BioSpecimen Exchange for Neurological Disorders (BioSEND)

Rochester UDALL Training Webinar

NINDS BioSEND

BioSEND Training Webinar Overview

1. Study Reminders
2. Site Equipment
3. UDALL 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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UDALL Biospecimen Collection Protocol

	BL
DNA (6ml)	X
Whole Blood (6ml)	X
Plasma (6 x 1ml)	X
Serum (6 x 1ml)	X
RNA (2 x 2.5ml)	X

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Requesting Kits

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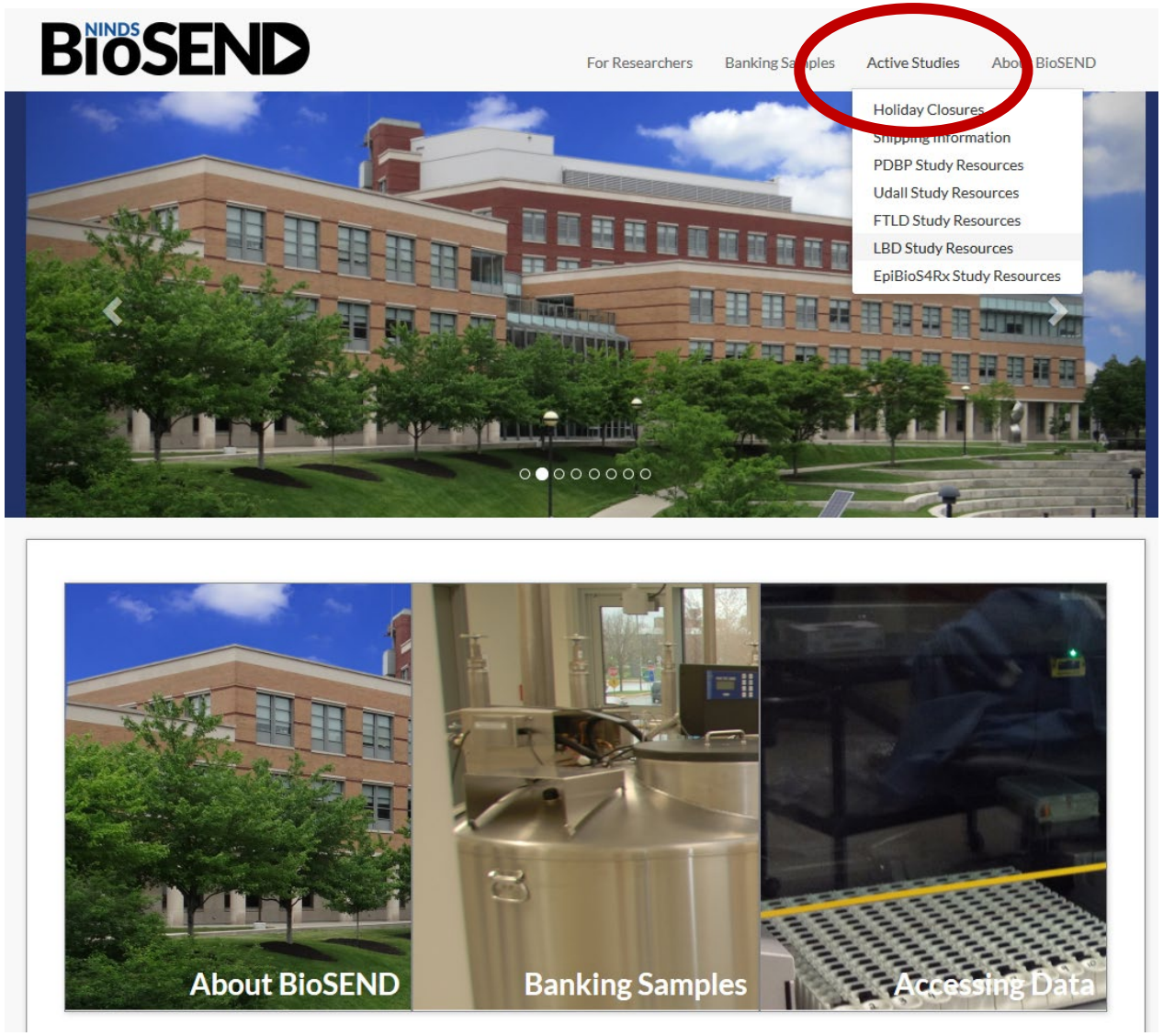

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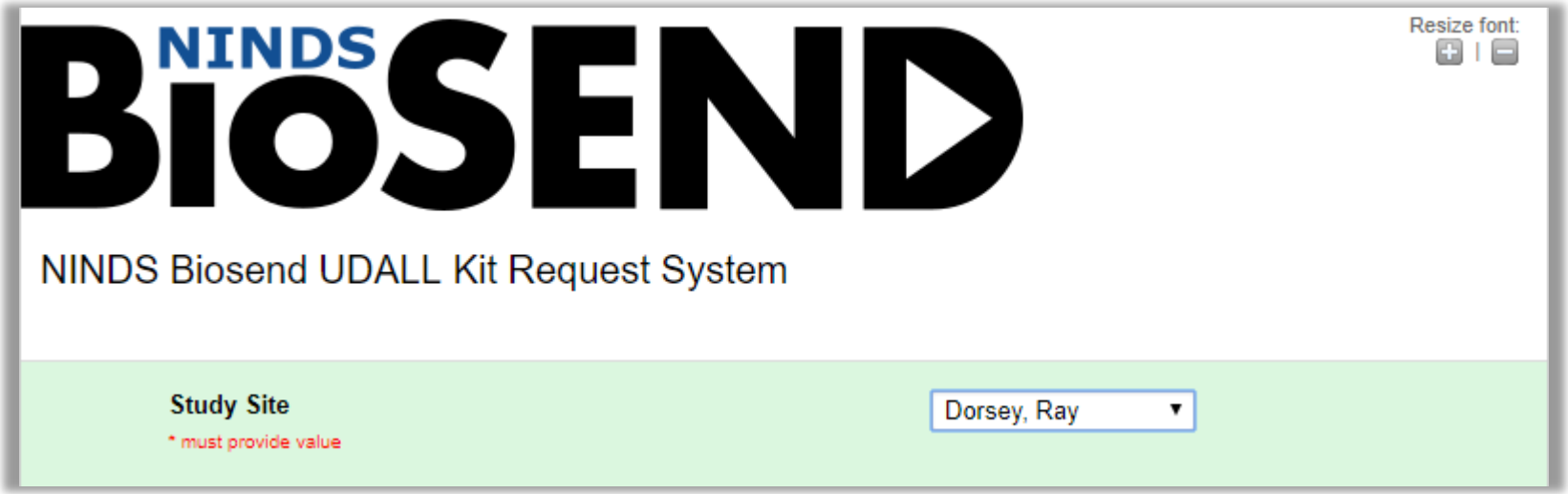
https://www.biosend.org/

