BioSpecimen Exchange for Neurological Disorders (BioSEND)

Parkinsonism Biomarker Subtypes Training Webinar

NINDS BioSEND

BioSEND Training Webinar Overview

1. Study Reminders
2. Site Equipment
3. PBS 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
- Pipettes and pipette tips
- 4°C Centrifuge
- -80°C Freezer
- Dry ice

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PBS Biospecimen Collection Protocol

	BL	12M
Plasma (6 x 1ml)	X	X
Serum (6 x 1ml)	X	X
RNA (2 x 2.5ml)	X	X
Whole Blood for DNA (ambient, 1x 6ml)	X	X
Whole Blood (frozen, 1x 6ml)	X	X
CSF (10 x 1ml)	Optional	Optional

CSF collection is OPTIONAL at either visit for all subjects. Please note that 2 CSF kits are provided at study launch, but NOT as part of standard kits. Please monitor supply to ensure availability.

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Requesting Kits

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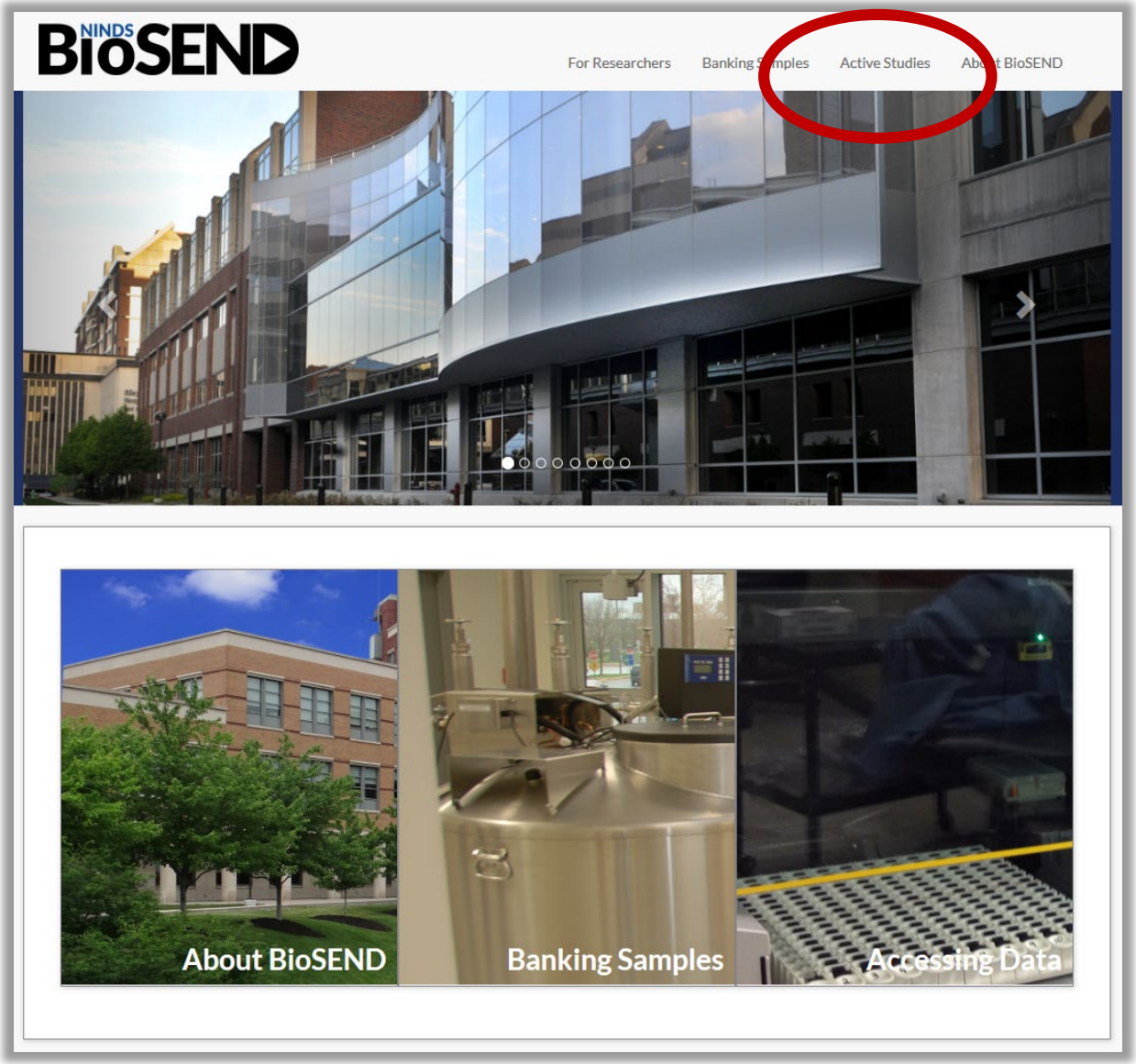
NINDS BioSEND Website



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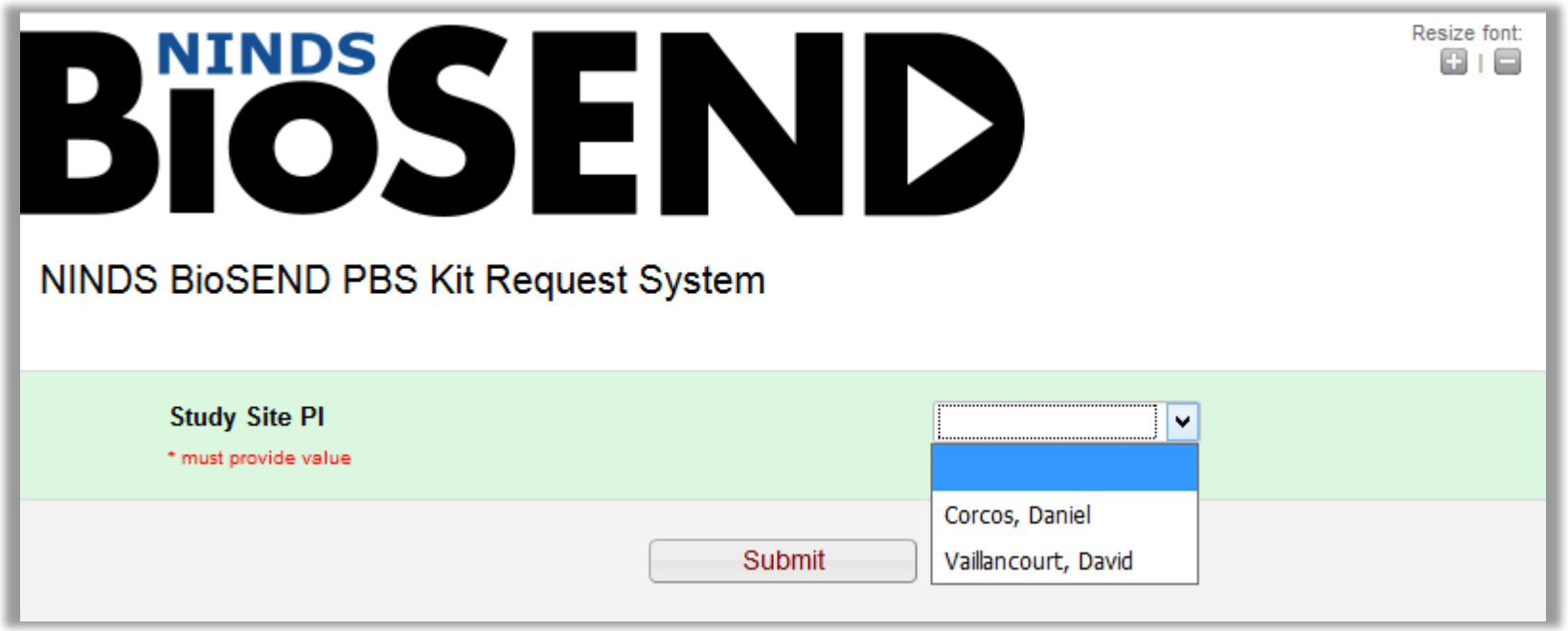
https://www.biosend.org/

