BioSpecimen Exchange for Neurological Disorders (BioSEND)

EpiBioS4Rx Training Webinar

NINDS BioSEND

BioSEND Training Webinar Overview

1. Study Reminders
2. Site Equipment
3. EpiBioS4Rx 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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EpiBioS4Rx Biospecimen Collection Protocol

	D1	D3	D5	D15	D30	D90	D180
DNA from Buffy coat	X	X	X	X	X	X	X
Plasma (6 x 250ul)	X	X	X	X	X	X	X
RNA (2 x 2.5ml)			X	X			

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Study Visit Protocol

- Due to the small window between visits, a kit will contain all supplies required to collect an entire subject's longitudinal visits
- BioSEND will send 4 kits at study startup, plus a supplemental kit which contains extra components of all kit materials
  - Supplemental EXCLUDES labels
- Sites are encouraged to frequently review kit inventory to ensure adequate supply

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Study Identifiers

Study ID	ST-Number
• Format: #_##_####	• Format: ST-1#######
• Example: 3_17_0000	• Example: ST-10001234
• Middle two-digit number = site number	• To be used in reference to BioSEND
• To be used for clinical data capture	• Present on all sample labels

- A subject MUST have the SAME Study ID and ST-Number for the duration of the study
- Example: Study ID 3_17_0000 = ST-10001234 for ALL longitidunal visits for that subject
- BOTH identifiers to be included on the Biosample shipping form

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Requesting Kits

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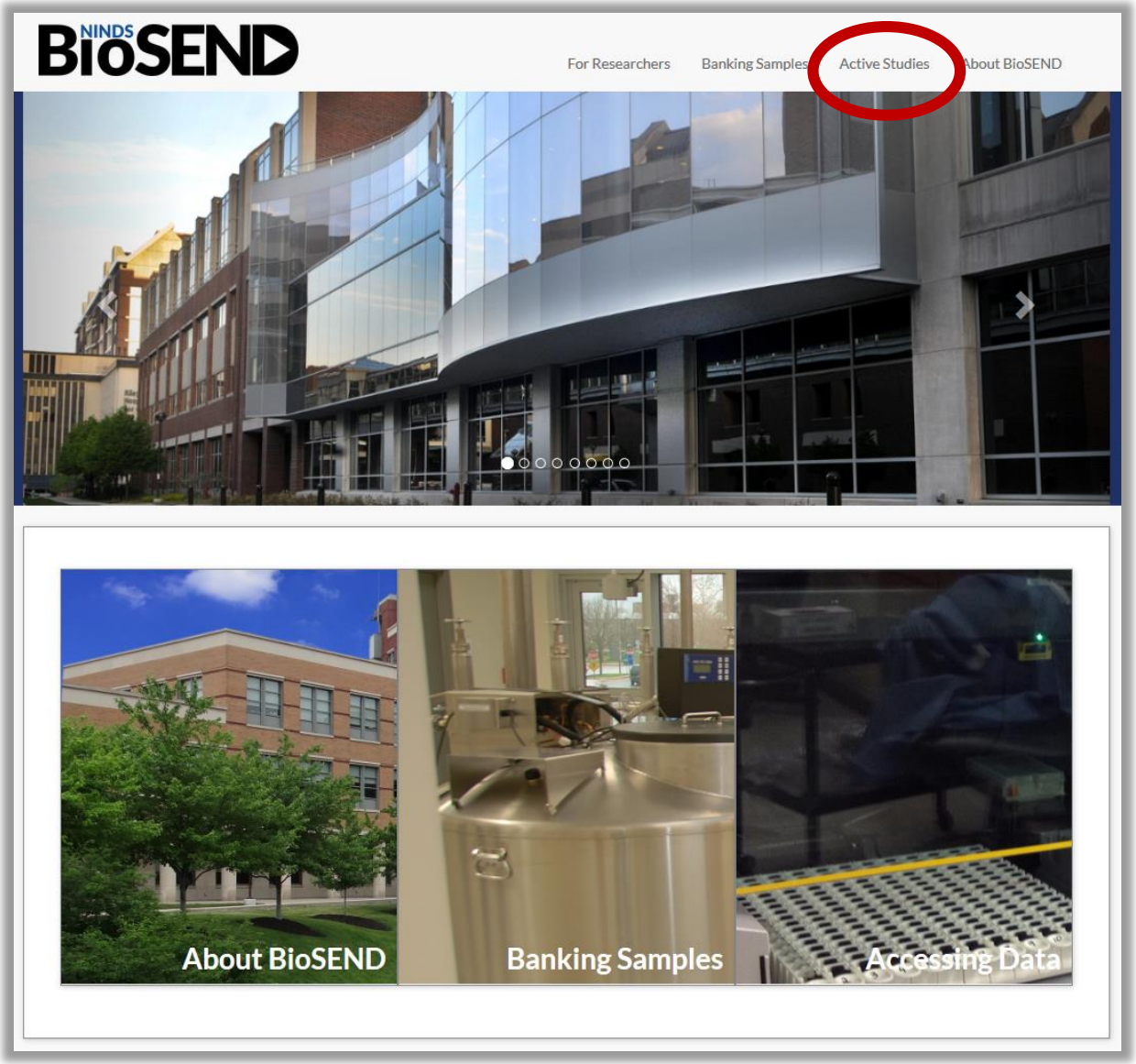
NINDS BioSEND Website



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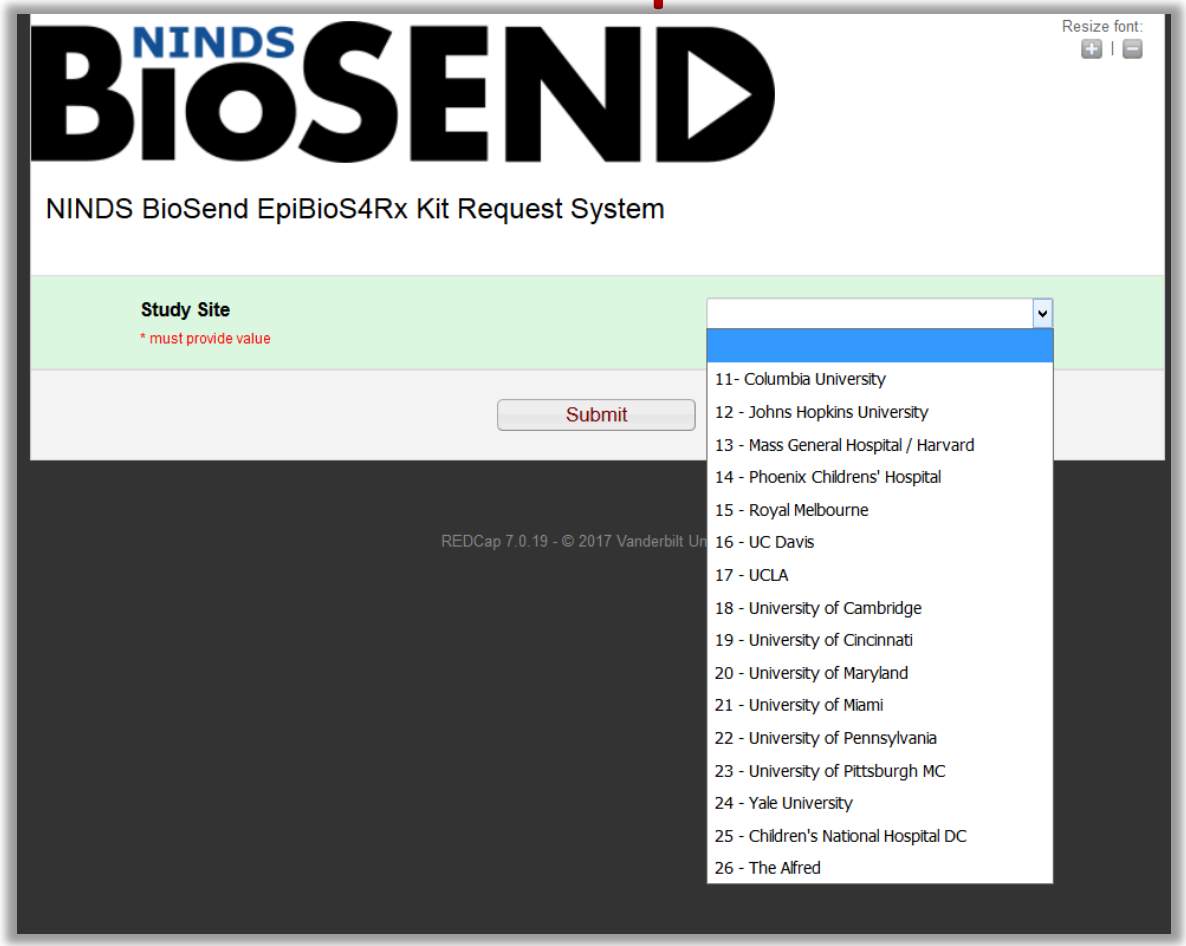
https://www.biosend.org/