BioSEND Kit Request Module



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

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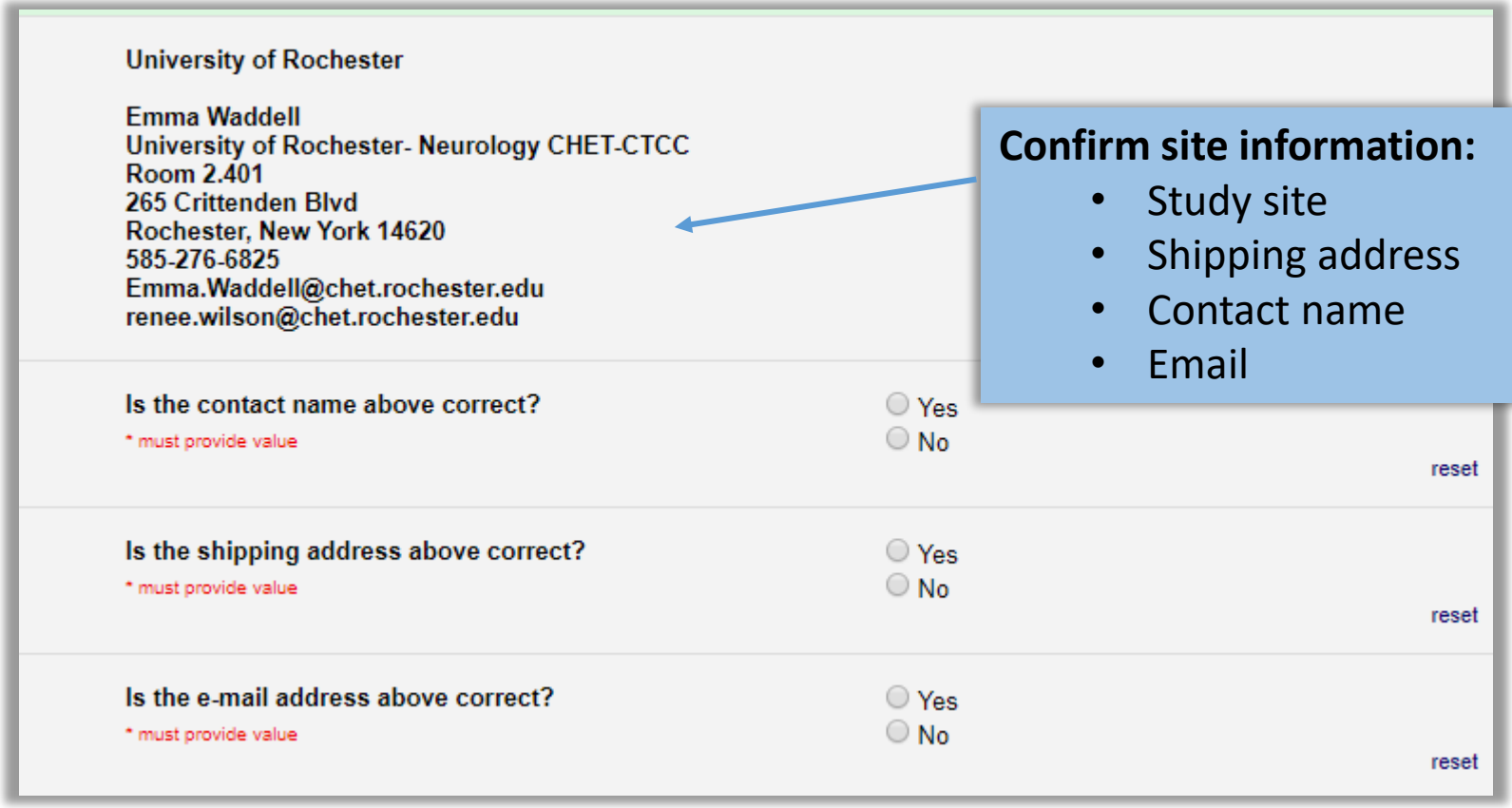
BioSEND Kit Request Module



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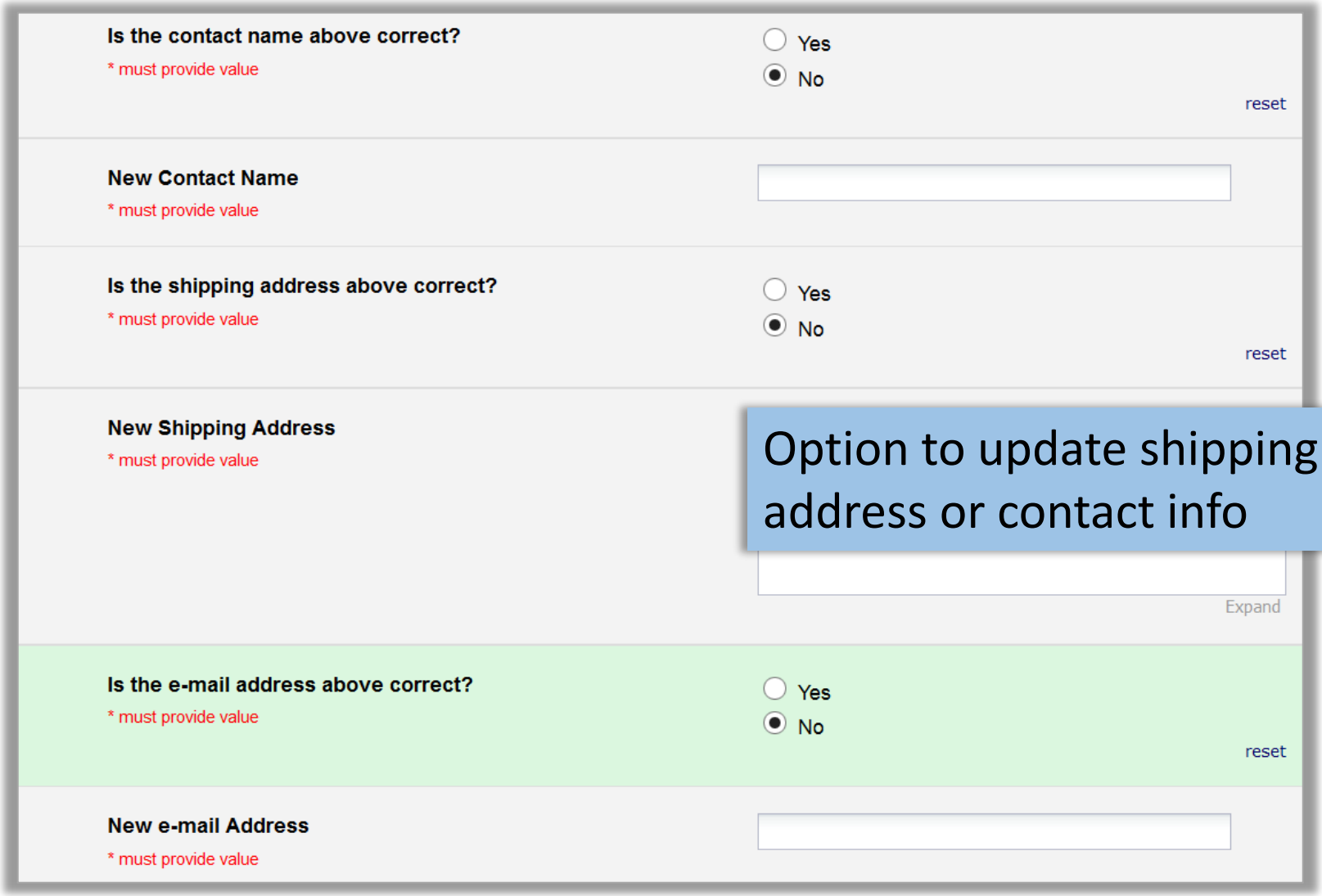
BioSEND Kit Request Module



