BioSpecimen Exchange for Neurological Disorders (BioSEND)

Training Webinar

CRC-SCA

NINDS BioSEND

BioSEND Training Webinar Overview

1. Study Reminders
2. Site Equipment
3. Biospecimen Collection Protocol
4. Kits & Samples
   - Requesting Kits
   - Labels
   - Sample Collection & Processing
   - Sample Shipment
5. BioSEND Website
6. Contact Information

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Study Reminders

Please remember…

- Biospecimens are limited, valuable resources.
- Standardization and quality are key! 
- Reference the BioSEND Manual of Procedures as needed.
- Do not replace or supplement any kit components without first receiving approval from BioSEND.

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Site Equipment

The following items are to be supplied by the site:

- Personal protective equipment
- Alcohol prep pads
- Butterfly needles and hubs
- Tourniquet
- Gauze pads
- Bandages
- Sharps bin and lid
- Microcentrifuge tube rack
- Test tube rack
- Pipettes and pipette tips
- 4°C Centrifuge
- -80°C Freezer
- Crushed/wet ice
- Dry ice

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Biospecimen Collection Protocol

All specimen types FROZEN

	BL
Serum (6 x 1ml)	X
Plasma (6 x 1ml)	X
Buffy Coat (2 aliquots)	X
CSF (10 x 1ml)	X

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Requesting Kits

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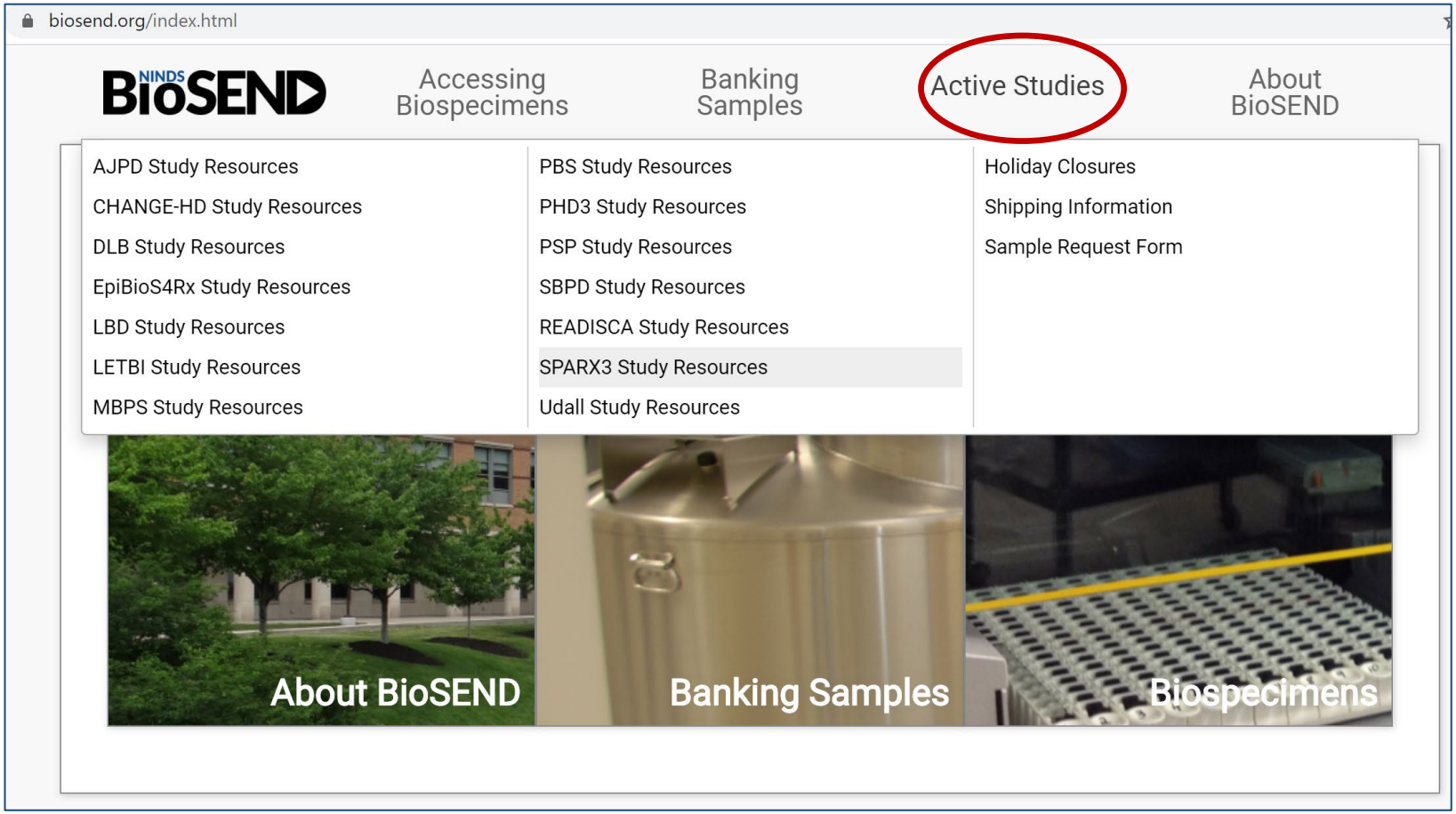
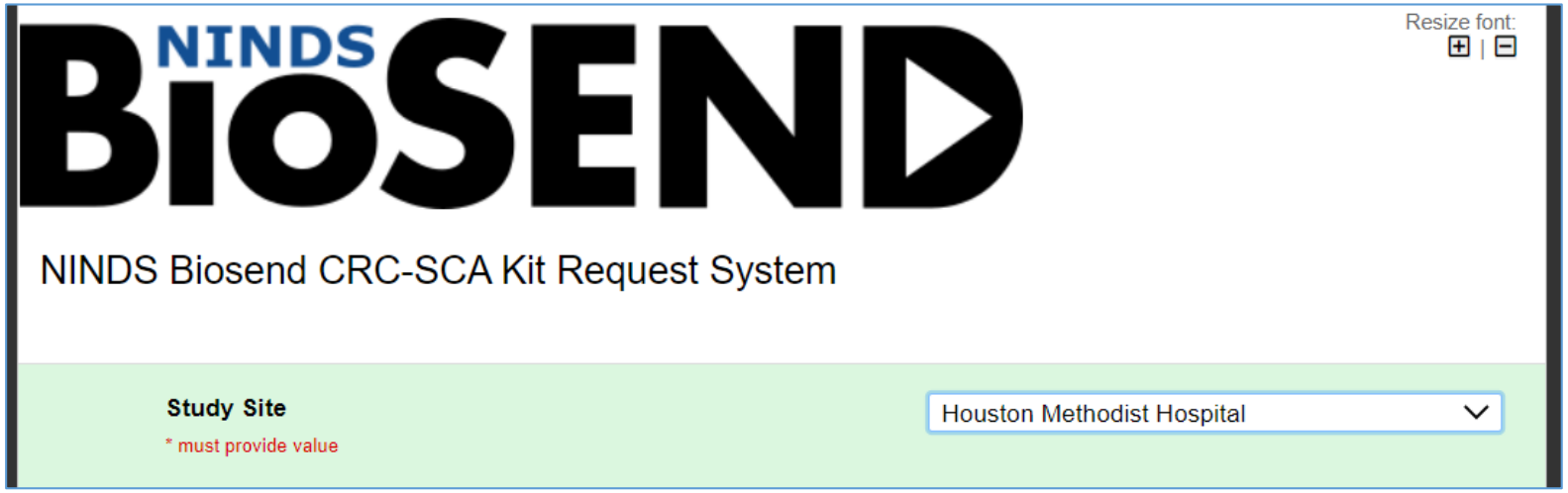
NINDS BioSEND Website



https://www.biosend.org/

BioSEND

BioSEND Kit Request Module



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

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BioSEND Kit Request Module

University of Colorado

Katherine Balfany
University of Colorado Denver Anschutz Medical Campus
Department of Physical Therapy
13121 E 17th Ave. Mail Stop C244
Aurora, CO 80045

Katherine.balfany@cuanschutz.edu
Phone Office: 303.724.9101
Phone Cell: 608.317.6222

