BioSpecimen Exchange for Neurological Disorders (BioSEND)

Dissecting Oligogenic Biomarkers in Ashkenazi Jews with Parkinson's Disease

Training Webinar- AJPD

NINDS BioSEND

BioSEND Training Webinar Overview

1. Study Reminders
2. Site Equipment
3. Biospecimen Collection Protocol
4. Study Visit Protocol
5. 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
- Crushed ice
- 4°C Centrifuge
- -80°C Freezer
- Dry ice

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Biospecimen Collection Protocol

All specimen types FROZEN

	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 15ml)	X	X	X	X	X

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Study Visit Protocol

- After subject completes baseline visit, BioSEND sets up automated kit sending schedule for subject's subsequent visits
- Schedule gives 2 month window around the longitudinal study visit target (1 month on either side)
- BioSEND will send kits prior to start of study window
  - Reduces effort for study coordinators
  - Sites only need to order kits if visit will occur **AHEAD** of the study visit window
- All study visit target dates are determined from Baseline Visit (not from last study visit date)

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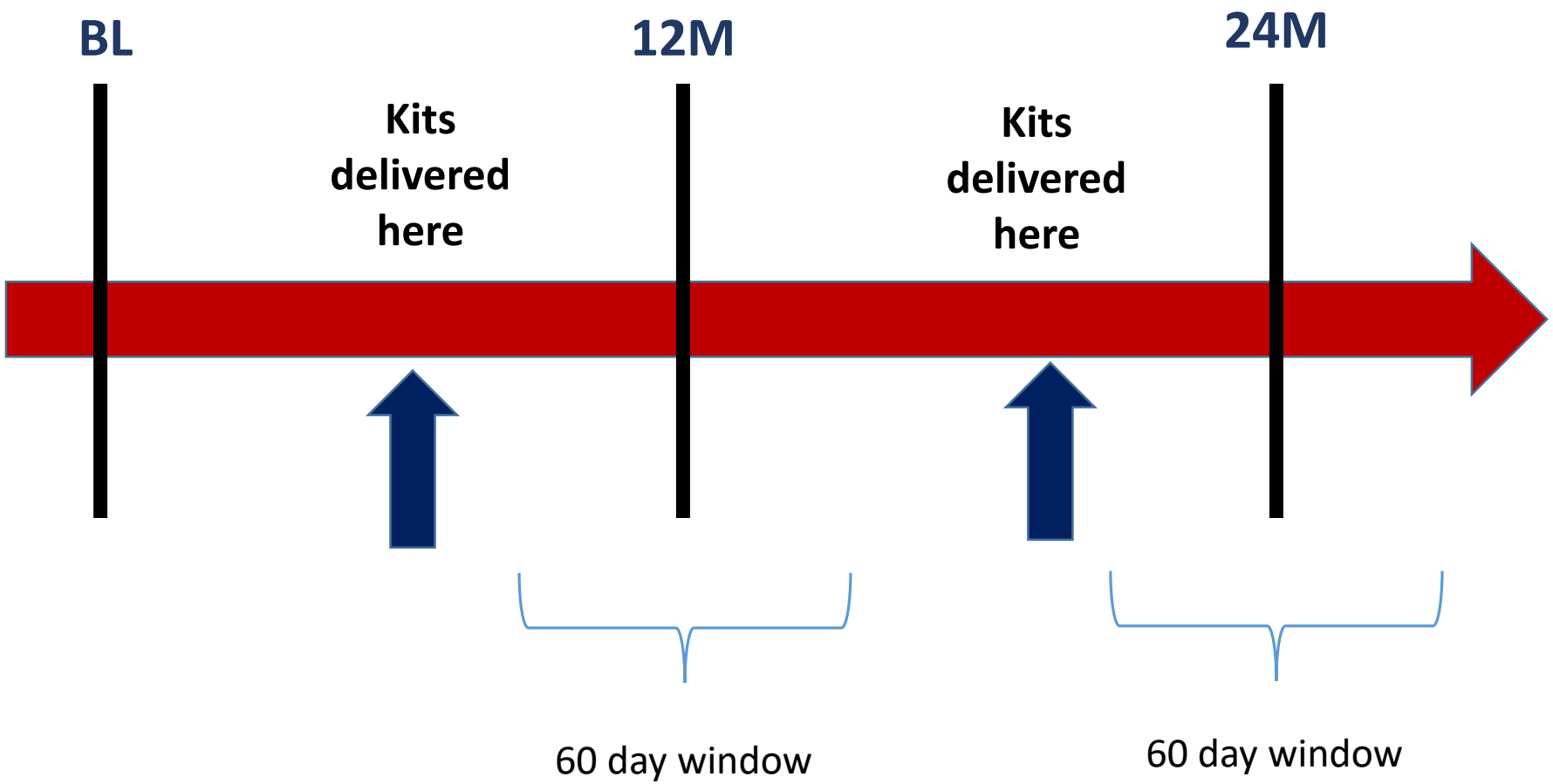
Study Visit Protocol



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Requesting Kits

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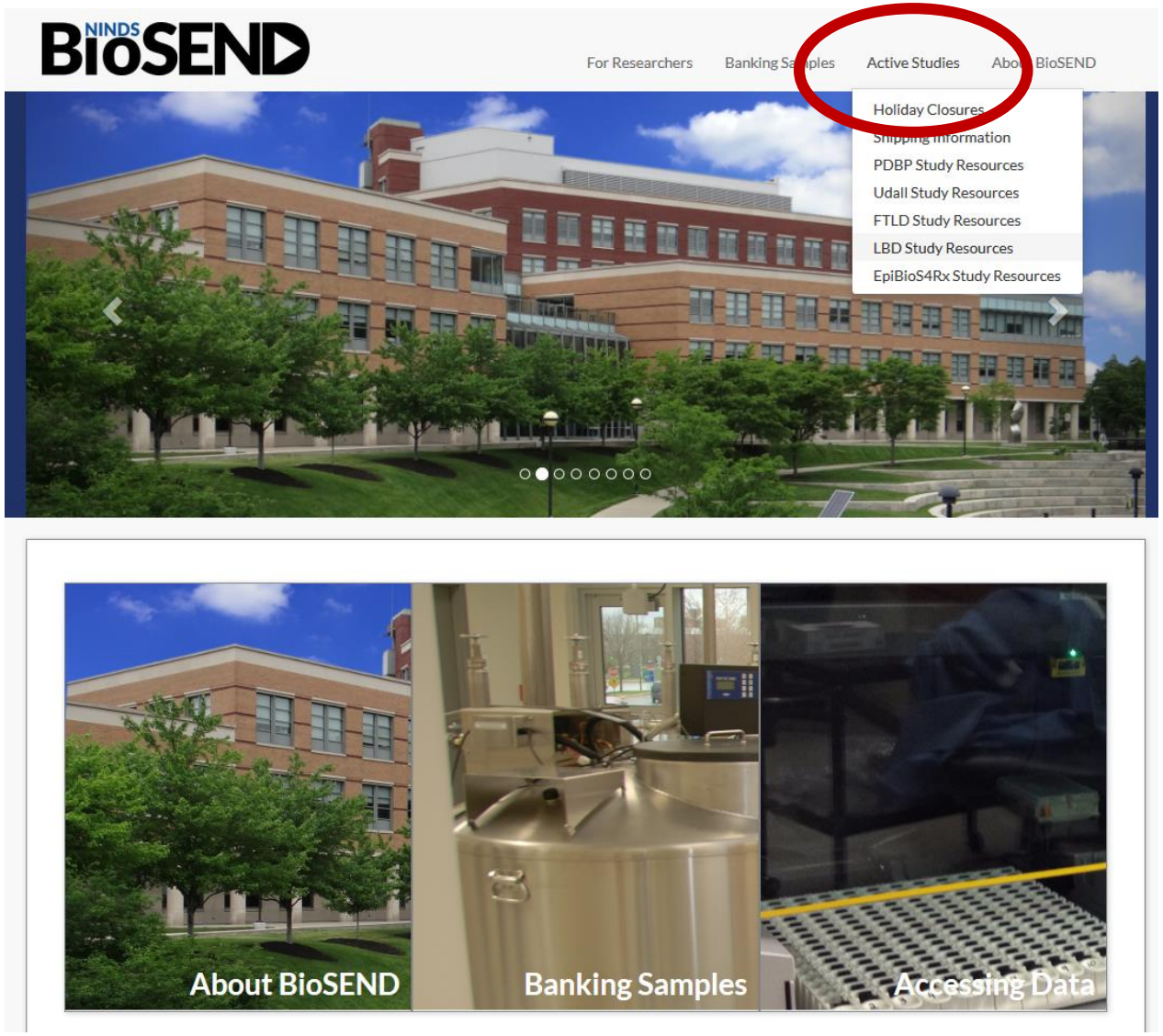
NINDS BioSEND Website



