BioSpecimen Exchange for Neurological Disorders (BioSEND)

READISCA (SCA) Training
Webinar



BioSEND Training Webinar Overview

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



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.



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



SCA Biospecimen Collection Protocol

	BL	12M	24M	36M	48M
DNA ** (6ml)	X				
Plasma (6 x 1ml)	X	X	X	X	X
Buffy Coat (2 aliquots)	X	X	X	X	X
CSF * (10 x 1ml)	X				X

- *CSF will be collected at two timepoints throughout the study. They will not necessarily occur during the baseline visit and year 5 follow up visit.
- **DNA collection will occur only once, at either the first or the second CSF collection visit.



Variable CSF and DNA collection

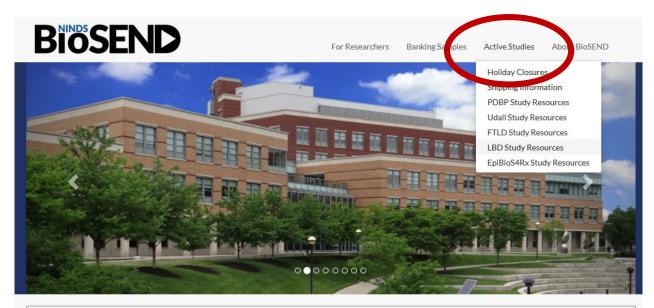
- To account for CSF and DNA to be collected at any visit, ALL kits will include CSF and DNA labels
- CSF and DNA kits will be ordered independently
- Blood kits will contain labels for ALL sample types