BioSEND Kit Request Module



- http://kits.iu.edu/epibios4rx **Choose your site number and location from the drop down menu**

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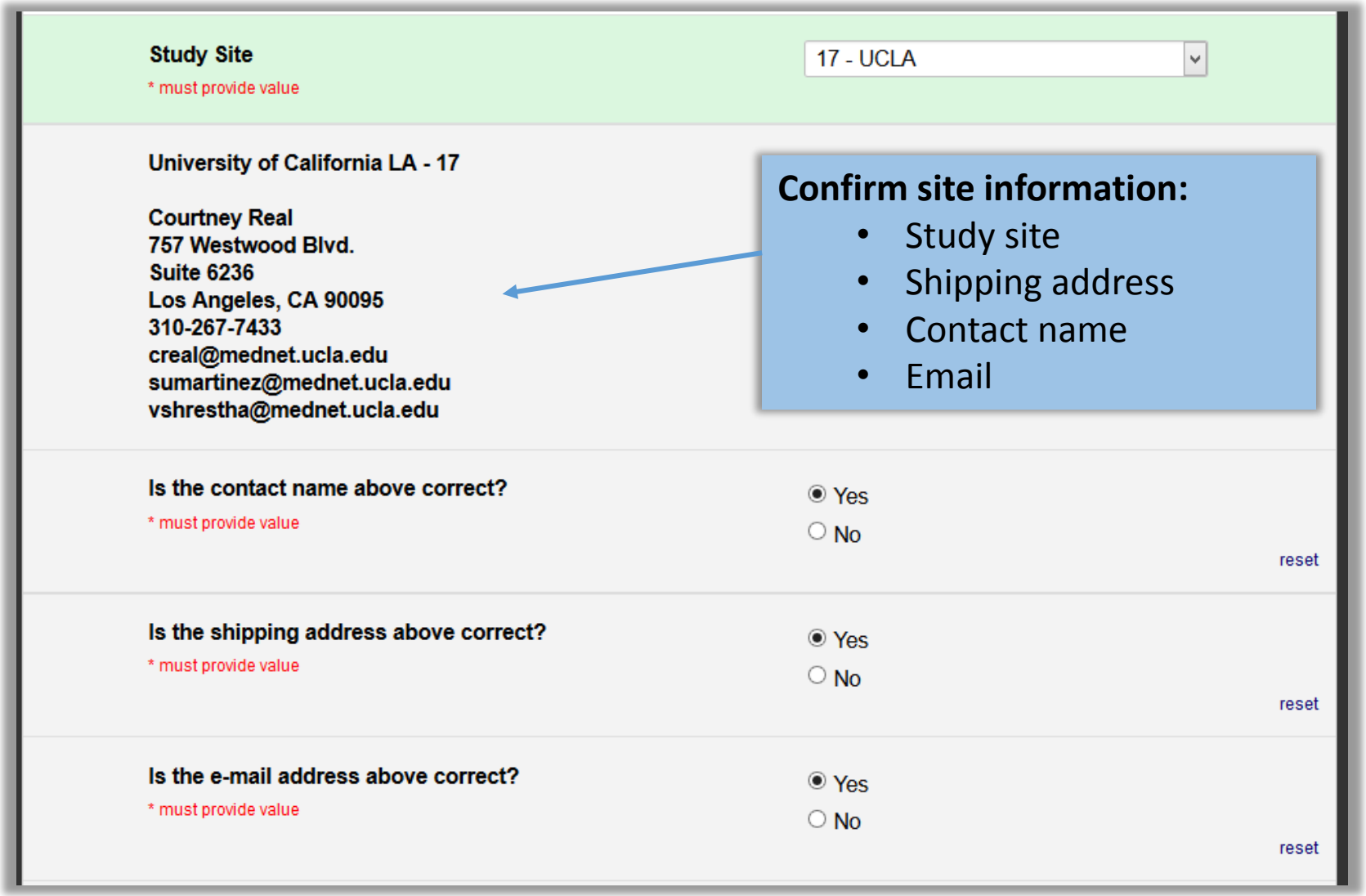
BioSEND Kit Request Module



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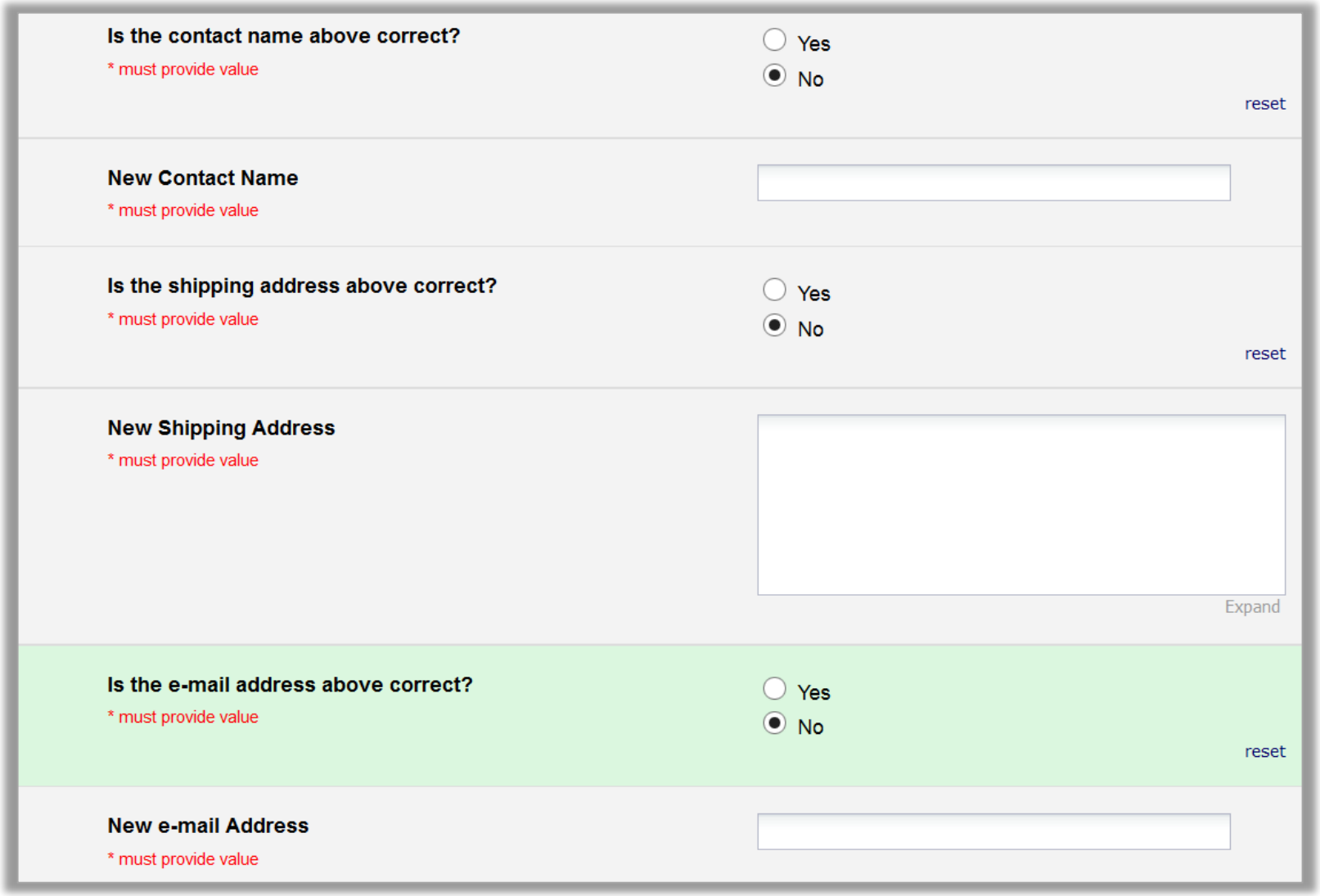
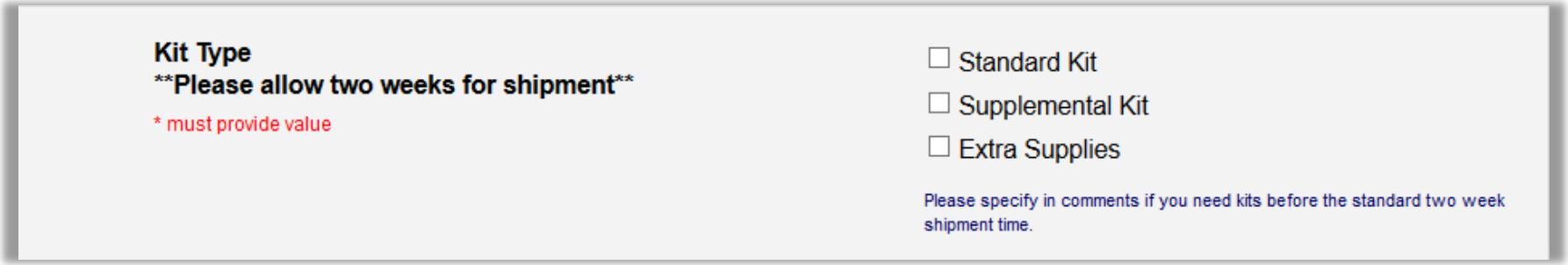
BioSEND Kit Request Module