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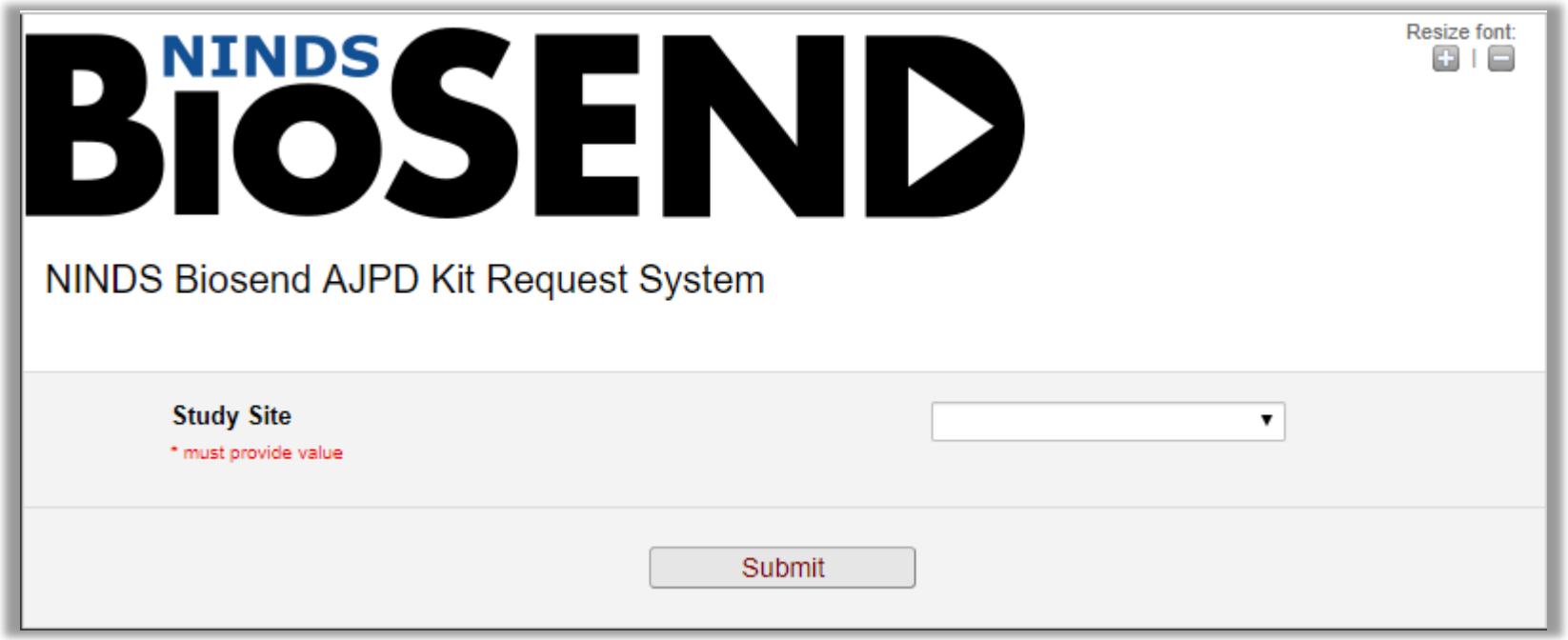
https://www.biosend.org/

BioSEND Kit Request Module



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

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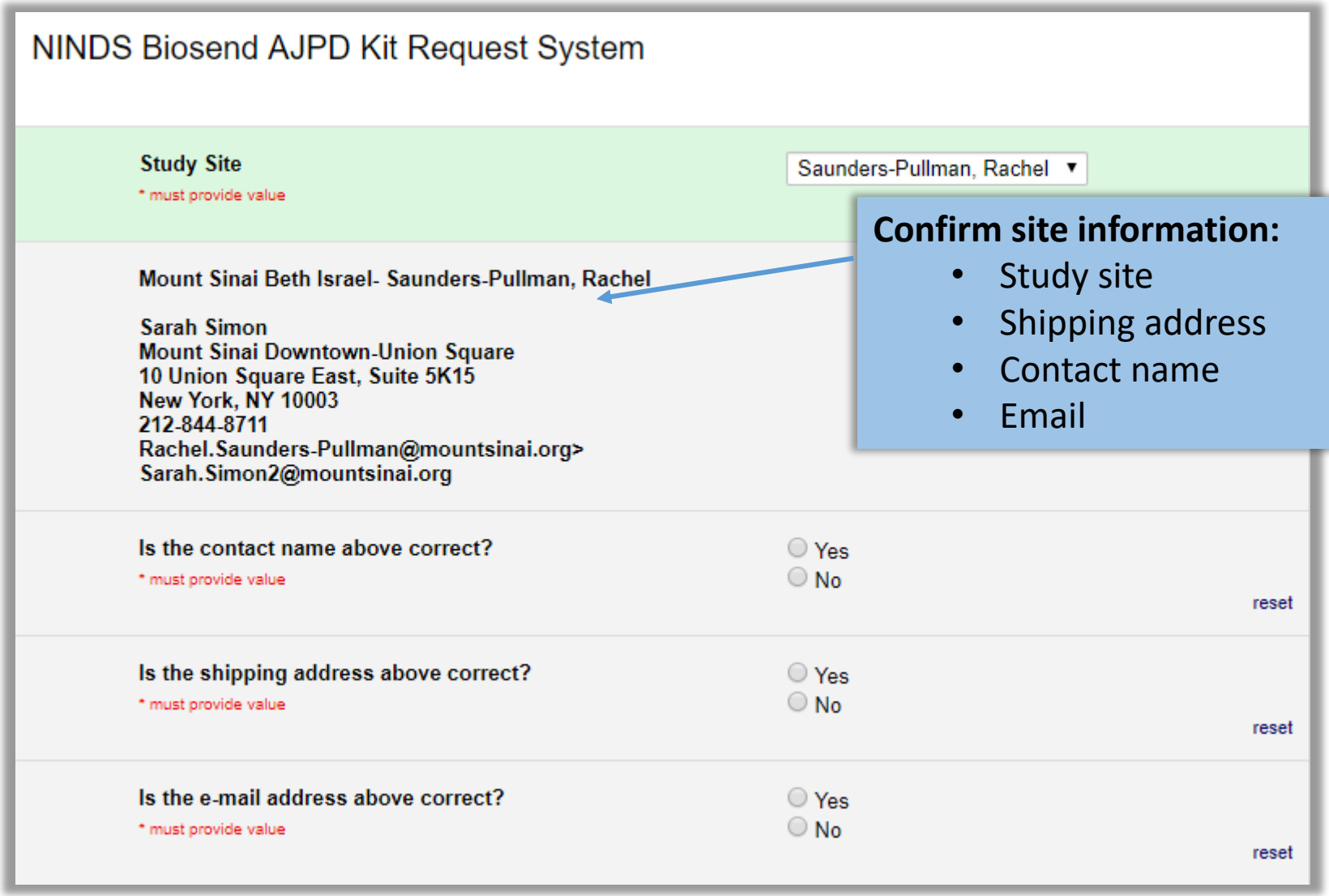
BioSEND Kit Request Module



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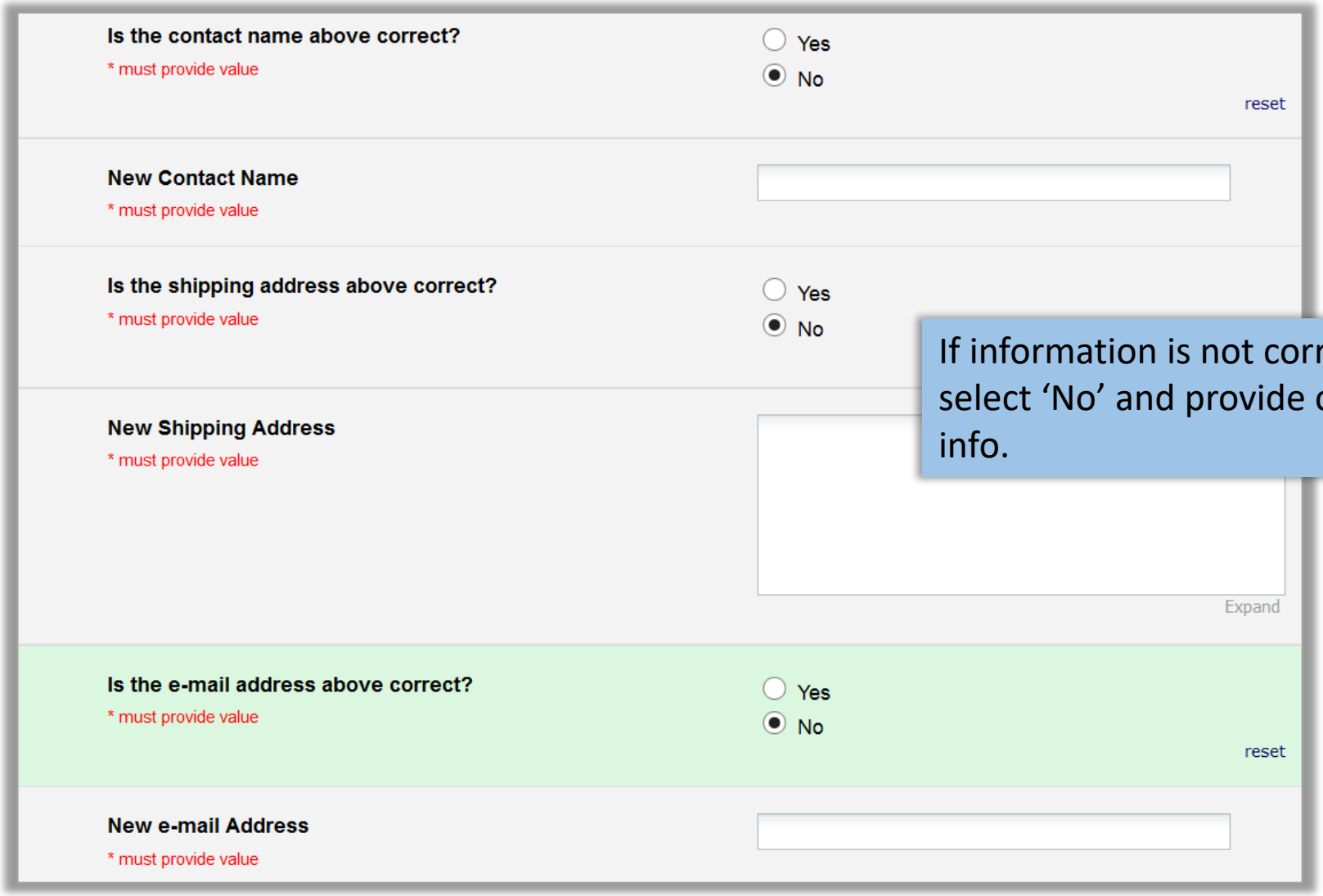
BioSEND Kit Request Module



If information is not correct, select 'No' and provide correct info.