Confirm site information:

- Study site
- Shipping address
- Contact name
- Email

Is the contact name above correct?
* must provide value
○ Yes
○ No
reset

Is the shipping address above correct?
* must provide value
○ Yes
○ No
reset

Is the e-mail address above correct?
* must provide value
○ Yes
○ No
reset

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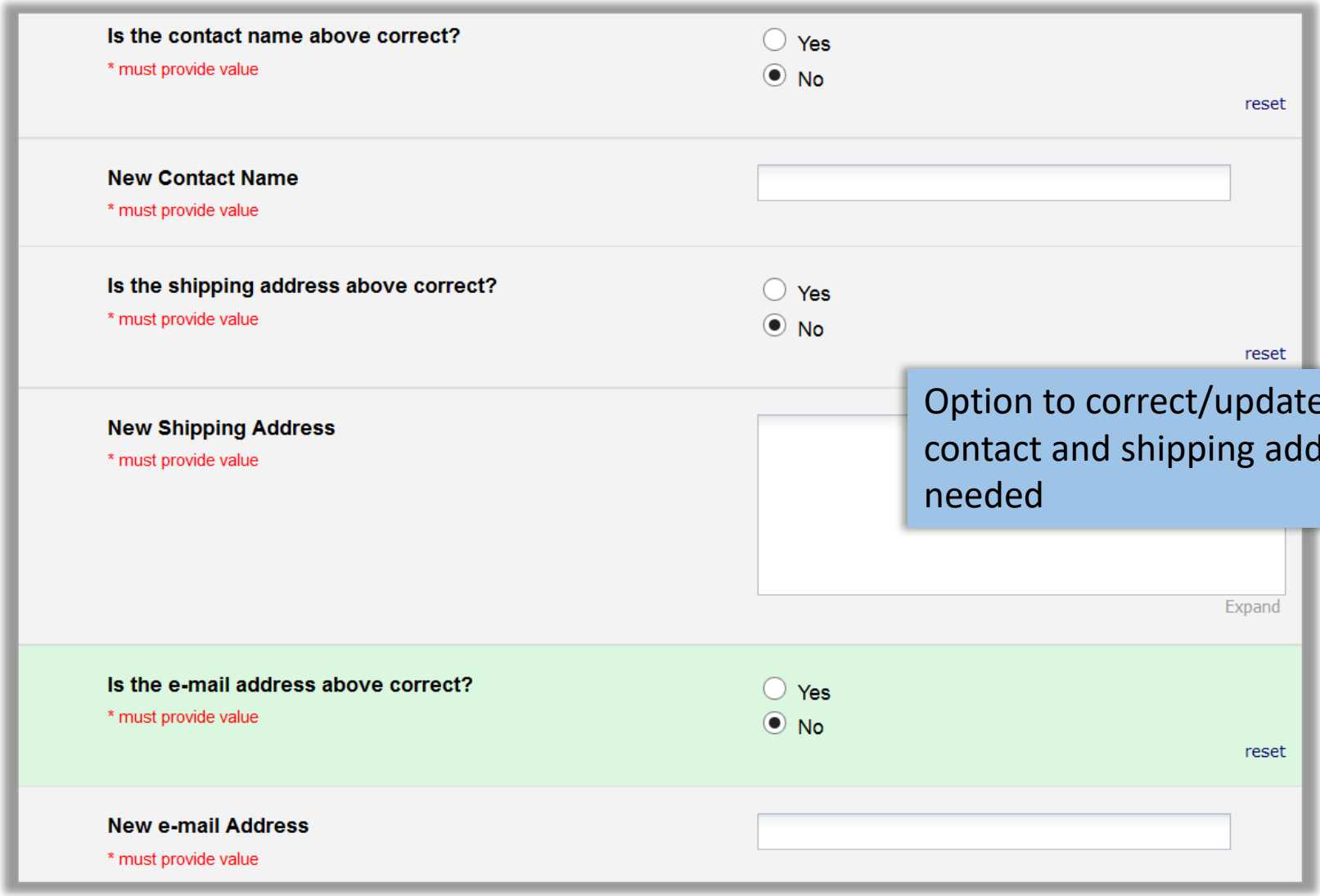
BioSEND Kit Request Module



Option to correct/update contact and shipping address, if needed

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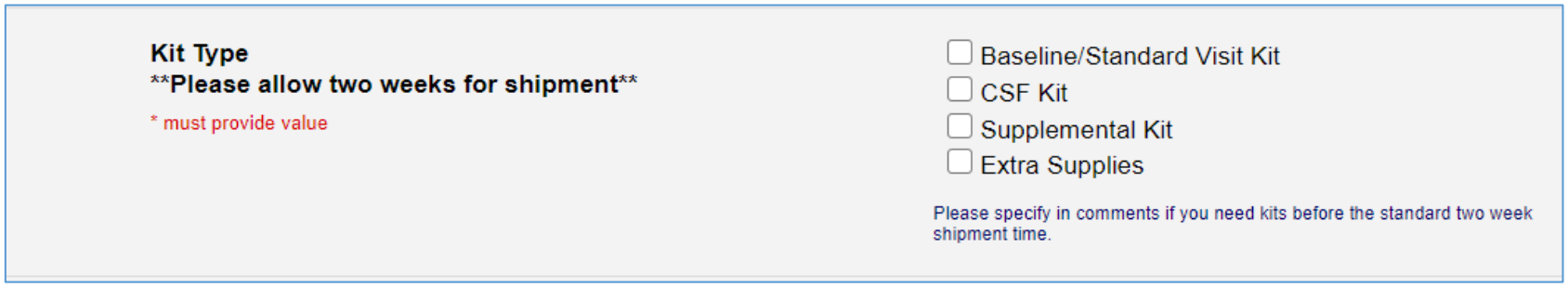
BioSEND Kit Request Module: Kit Type



Multiple kit types available

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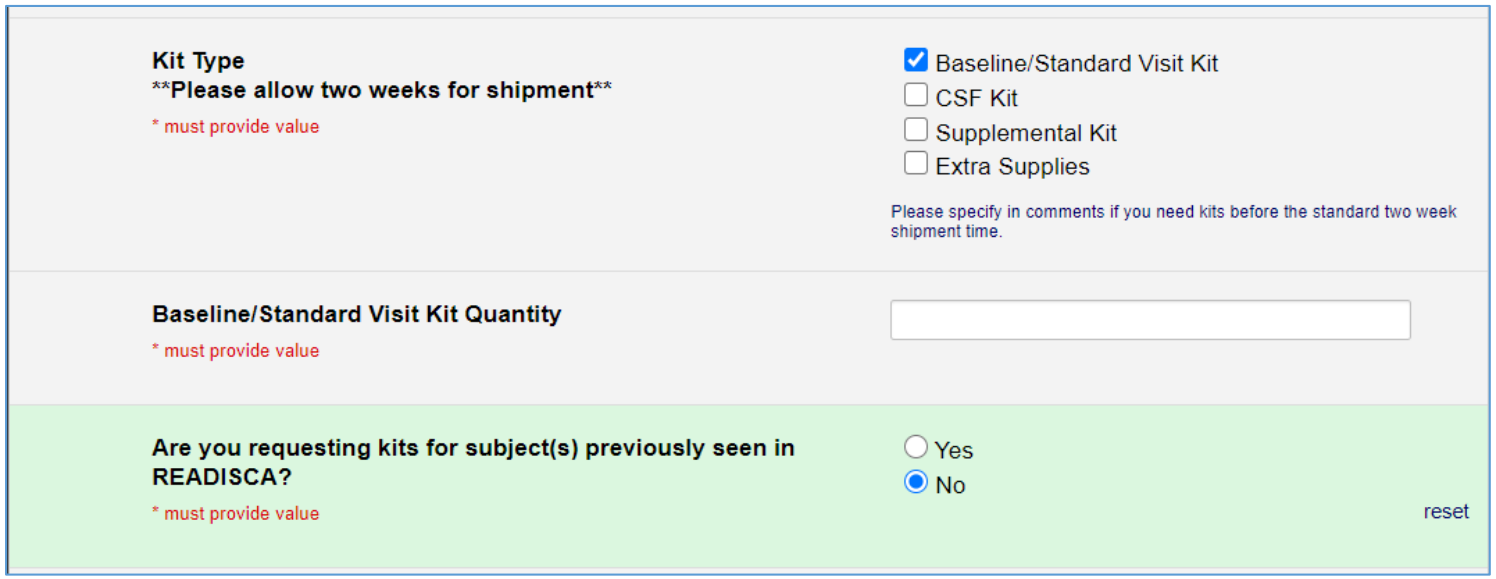
BioSEND Kit Request Module: Baseline Kit