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

BioSEND Kit Request Module: Kit Type



- Select the type of kit that you'd like to order

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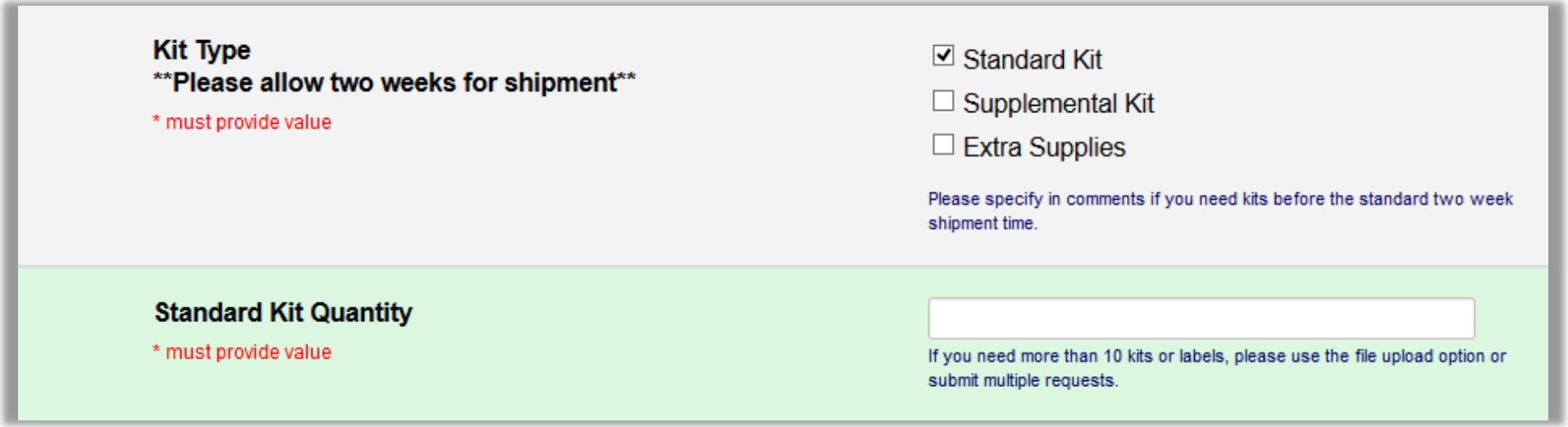
BioSEND Kit Request Module: Standard Kit



- BioSEND creates ST numbers for standard kits
- Enter kit quantity

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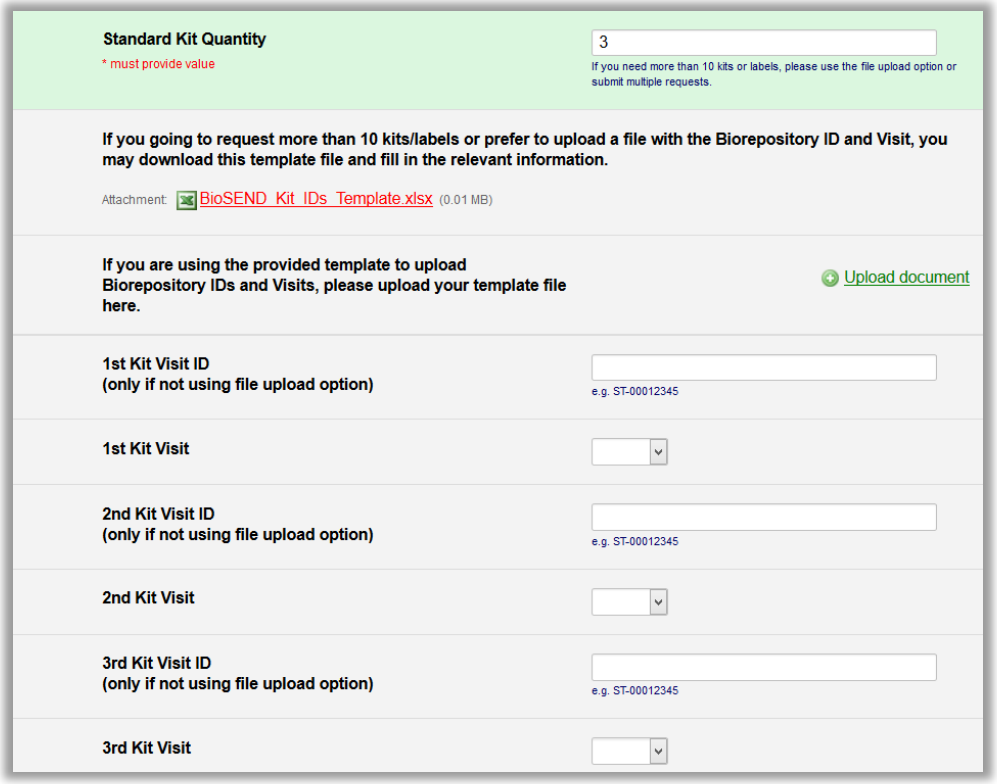
BioSEND Kit Request Module: Requesting Specific Subjects



- Since Standard kits contain all materials for longitudinal visits for a subject, sites DO NOT need to request specific subjects unless replacement labels are needed.