BioSEND Kit Request Module



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

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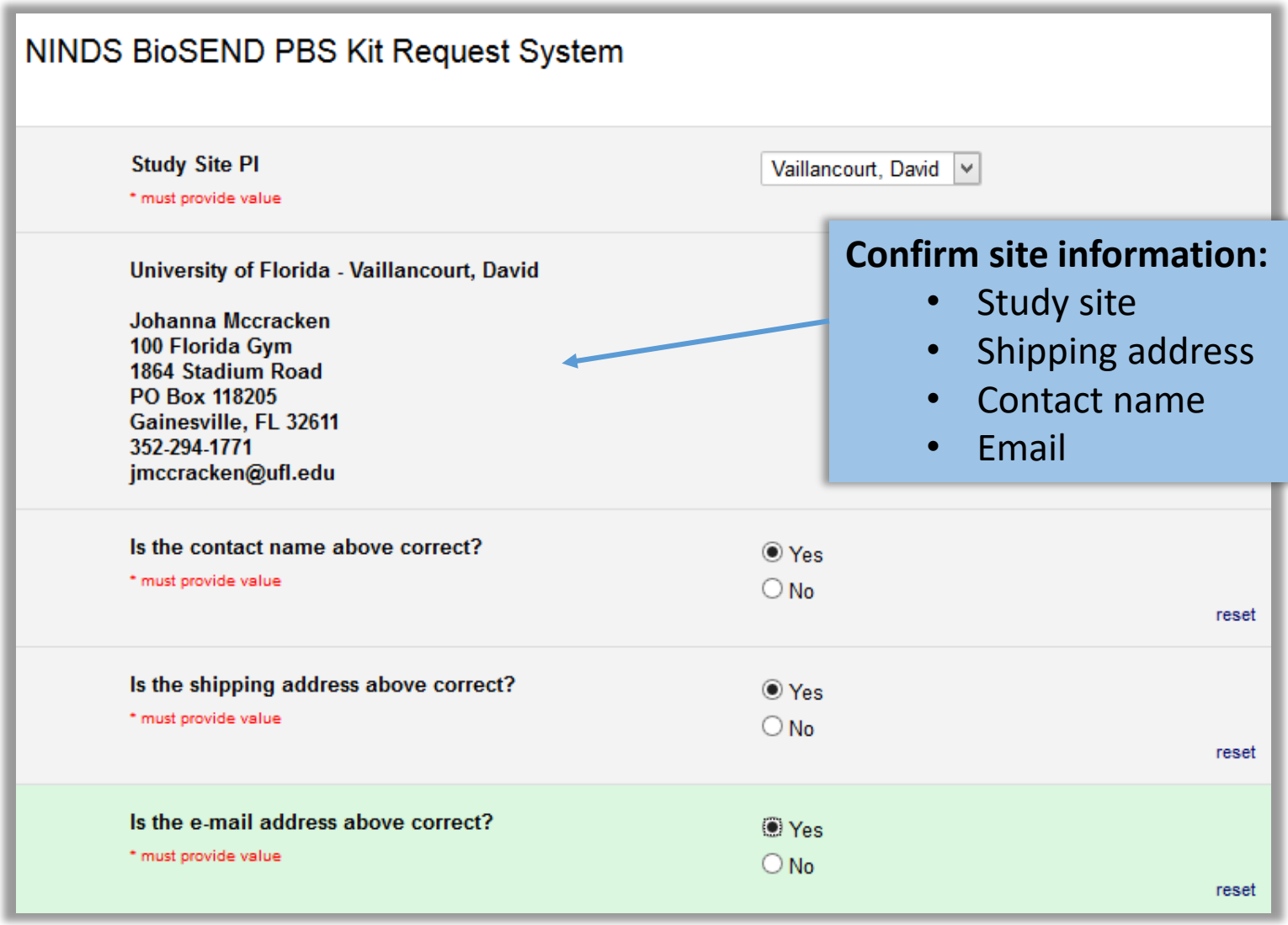
BioSEND Kit Request Module



Confirm site information:

- Study site
- Shipping address
- Contact name
- Email

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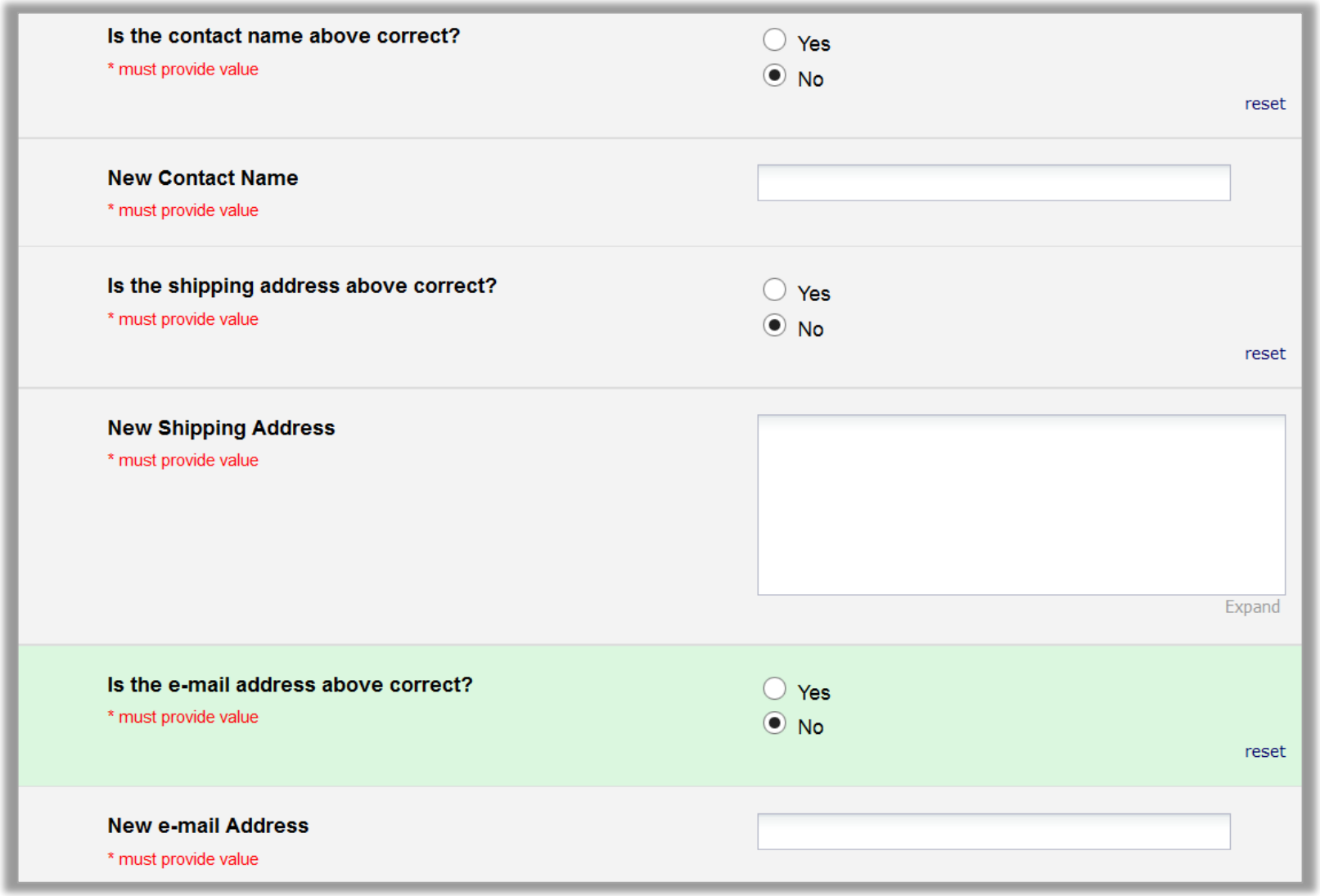
BioSEND Kit Request Module



- Is the information correct?
- Provide the correct information if needed

BioSEND Kit Request Module: Kit Type

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

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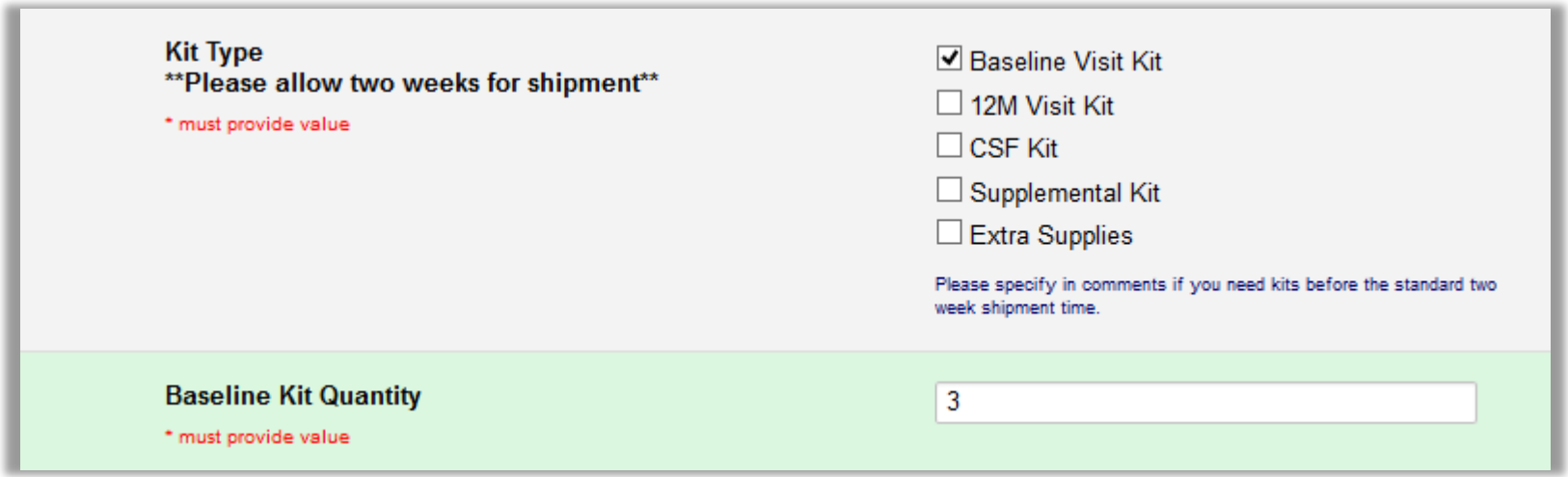
BioSEND Kit Request Module: Baseline



- BioSEND creates ST numbers (blinded subject number) for baseline kits
- Enter kit quantity