- BioSEND creates ST-Numbers for baseline kits that serve as the biorepository subject identifier
- If subject was not seen in READISCA, BioSEND will generate a new ST-Number for your kit(s)

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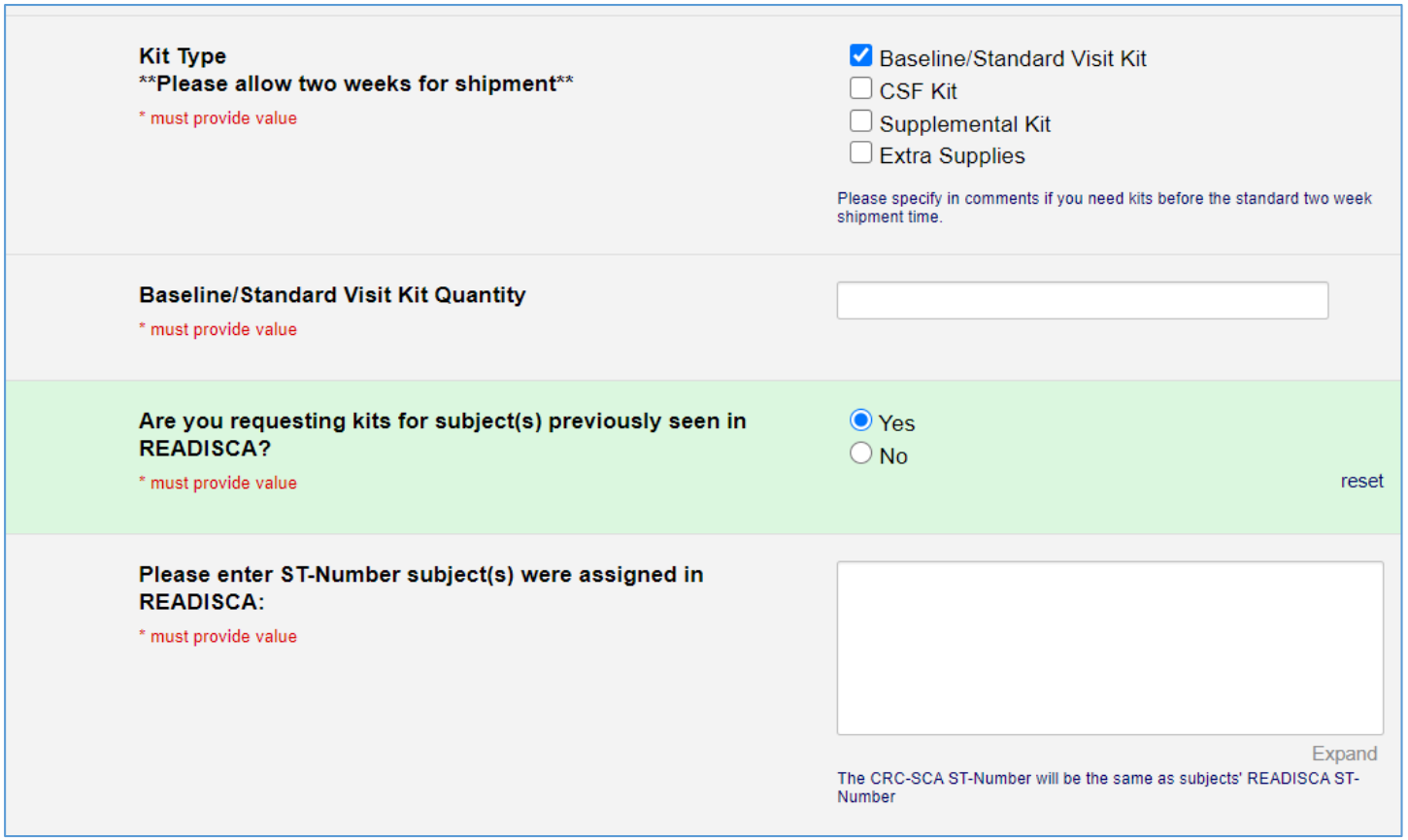
BioSEND Kit Request Module: Baseline Kit



- If subject was seen in READISCA and already has an ST-Number, the same ST-Number will be used for the subject's CRC-SCA visit

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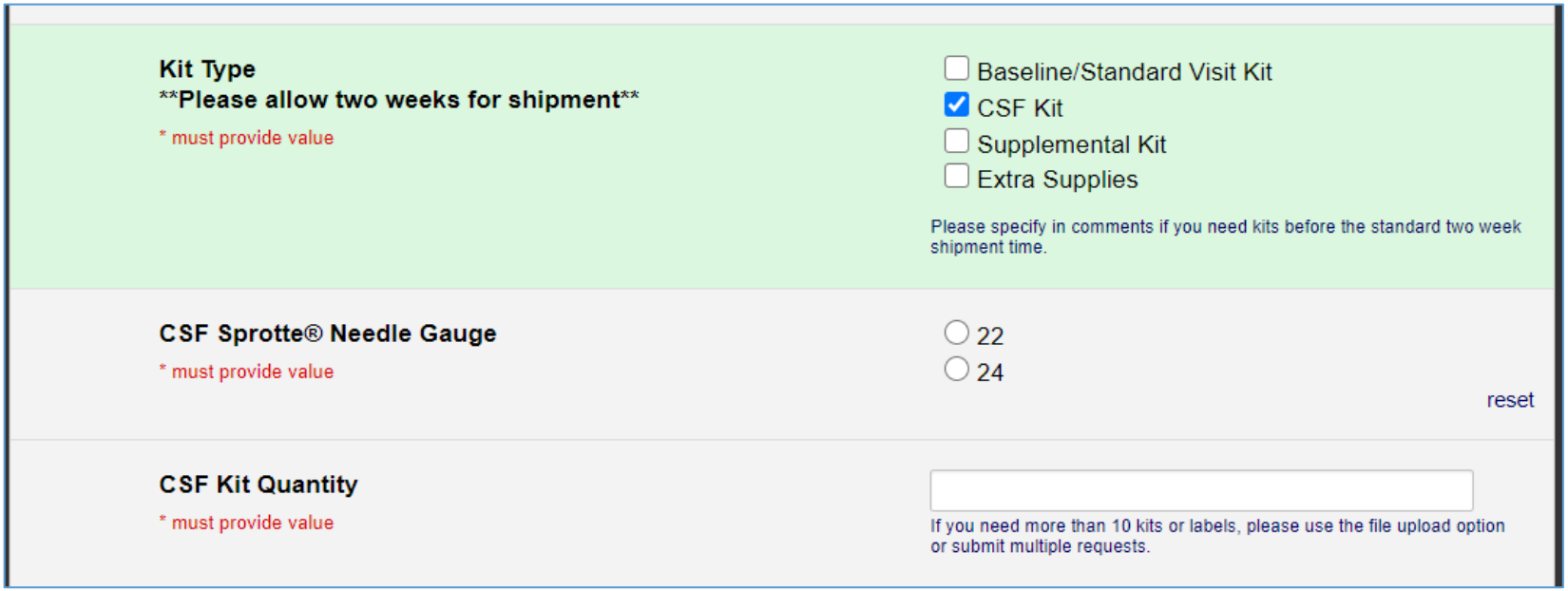
BioSEND Kit Request Module: CSF Kit