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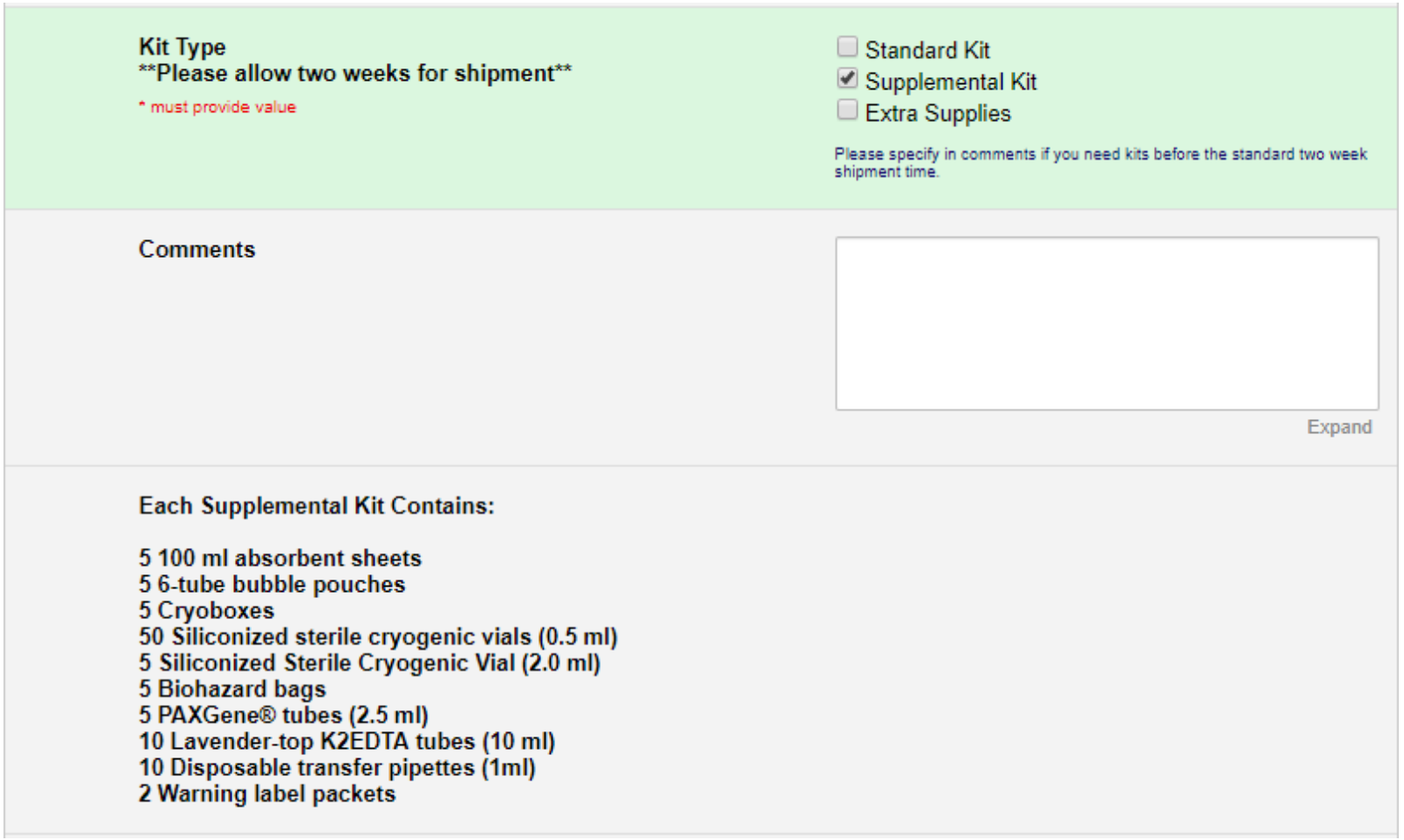
BioSEND Kit Request Module: Supplemental Kit



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

* must provide value

- [ ] Standard Kit
- [x] Supplemental Kit
- [ ] Extra Supplies

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

Comments

Expand

Each Supplemental Kit Contains:

5 100 ml absorbent sheets
5 6-tube bubble pouches
5 Cryoboxes
50 Siliconized sterile cryogenic vials (0.5 ml)
5 Siliconized Sterile Cryogenic Vial (2.0 ml)
5 Biohazard bags
5 PAXGene® tubes (2.5 ml)
10 Lavender-top K2EDTA tubes (10 ml)
10 Disposable transfer pipettes (1ml)
2 Warning label packets

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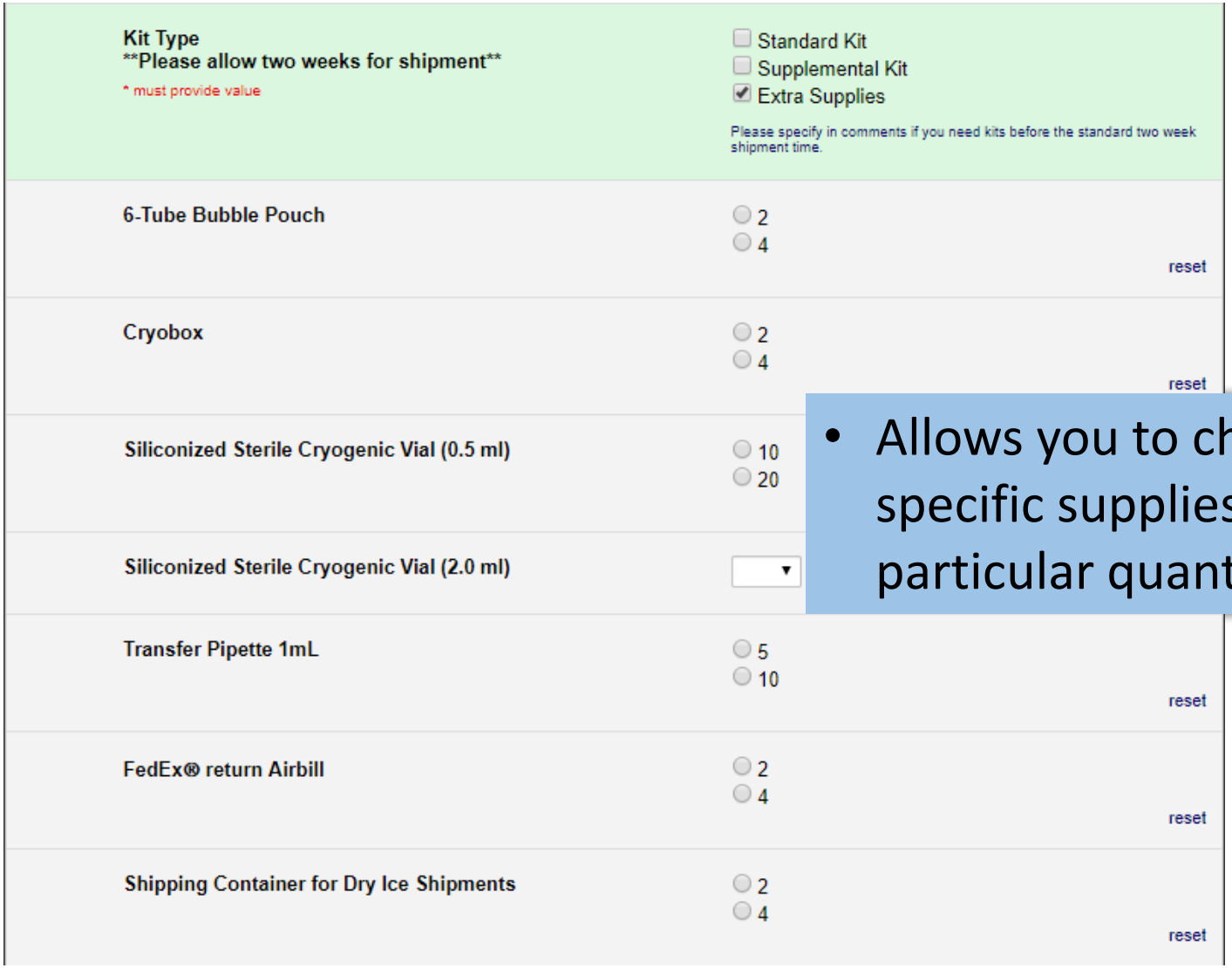
- Contains a variety of extra kit components