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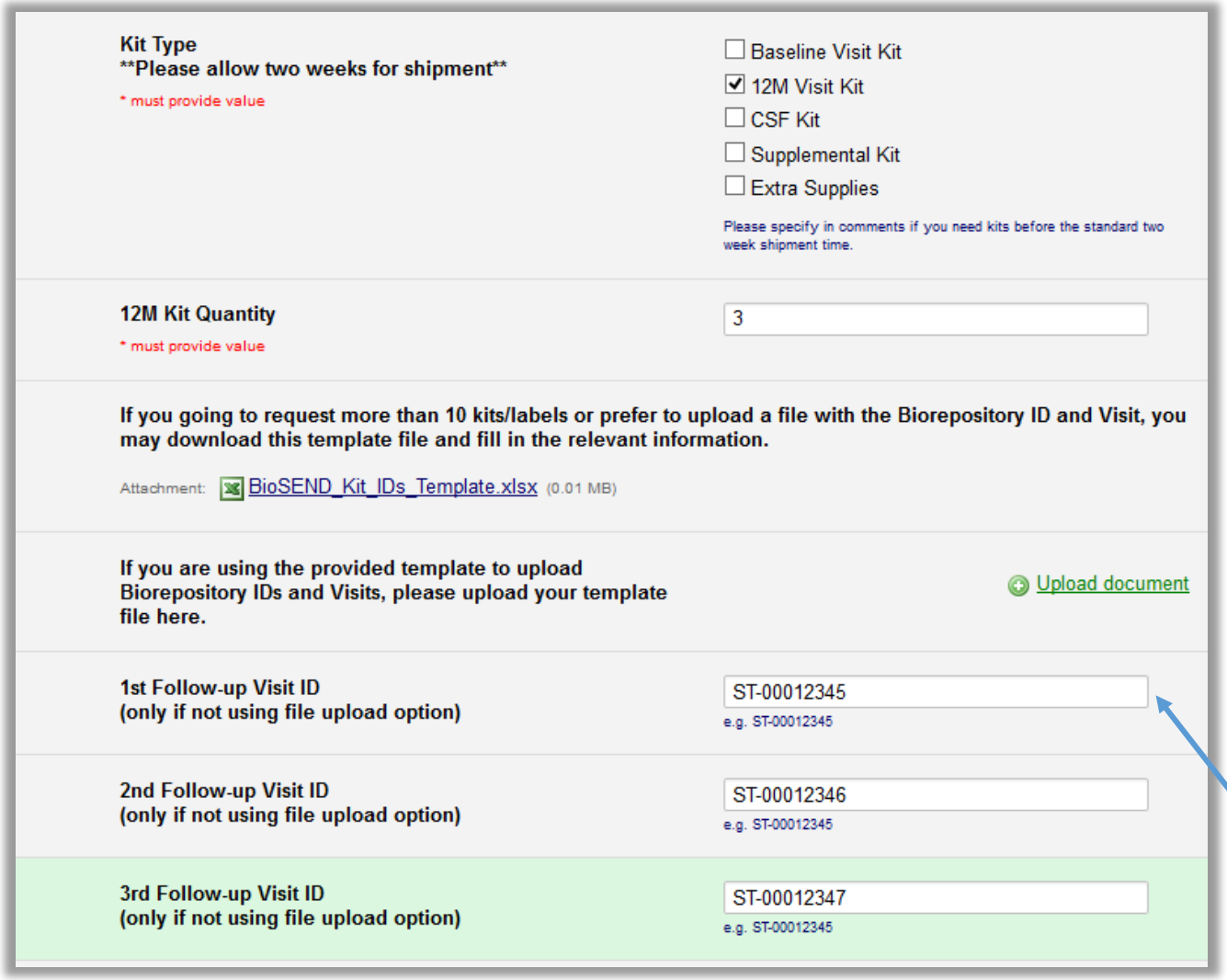
BioSEND Kit Request Module: 12M



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

- ☐ Baseline Visit Kit
- ☑ 12M Visit Kit
- ☐ CSF Kit
- ☐ Supplemental Kit
- ☐ Extra Supplies

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

12M Kit Quantity
* must provide value
3

If you going to request more than 10 kits/labels or prefer to upload a file with the Biorepository ID and Visit, you may download this template file and fill in the relevant information.

Attachment: BioSEND_Kit_IDs_Template.xlsx (0.01 MB)

If you are using the provided template to upload Biorepository IDs and Visits, please upload your template file here.
Upload document

1st Follow-up Visit ID
(only if not using file upload option)
ST-00012345
e.g. ST-00012345

2nd Follow-up Visit ID
(only if not using file upload option)
ST-00012346
e.g. ST-00012345

3rd Follow-up Visit ID
(only if not using file upload option)
ST-00012347
e.g. ST-00012345

- Enter kit quantity

- For 12M kits, specific subject needs to be specified so correct labels are included
- Up to 10 subjects can be specified.

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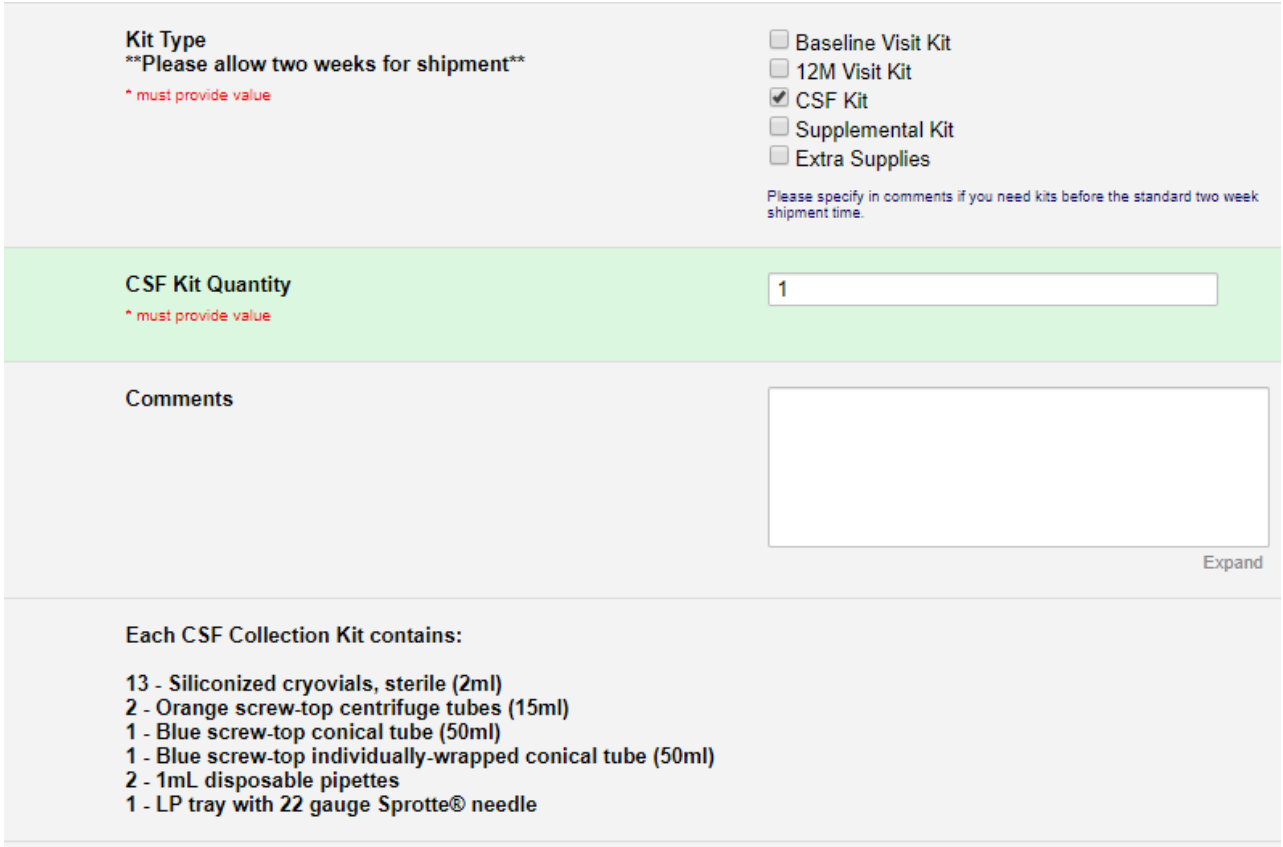
BioSEND Kit Request Module: CSF Kit



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

- [ ] Baseline Visit Kit
- [ ] 12M Visit Kit
- [x] CSF Kit
- [ ] Supplemental Kit
- [ ] Extra Supplies

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

CSF Kit Quantity
* must provide value

1

Comments

Expand

Each CSF Collection Kit contains:

13 - Siliconized cryovials, sterile (2ml)
2 - Orange screw-top centrifuge tubes (15ml)
1 - Blue screw-top conical tube (50ml)
1 - Blue screw-top individually-wrapped conical tube (50ml)
2 - 1mL disposable pipettes
1 - LP tray with 22 gauge Sprotte® needle

- Subjects do not need specified for CSF kits, as CSF labels will be provided for all subject visits

- 2 kits provided as standard, but will otherwise only be provided if requested
- Monitor supply to ensure availability