Requesting Kits



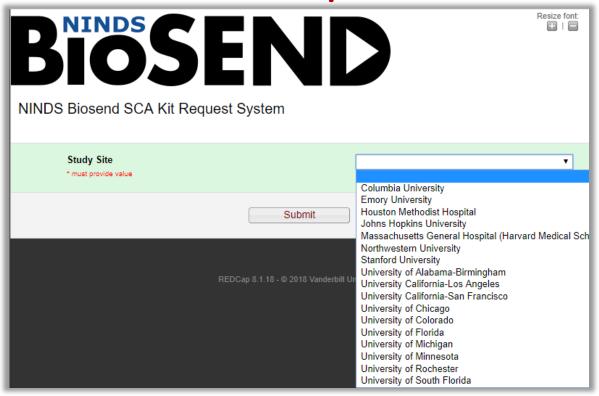
NINDS BioSEND Website







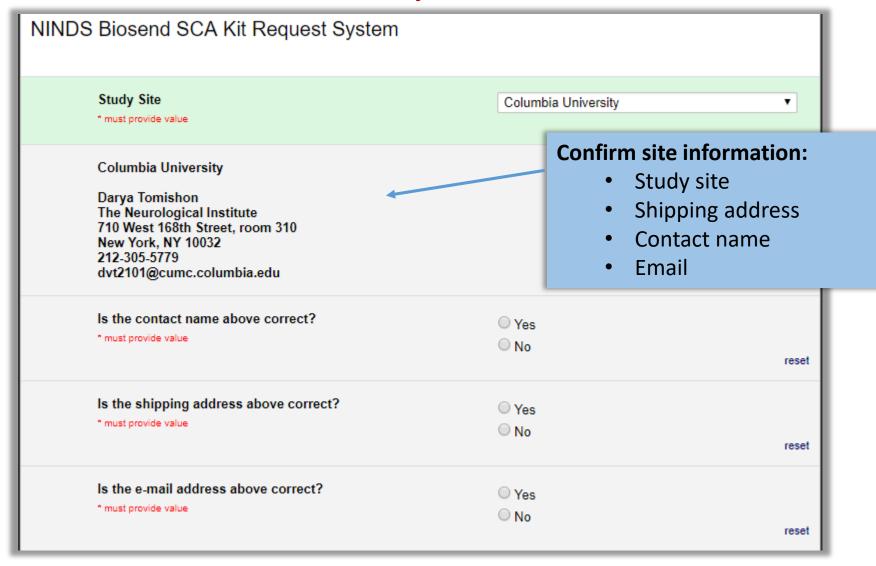
BioSEND Kit Request Module



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

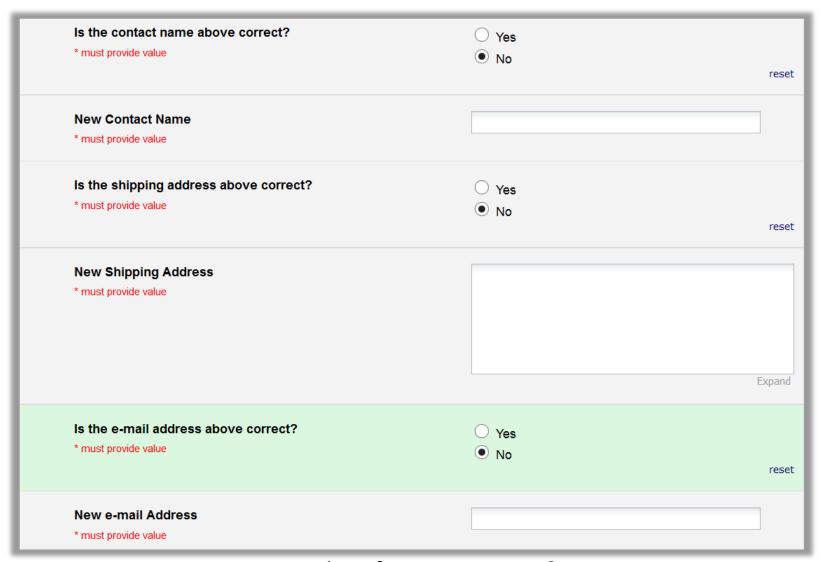


BioSEND Kit Request Module





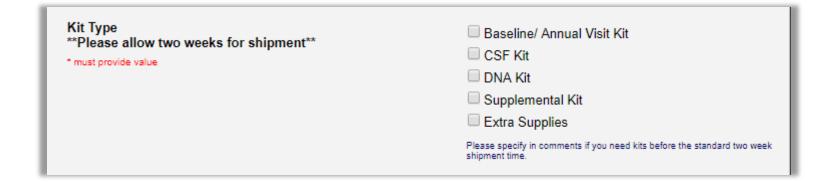
BioSEND Kit Request Module





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

BioSEND Kit Request Module: Kit Type





BioSEND Kit Request Module: Baseline Kit

Kit Type **Please allow two weeks for shipment** * must provide value	■ Baseline/ Annual Visit Kit ■ CSF Kit ■ DNA Kit ■ Supplemental Kit ■ Extra Supplies Please specify in comments if you need kits before the standard two were shipment time.	
Baseline Visit Kit Quantity * must provide value	If you need more than 10 kits or labels, please use the file upload option or submit multiple requests.	
Annual Visit Kit Quantity		
* must provide value	If you need more than 10 kits or labels, please use the file upload option or submit multiple requests.	

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

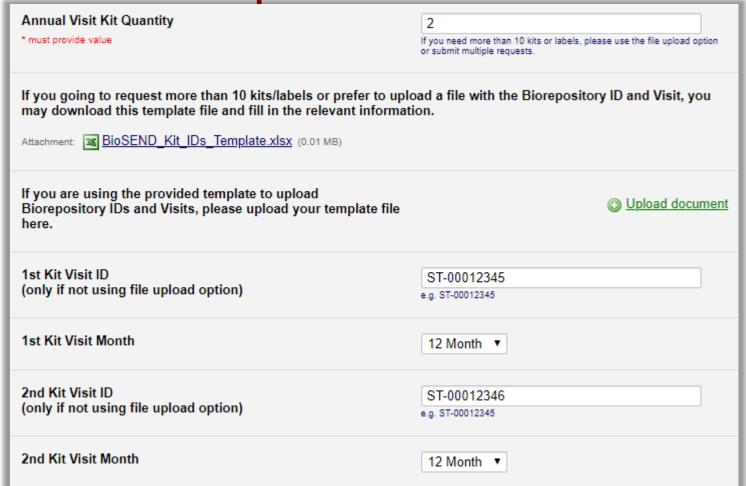


BioSEND Kit Request Module: Baseline Kit

Please note: the ST number provided in a BL kit can be used for any subject's BL visit. This ST number is a subject identifier, and will need to stay linked to the patient through the entirety of the study.



BioSEND Kit Request Module: Annual Visits



•If requesting a follow-up visit kit, you must provide us with time point and ST number of the kit you are requesting. Not doing so will delay your shipment, as we cannot complete your request without this information.



BioSEND Kit Request Module: CSF Kit

Kit Type **Please allow two weeks for shipment** * must provide value	■ Baseline/ Annual Visit Kit ■ CSF Kit ■ DNA Kit ■ Supplemental Kit ■ Extra Supplies Please specify in comments if you need kits before the standard two week shipment time.
CSF Sprotte® Needle Gauge * must provide value	● 22○ 24reset
CSF Kit Quantity * must provide value	2 If you need more than 10 kits or labels, please use the file upload option or submit multiple requests.
Comments	Expand
CSF Collection Kit Contents: 11 - Siliconized cryovials, sterile (2ml) 2 - Blue screw-top centrifuge tubes (15ml) 2 - Blue screw-top conical tube (50ml) 1 - LP tray with 22 gauge Sprotte® needle	

- Select needle gauge and enter kit quantity
- Can be used for any subject, do not need to specify
- Labels will be in BL/Annual Kit