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BioSEND Kit Request Module: Kit Type

Multiple ordering options

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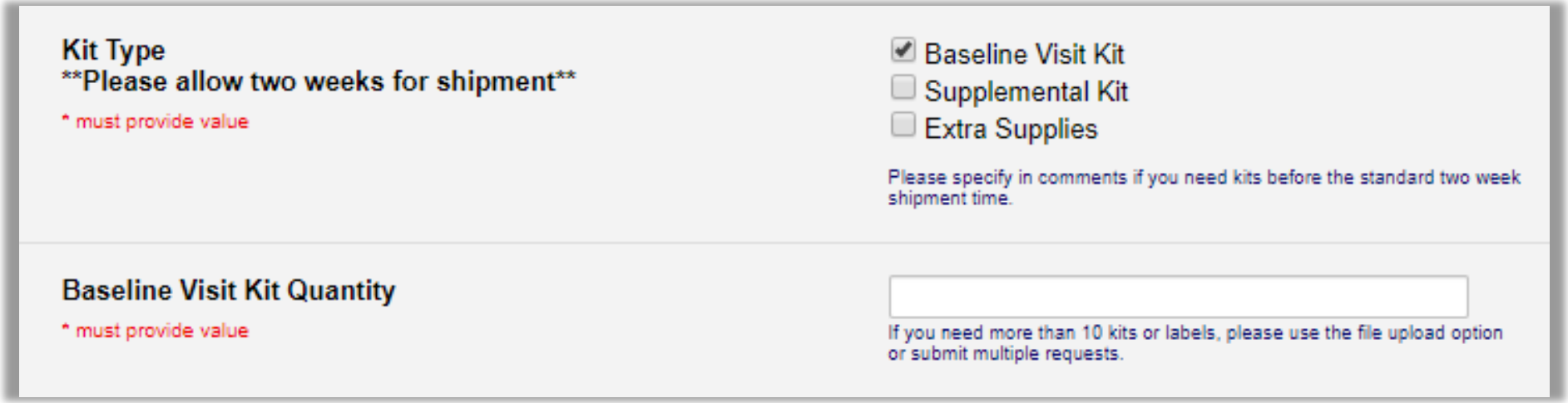
BioSEND Kit Request Module: Baseline Kit



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

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BioSEND Kit Request Module: Supplemental Kit

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

* must provide value

- [ ] Baseline Visit 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

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)
2 Biohazard bags
2 Vacutainer - PAXgene™ tubes (2.5 ml)
2 Monoject- Lavender-top EDTA tubes (10 ml)
2 Vacutainer - Purple-top EDTA tubes (6 ml)
2 - Vacutainer - Red-top serum tube (10 ml)
2 Disposable transfer pipettes (1ml)
2 Warning label packets

Contains a variety of extra kit pieces

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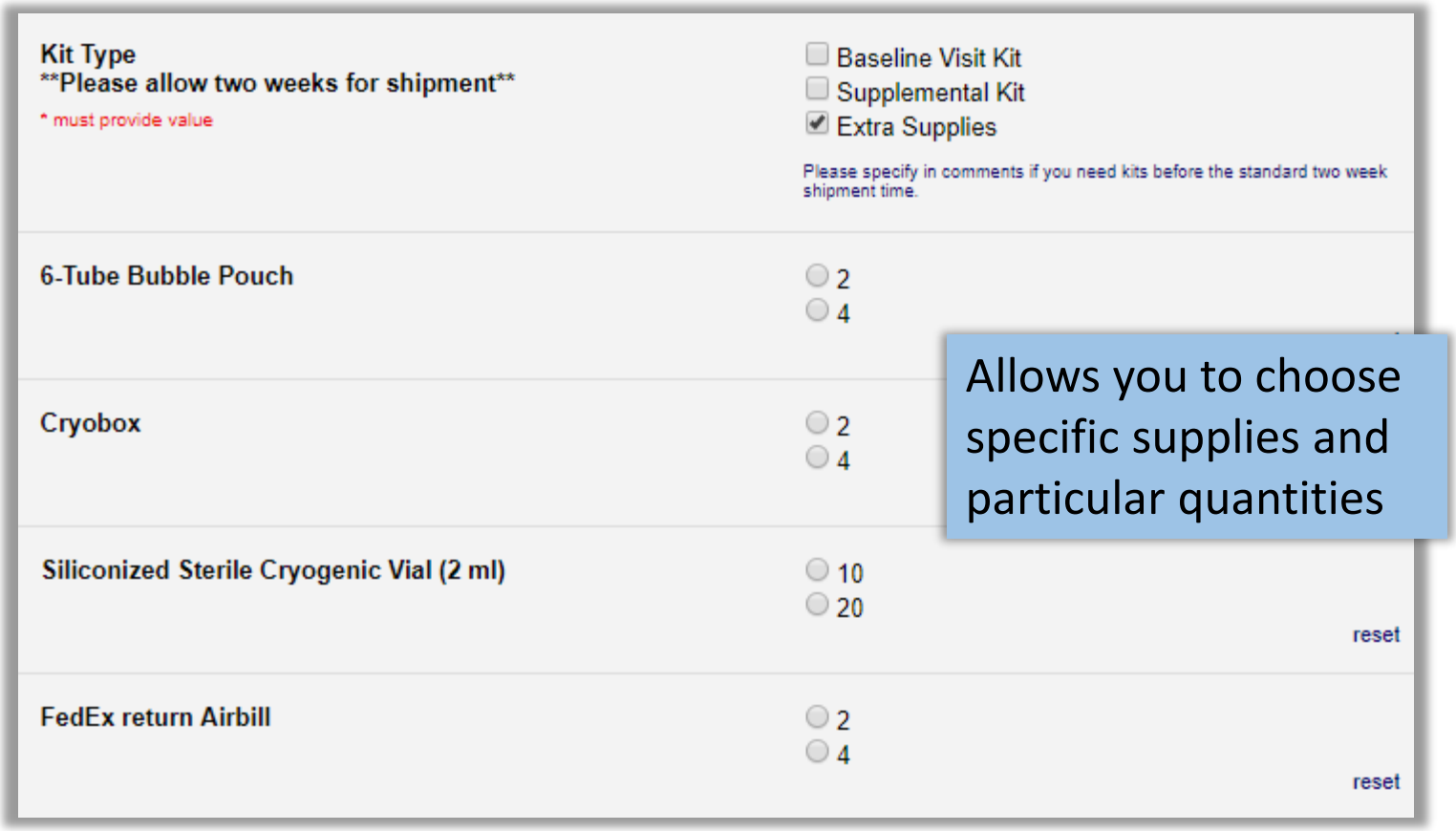
BioSEND Kit Request Module: Extra Supplies