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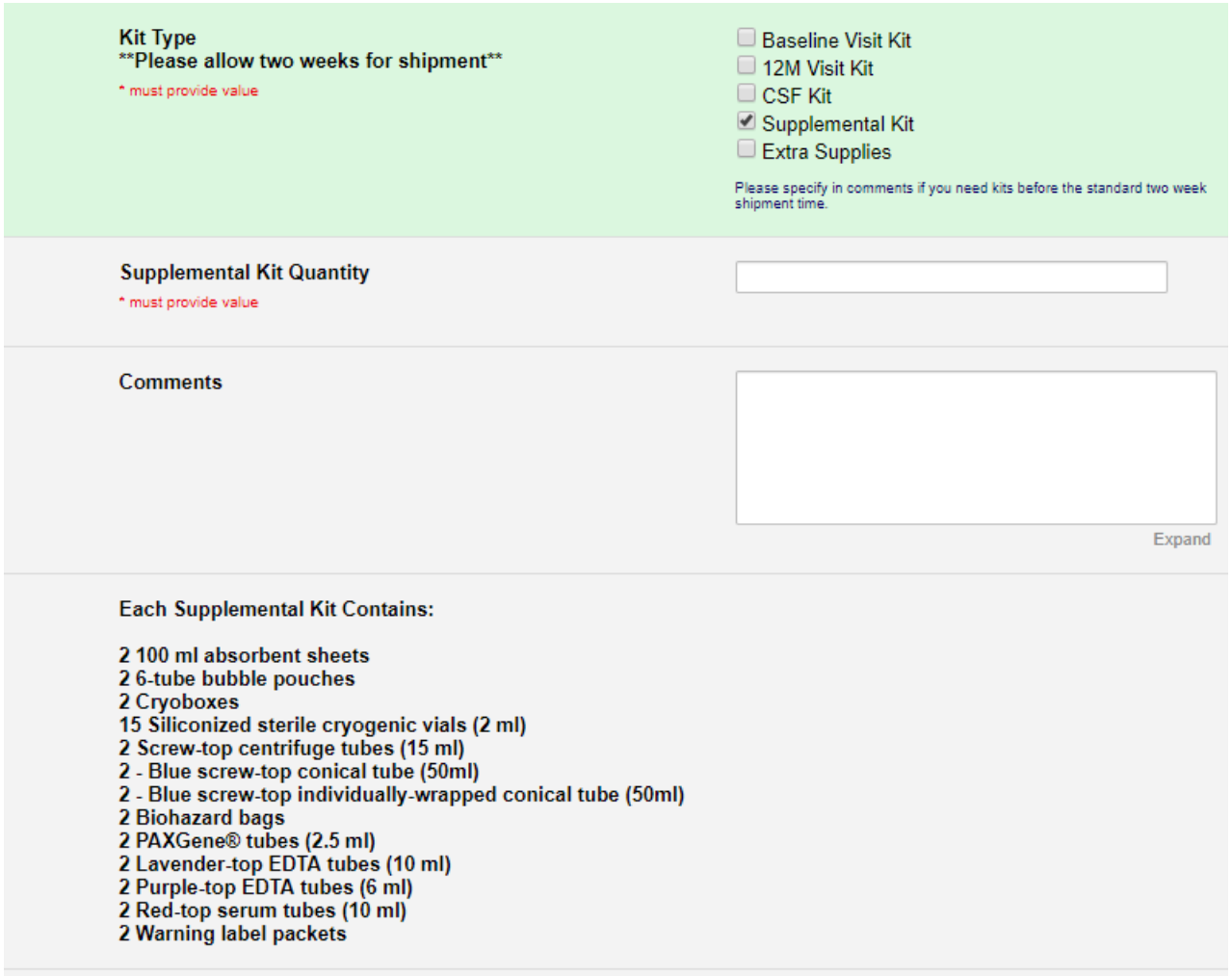
BioSEND Kit Request Module: Supplemental Kit



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

- [ ] Baseline Visit Kit
- [ ] 12M Visit Kit
- [ ] CSF Kit
- [x] 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

Comments

Expand

Each Supplemental Kit Contains:

2 100 ml absorbent sheets
2 6-tube bubble pouches
2 Cryoboxes
15 Siliconized sterile cryogenic vials (2 ml)
2 Screw-top centrifuge tubes (15 ml)
2 - Blue screw-top conical tube (50ml)
2 - Blue screw-top individually-wrapped conical tube (50ml)
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 Warning label packets

- Provided with first shipment
- 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
- ☐ 12M Visit Kit
- ☐ CSF 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
Cryobox	○ 2 ○ 4
Siliconized Sterile Cryogenic Vial (2 ml)	○ 10 ○ 20 reset
FedEx® return Airbill	○ 2 ○ 4 reset

- 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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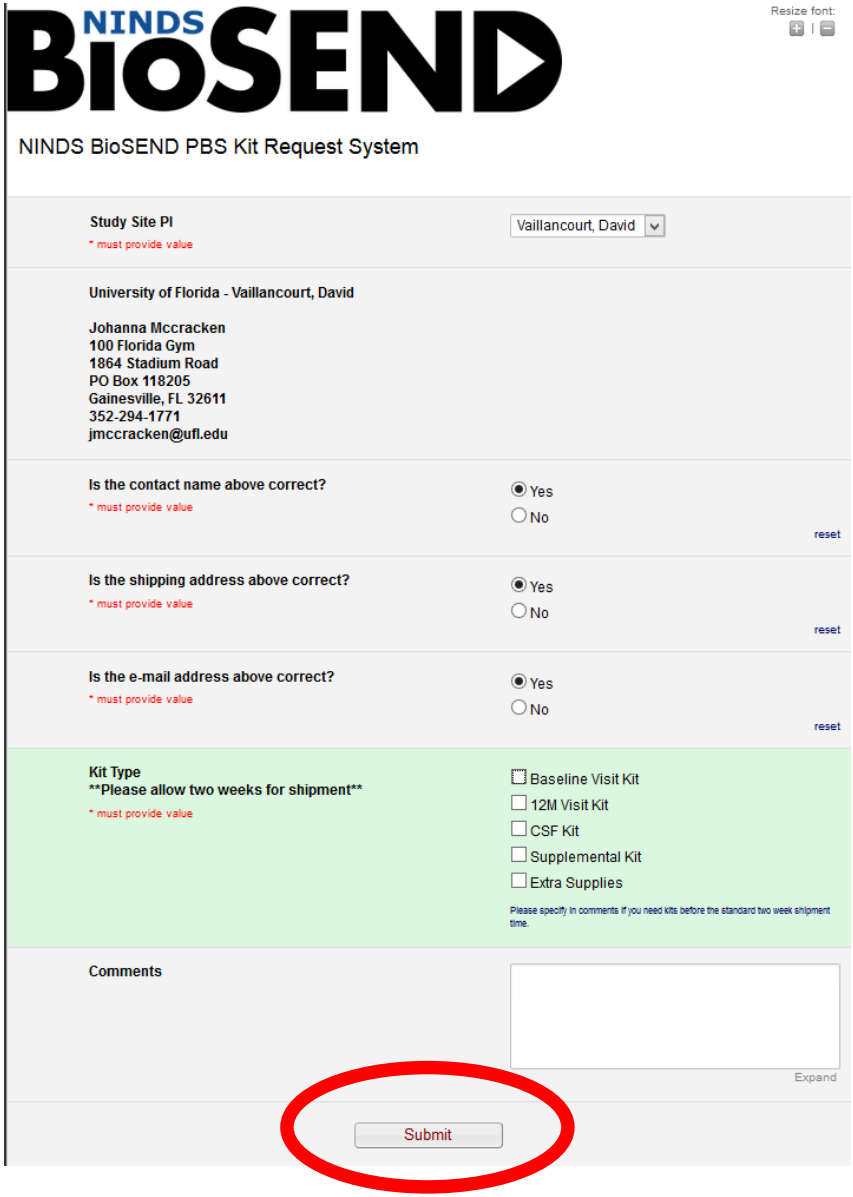
BioSEND Kit Request Module: Submit

- 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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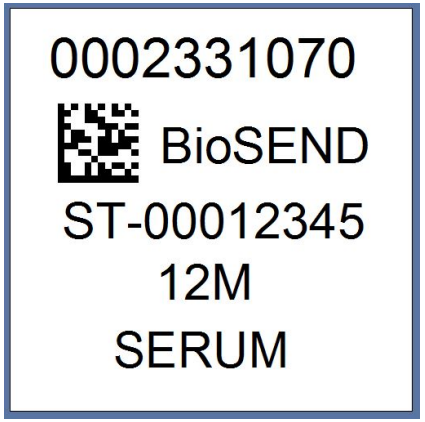
Labels

Types of Labels

Case Label

Identify study and PI

Specimen Label



Identify individual biospecimens

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Case Label

Subject Number

Study:Investigator:Visit

Biorepository Name

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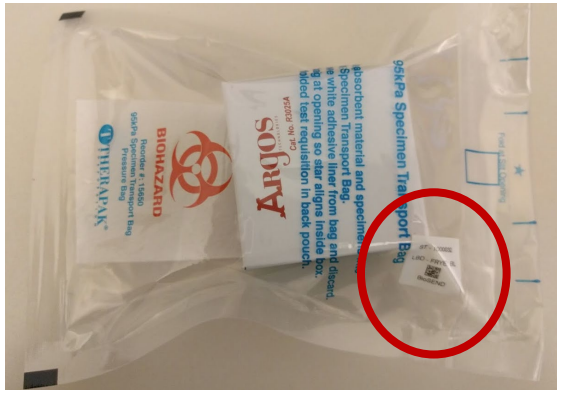
Case Labels