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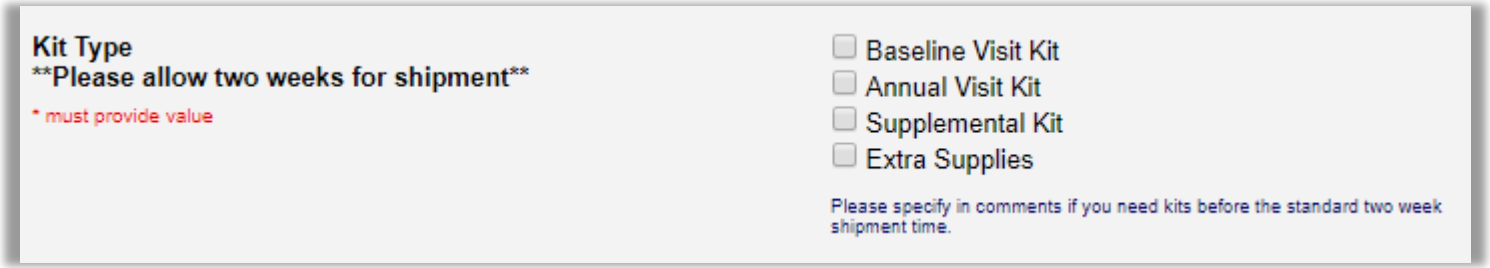
BioSEND Kit Request Module: Kit Type



Typically, you will only need to order kits for the Baseline visit

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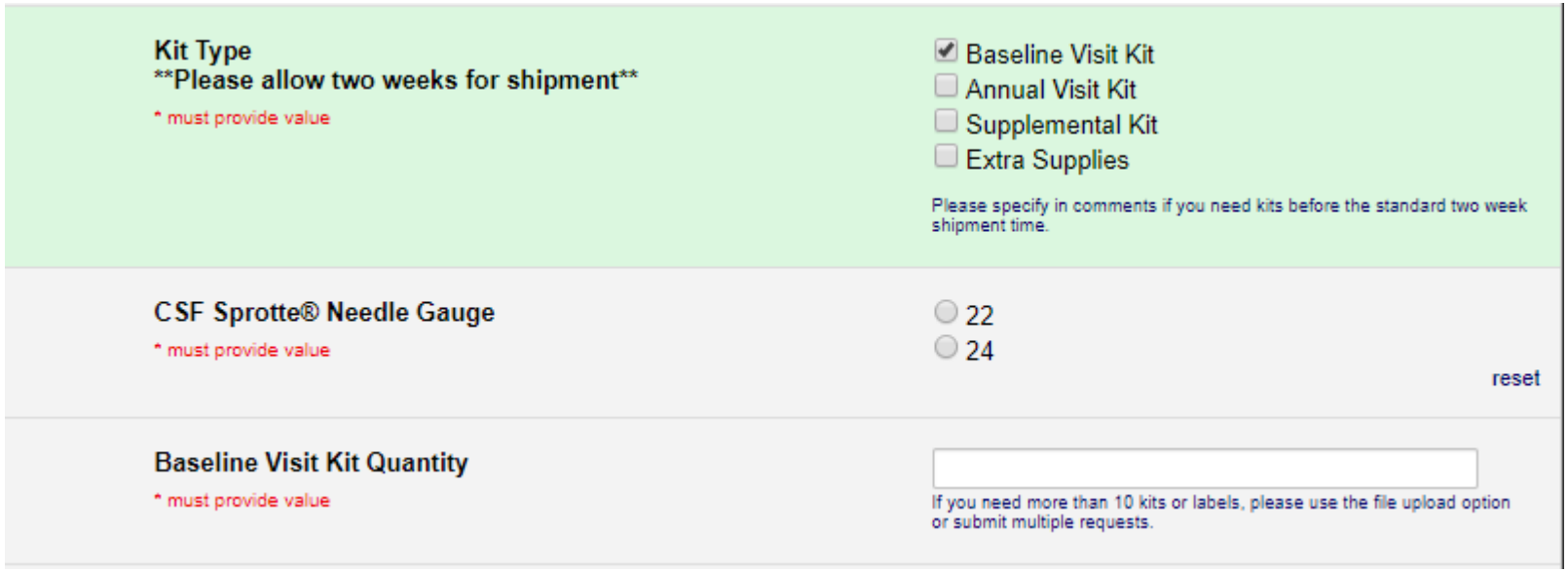
BioSEND Kit Request Module: Baseline Kit



- BioSEND creates ST numbers for baseline kits
- Enter preferred CSF needle gauge
- Enter kit quantity

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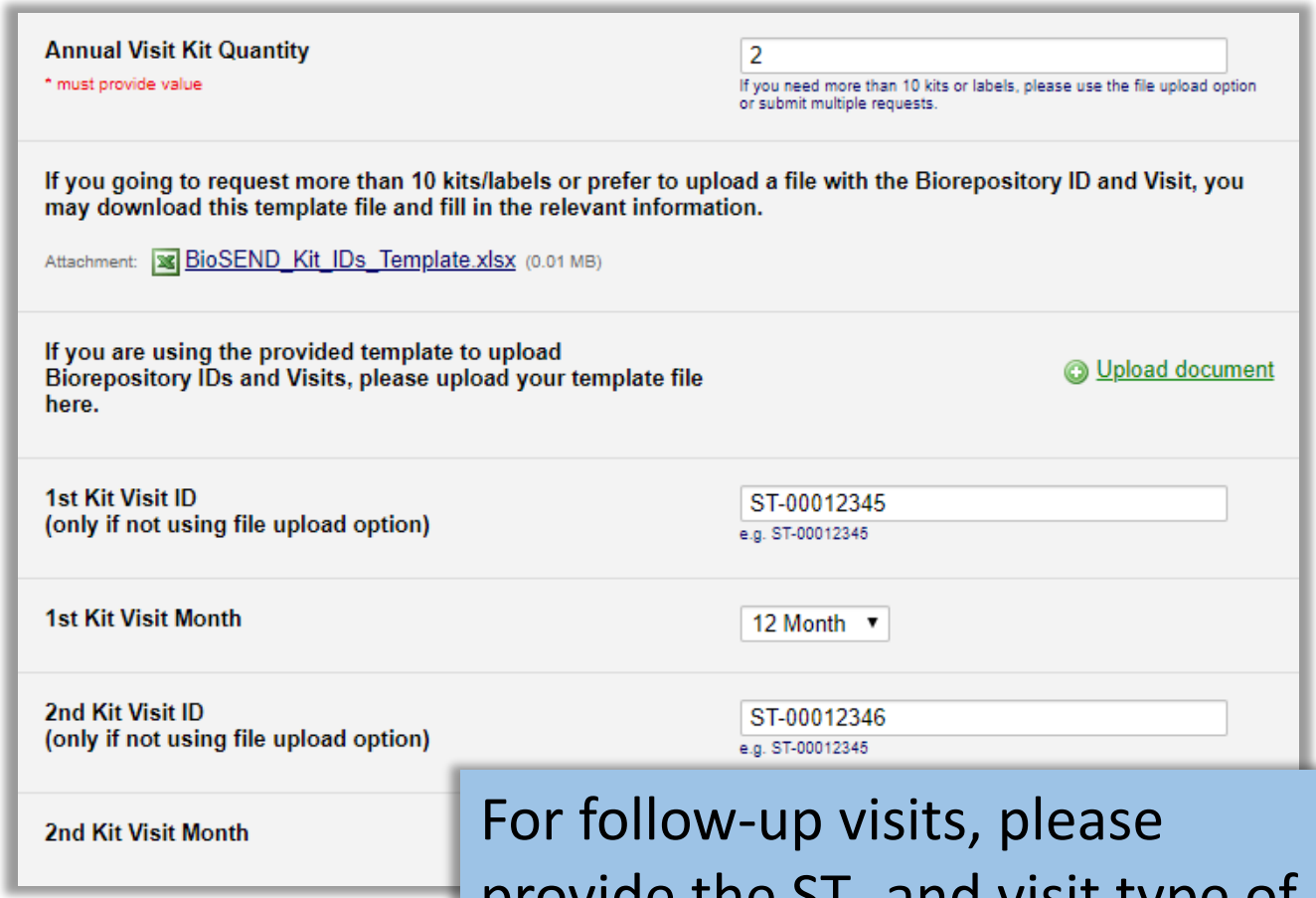
BioSEND Kit Request Module: Annual Visits

Only use this if the subject will be seen ahead of a study visit window



For follow-up visits, please provide the ST- and visit type of the kit(s) you are requesting. **We cannot complete your request without this information.**