- CSF labels are included with the specimen labels in all Baseline kits

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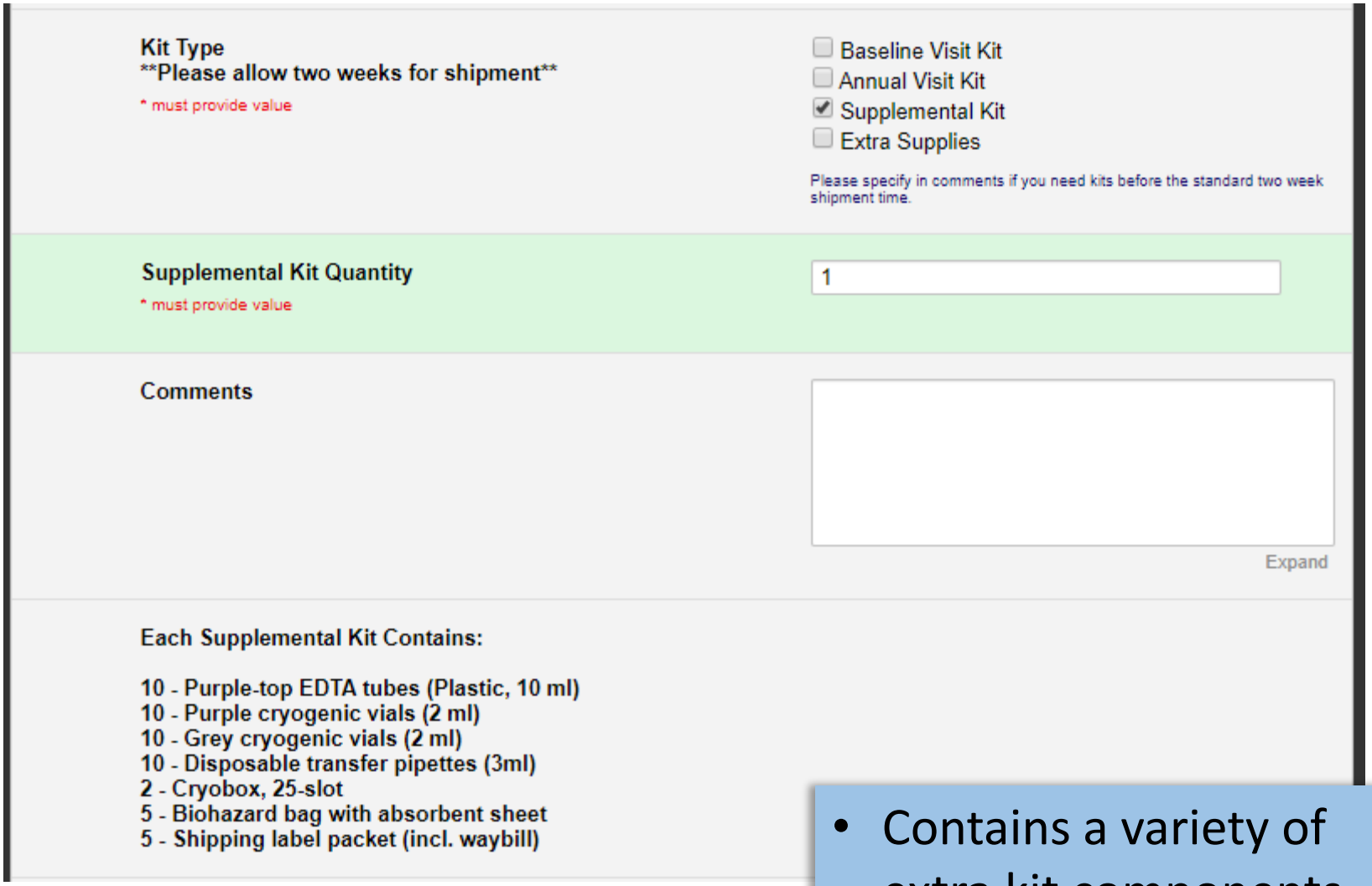
BioSEND Kit Request Module: Supplemental Kit



- Contains a variety of extra kit components

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BioSEND Kit Request Module: Extra Supplies

Kit Type
****Please allow two weeks for shipment****

* must provide value

- ☐ Baseline Visit Kit
- ☐ Annual Visit Kit
- ☐ Supplemental Kit
- ☑ Extra Supplies

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

Resealable Tube Pouches	○ 2 ○ 4	reset
Cryobox	○ 2 ○ 4	reset
Cryogenic Vial (2 ml) - Grey	○ 10 ○ 20	reset
Cryogenic Vial (2 ml) - Purple	○ 10 ○ 20	reset
FedEx® return Airbill	○ 2 ○ 4	reset
Shipping Container for Dry Ice Shipments (includes shipping labels & airbill)	○ ○	

- Allows you to choose specific supplies and particular quantities

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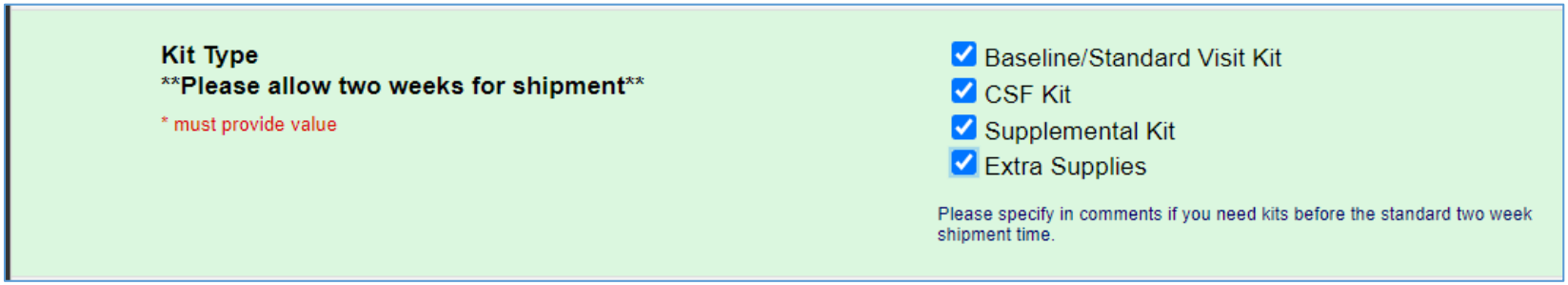
BioSEND Kit Request Module: Multiple Orders



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

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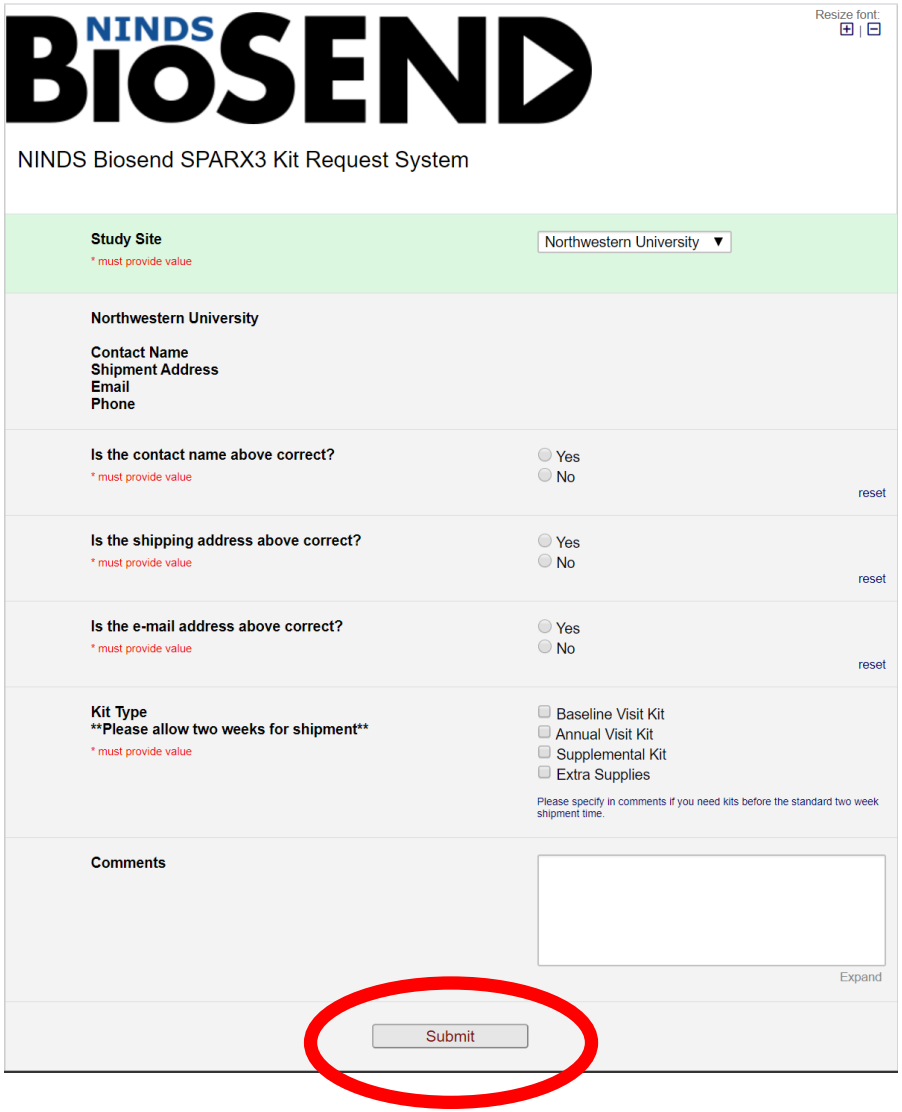
BioSEND Kit Request Module: Submit