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 the shipping canisters.
- *On the lid of frozen shippers*

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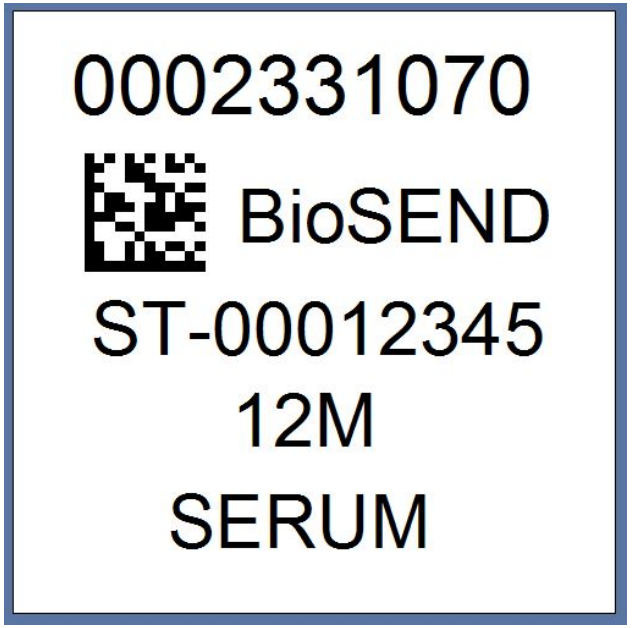
Collection and Aliquot Tube Label



Biospecimen Number

Repository Name

Subject Number

Visit Type

Specimen Type

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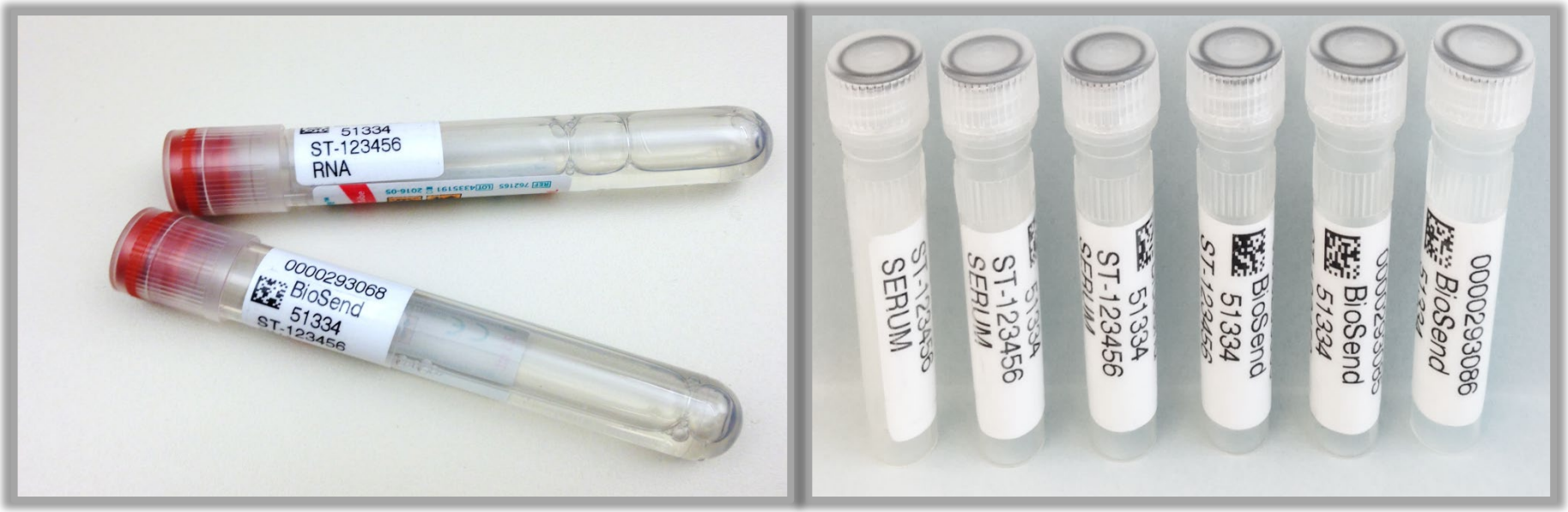
Collection and Aliquot Tube Label

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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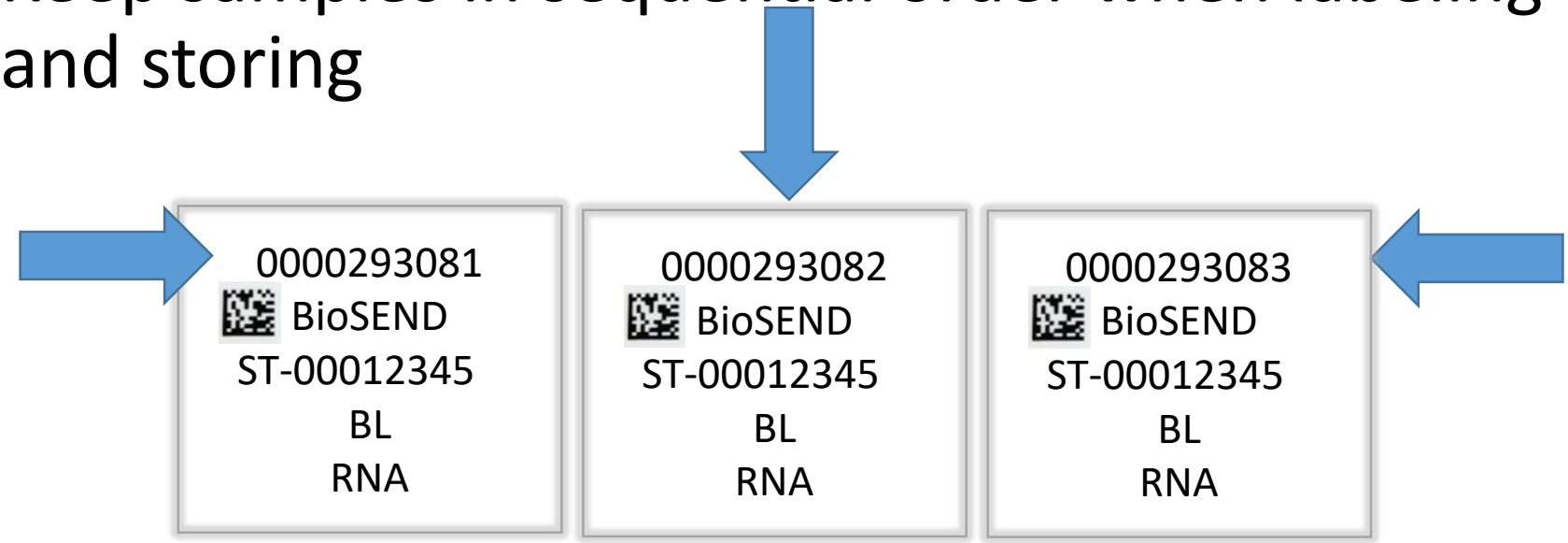
Aliquot Labels

- Keep samples in sequential order when labeling and storing



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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, whole blood, and urine 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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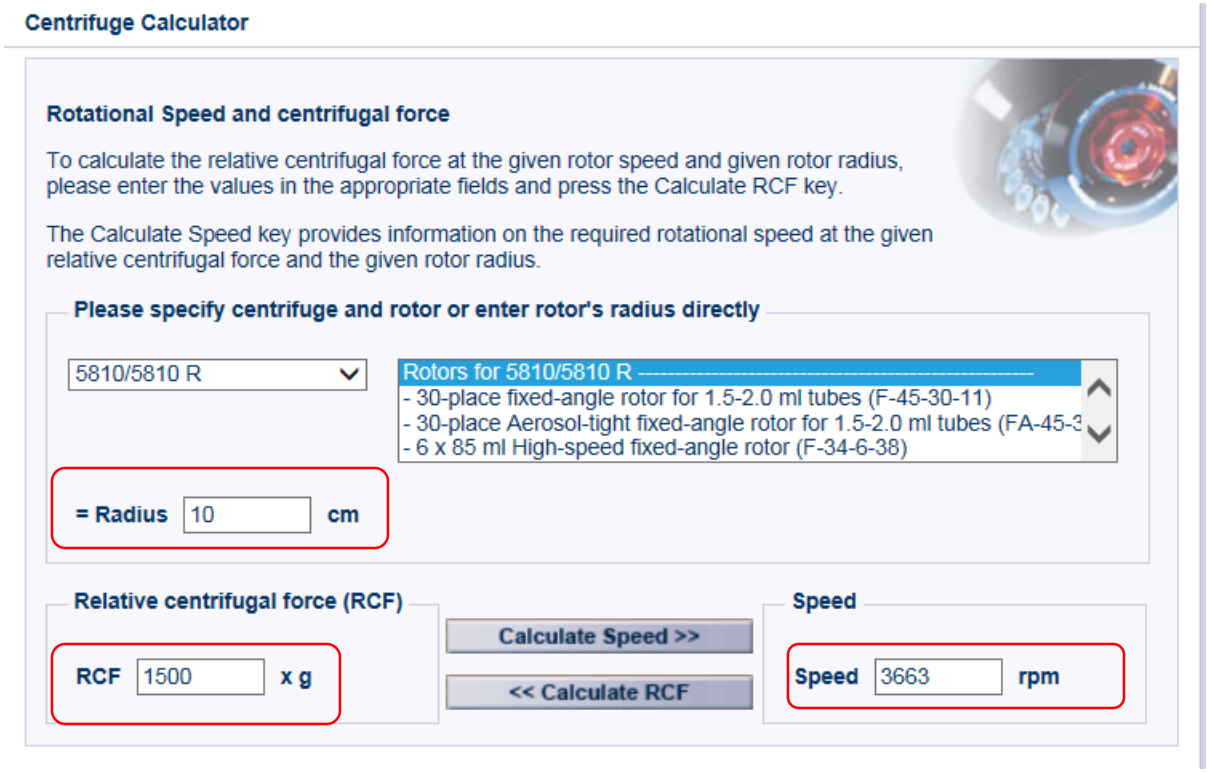
Calculating Centrifugation Speed