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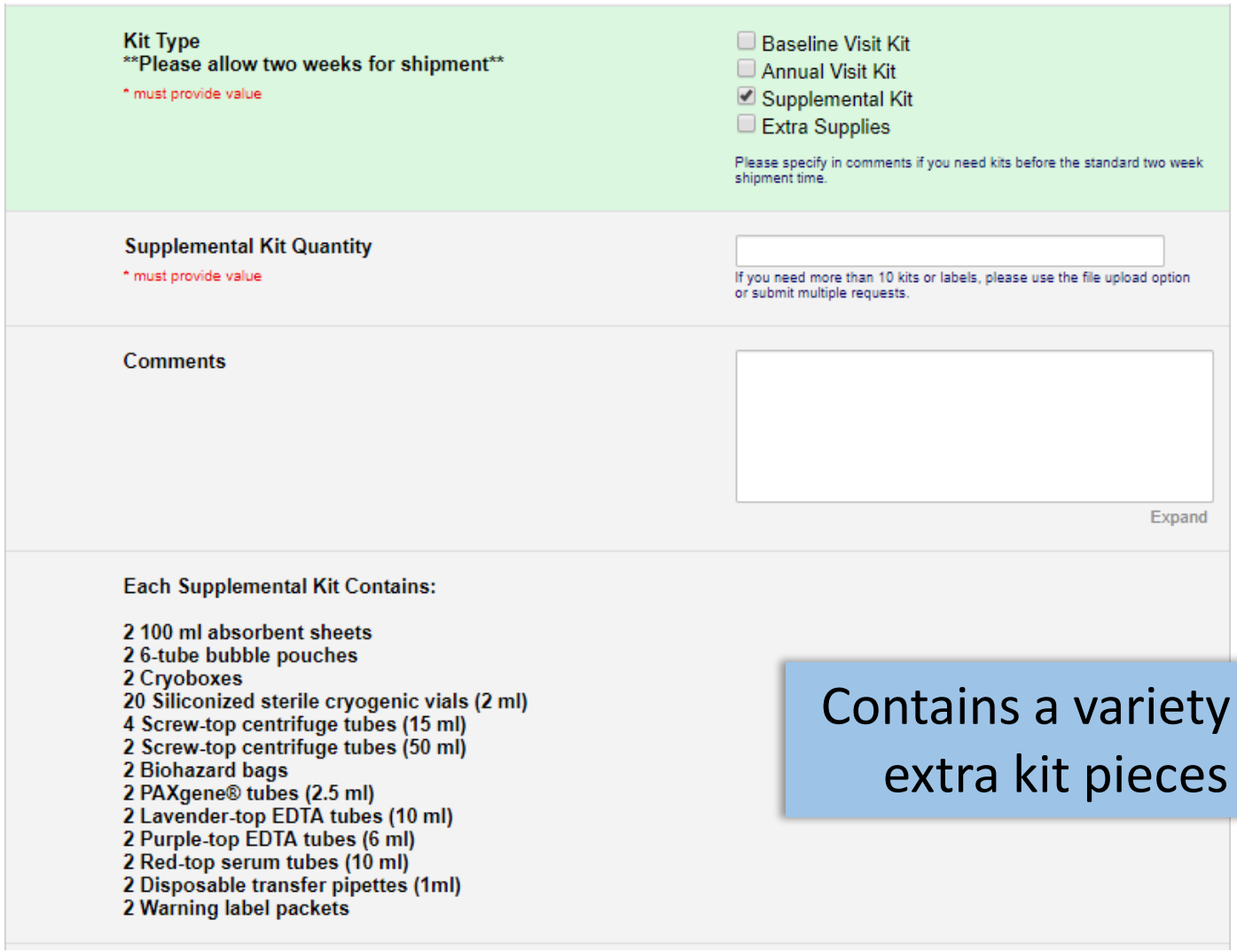
BioSEND Kit Request Module: Supplemental Kit



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.

Supplemental 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 Supplemental Kit Contains:

2 100 ml absorbent sheets
2 6-tube bubble pouches
2 Cryoboxes
20 Siliconized sterile cryogenic vials (2 ml)
4 Screw-top centrifuge tubes (15 ml)
2 Screw-top centrifuge tubes (50 ml)
2 Biohazard bags
2 PAXgene® tubes (2.5 ml)
2 Lavender-top EDTA tubes (10 ml)
2 Purple-top EDTA tubes (6 ml)
2 Red-top serum tubes (10 ml)
2 Disposable transfer pipettes (1ml)
2 Warning label packets

Contains a variety of extra kit pieces

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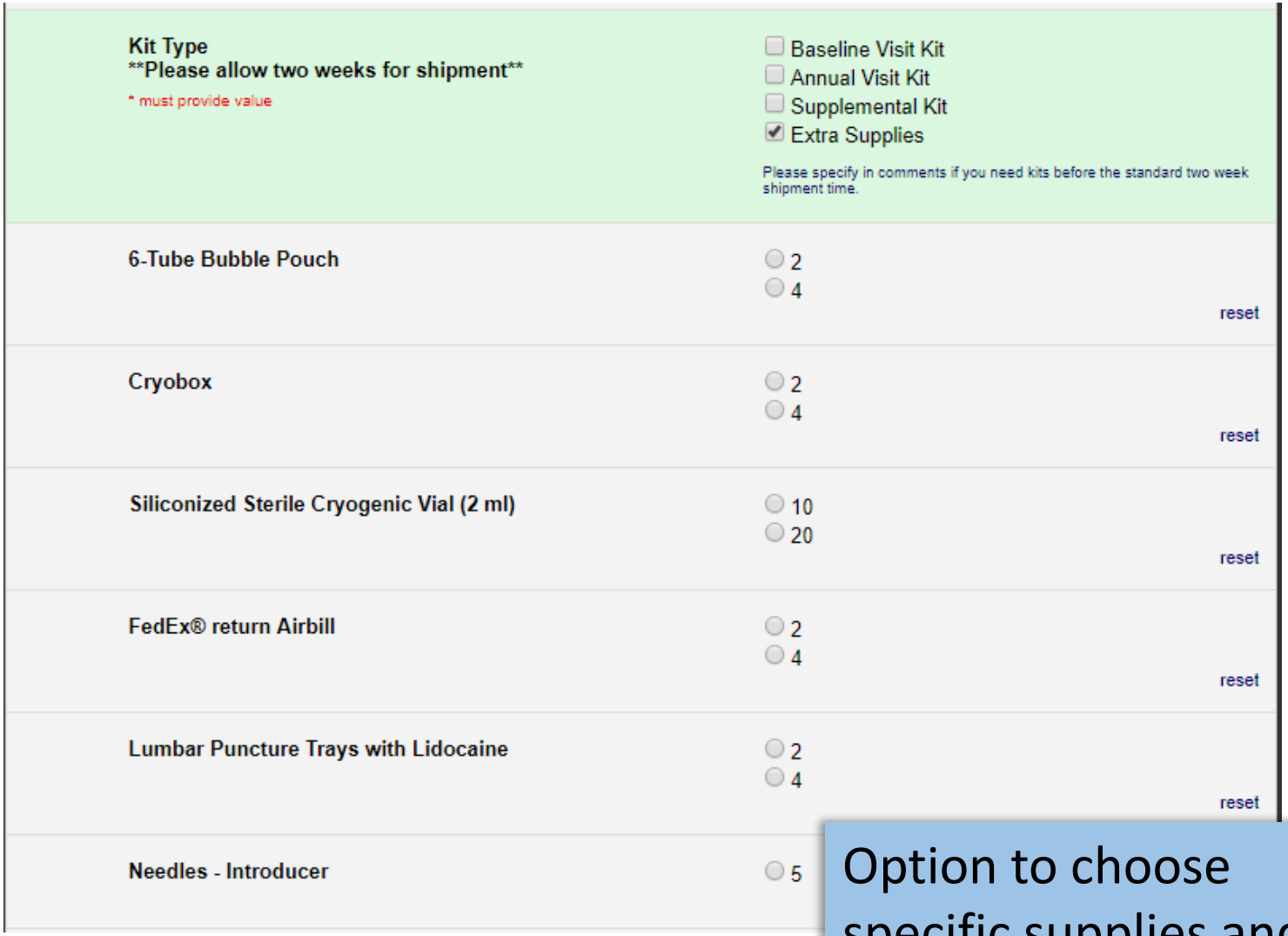
BioSEND Kit Request Module: Extra Supplies



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.

6-Tube Bubble Pouch	○ 2 ○ 4	reset
Cryobox	○ 2 ○ 4	reset
Siliconized Sterile Cryogenic Vial (2 ml)	○ 10 ○ 20	reset
FedEx® return Airbill	○ 2 ○ 4	reset
Lumbar Puncture Trays with Lidocaine	○ 2 ○ 4	reset
Needles - Introducer	○ 5	

Option to choose specific supplies and particular quantities

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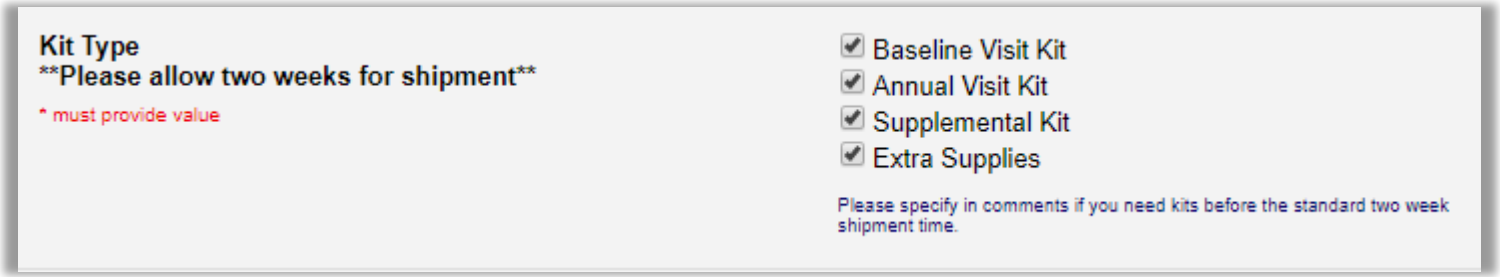
BioSEND Kit Request Module: Multiple Orders