- Click “Submit” to turn in your request.
- The BioSEND staff will notify you that your request has been received and address any issues.



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Labels

Types of Labels

Case Label

ST-10000123:
CRC-SCA:BL

BioSend

Identify study and Visit

Specimen Label

0001234567

BioSEND
ST-10001234
BL
PLASMA

For collection tubes

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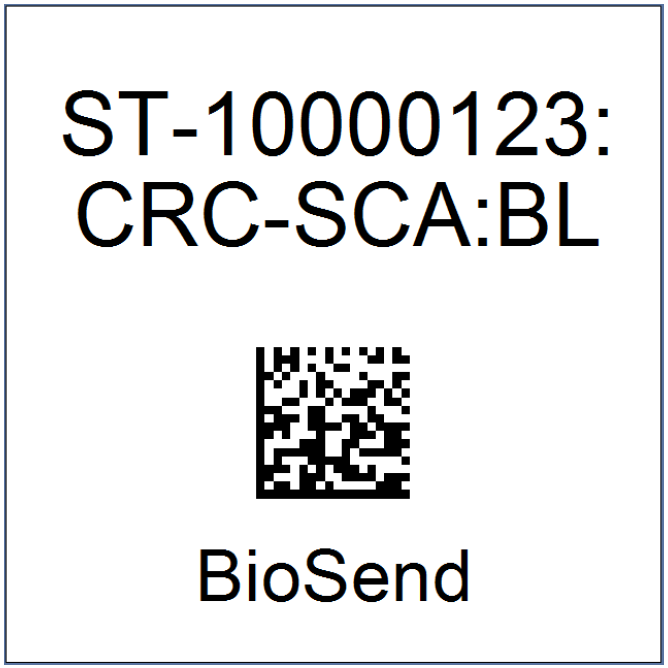
Case Label



Subject Number

Study and Visit

Biorepository Name

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Case Labels

Case labels are placed:

- On the biohazard bag of the cryobox.
- *On the lid of frozen shippers*

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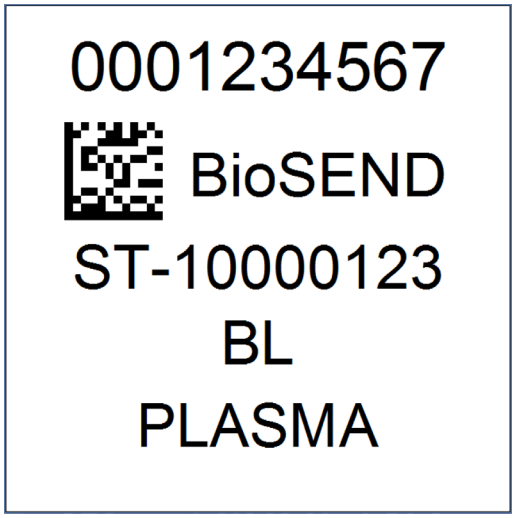
Specimen Label



Unique Barcode

Subject Number

Visit Type

Specimen Type

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Sample Collection & Processing

Reminders:

- Collection of biospecimen blood samples should be from subjects who have been *fasting for 8 hours* or more. If fasting is not feasible, follow suggested low-fat diet.

- G force ≠ RPM

- All specimens should be frozen and stored **UPRIGHT**
  - For plasma and buffy coat aliquots, please freeze samples upright in the cryobox provided

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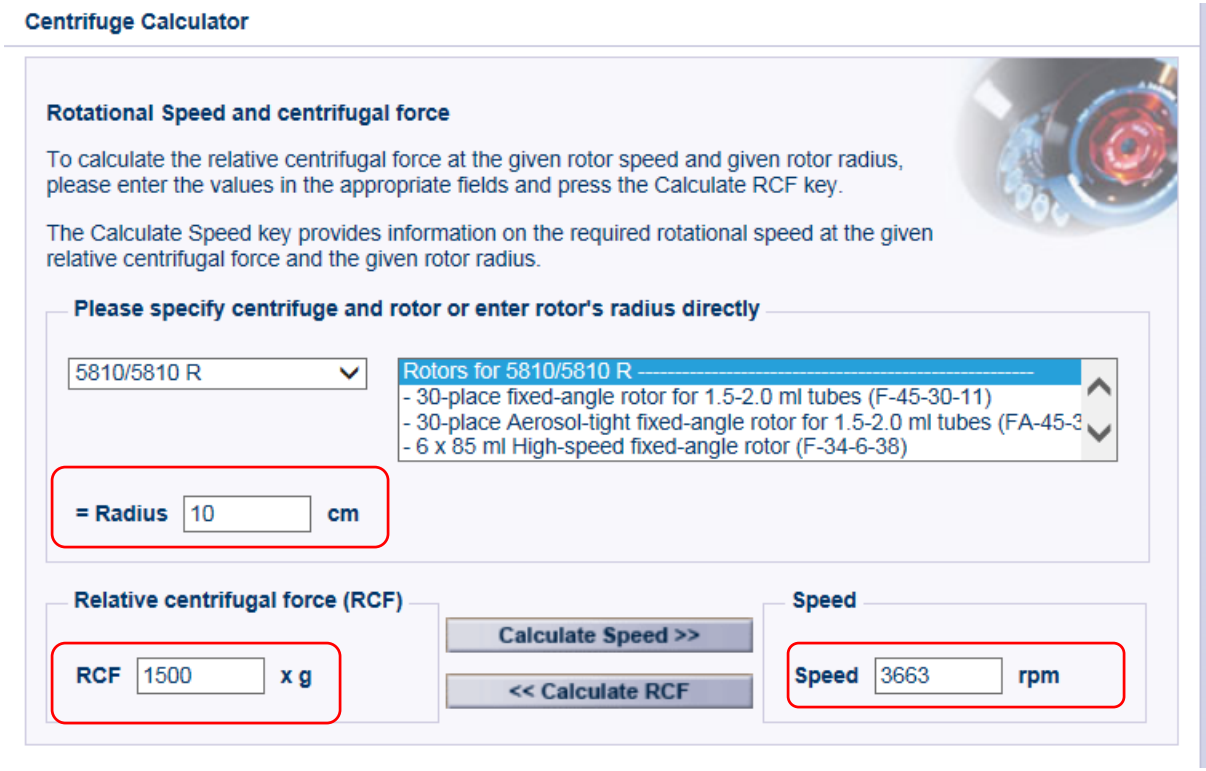
Calculating Centrifugation Speed

https://www.eppendorf.com/CA-en/centrifuge-speed-calculator/



The 3663 rpm speed was calculated using a hypothetical radius of 10 cm and a RCF of 1500 x g.