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

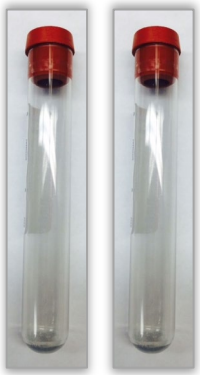


The 3663 rpm speed was calculated using a hypothetical radius of 10 cm and a RCF of 1500 x g.

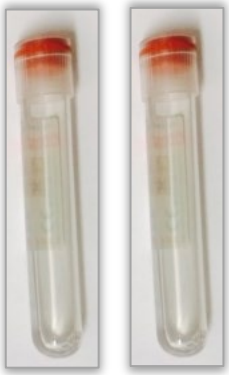
Refer to Appendix F in the BioSEND Biologics Manual for further information regarding the centrifugation of samples

Order of Blood Specimen Collection

1. Serum **10 ml (red top)** blood collection for serum
2. PAXgene® tube for RNA
3. EDTA **6 ml (purple top) ambient** whole blood collection for DNA
4. EDTA **6 ml (purple top) frozen** whole blood collection for banking
5. EDTA **10 ml (lavender top**) blood collection for plasma



Serum



PAXgene (RNA)

6ml EDTA (ambient DNA)

6ml EDTA (Frozen WB)

10ml EDTA (Plasma)

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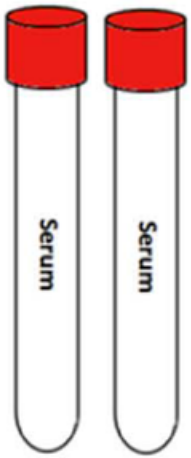
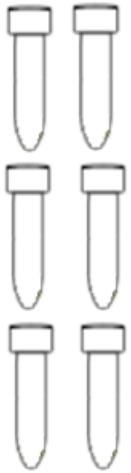
Serum Preparation (10ml Red Top Tube)

Step One



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

Step Two

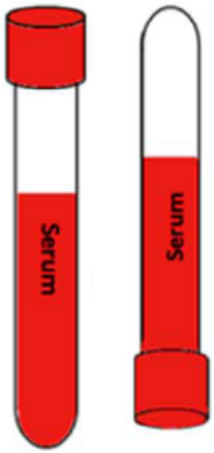


- Label 6 cryovials with pre-printed subject labels prior to blood draw.
- Pre-chill cryovials on wet ice for 5 minutes or longer.

Step Three

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

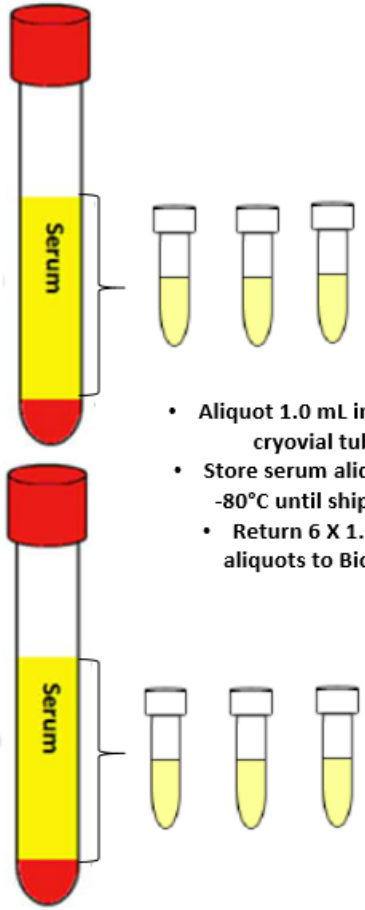
Step Four



- 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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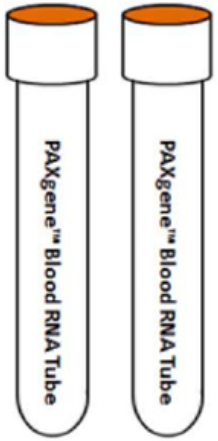
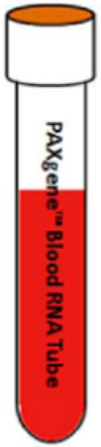
PAXgene™ Preparation (2.5ml Tube)

Step One



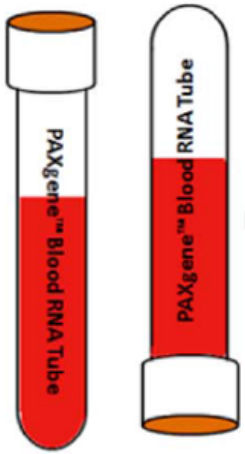
- Store tubes at room temperature.
- Label tubes with pre-printed subject labels prior to blood draw.

Step Two



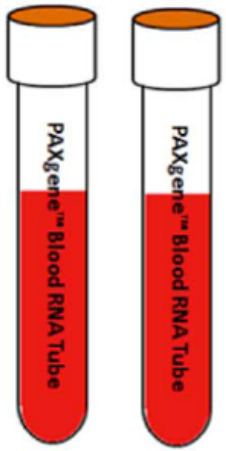
- Collect blood into *one* PAXgene Tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



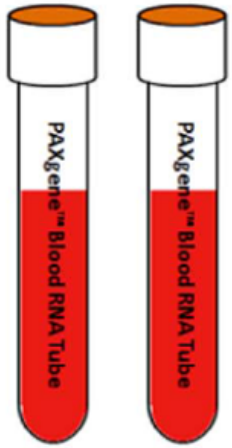
- Immediately after blood draw, invert tubes 8-10 times to mix samples.
- **Repeat steps two and three for second tube.**

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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DNA Preparation (6ml Lavender Top Tube)

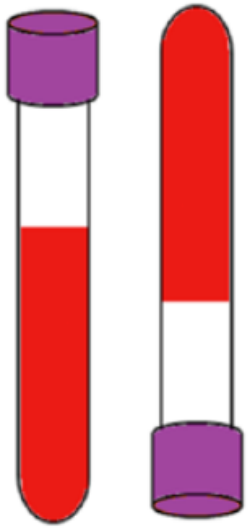
Step One

- 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

- Hold the specimen at room temperature until shipment to BioSend.
- Needs to be received by BioSend within 5 days of collection

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Frozen Whole Blood Preparation (6ml 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 to BioSEND

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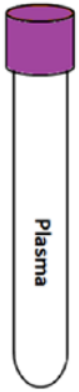
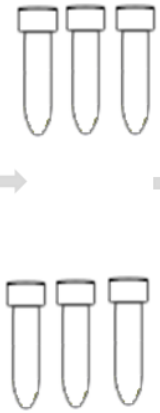
Plasma Preparation (10ml Lavender Top Tube)

Step One



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

Step Two

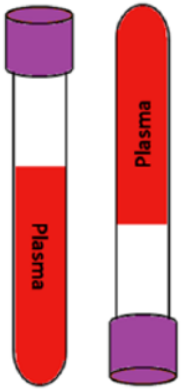


- Label 6 cryovials for plasma with pre-printed subject labels prior to blood draw.
- Pre-chill cryovials on wet ice for 5 minutes or longer.

Step Three

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

Step Four

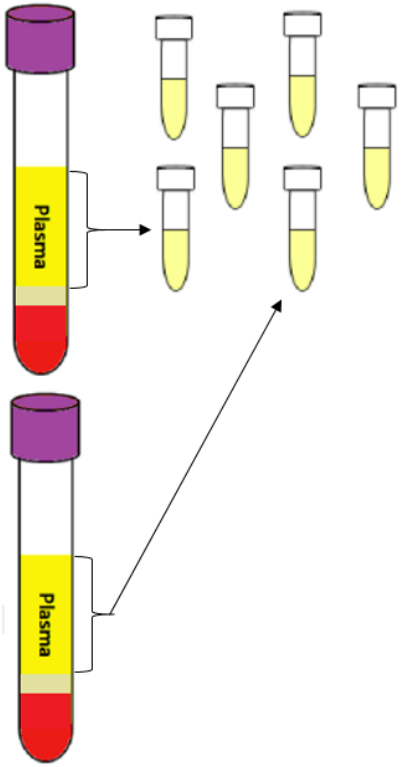


- Immediately after blood draw, invert tubes 8-10 times to mix samples.
- Repeat Steps 3 and 4 for second tube.