BioSEND Kit Request Module: Extra Supplies



- Allows you to choose specific supplies and particular quantities

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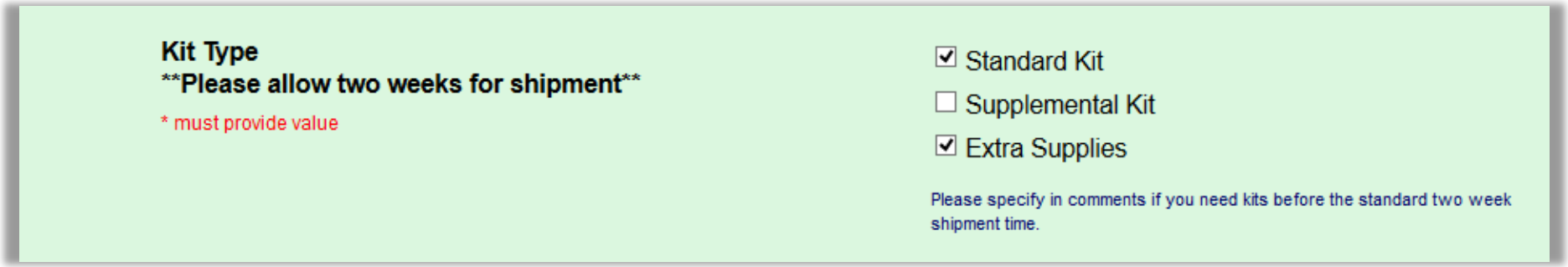
BioSEND Kit Request Module: Multiple Orders



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

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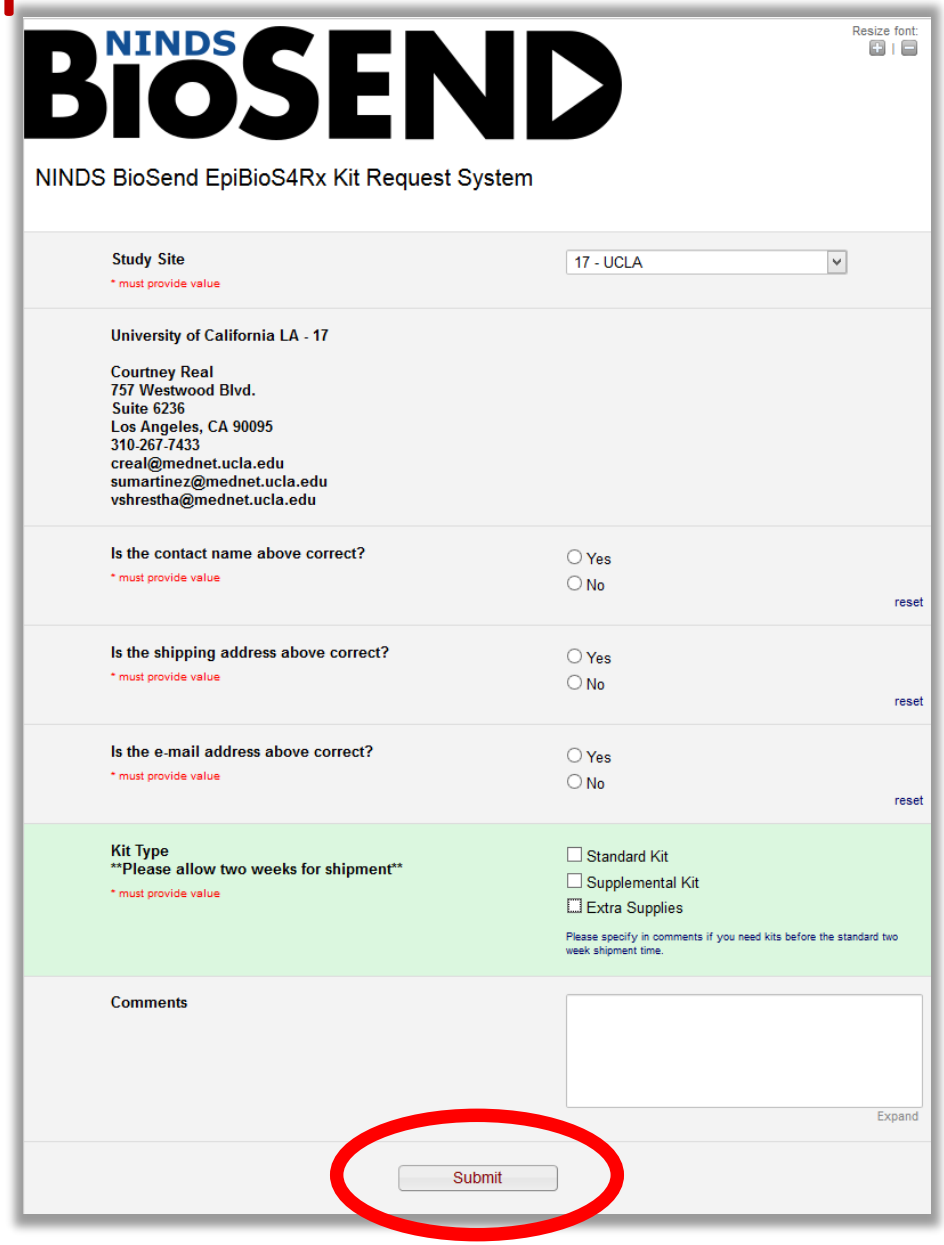
BioSEND Kit Request Module: Submit

- 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 site number

Specimen Label

Identify individual biospecimens

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Case Label

Subject Number

Study – Site number

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

Sample Identifier

Biorepository Name

Subject Number

Visit Type

Specimen Type

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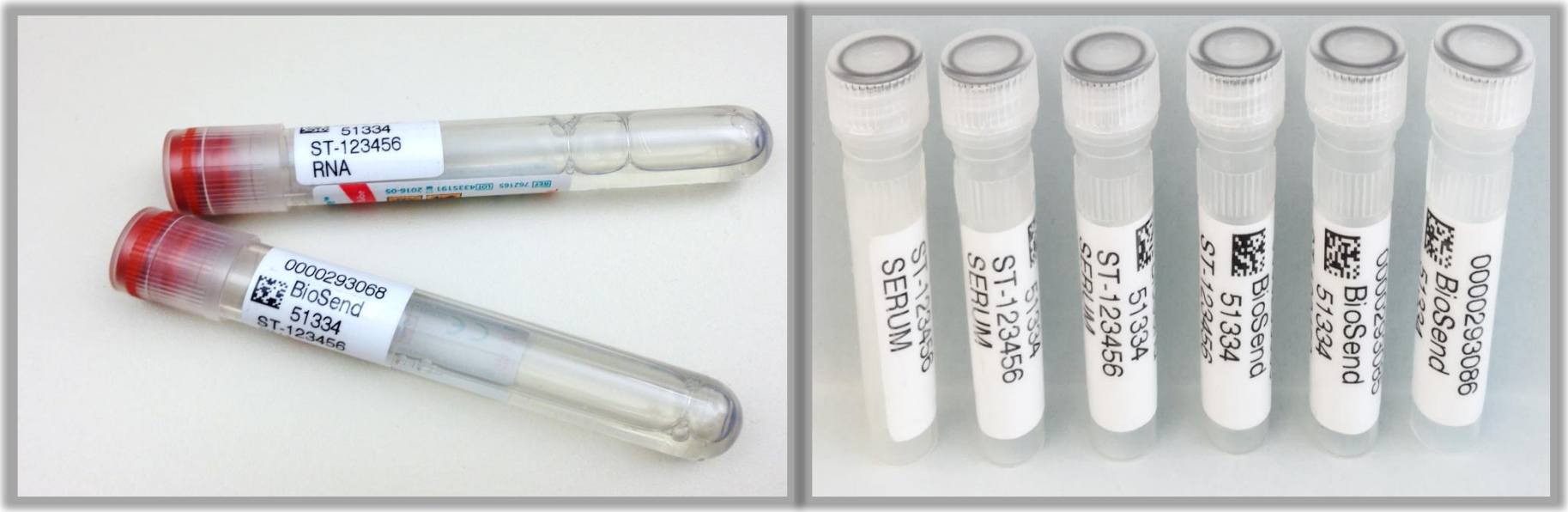
Collection and Aliquot Tube Label