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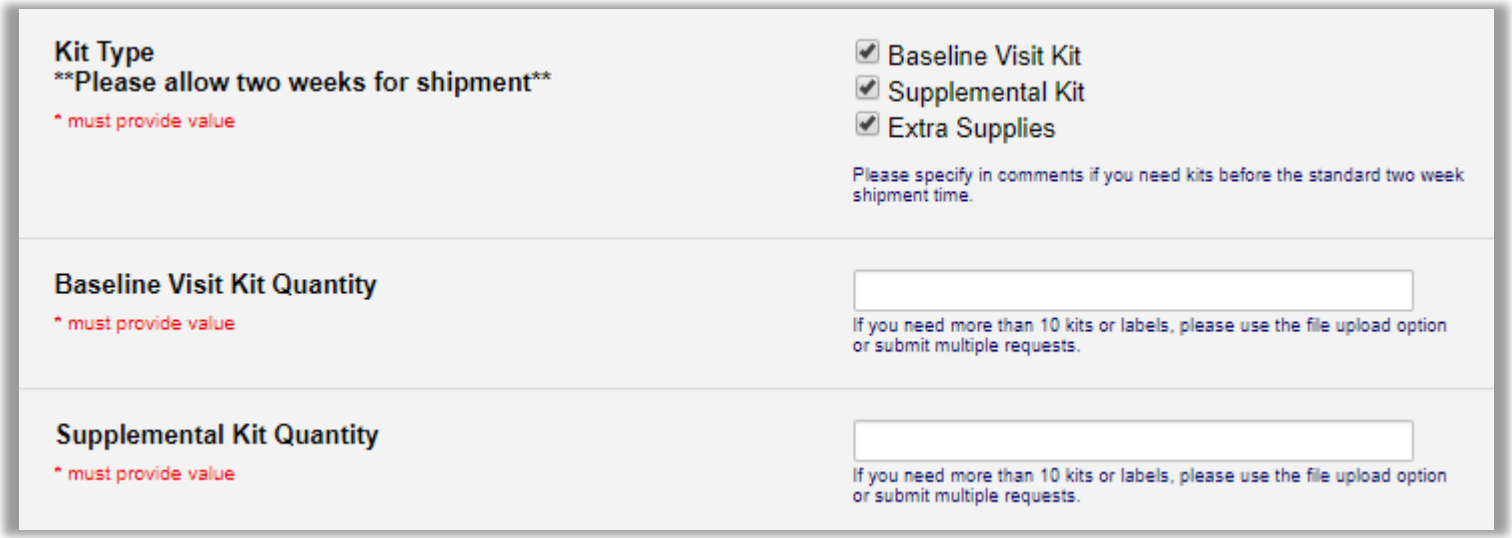
BioSEND Kit Request Module: Multiple Orders



You can order more than one type of kit and/or supply in a single kit request

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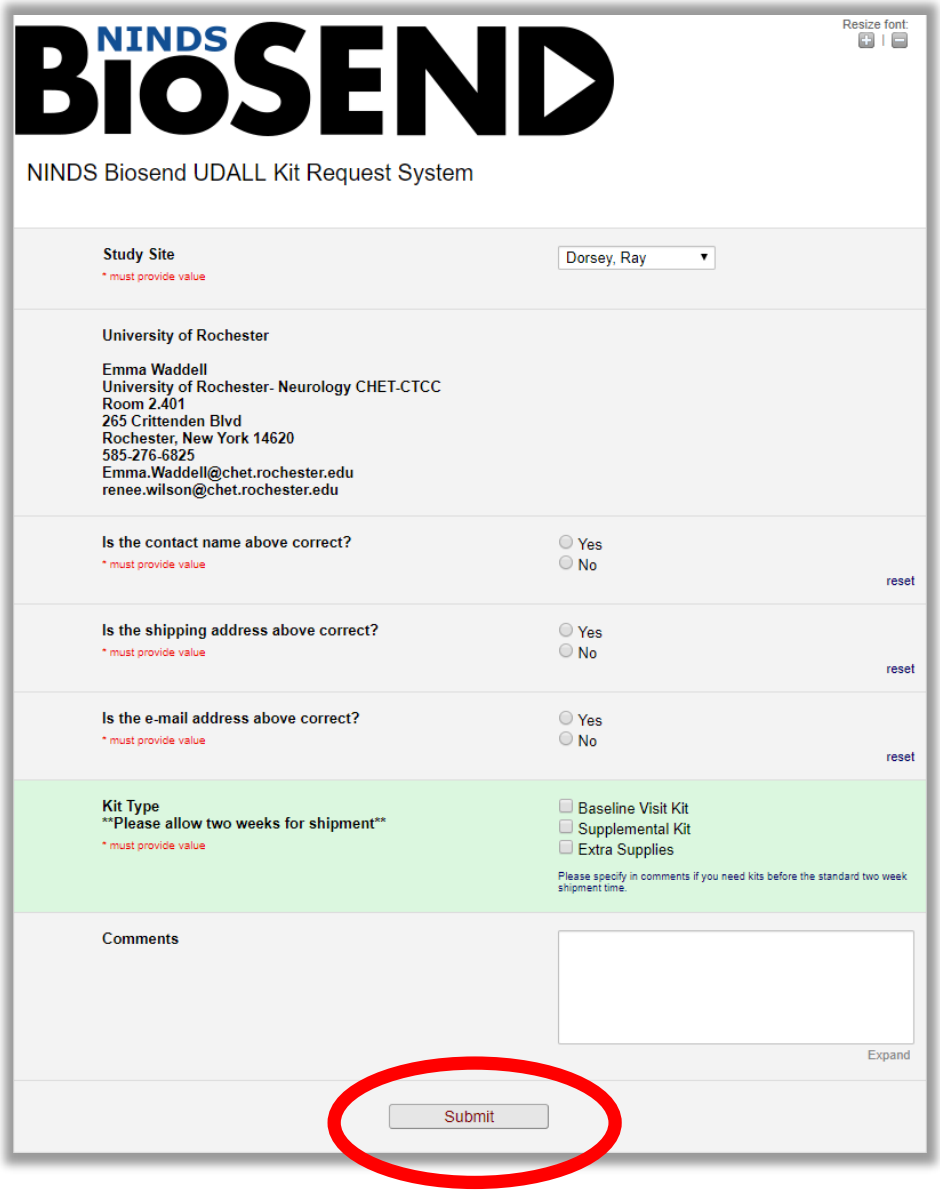
BioSEND Kit Request Module: Submit

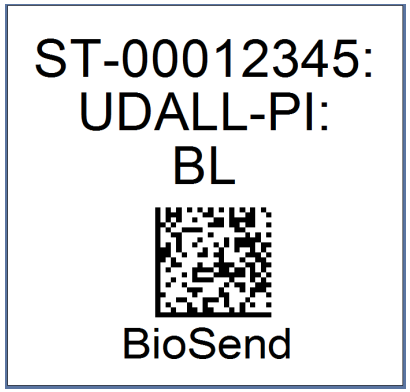
- Click “Submit” to turn in your request.
- The BioSEND staff will notify you that your request has been received and address any issues.