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

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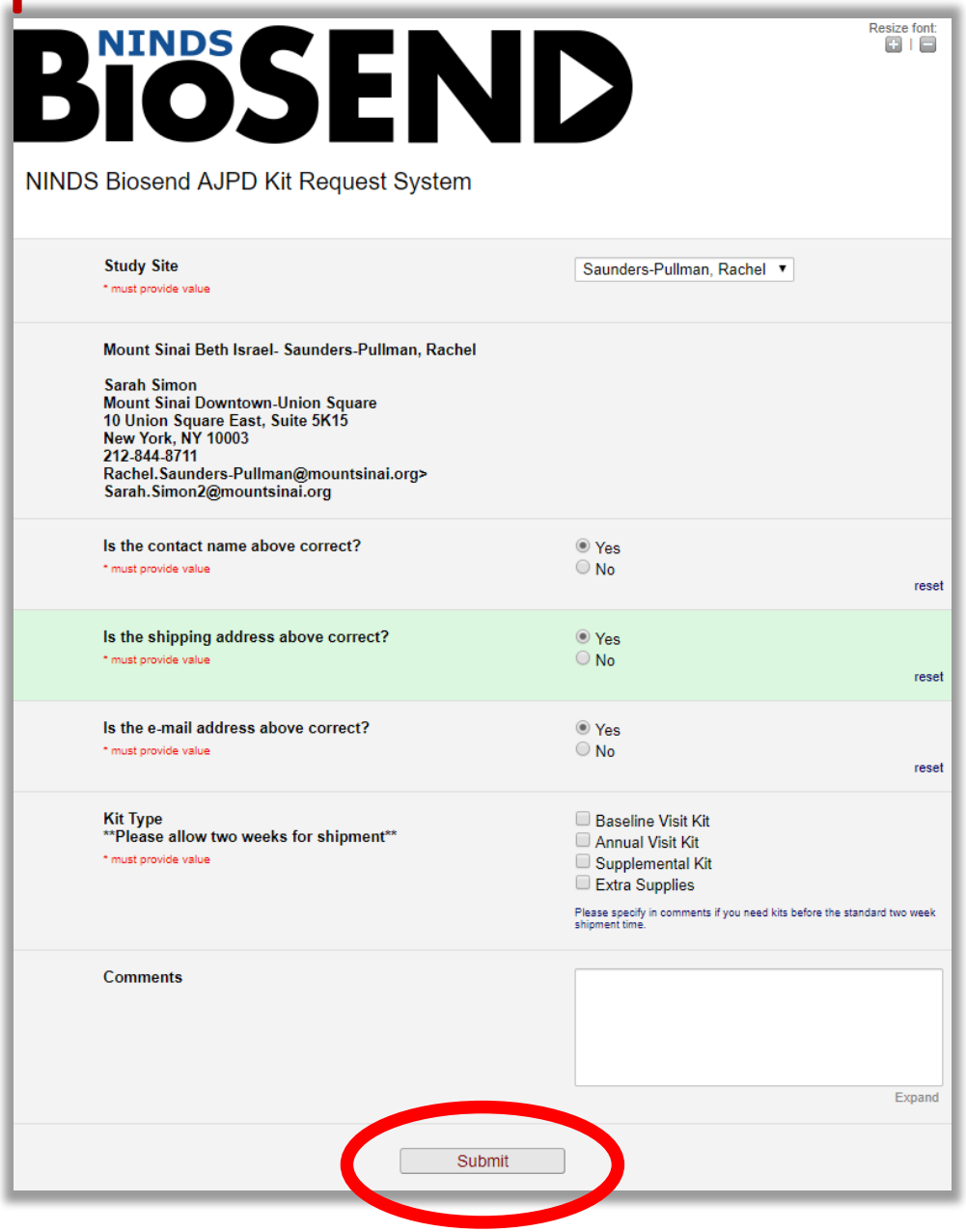
BioSEND Kit Request Module: Submit

- 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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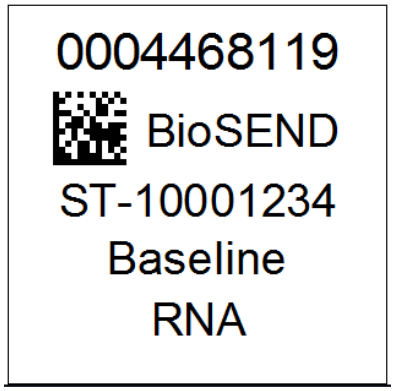
Labels

Types of Labels

Case Label

Identify study and PI

Specimen Label



Identify individual biospecimens

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Case Label

Subject Number

Study – PI

Visit

Biorepository Name

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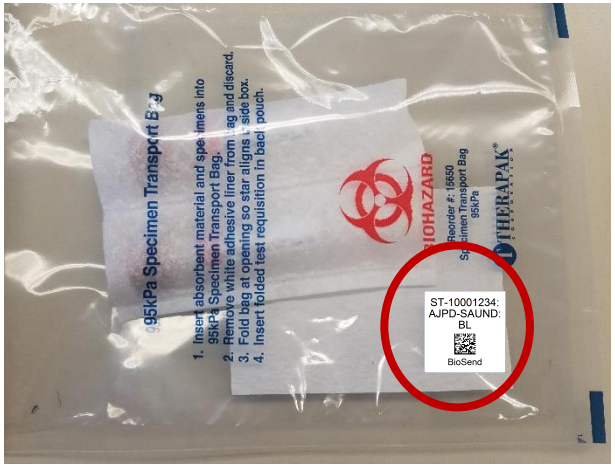
Case Labels

Case labels are placed:

- On the plastic biohazard bag of the cryovial transport box.
- On the plastic biohazard bag for the PAXgene® tubes.
- *On the lid of frozen shippers*



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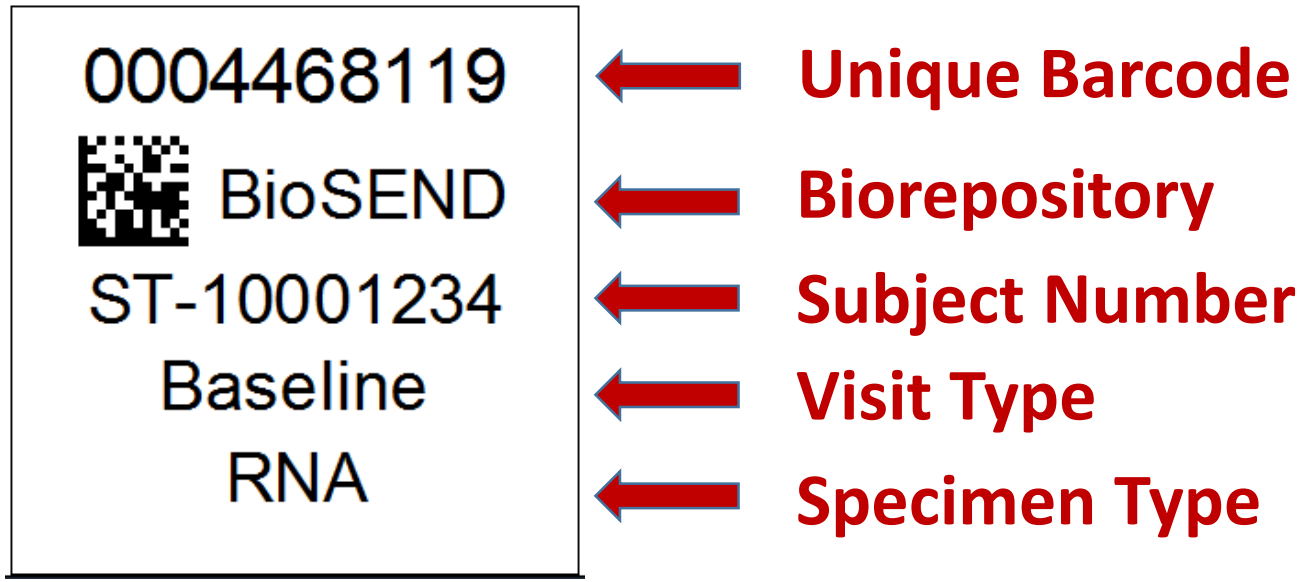
Collection and Aliquot Tube Label



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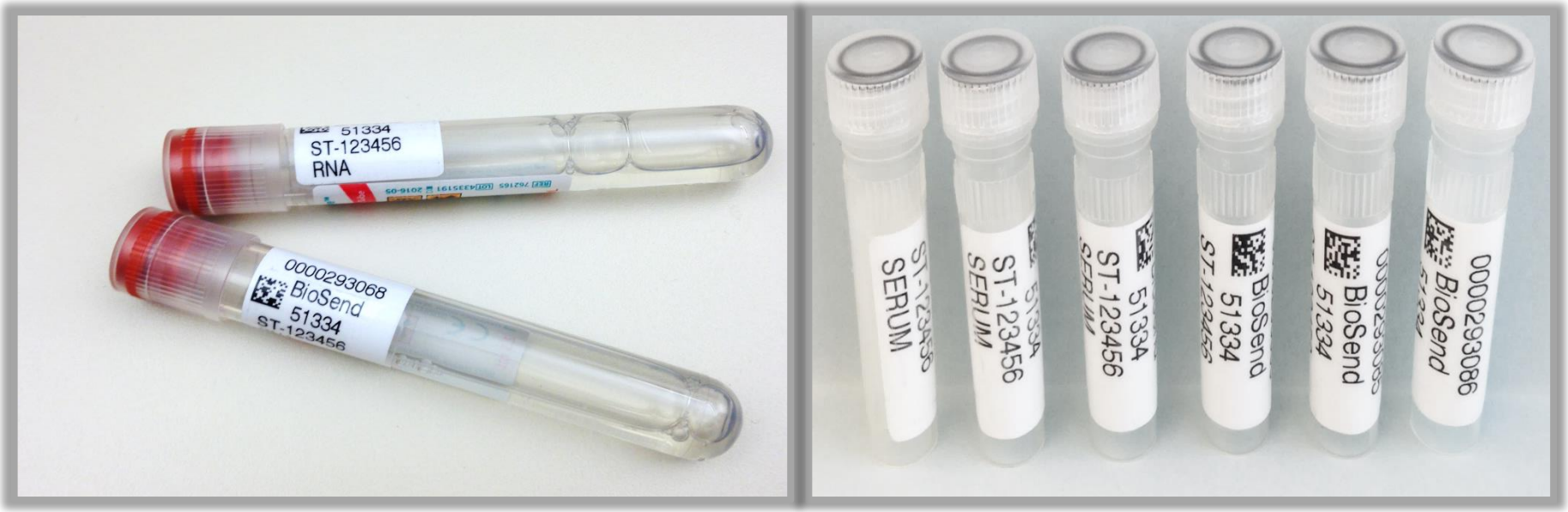
Collection and Aliquot Tube Label

Collection and Aliquot Tube Labels are placed on:

- All collection tubes
- All aliquot tubes (cryovials)

Be sure that the tube has the correct specimen type on the label



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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 RNA and whole blood specimens, please freeze samples upright in a non-styrofoam rack
  - For plasma, buffy coat, serum, and CSF aliquots, please freeze samples upright in the cryobox provided

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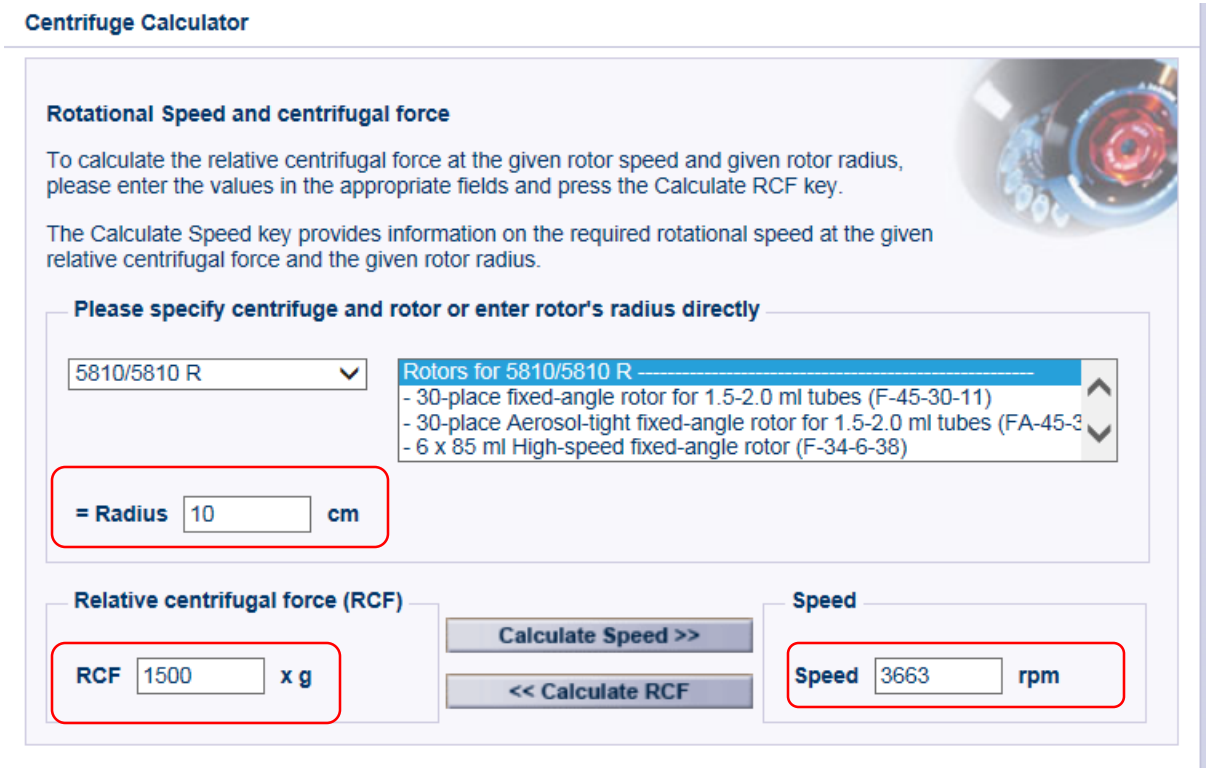
Calculating Centrifugation Speed

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. PAXgene® tube for RNA
3. EDTA **10 ml (lavender top**) blood collection for plasma and buffy coat
4. EDTA **6 ml (purple top)** blood collection for frozen Whole Blood

Serum

PAXgene (RNA)

EDTA (Plasma)

EDTA (WB)

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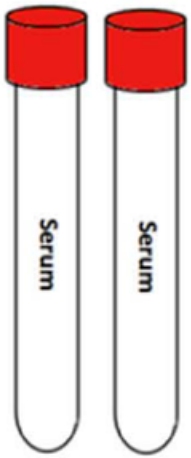
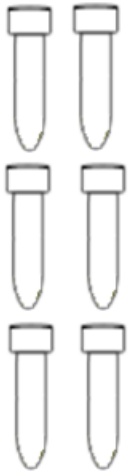
Serum Preparation (10ml Red Top Tube)

Step One



- Store tubes at room temperature.
- Label 2 tubes with pre-printed subject labels prior to blood draw.

Step Two

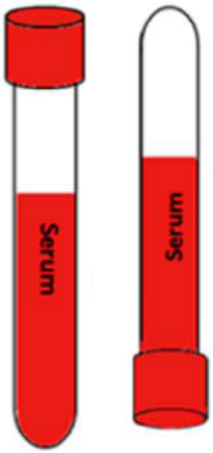


- Label 6 cryovials with pre-printed subject labels prior to blood draw.
- Pre-chill cryovials on wet ice for 5 minutes or longer.

Step Three

- Collect blood in Serum Tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

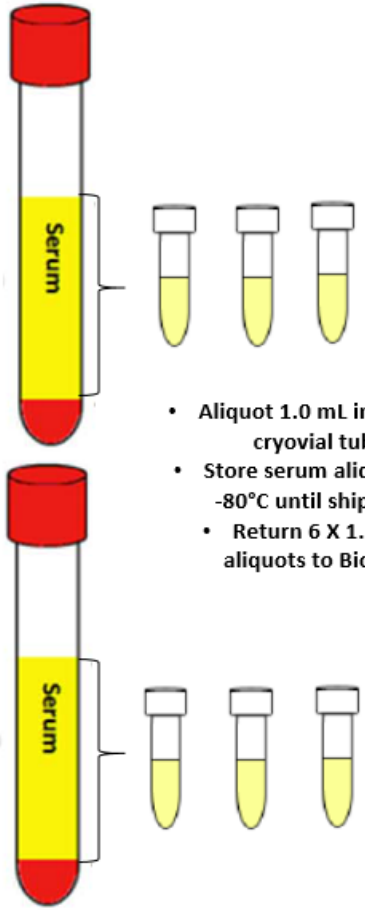
Step Four



- Immediately after blood draw, invert tubes 8-10 times to mix samples.
- ***Repeat steps three and four for second tube.***

Step Five

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



- Aliquot 1.0 mL into each cryovial tube.
- Store serum aliquots at -80°C until shipment.
- Return 6 X 1.0 mL aliquots to BioSend

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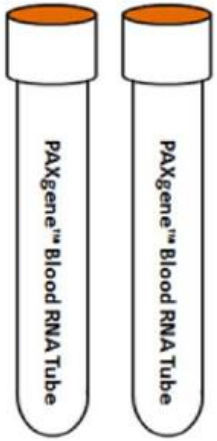
PAXgene™ Preparation (2.5ml Tube)

Step One

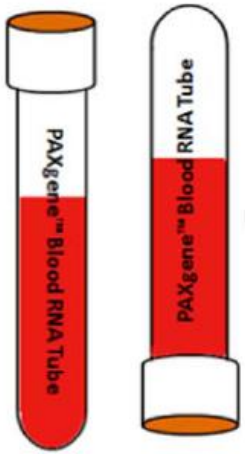


- Store tubes at room temperature.
- Label tubes with pre-printed subject labels prior to blood draw.

Step Two

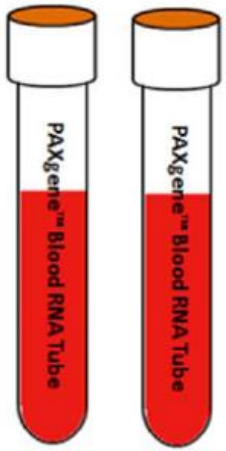
- Collect blood into *one* PAXgene Tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



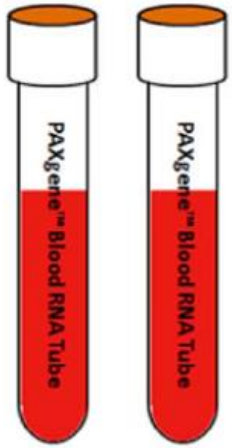
- Immediately after blood draw, invert tubes 8-10 times to mix samples.
- **Repeat steps two and three for second tube.**

Step Four



- Incubate tubes upright at room temperature for 24 hours before freezing the samples.

Step Five



- After 24 hour incubation at room temperature, store tubes upright in a -80°C in a wire rack until shipment.

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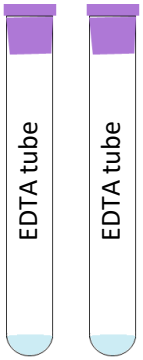
Plasma Preparation –10 ml EDTA (Purple Top) Tube

Step One

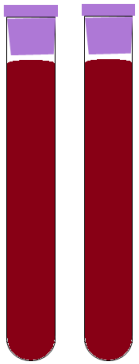


- Store tubes at room temperature.
- Label tubes with preprinted plasma labels prior to blood draw.

Step Two

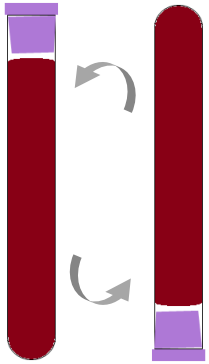
- Label 6 cryotubes with plasma aliquot labels.
- Pre-chill cryotubes on wet ice for at least 5 minutes

Step Three



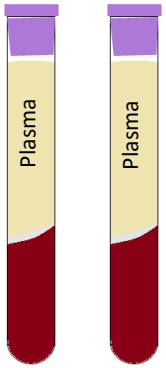
- Collect blood in EDTA tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Four



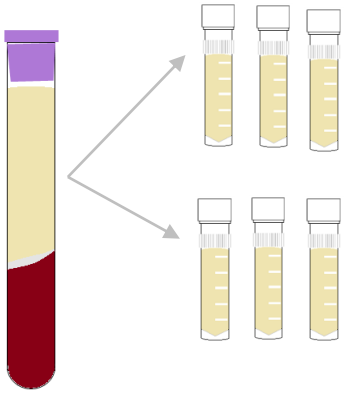
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

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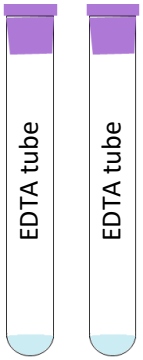
Buffy Coat Preparation –10 ml EDTA (Purple Top) Tube

Step One

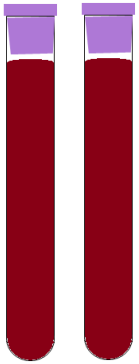


- Store tubes at room temperature.
- Label tubes with preprinted plasma labels prior to blood draw.