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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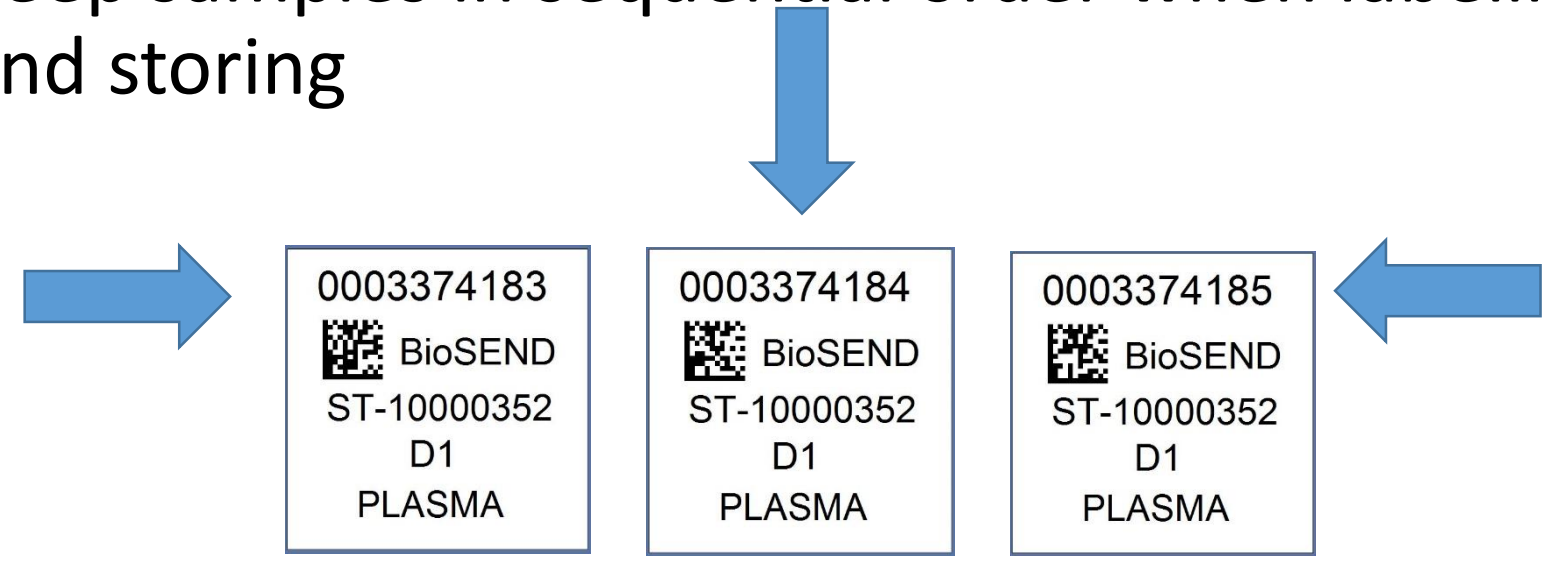
Aliquot Labels

- Keep samples in sequential order when labeling and storing



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Sample Collection & Processing

Reminders:

- G force ≠ RPM

- All specimens should be frozen and stored **UPRIGHT**
  - For RNA, please freeze samples upright in a non-styrofoam rack
  - For plasma and buffy coat, please freeze samples upright in the cryobox provided

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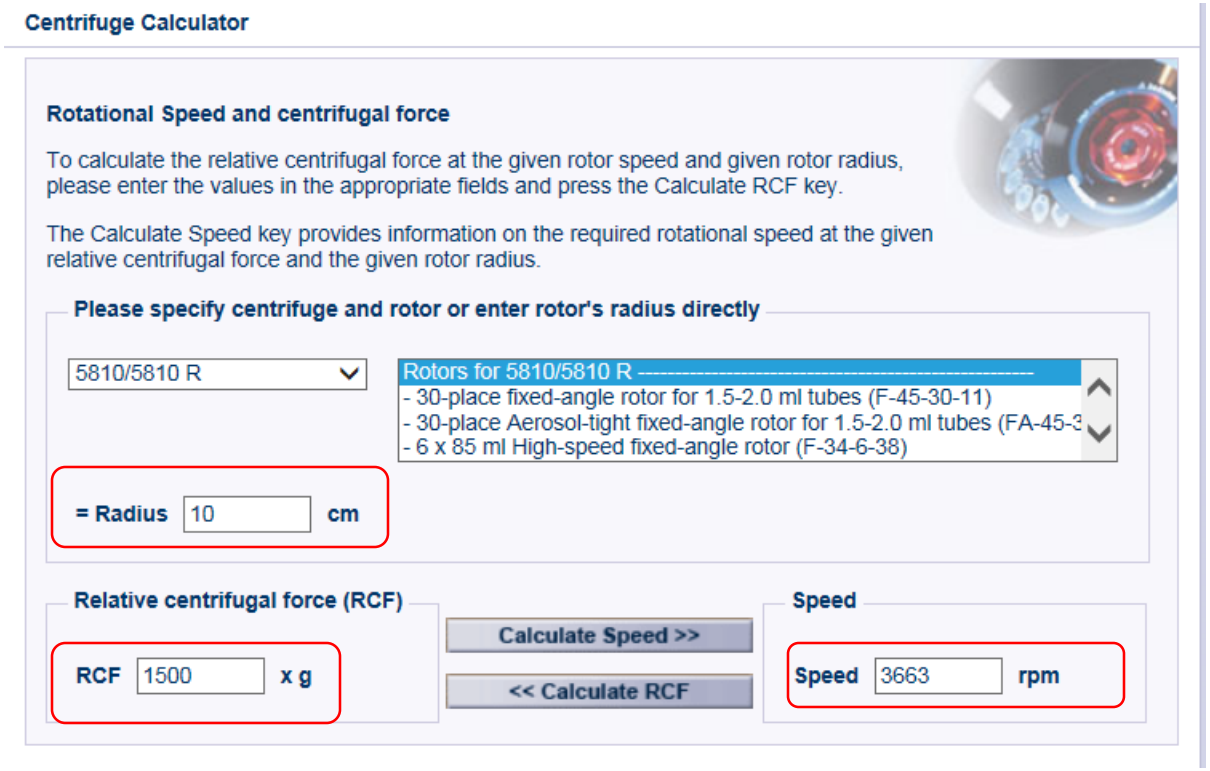
Calculating Centrifugation Speed

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. PAXgene® tube for RNA
2. K2 EDTA **10 ml (lavender top**) blood collection for plasma and buffy coat

PAXgene®
(RNA)

10ml K2EDTA
(Plasma, Buffy Coat)

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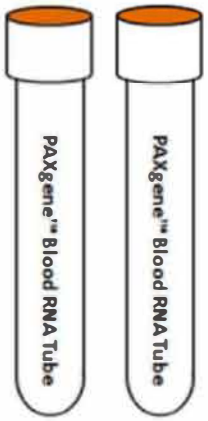
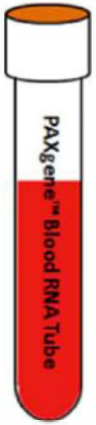
PAXgene™ Preparation (2.5ml Tube)

Step One



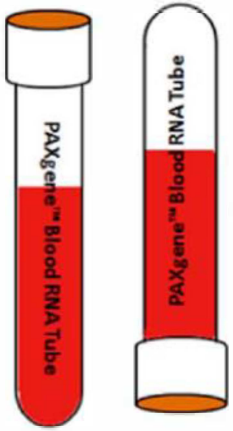
- Store tubes at room temperature.
- Label tubes with pre-printed subject labels prior to blood draw.

Step Two



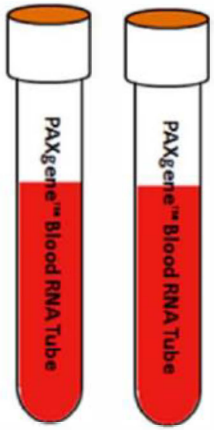
- Collect blood into *one* PAXgene® Tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



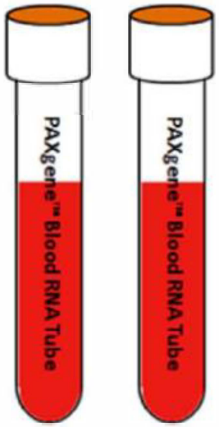
- Immediately after blood draw, invert tubes 8-10 times to mix samples.
- Repeat steps two and three for second tube.