Labels

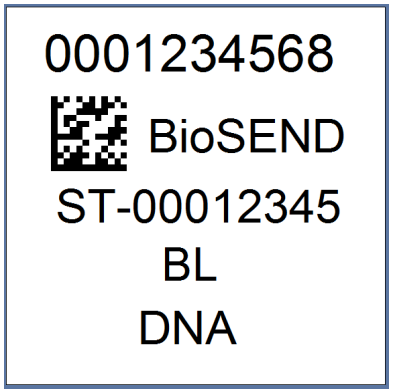
Types of Labels

Case Label



Identify study

Specimen Label



Identify individual biospecimens

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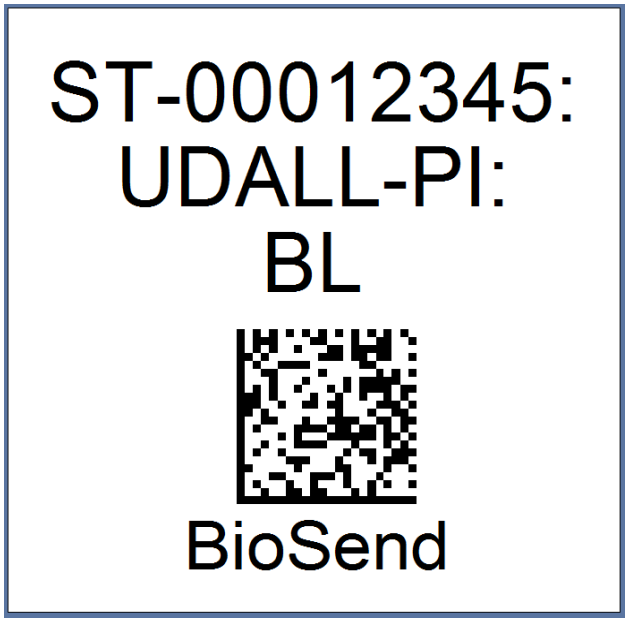
Case Label



Subject Number

Study

Visit

Biorepository Name

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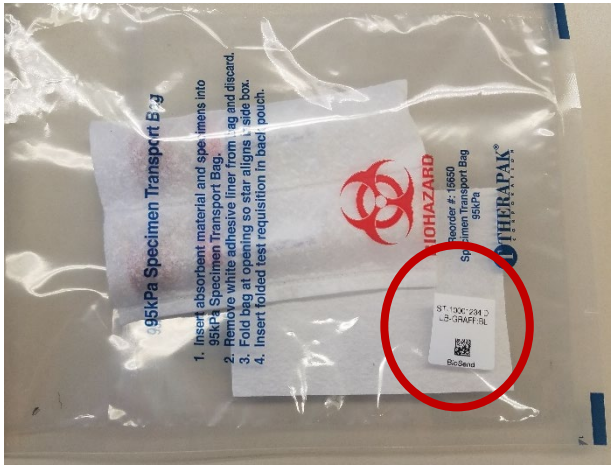
Case Labels

Case labels are placed:

- On the plastic biohazard bag of the cryovial transport box.
- On the plastic biohazard bag for the PAXgene™ and whole blood (EDTA) tubes.
- *On the lid of frozen shippers*



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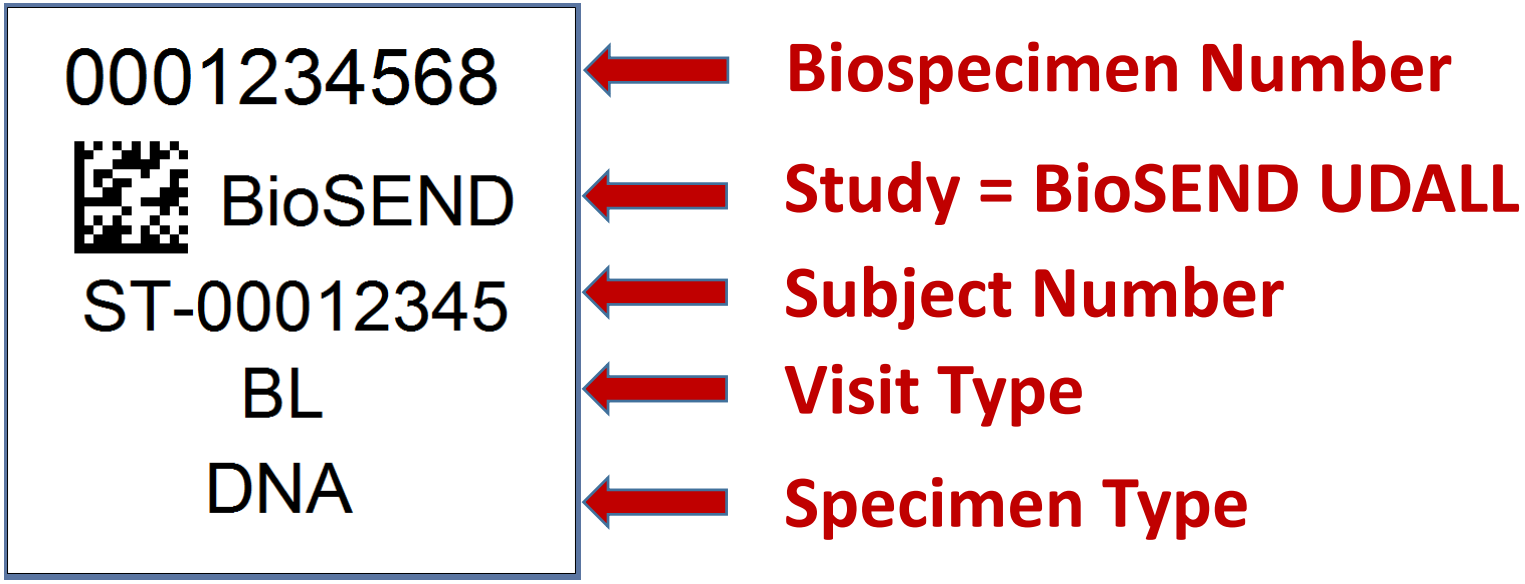
Collection and Aliquot Tube Label