Step Five

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

Step Six and Seven



- Aliquot 1.0mL of plasma into 6 cryovial tubes.
- Store plasma aliquots at -80°C until shipment.
- Return 6 X 1.0 mL plasma aliquots to BioSend

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Lumbar Puncture Procedure

- LP is Optional
- 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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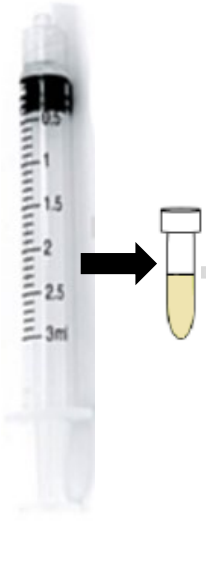
CSF Preparation Processing

Step One

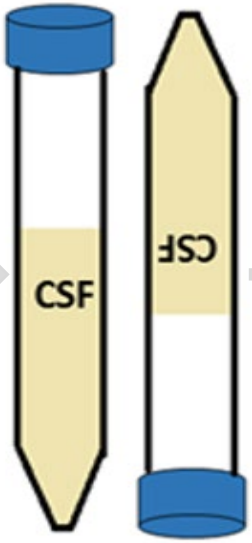


- Collect CSF into the 3 mL luer lock syringe or by gravitational pull.
- Dispense 1-2 mL in a cryovial.
- Send to local lab for testing.

Step Two

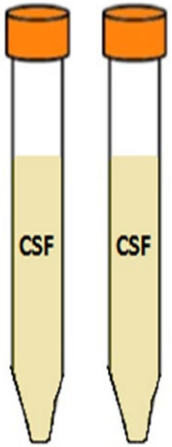
- Collect CSF into 5 mL luer lock syringe or by gravitational pull.
- Collect approved volume into the wrapped 50 mL conical tube.

Step Three



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

Step Four

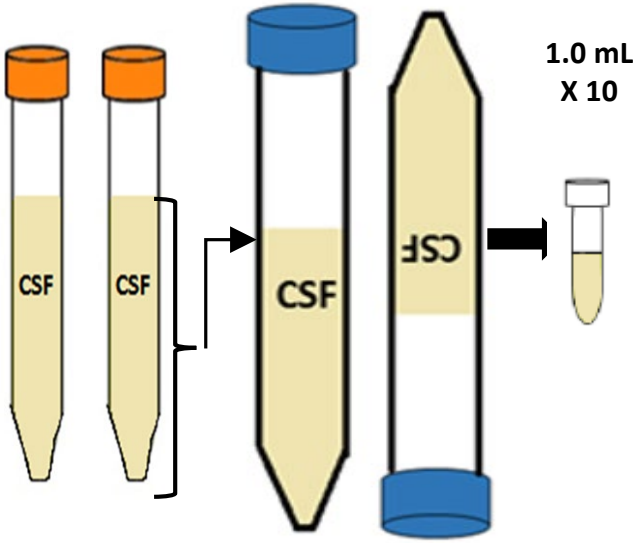


- Transfer CSF into 2 15 ml conical tubes.
- Within 15 minutes of collection, centrifuge samples at room temperature at 2000 x g for 10 minutes.

Step Five

- Label tubes with pre-printed subject labels prior to collection.

Step Six



- Using a clean transfer pipette, transfer CSF from both 15 ml conical tubes into a NEW unwrapped 50mL conical tube leaving the debris in the bottom.
- Mix the 50 ml conical tube gently by inverting 3-4 times.
- Aliquot 1.0 ml into 10 cryovials, Aliquot residual mL in last cryovial (for site use).
- Store CSF aliquots at -80°C until shipment.
- Return 10 1.0 mL aliquots to BioSend.

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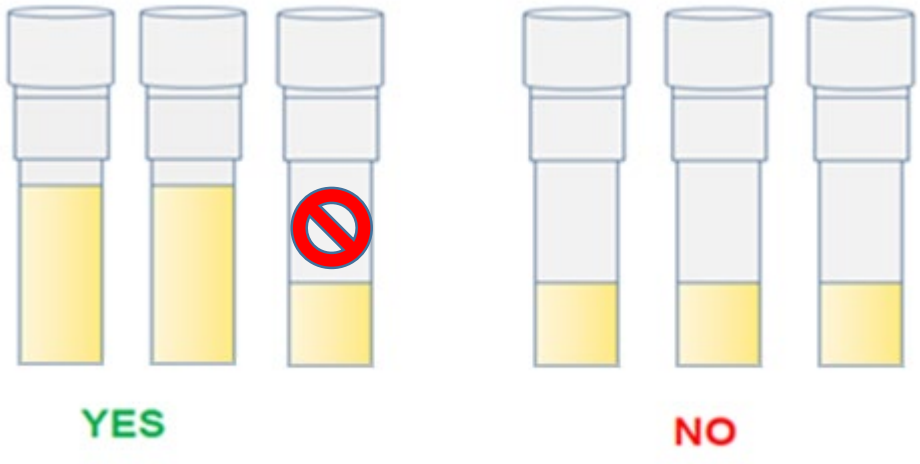
Serum, Plasma and CSF Aliquots

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



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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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Ambient Sample Shipment

- 6 ml purple (EDTA) whole blood for DNA collection tube
  - Collected at 6M visit

- **Monday – Thursday only via FedEx® Priority Overnight**

- Schedule FedEx® pickup

- Email Sample Record and Shipment Notification Form including the FedEx® tracking number **AHEAD OF SHIPMENT to BioSEND**

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Packaging Ambient Samples

- Insert tube into the tube sleeve.
- Insert the sleeve into the canister.
- Seal the canister tightly.
- Place case label on canister.
- Wrap the canister in the enclosed bubble wrap.
- Place canister into the cardboard box.

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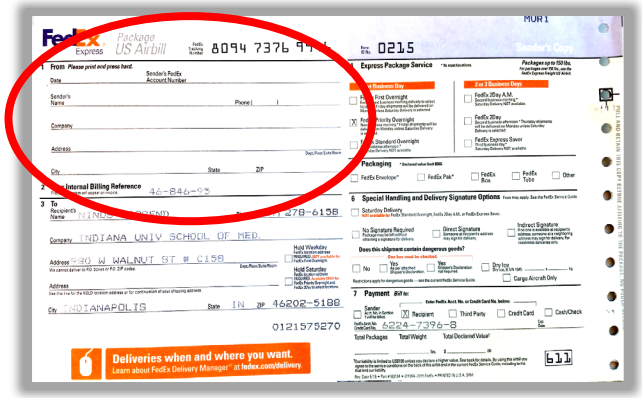
Labeling Ambient Sample Shipments

- Apply the UN3373 label to the outside of the cardboard box.
- Place the box and a copy of the PBS Sample Record and Shipment Notification Form in the Clinical Pak and seal the Pak.
- Complete the “From” portion of the air waybill with your name, address, and phone number.
- Apply the air waybill to the outside of the package.



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Shipping Ambient Samples

- Ship the sample(s) to BioSEND on the day of collection.
- If this is not possible, hold at room temperature until shipping can be arranged.
- ***Sample(s) must be received at BioSEND within 5 days of collection. Please do not ship these samples on Fridays.***

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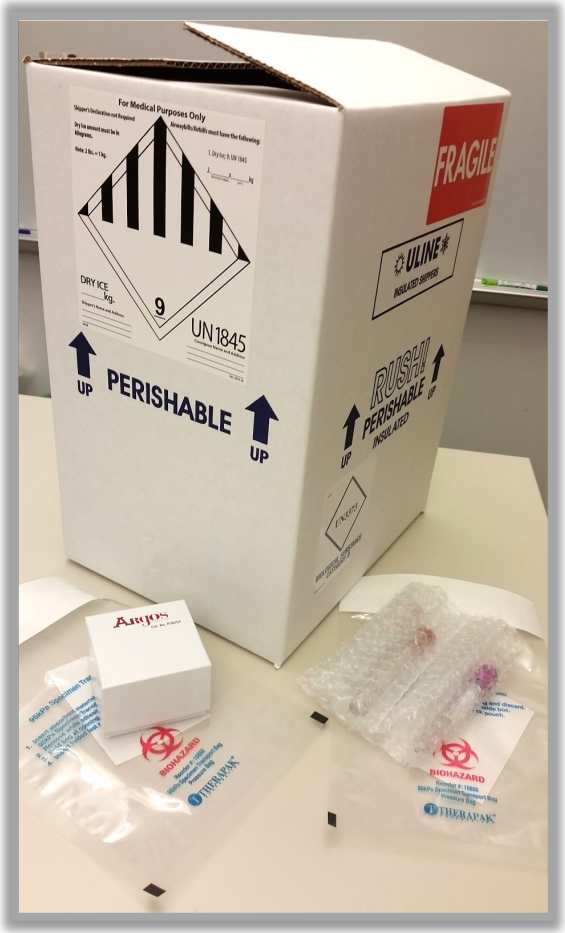
Frozen Samples