Step Two

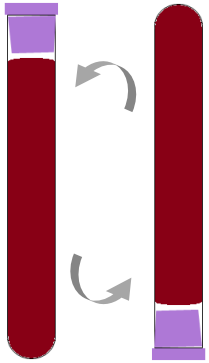
- Label 2 cryotubes with buffy coat aliquot labels.
- Pre-chill cryotubes on wet ice for at least 5 minutes.

Step Three



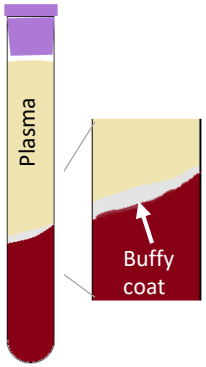
- Collect blood in EDTA tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Four



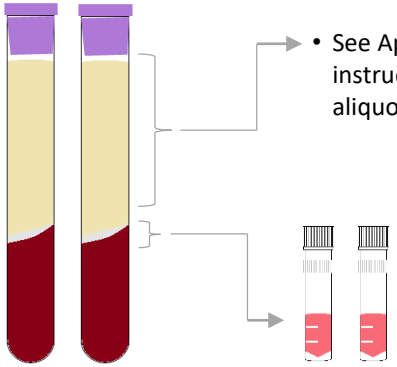
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Five



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

Step Six



- See Appendix B for instruction on aliquoting plasma
- 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 <u>upright</u> at -80°C until shipment.

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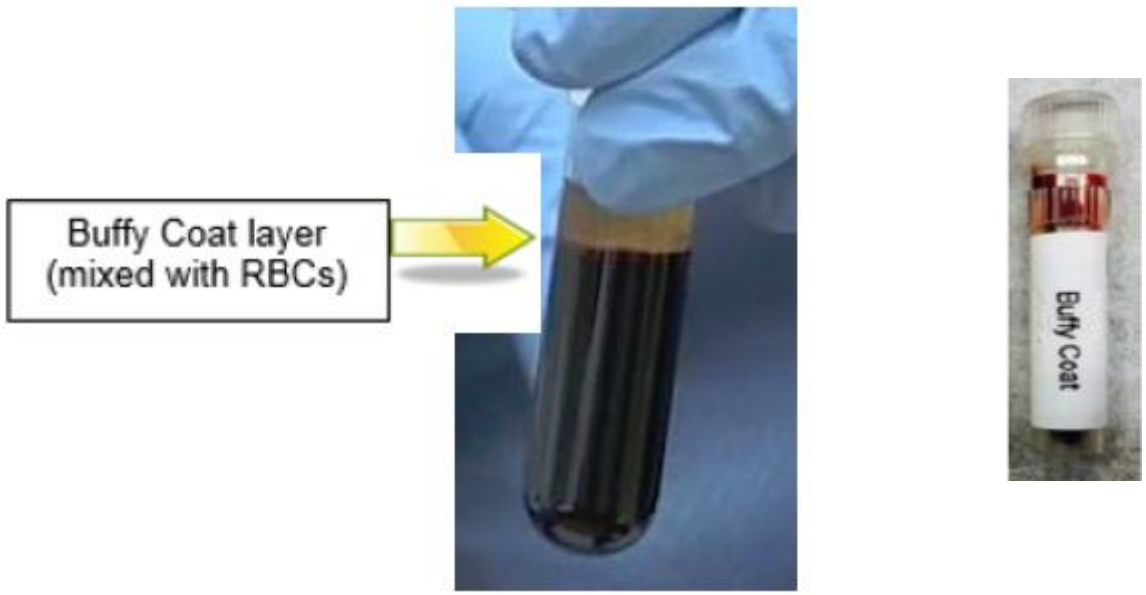
Buffy Coat Collection



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.

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Whole Blood (6 ml Lavender Top Tube)

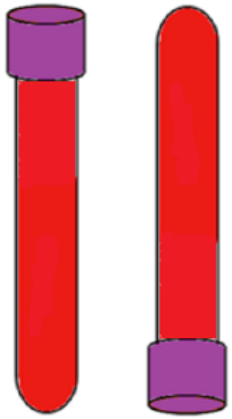
Step One

- Store tube at room temperature.
- Label tube with pre-printed labels prior to blood draw.

Step Two

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

Step Three



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

Step Four

- Transfer to -80°C freezer. Store upright and keep frozen until shipment BioSend.

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Lumbar Puncture Procedure

- 24g or 22g spinal needle provided in custom LP tray

- Prepare transfer and aliquot tubes (NOT in LP tray but in kit)
  - **Label first!**
  - **Do NOT** pre-chill aliquot tubes

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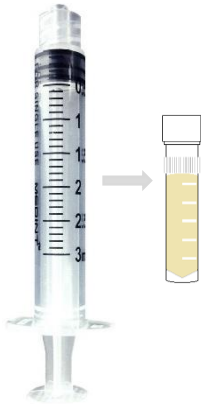
CSF Collection and Preparation (15-20 ml total)

Step One

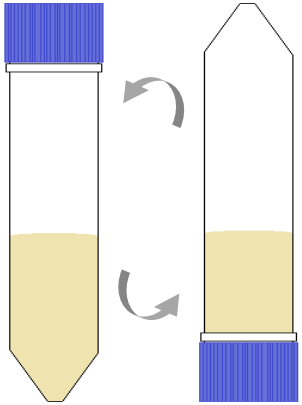


- Collect CSF into the 3ml luer lock syringe or by gravitational pull.
- Dispense 1-2 ml into cryovial.
- Send to local lab for testing.

Step Two

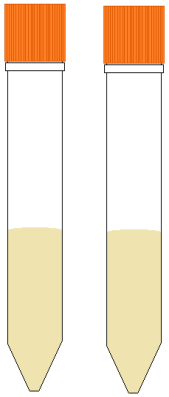
- Collect CSF into the 5ml luer lock syringe or by gravitational pull
- Transfer sample into 50 ml conical tube.

Step Three



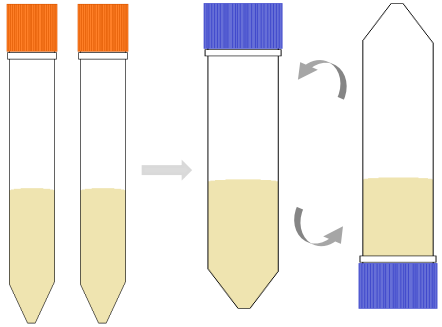
- Immediately after collection, gently invert the 50 ml conical tube 3-4 times to mix the sample.

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



1.0ml x 10 cryovials

- 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.

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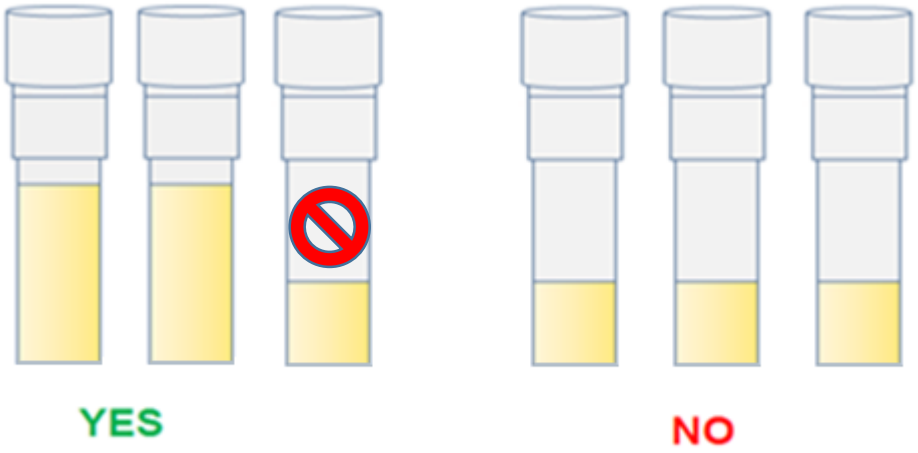
Serum, Plasma, Buffy Coat and CSF Aliquots

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



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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.