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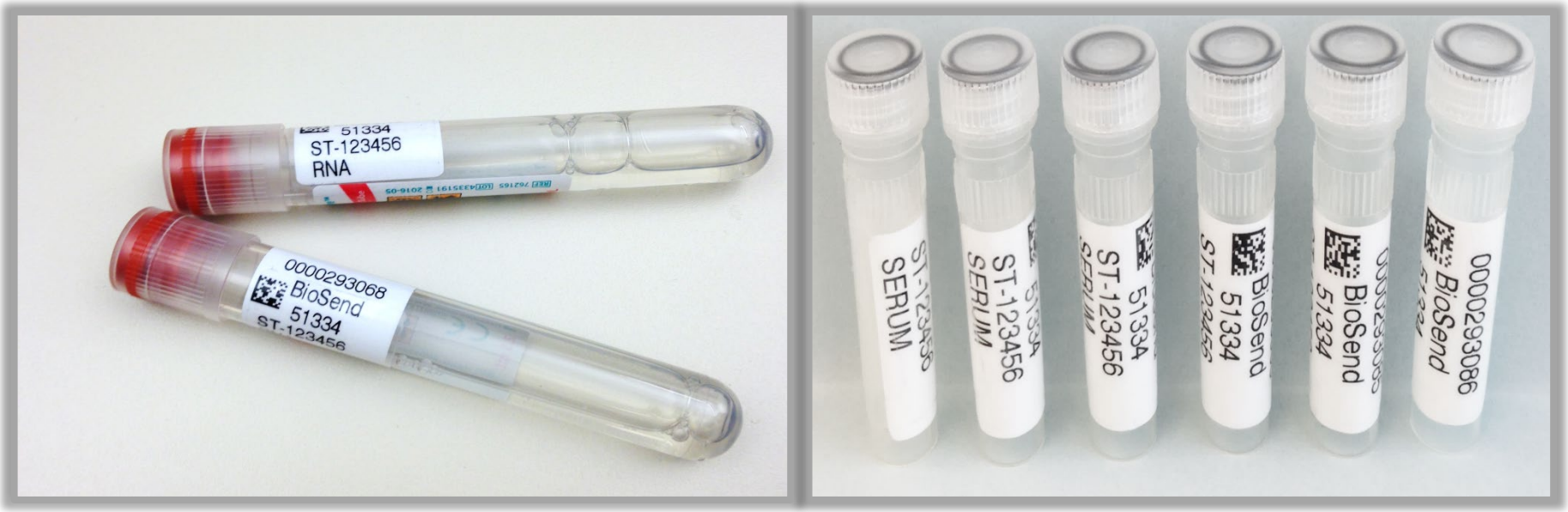
Collection and Aliquot Tube Label

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 plasma and CSF aliquots, please freeze samples upright in the cryobox provided

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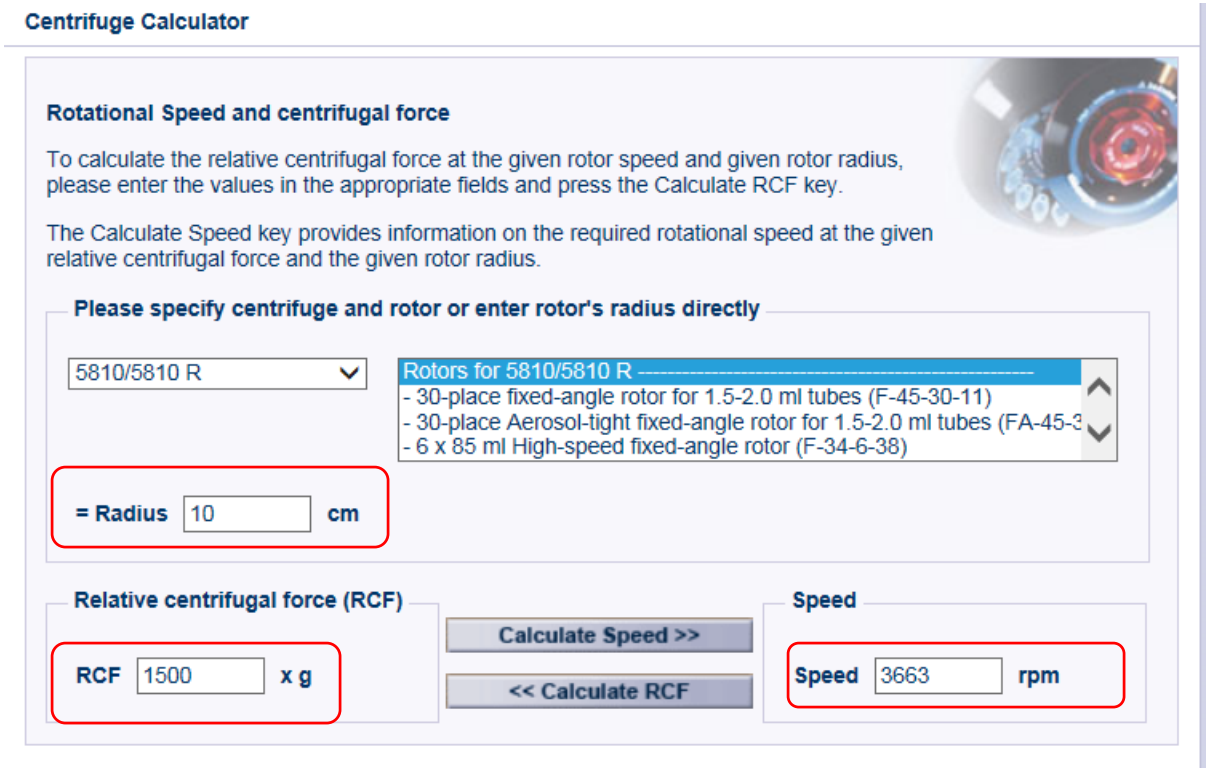
Calculating Centrifugation Speed

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

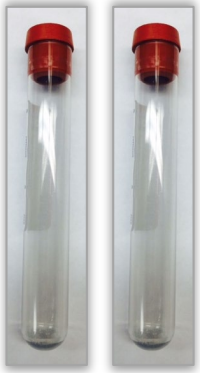


The 3663 rpm speed was calculated using a hypothetical radius of 10 cm and a RCF of 1500 x g.

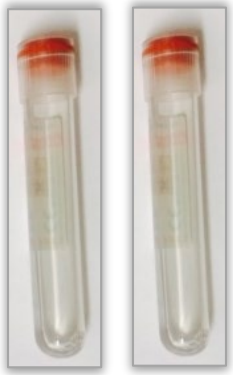
Refer to Appendix F in the BioSEND Biologics Manual for further information regarding the centrifugation of samples

Order of Specimen Collection

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



Serum



PAXgene ® (RNA)

EDTA (DNA)

EDTA (Plasma)

EDTA (WB)

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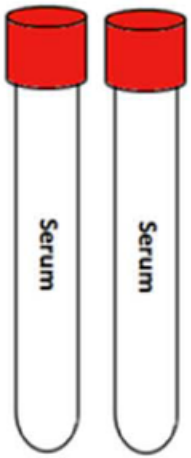
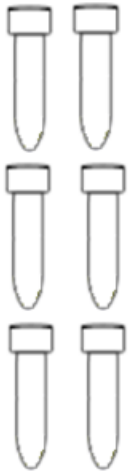
Serum Preparation (10ml Red Top Tube)

Step One



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

Step Two

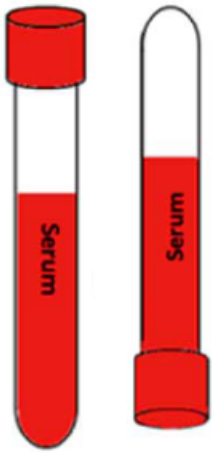


- Label 6 cryovials with pre-printed subject labels prior to blood draw.
- Pre-chill cryovials on wet ice for 5 minutes or longer.