- **All other samples are shipped frozen**
  - Plasma, serum, CSF, whole blood 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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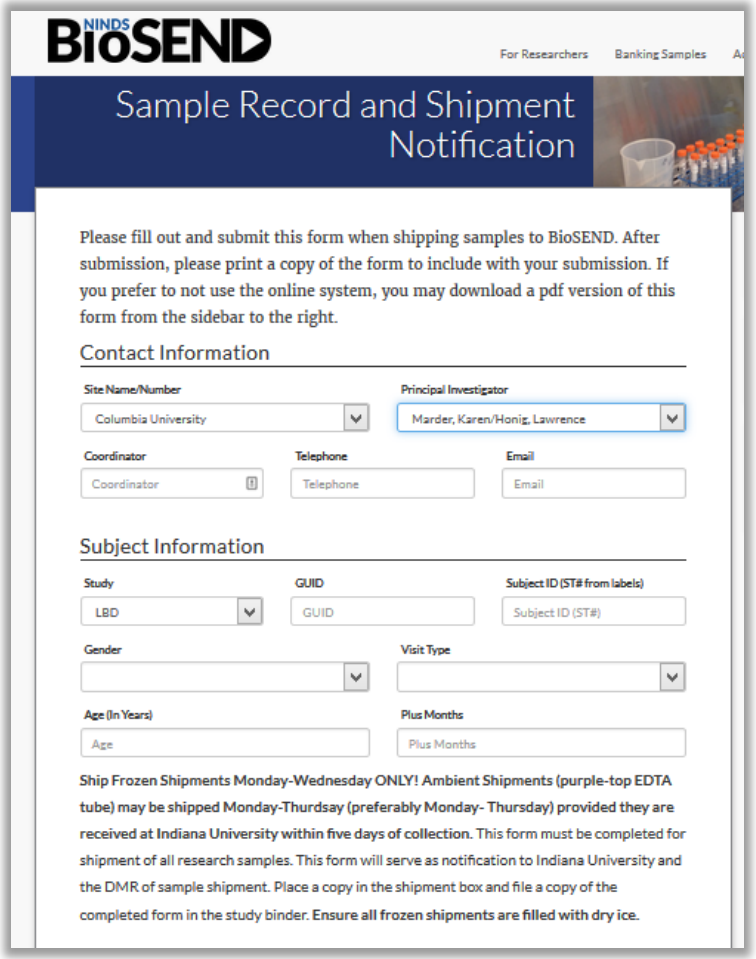
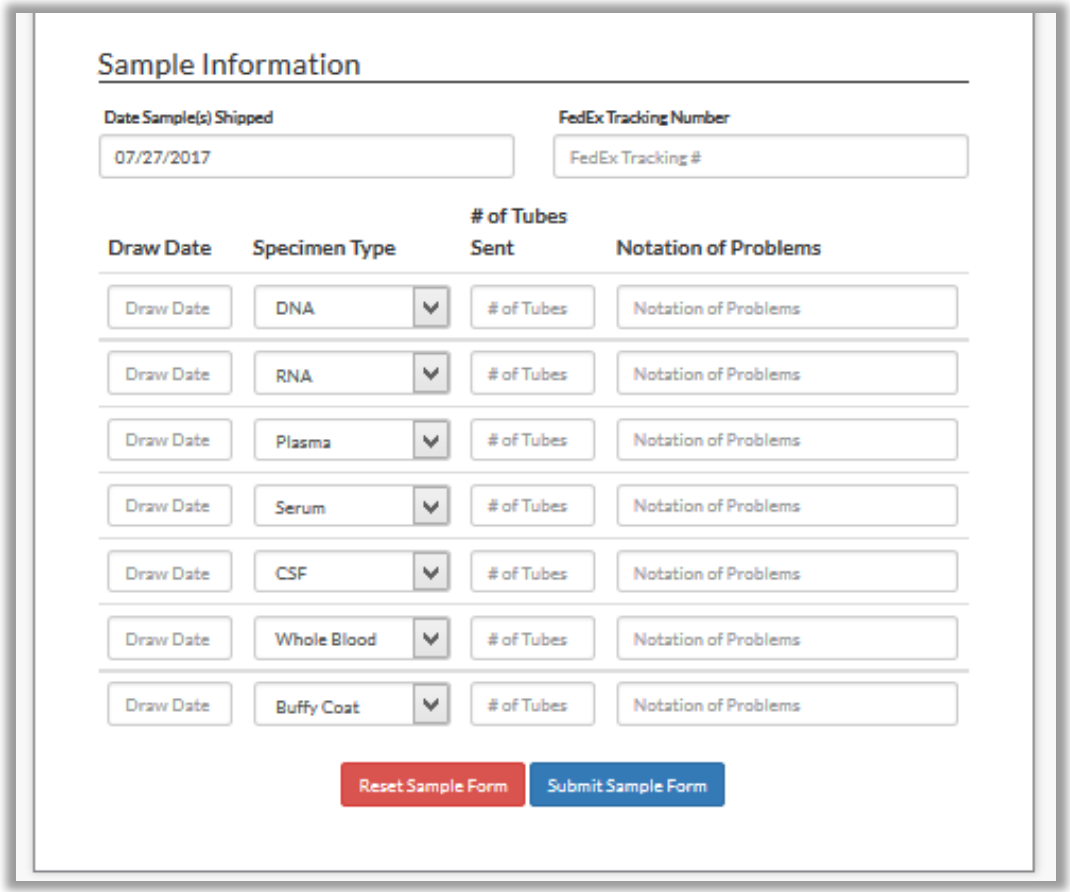
Sample Shipment Notification Form, Online





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BioSEND Website

- Basic information now but will be expanding to include more information

https://www.biosend.org

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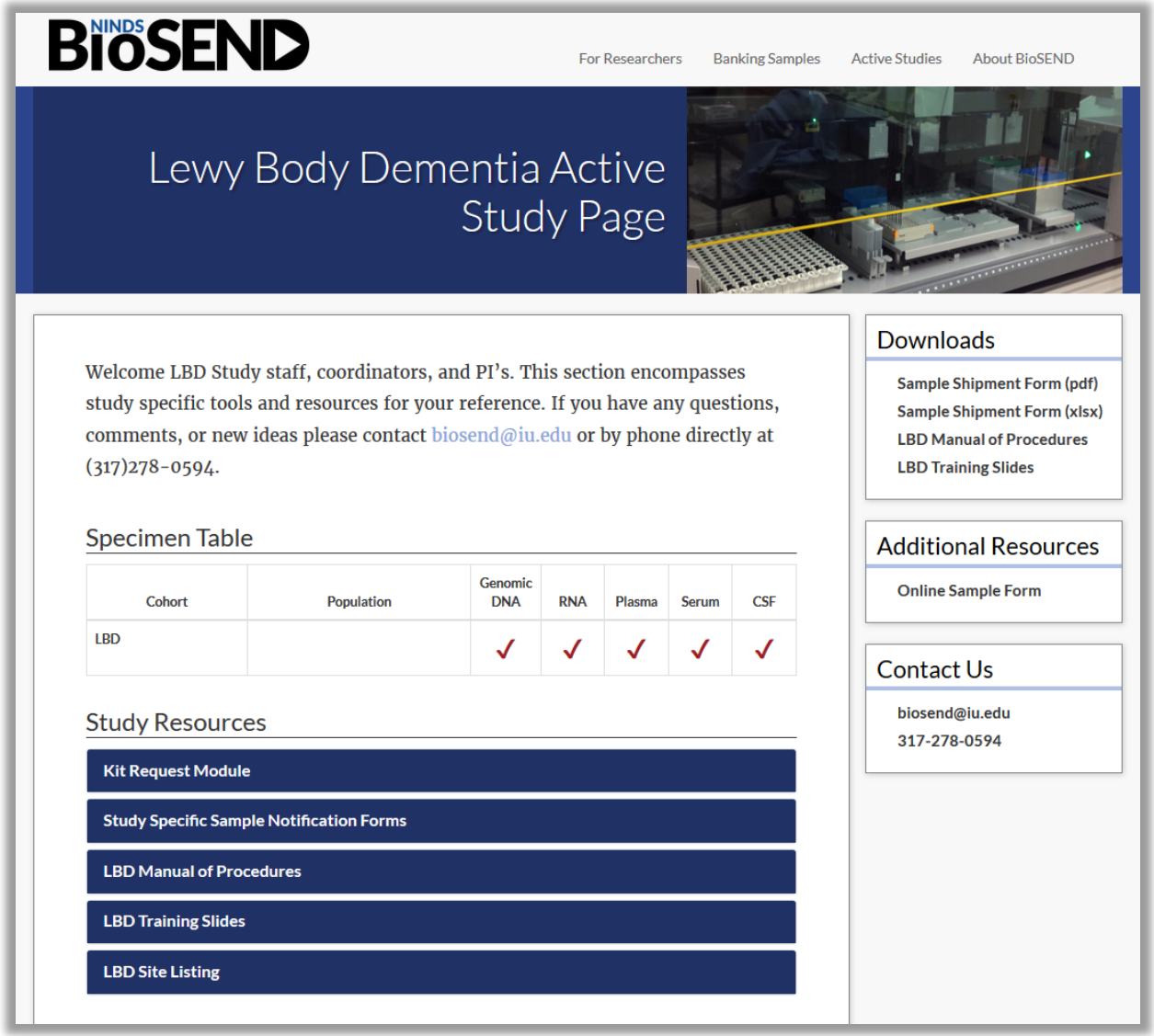
NINDS BioSEND Study Resources



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https://www.biosend.org/

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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