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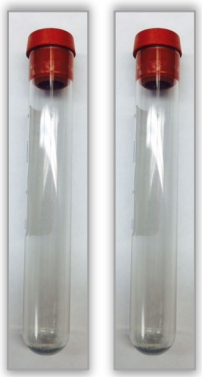
Order of Specimen Collection

1. Serum **10 ml (red top**) blood collection for serum
2. EDTA **10 ml (lavender top)** blood collection for plasma and buffy coat



Serum

EDTA, 10ml (Plasma and Buffy Coat)

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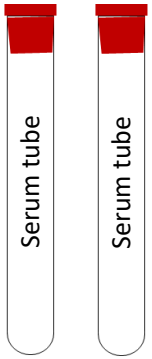
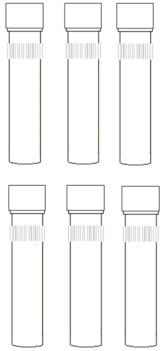
Serum Preparation –10 ml Serum (Red Top) Tube

Step One



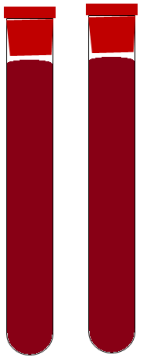
- Store tubes at room temperature.
- Label tubes with preprinted serum labels prior to blood draw.

Step Two



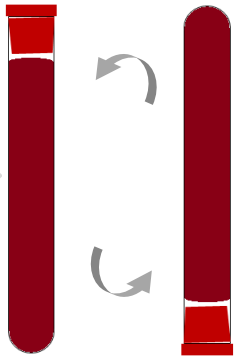
- Label 6 cryotubes with serum aliquot labels.
- Pre-chill cryotubes on wet ice for at least 5 minutes

Step Three



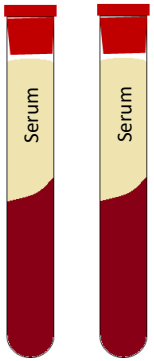
- Collect blood in serum tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Four



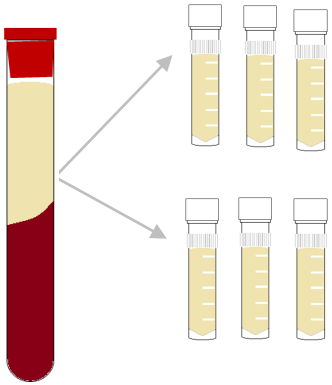
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

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.

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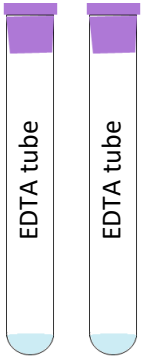
Plasma Preparation –10 ml EDTA (Purple Top) Tube

Step One

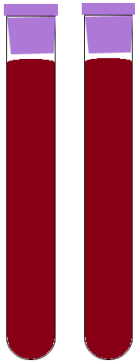


- Store tubes at room temperature.
- Label tubes with preprinted plasma labels prior to blood draw.

Step Two

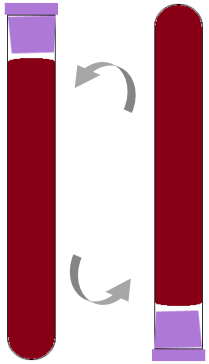
- Label 6 cryotubes with plasma aliquot labels.
- Pre-chill cryotubes on wet ice for at least 5 minutes

Step Three



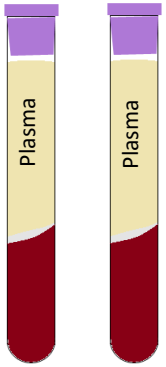
- Collect blood in EDTA tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Four



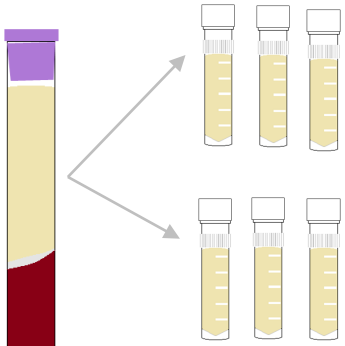
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

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.
- Store plasma aliquots upright at -80°C until shipment.

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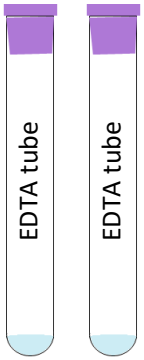
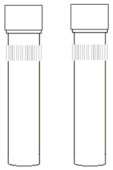
Buffy Coat Preparation –10 ml EDTA (Purple Top) Tube

Step One



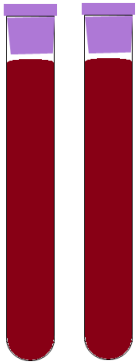
- Store tubes at room temperature.
- Label tubes with preprinted plasma labels prior to blood draw.

Step Two



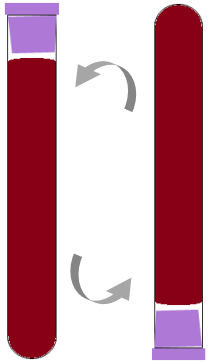
- Label 2 cryotubes with buffy coat aliquot labels.
- Pre-chill cryotubes on wet ice for at least 5 minutes.

Step Three



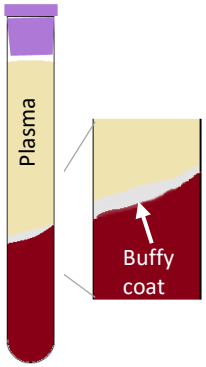
- Collect blood in EDTA tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Four



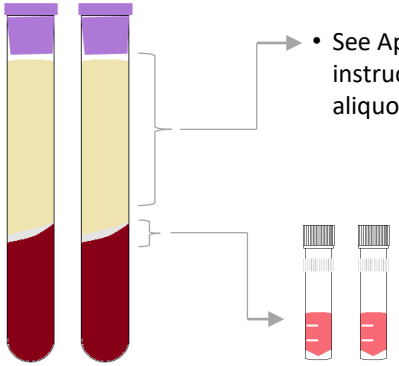
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

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, collect the buffy coat (may contain residual plasma and RBCs).
- Transfer the buffy coat from one EDTA into a cryotube. Transfer the buffy coat from the second EDTA into a second cryotube.
- Store buffy coat aliquots upright at -80°C until shipment.
- See Appendix B for instruction on aliquoting plasma

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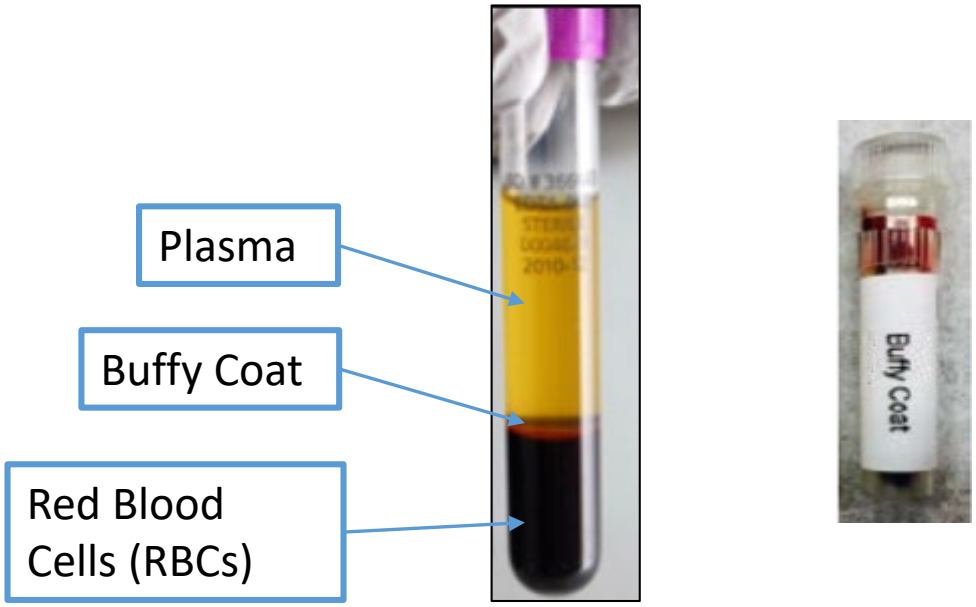
Buffy Coat Collection



Collect the buffy coat layer using the transfer pipet provided. Residual plasma as well as some RBCs will be included in this collection. A buffy coat will be reddish in color due to RBCs. Freeze buffy coats upright on dry ice or -80 freezer. Store in -80 freezer until shipment to BioSEND.