Step Three

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

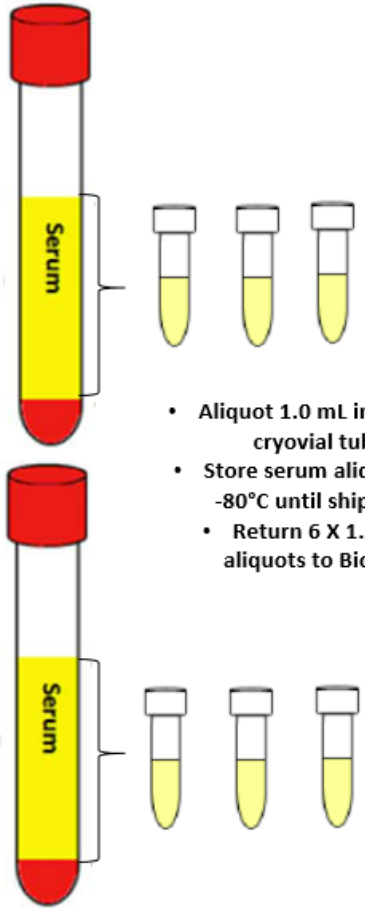
Step Four



- 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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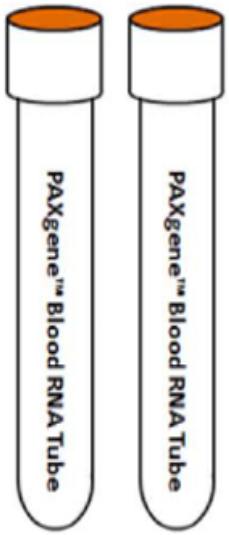
PAXgene™ Preparation (2.5ml Tube)

Step One

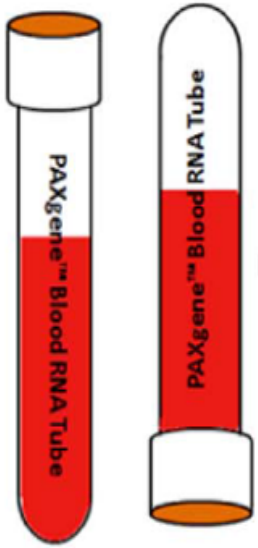


- Store tubes at room temperature.
- Label tubes with pre-printed subject labels prior to blood draw.

Step Two

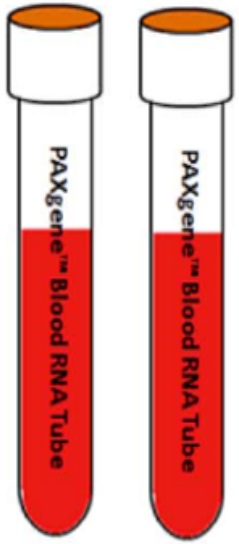
- Collect blood into *one* PAXgene Tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



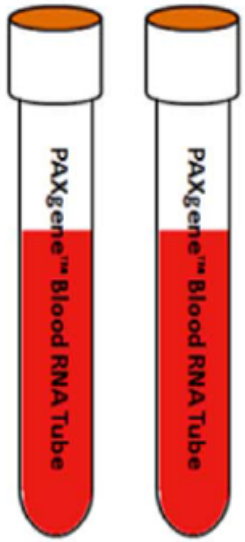
- Immediately after blood draw, invert tubes 8-10 times to mix samples.
- **Repeat steps two and three for second tube.**

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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DNA Preparation (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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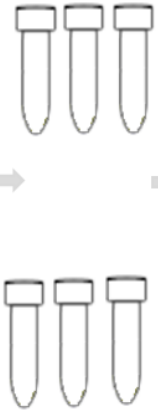
Plasma Preparation (10ml Lavender Top Tube)

Step One

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

Step Two

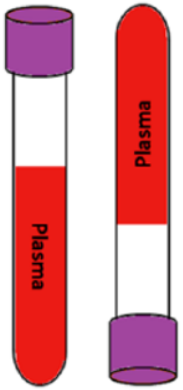


- Label 6 cryovials for plasma with pre-printed subject labels prior to blood draw.
- Pre-chill cryovials on wet ice for 5 minutes or longer.

Step Three

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

Step Four

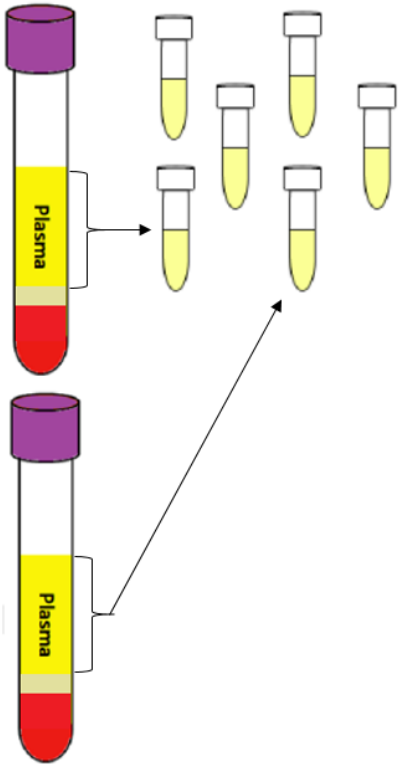


- Immediately after blood draw, invert tubes 8-10 times to mix samples.
- Repeat Steps 3 and 4 for second tube.

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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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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Plasma & Serum Aliquots

- Fill cryovials to 1ml
- Over-filled vials may burst in freezer
- Ship material to BioSEND
  - 6 Plasma aliquots
  - 6 Serum aliquots
  - 2 x PAXGene tubes
  - 2 x EDTA (whole blood, DNA)
- Do NOT send residual volumes to BioSEND

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Blood Collection: Troubleshooting

Issue #1: Blood draw 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 blood 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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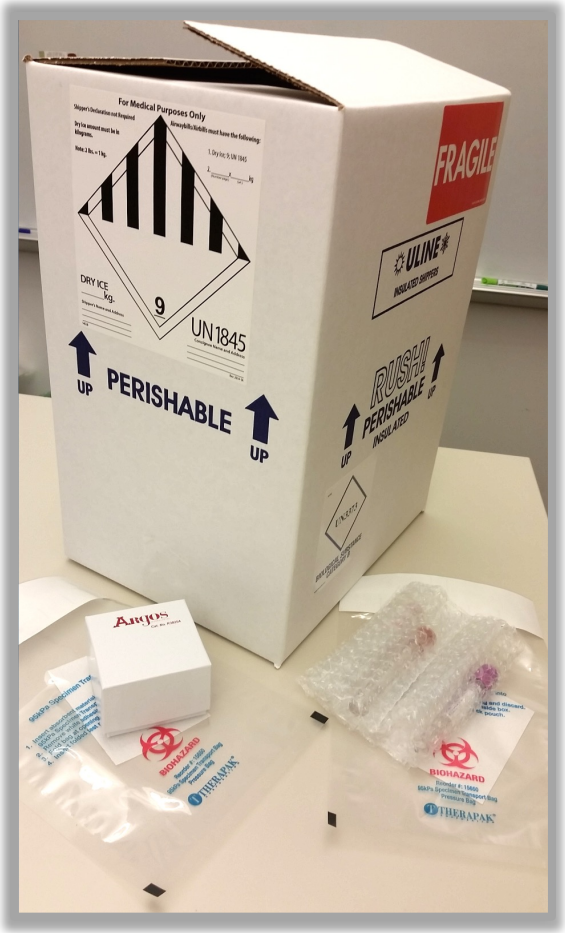
Frozen Samples

