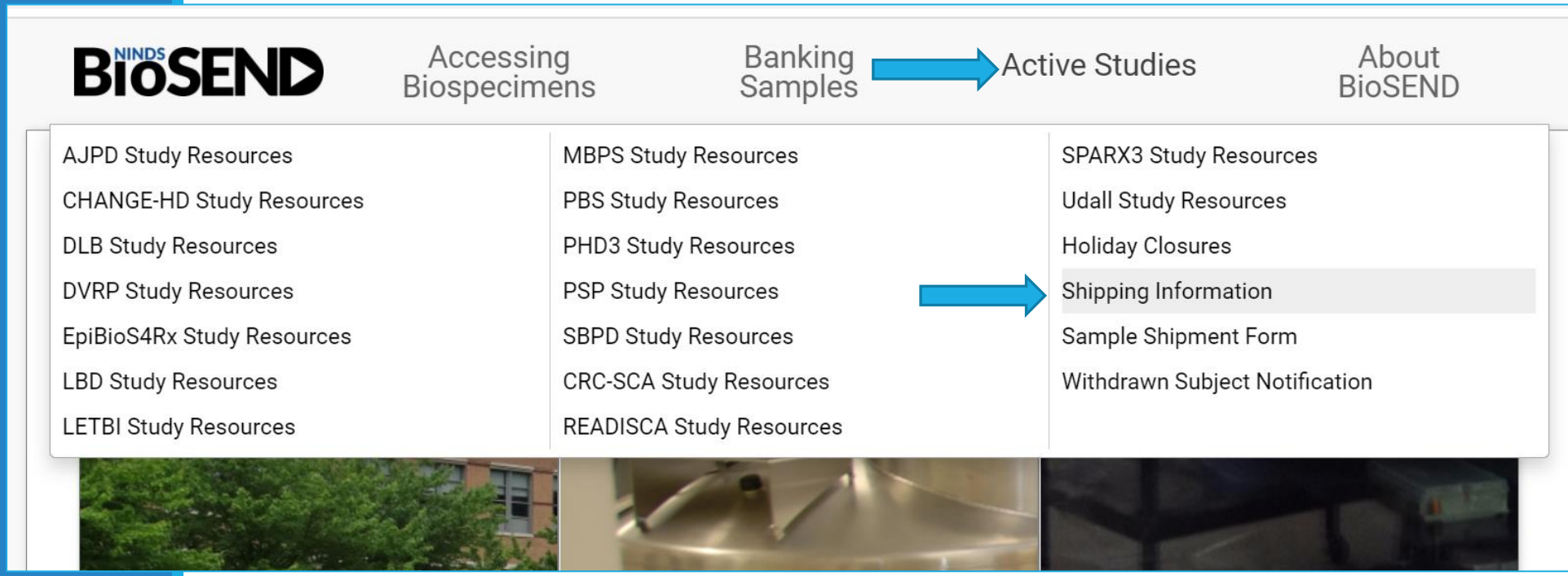
Packing and Shipping Frozen Samples

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



Shipping Samples

UPS resources available on BioSEND website



Shipping Samples

Links on this page to generate airwaybills, schedule pickups, request account, and view a guide for using the UPS ShipExec Thin Client system.

Shipping Information

Shipping Address

BioSEND
Indiana University School of Medicine
351 West 10th Street
TK-217
Indianapolis, IN 46202

UPS Shipping Resources

To generate air waybills and schedule UPS pickups for shipments to BioSEND, please visit the UPS ShipExec™ Thin Client website.

For instructions on how to use the UPS ShipExec™ Thin Client website, please refer to the BioSEND UPS ShipExec™ Thin Client Guide

To request a new user account for UPS ShipExec™ Thin Client or to request an update to your site's address in the system, please use this form to submit your request.