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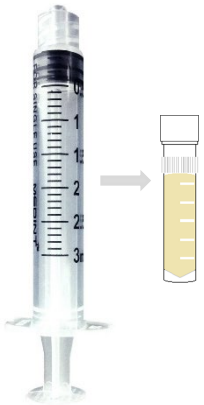
CSF Collection and Preparation

Step One

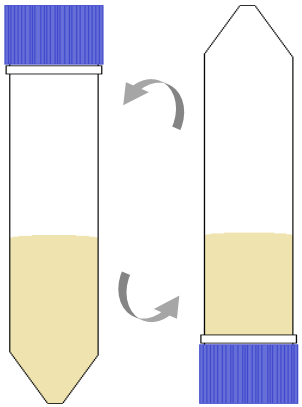


- Collect CSF into the 3ml luer lock syringe or by gravitational pull.
- Dispense 1-2 ml into cryovial.
- Send to local lab for testing, if applicable.

Step Two

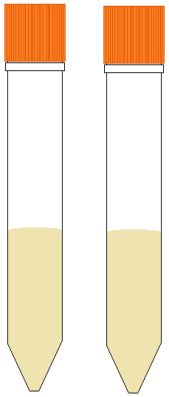
- Collect CSF into the 5ml luer lock syringe or by gravitational pull
- Transfer sample into 50 ml conical tube.

Step Three



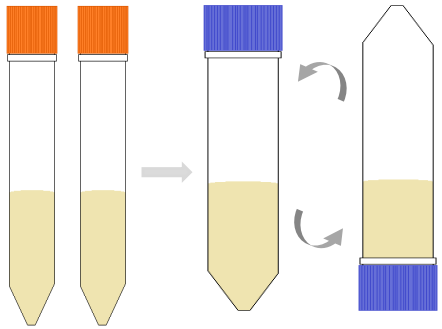
- Immediately after collection, gently invert the 50 ml conical tube 3-4 times to mix the sample.

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.

1.0ml x 10 cryovials

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Serum, Plasma, and CSF Aliquots

- Fill plasma cryovials to 1 ml
- Over-filled vials may burst in freeze
- Do NOT send residual volumes to BioSEND
- Ship material to BioSEND
  - 6 Plasma aliquots
  - 6 Serum aliquots
  - 2 Buffy Coat
  - 10 CSF aliquots

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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**

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Blood Collection: Troubleshooting

Issue #2: Hemolyzed (pink/red) plasma

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

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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

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
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)

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Sample Shipment

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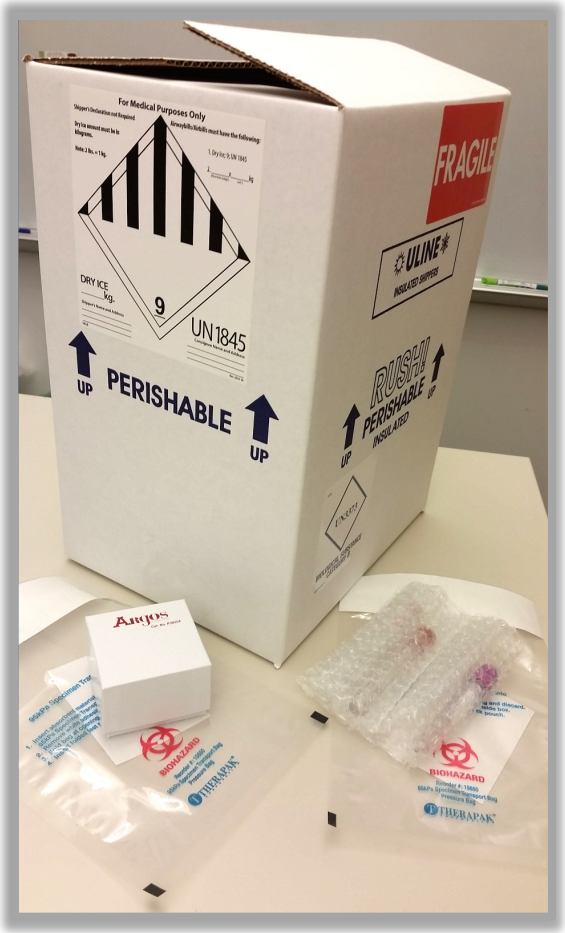
Frozen Samples

- **All samples are shipped frozen**
  - Plasma, buffy coat, and whole blood
- **Ship Monday-Wednesday Only via FedEx Priority Overnight**
- Schedule FedEx® pickup
- Email Sample Record and Shipment Notification Form including FedEx® tracking number **AHEAD OF SHIPMENT**



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Packaging and Shipping Frozen Samples

1. Place serum/plasma/buffy coat/CSF cryovials in cryobox and seal lid. Label cryobox with case label.

2. Place cryobox in biohazard bag with absorbent sheet, seal, and label with case label.

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Packaging and Shipping Frozen Samples

Place approx. 2-3 inches of dry ice in bottom of shipper

Place biohazard bags upright in box

FILL dry ice to top of box

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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 the tubes are securely placed into the bubble wrap pouch and are placed in a separate bag from the boxed aliquots.
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

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Shipping Frozen Samples

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

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

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Sample Shipment Notification Form

Sample Record and Shipment Notification

Study:

Site Name: Principal Investigator:

Coordinator: Telephone: Email:

Please list only ONE subject per Sample Record Summary and Shipment Notification Form

Clinical ID: Subject ID (ST# from pre-printed labels):

Gender: Visit Type:

Age in Years: Plus Months:

Instructions: Ship Frozen Shipments Monday- Wednesday ONLY! Ambient Shipments (purple-top EDTA tube) may be shipped Monday- Thursday (preferably Monday- Wednesday) provided they are received at Indiana University within five days of collection. This form must be completed for shipment of all research samples. Notify Indiana University (email preferred) and the DMR in advance of shipment using contact information below. Place a copy in the shipment box and file a copy of the completed form in the study binder. **Ensure all frozen shipments are completely filled with dry ice.**

Date Sample(s) Shipped: FedEx Tracking Number:

In the table below, please indicate the date of specimen collection and number of tubes/aliquots submitted.

Completed by Submitter/Site			
Dates of Draw	Specimen Type	Number of Tubes/ Aliquots sent to BioSEND	Notation of Problems
	DNA		
	RNA		
	Buffy Coat		
	Plasma		
	Serum		
	CSF		
	Whole Blood		

Contact Information: Indiana University; Email: biosend@iu.edu **Ph:** 317-278-0594