- **All samples are shipped frozen**
  - Plasma, Serum, Whole Blood for banking and DNA extraction, 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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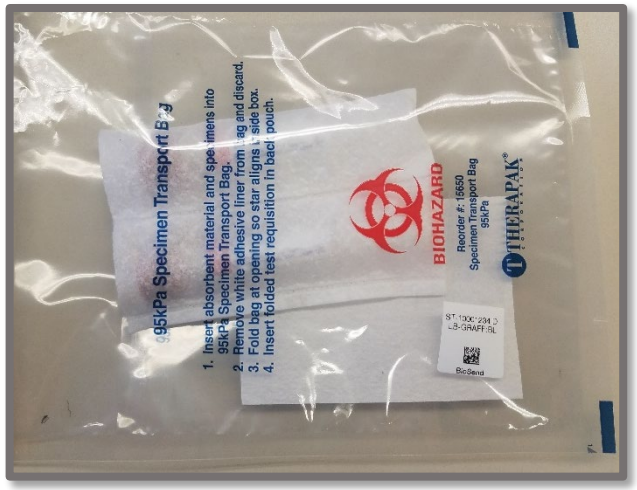
Packaging and Shipping Frozen Samples



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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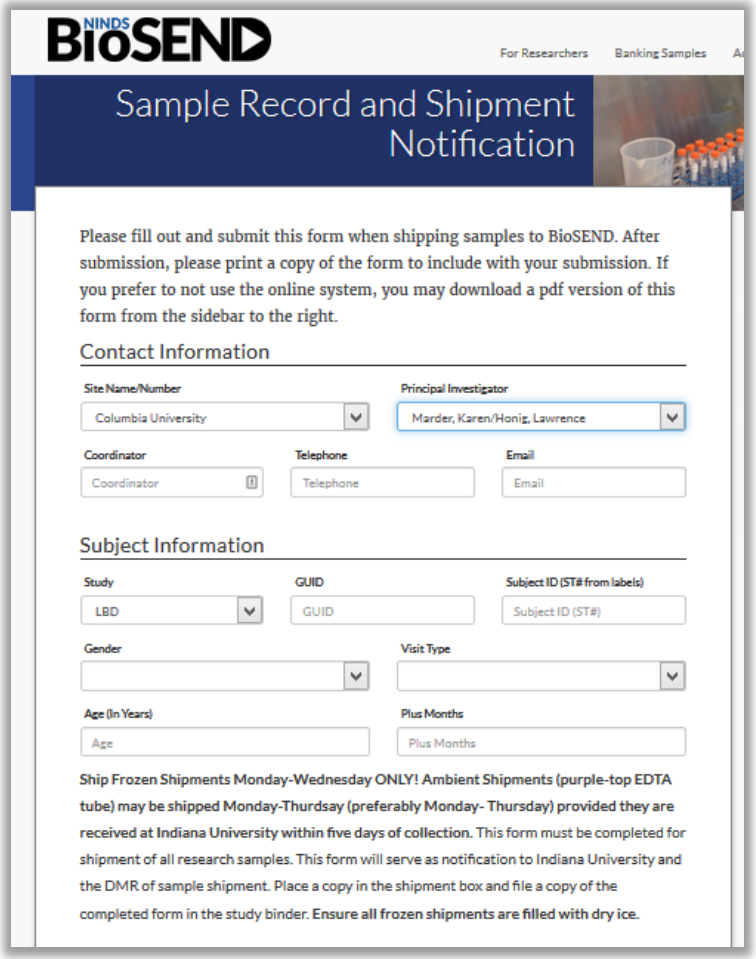
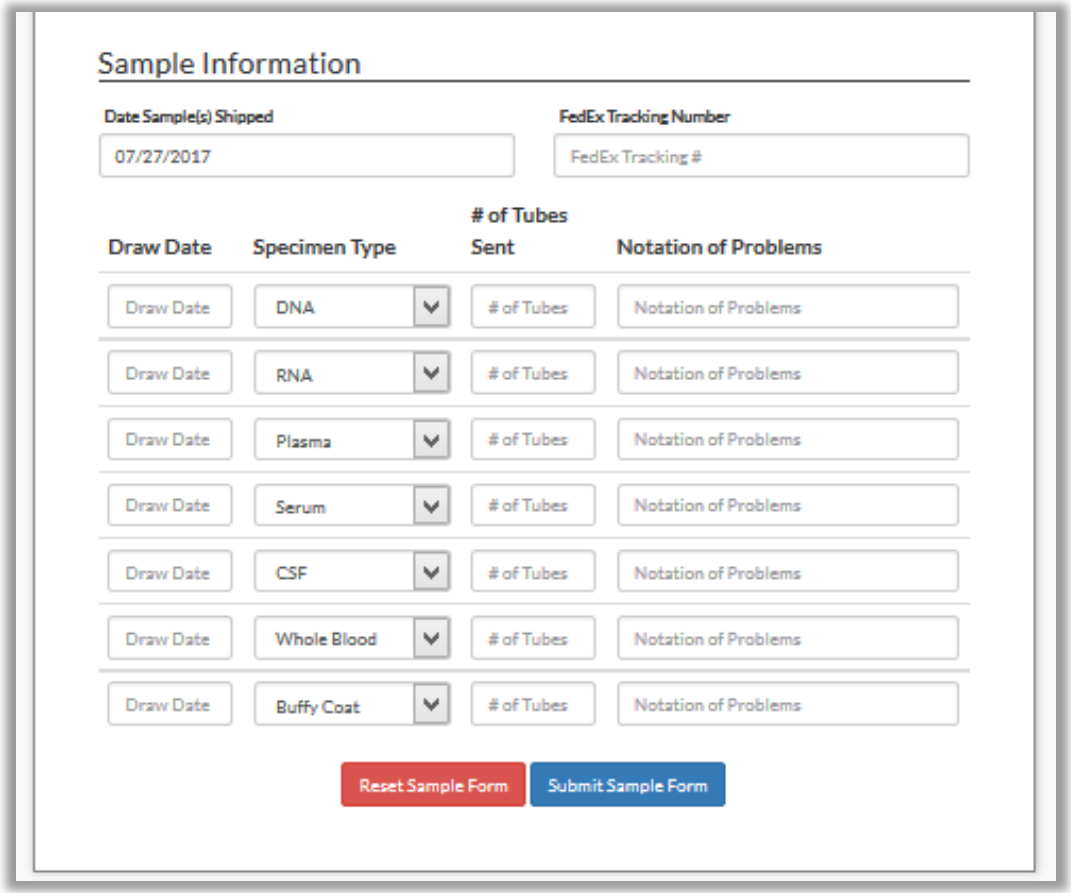
Sample Shipment Notification Form, Online





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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?

  Project Manager: Claire Wegel (cwegel@iu.edu)

  - Email: biosend@iu.edu

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Questions?

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