Step Four



- Incubate tubes upright at room temperature for 2 hours before freezing the samples.

Step Five



- After 2 hour incubation at room temperature, store tubes upright in a -80°C in a wire rack until shipment.

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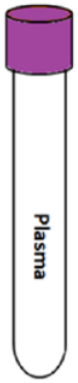
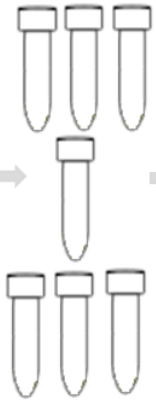
Plasma and Buffy Coat Preparation (10ml Lavender Top Tube)

Step One



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

Step Two

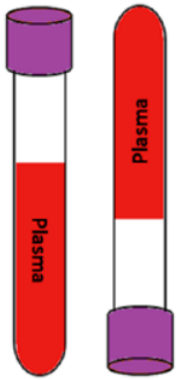


- Label 6 0.5mL cryovials for plasma and 1 2.0mL cryovial for buffy coat with pre-printed subject labels prior to blood draw.
- Pre-chill cryovials on wet ice for 5 minutes or longer.

Step Three

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

Step Four

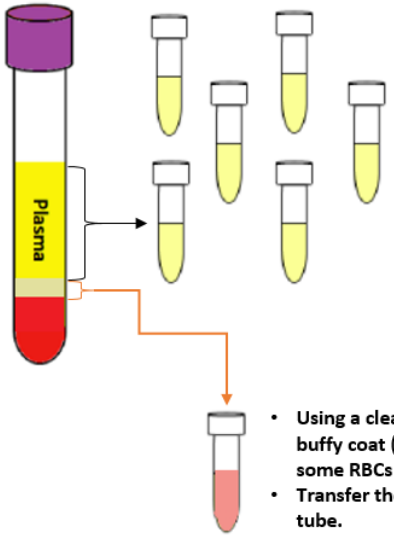


- Immediately after blood draw, invert tubes 8-10 times to mix samples.

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 250 uL of plasma into each 0.5mL cryovial tube.
- Store plasma aliquots at -80°C until shipment.
- Return 6 X 250 uL aliquots to BioSend

- Using a clean transfer pipette, collect the buffy coat (may have residual plasma and some RBCs included).
- Transfer the buffy coat into a 2.0mL cryovial tube.
- Store buffy coat aliquot at -80°C until shipment.
- Return 1 X 2.0 mL aliquot to BioSend.

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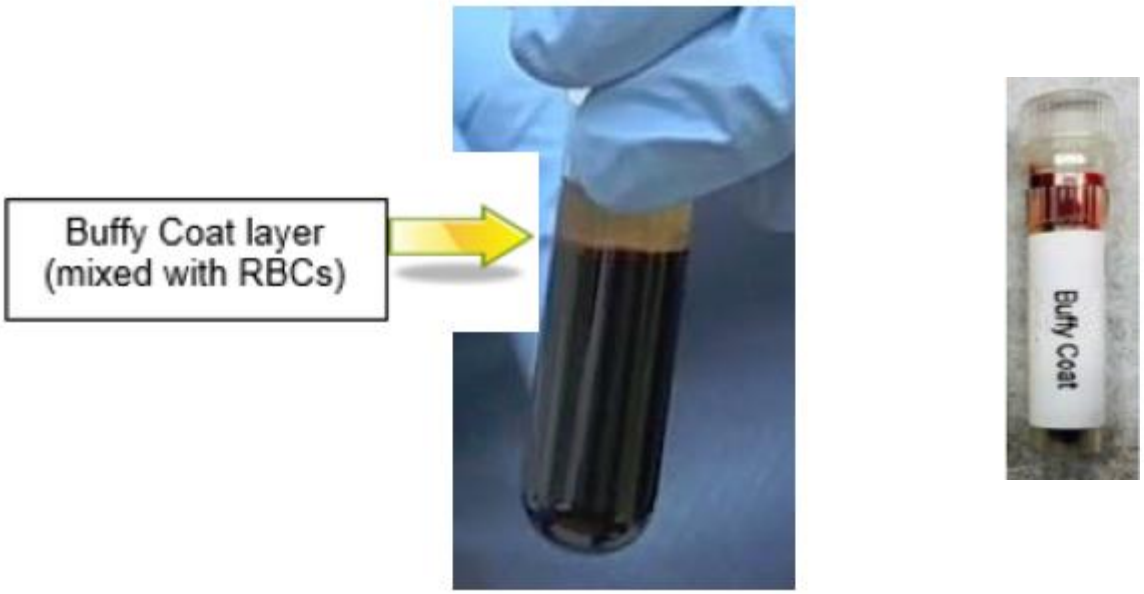
Buffy Coat Collection



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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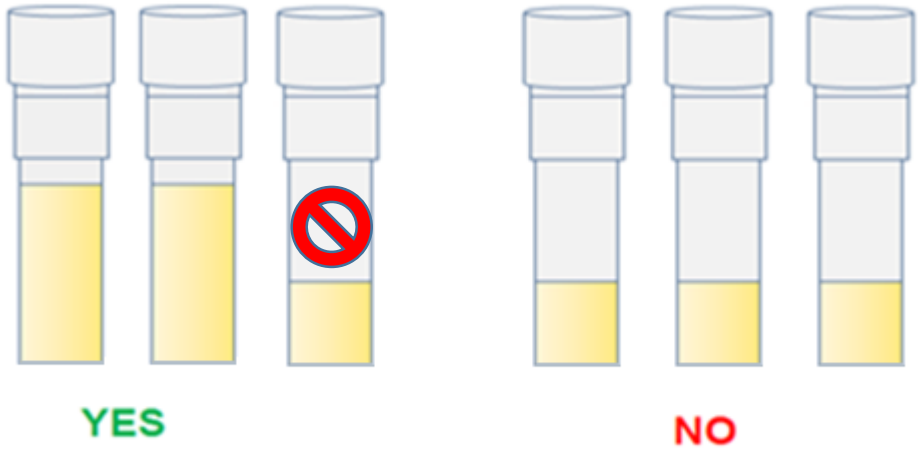
Plasma and Buffy Coat Aliquots

- Fill Plasma cryovials to 250ul
- Over-filled vials may burst in freezer
- Ship material to BioSEND
  - 6 Plasma aliquots
  - 1 Buffy Coat aliquot
- Do NOT send residual volumes to BioSEND