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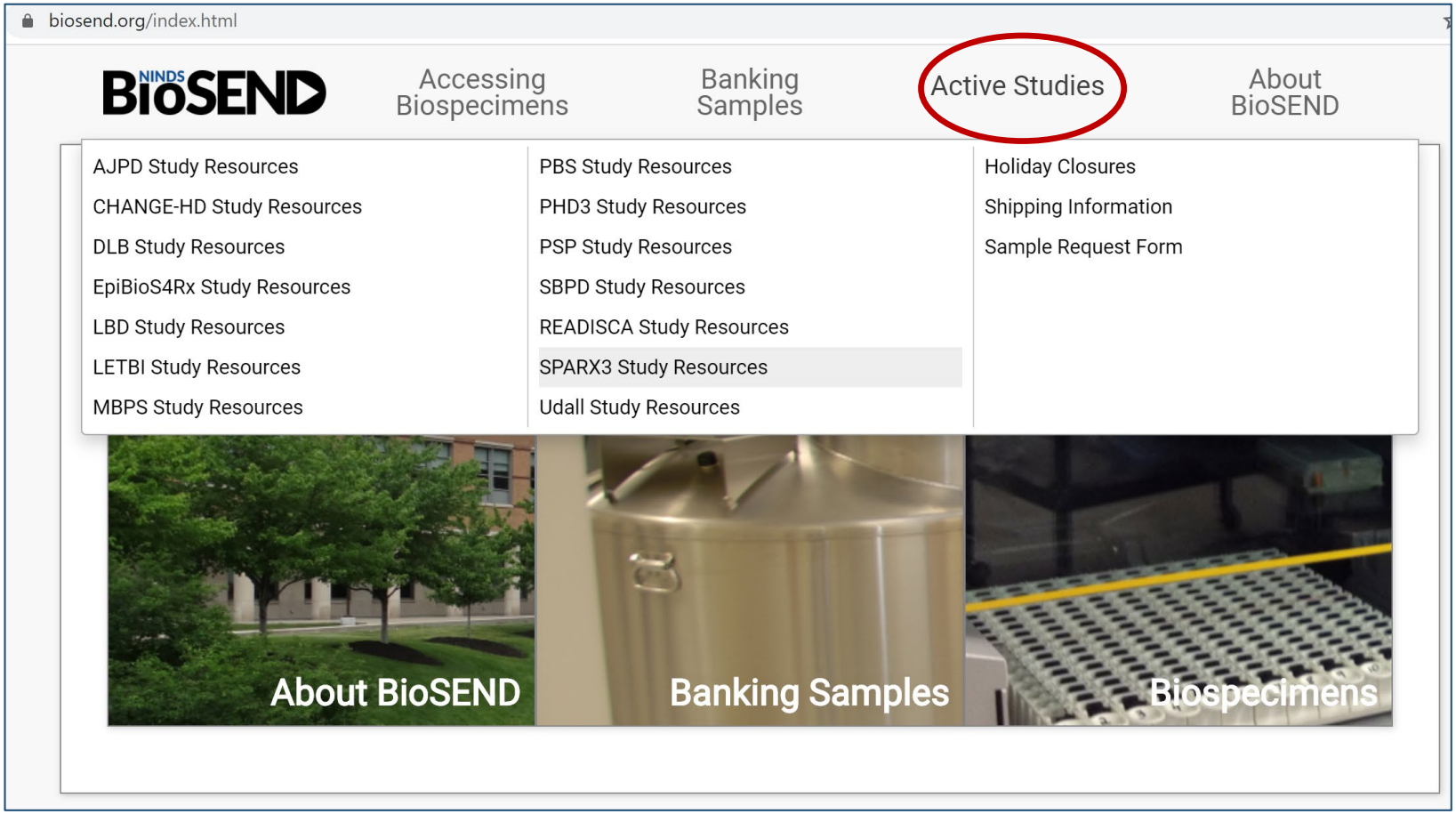
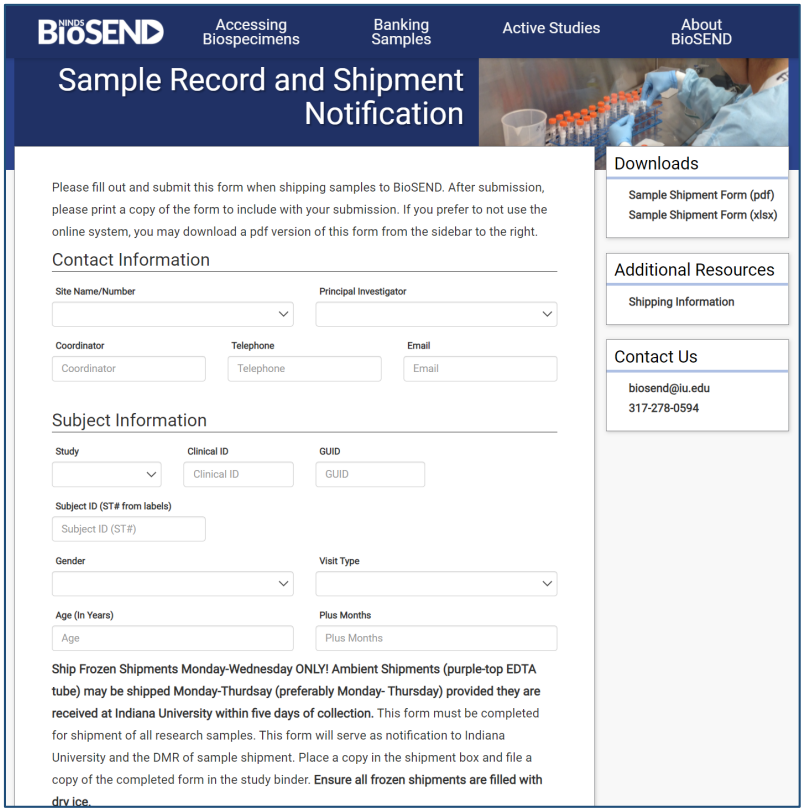
NINDS BioSEND Website

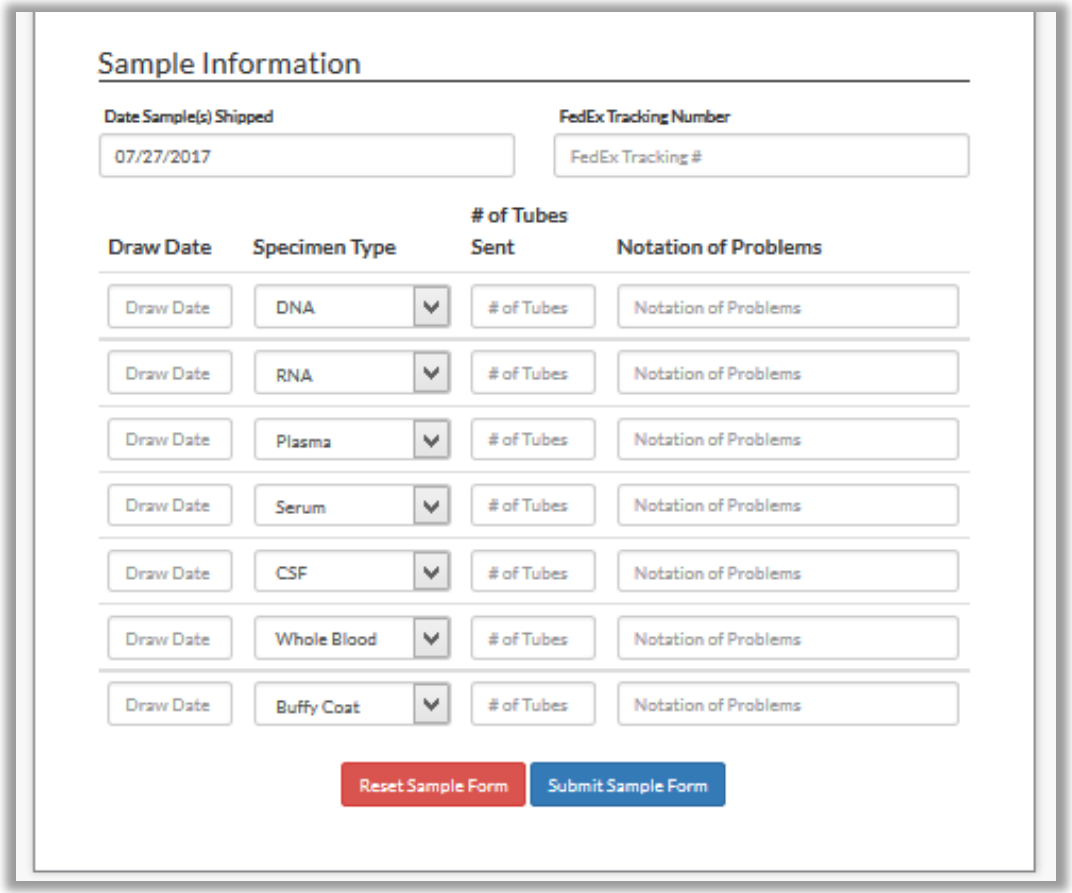


https://www.biosend.org/

BioSEND

Sample Shipment Notification Form, Online





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Holiday 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 Day

Note that BioSEND has extended closures surrounding the Thanksgiving, Christmas, and New Year's Holidays. BioSEND will send notification of these dates prior to the holiday season.

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BioSEND Contact Information

- Questions?

  Please contact:

  - General Email: biosend@iu.edu
  - Project Manager: Claire Wegel (cwegel@iu.edu)
  - Coordinator: Carolyn Dunifon (cdunifon@iu.edu)

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Questions?

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