Additional Resources

Sample Submission Form
UPS ShipExec™ Guide

Contact Us

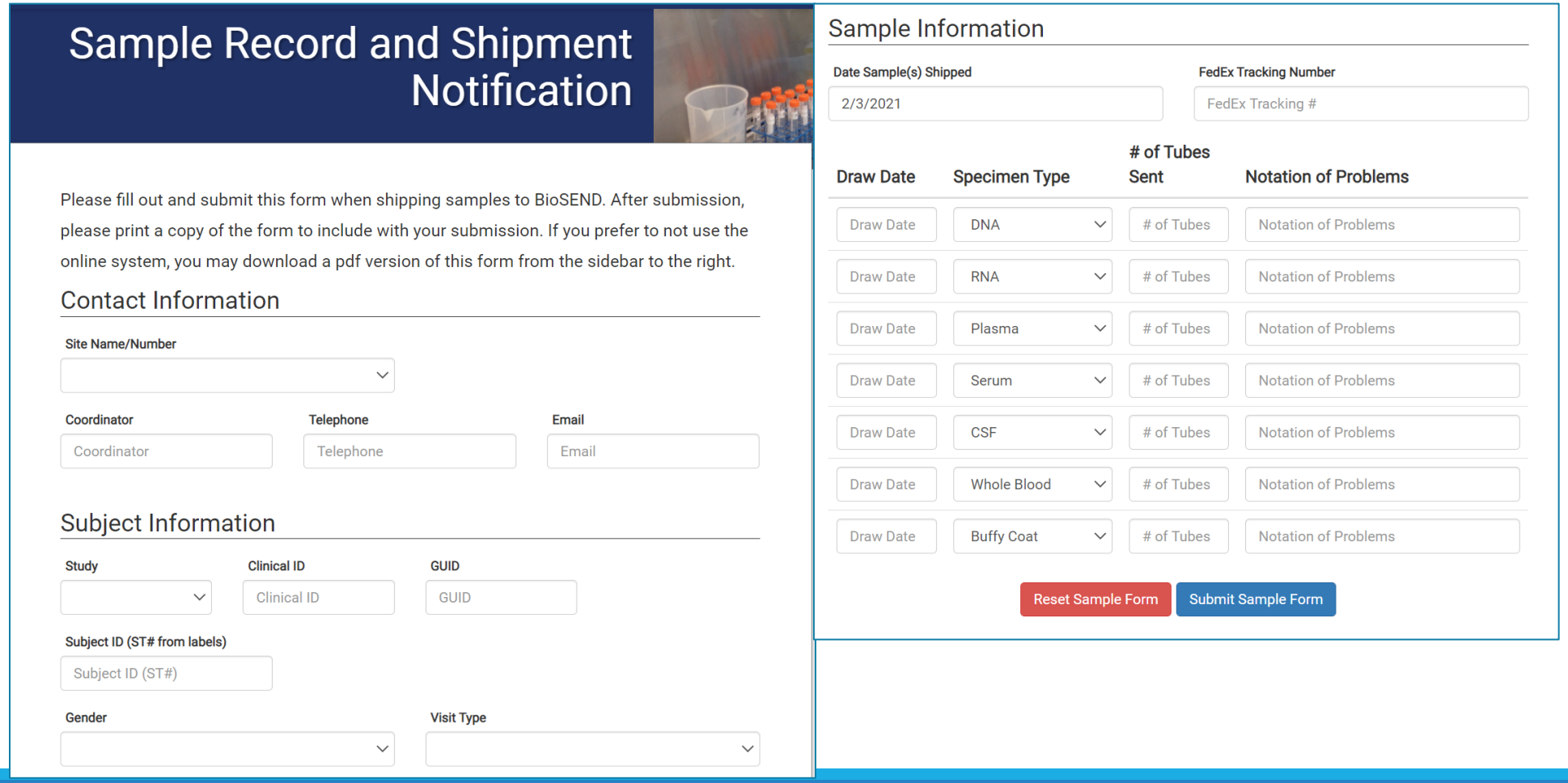
biosend@iu.edu
317-278-0594

Shipping Samples: Frozen

Please notify BioSEND ahead of shipment

- Email biosend@iu.edu with copy of Sample Form and tracking number
- OR use Online Sample form on biosend.org

Shipping Samples: Sample Form



Shipping Frozen Samples

Hold packaged samples in a -80°C freezer until pickup.

Samples should be received at BioSEND within 2 weeks of collection.

Packaging & Shipping Troubleshooting

Issue: Broken/Damaged Tubes

Cause	Preventative Action
Over filling tubes	Fill tubes to suggested volume. If any sample still remains, place in an additional tube
Improper packaging	Ensure any tubes are securely placed into the bubble wrap pouch and are placed in a separate bag from the cryobox
Rough shipping conditions	Extra bubble wrap may be needed to pad blood tubes
Extreme changes in temperature (ambient→freezer; freezer→dry ice)	Wrapping the tubes in bubble wrap before freezing may help slow the cooling process

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
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. Please reach out to BioSEND if you an unsure if it is safe to ship.

Non-Conformance Reporting

Most common non-conformance issues:

- Samples shipped for weekend/holiday delivery
- Sample form incomplete/inaccurate
- Unlabeled or mislabeled tube(s)
- Sample hemolysis

Contacts

Indiana University

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