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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 tubes from supplemental kit nearby during blood draw to replace "bad" tubes**

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

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Blood Collection: Troubleshooting

Issue #2: Hemolyzed (pink/red) serum and 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) serum and 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
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)

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Sample Shipment

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Frozen Samples

- **All samples are shipped frozen**
  - Plasma, buffy coat, serum, whole blood, CSF, urine, and PAXgene®
- **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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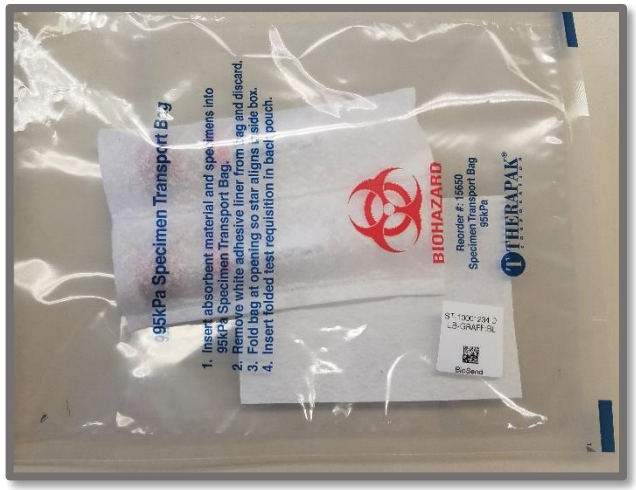
Packaging and Shipping Frozen Samples



Pack bags, place upright & side-by-side

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 plasma, serum, and CSF.
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

GUID: 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
Data Management Resource (DMR); Email: PDBP-OPS@mail.nih.gov

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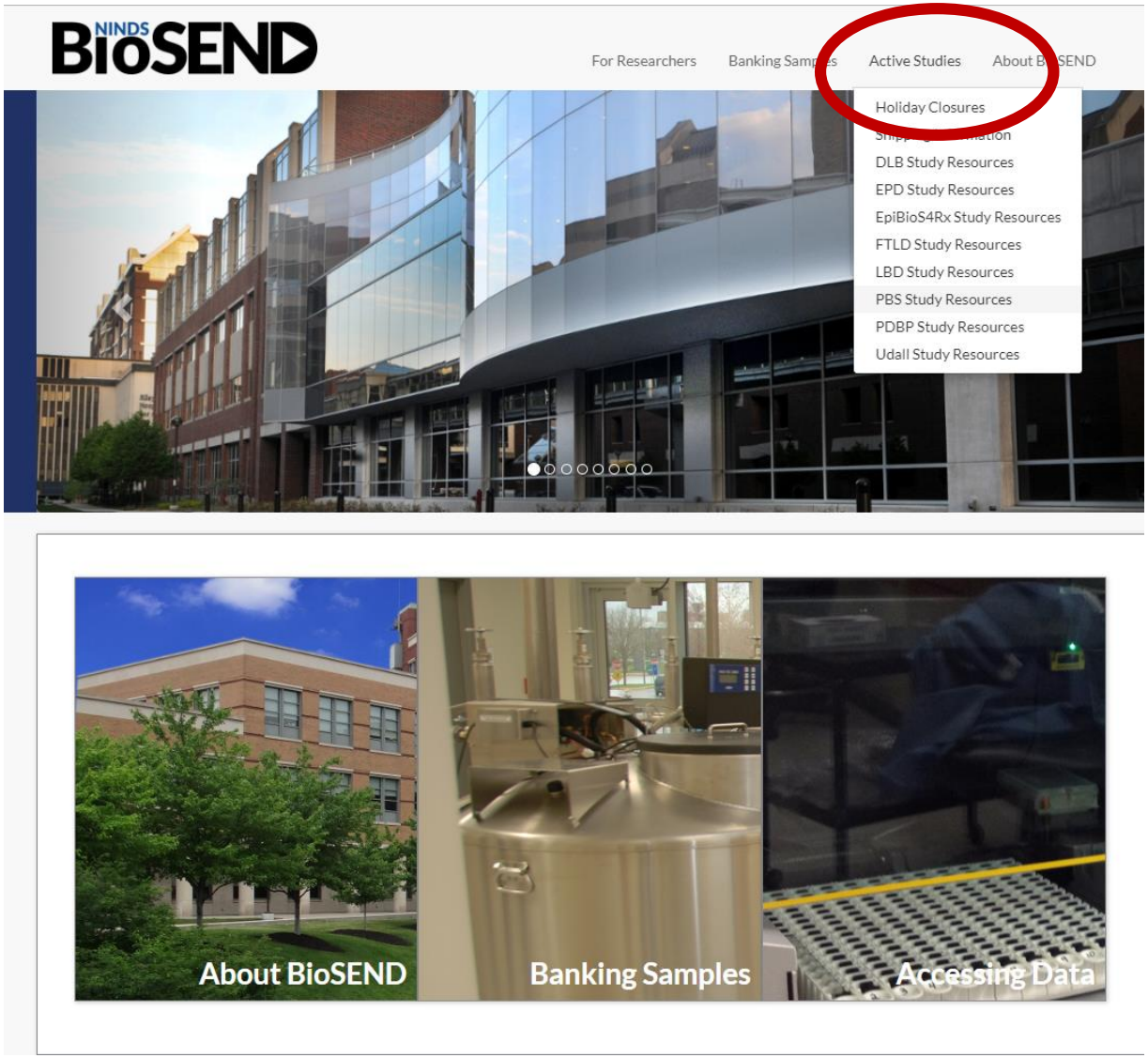
NINDS BioSEND Website



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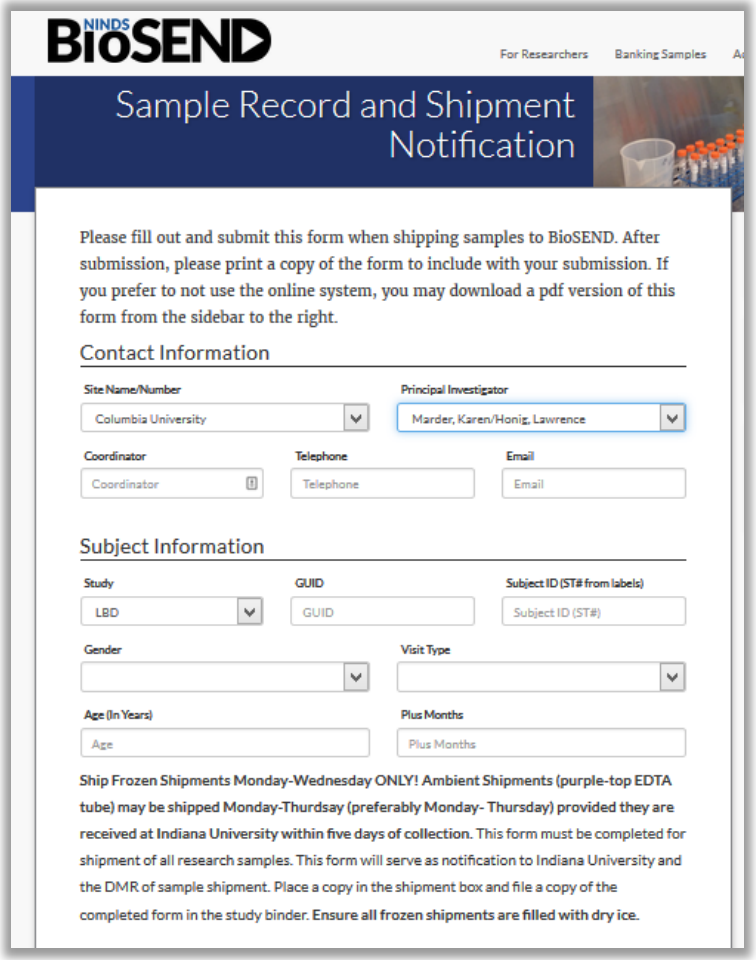
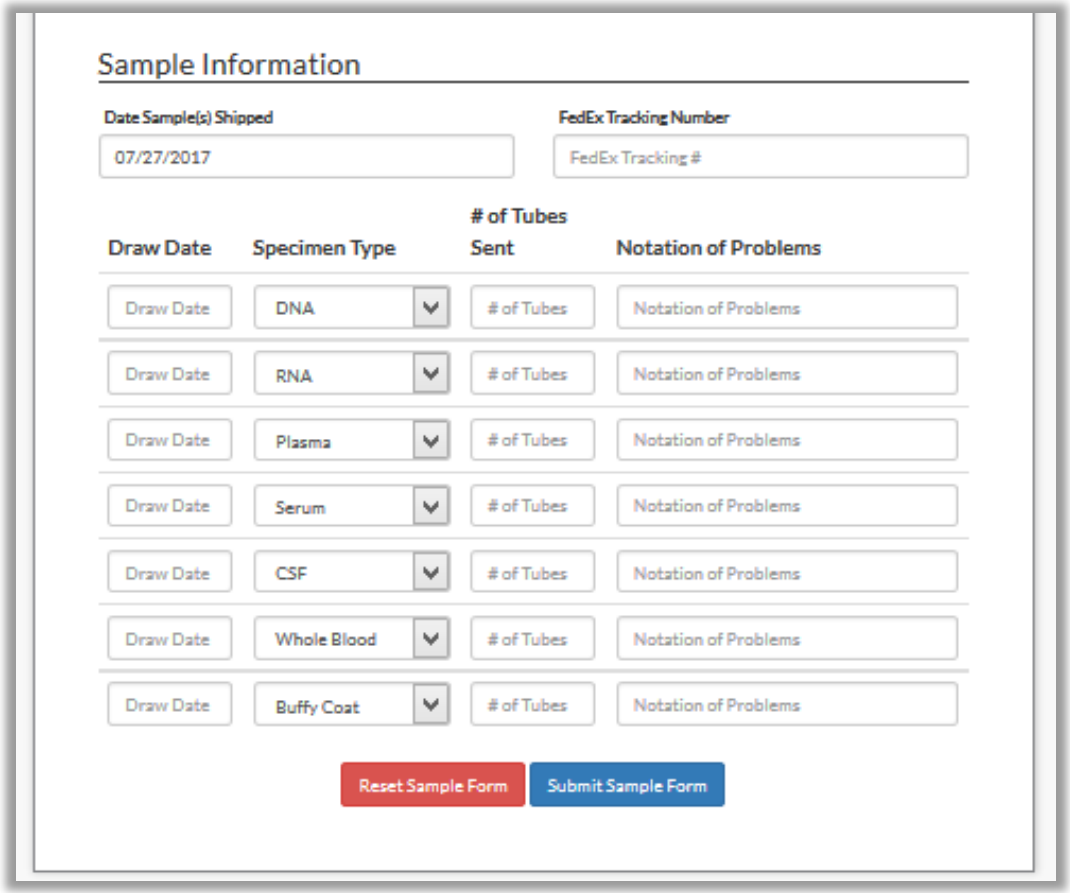
https://www.biosend.org/

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

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BioSEND Contact Information

- Questions?

  Please contact: Claire Wegel (cwegel@iu.edu)
  - Email: biosend@iu.edu

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Questions?

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