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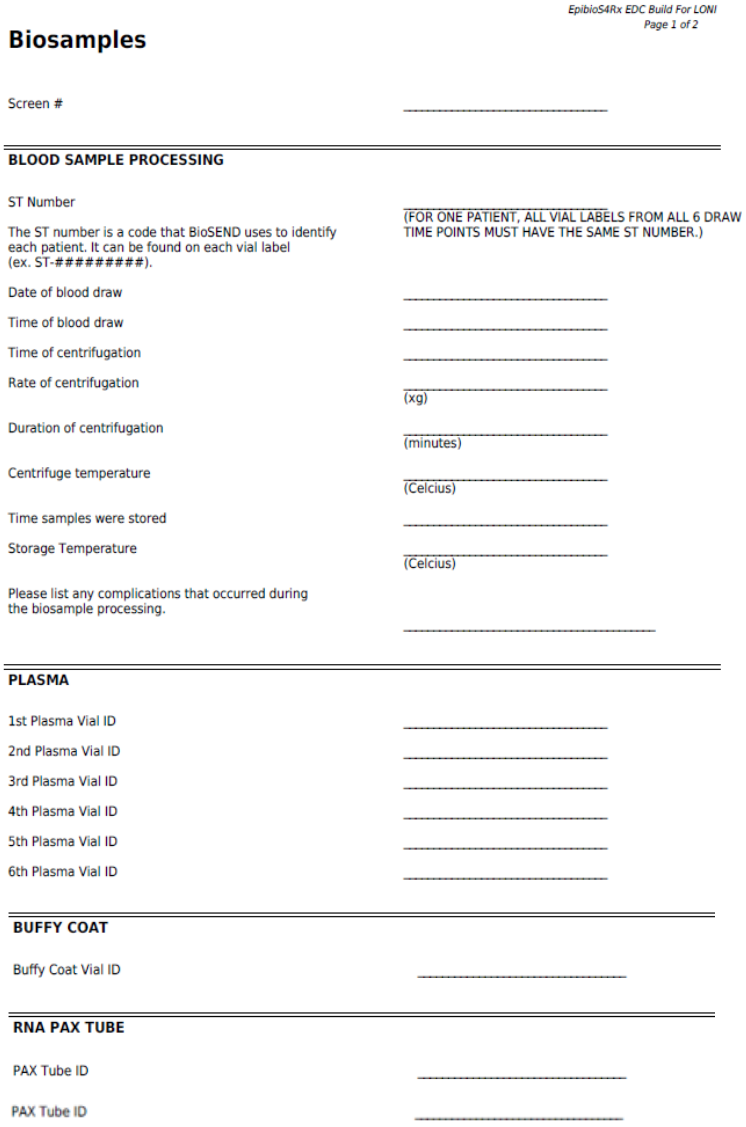
Biosample processing data collection

- All biosample processing details should be recorded in the biosample form in Redcap
- Record Patient number (ST-number), collection date/time, processing details, and vial IDs



Biosamples

Screen # _____

BLOOD SAMPLE PROCESSING

ST Number _____
(FOR ONE PATIENT, ALL VIAL LABELS FROM ALL 6 DRAW TIME POINTS MUST HAVE THE SAME ST NUMBER.)

The ST number is a code that BioSEND uses to identify each patient. It can be found on each vial label (ex. ST-#########).

Date of blood draw _____

Time of blood draw _____

Time of centrifugation _____

Rate of centrifugation _____ (xg)

Duration of centrifugation _____ (minutes)

Centrifuge temperature _____ (Celcius)

Time samples were stored _____

Storage Temperature _____ (Celcius)

Please list any complications that occurred during the biosample processing.
_____

PLASMA

1st Plasma Vial ID _____

2nd Plasma Vial ID _____

3rd Plasma Vial ID _____

4th Plasma Vial ID _____

5th Plasma Vial ID _____

6th Plasma Vial ID _____

BUFFY COAT

Buffy Coat Vial ID _____

RNA PAX TUBE

PAX Tube ID _____

PAX Tube ID _____

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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 vacutainer 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) plasma

Cause: Blood Collection Methods	Corrective Action
Improper venipuncture site	Draw from median cubital, basalic, and cephalic veins from antecubital region of arm
Prolonged tourniquet use	Tourniquet should be released after no more than 1 min, excessive fist clenching should be avoided
Not allowing alcohol to dry on skin before venipuncture	Without touching, allow the venipuncture site to air dry
Lumen of needle too close to inner wall of vein (indicated by slow blood flow)	
Use of too large/small bore needle resulting in excess force applied to blood	Avoid using too small/large needle. Needle size dependent on the subject's physical characteristics & amount of blood to be drawn. Most commonly used sizes are 19 – 23.
Pulling/pushing plunger too fast while drawing/transferring blood	Avoid drawing the syringe plunger too forcefully when collecting blood
	Ensure all blood collection assemblies are fitted securely, to avoid frothing

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

Blood Collection: Troubleshooting

Issue #2: Hemolyzed (pink/red) plasma

Cause: Sample Processing Methods	Corrective Actions
Vigorous mixing/shaking	Gently invert blood collection tube when mixing additive with specimen, follow guidelines in Biologics Manual regarding number of times to invert each type of tube
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, PAXgene®
- **Ship Monday-Wednesday Only via FedEx® Priority Overnight**
- Schedule FedEx® pickup
- Email Biosample Shipping Record 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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Shipping Schedule

- For first subject at each site acute samples (D1-D15) should be shipped upon completion of D15 collection
- Bulk shipping will be used for D30-D180 samples on a quarterly basis

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Quarterly Shipping Schedule

- Domestic Sites, M-W of designated week
- International, Monday ONLY

EpiBioS4Rx BioSEND DOMESTIC SHIPPING SCHEDULE			
2017			
Q4	10/16/2017	-	10/18/2017
2018			
Q1	1/22/2018	-	1/24/2018
Q2	4/16/2018	-	4/18/2018
Q3	7/16/2018	-	7/18/2018
Q4	10/15/2018	-	10/17/2018
2019			
Q1	1/14/2019	-	1/16/2019
Q2	4/15/2019	-	4/17/2019
Q3	7/15/2019	-	7/17/2019
Q4	10/21/2019	-	10/23/2019
2020			
Q1	1/13/2020	-	1/15/2020
Q2	4/20/2020	-	4/22/2020
Q3	7/13/2020	-	7/15/2020
Q4	10/19/2020	-	10/21/2020

INTERNATIONAL SHIPPING SCHEDULE	
2017	
Q4	10/16/2017
2018	
Q1	1/22/2018
Q2	4/16/2018
Q3	7/16/2018
Q4	10/15/2018
2019	
Q1	1/14/2019
Q2	4/15/2019
Q3	7/15/2019
Q4	10/21/2019
2020	
Q1	1/13/2020
Q2	4/20/2020
Q3	7/13/2020
Q4	10/19/2020

- Additional shipments due to capacity concerns available upon request

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Biosample Shipping Record

STUDY: EPIBIOS4RX

SHIP FROZEN SHIPMENTS MONDAY - WEDNESDAY ONLY!
Ensure all frozen shipments are completely filled with dry ice.

This form must be completed for shipment of all research samples.

Prior to shipping, email a PDF copy of this completed form to BioSEND at biosend@iu.edu.
Also place a hard copy of this form in the shipment box and in the study binder.

Site Name: _____

Site # _____
(The site # is the 2 digit number in your patient's study ID. Ex. 17 for UCLA, study ID = 3_17_0000)

Principal Investigator: _____

Coordinator Name: _____

Coordinator Telephone: _____

Coordinator Email: _____

PATIENT INFORMATION

Patient's Study ID: _____
(ex. 3_17_0000)

ST Number _____
(FOR ONE PATIENT, ALL VIAL LABELS FROM ALL 6 DRAW TIME POINTS MUST HAVE THE SAME ST NUMBER.)

Patient's Gender ◯ Male ◯ Female ◯ Unknown ◯ Not reported

Patient's Age _____
(years)

- Complete 1 form/subject shipped
- Input Coordinator and Patient information

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Biosample Shipping Record

SHIPPING INFORMATION:

Date samples were shipped? _____

Time samples were shipped? _____

FedEx Tracking #: _____

Which visit days for this patient are included in the shipment?

- ☐ Day 1
- ☐ Day 3
- ☐ Day 5
- ☐ Day 15
- ☐ Day 30
- ☐ Day 90
- ☐ Day 180

Notation of storage or shipping problems

_____

DAY 1

Blood draw date _____

# of plasma vials _____

# of buffy coat vials _____

DAY 3

Blood draw date _____

# of plasma vials _____

# of buffy coat vials _____

DAY 5

Blood draw date _____

# of plasma vials _____

# of buffy coat vials _____

# of PAX tubes _____

- Input date and time of sample shipment
- FedEx Tracking #
- Which visits are being shipped
- Any issues
- Collection Date
- Number of vials per sample type
- Email to biosend@iu.edu **AHEAD** of shipment
- Print and include in physical shipment

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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

NINDS BioSEND

BioSEND Contact Information

- Questions?

  Please contact: Claire Wegel, BioSEND Project Manager (cwegel@iu.edu)
  - Email: biosend@iu.edu
  - Phone: 317-278-0594

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Questions?

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