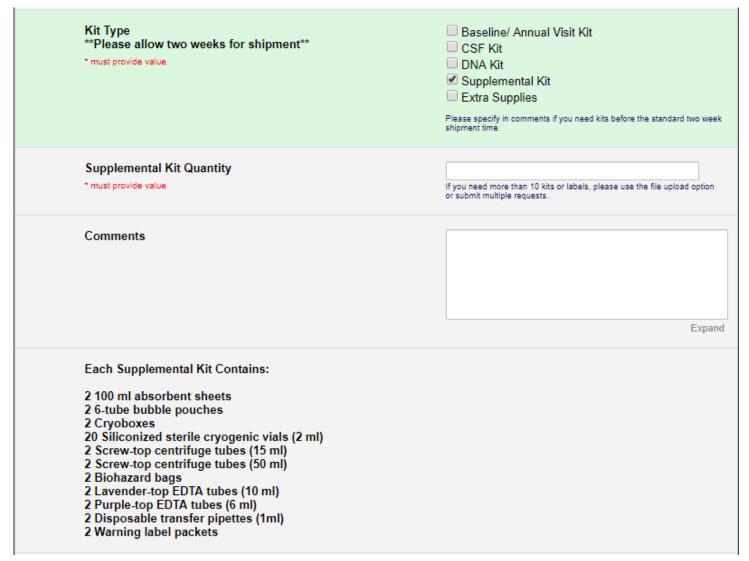
BioSEND Kit Request Module: DNA Kit

Kit Type **Please allow two weeks for shipment** * must provide value	■ Baseline/ Annual Visit Kit ■ CSF Kit ■ DNA Kit ■ Supplemental Kit ■ Extra Supplies Please specify in comments if you need kits before the standard two week shipment time.
DNA Kit Quantity * must provide value	2 If you need more than 10 kits or labels, please use the file upload option or submit multiple requests.
Comments	Expand
DNA Collection Kit Contents: 1 - Purple-top EDTA tube (6 ml) 1 - FedEx® return Airbill 1 - Ambient shipper 1 - FedEx® ClinPak	

- Enter kit quantity
- Can be used for any subject, do not need to specify
- Labels will be in BL/Annual Kit



BioSEND Kit Request Module: Supplemental Kit





Contains a variety of extra kit pieces

BioSEND Kit Request Module: Extra Supplies

Kit Type **Please allow two weeks for shipment** * must provide value	Baseline/ Annual Visit Kit CSF Kit DNA Kit Supplemental Kit Extra Supplies Please specify in comments if you need kits before the s	tandard two week
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
RIOSFNID	 Allows you to choose specific supplies and particular quantities 	

BioSEND Kit Request Module: Multiple Orders

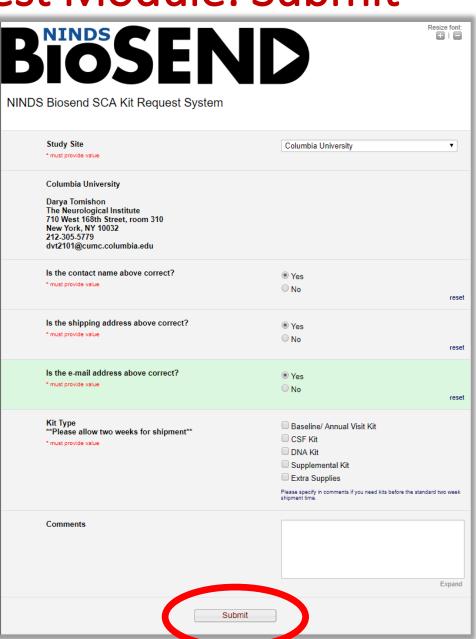


 You can order more than one type of kit in a single kit request



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

Specimen Label

ST-10000123: SCA:BL



BioSend

0003456124
BioSEND

ST-10000123

BL

DNA

Identify study

Identify individual biospecimens



Case Label

ST-10000123: SCA:BL

Subject Number

Study and Visit



BioSend



Biorepository Name



Case Labels

Case labels are placed:

- On the plastic biohazard bag of the cryovial biohazard bag.
- On the lid of frozen shippers.







Collection and Aliquot Tube Label

O003456124 ← Biospecimen Barcode
BioSEND ← Biorepository

ST-10000123 ← Subject Number

BL ← Visit Type

DNA ← Specimen Type



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





Sample Collection & Processing

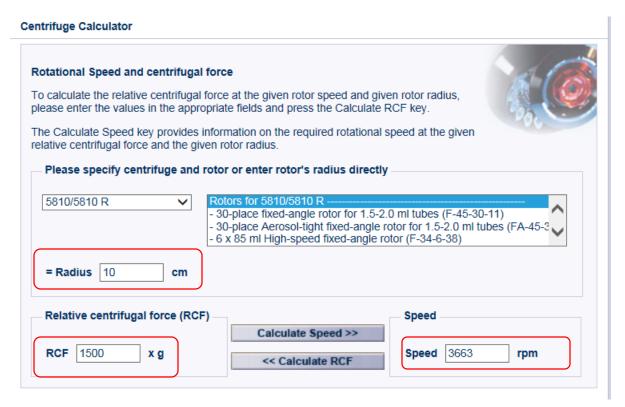
Reminders:

- Collection of biospecimen blood samples should be from subjects who have been <u>fasting for 8 hours</u> or more. If fasting is not feasible, follow suggested low-fat diet.
- G force ≠ RPM
- All specimens should be frozen and stored <u>UPRIGHT</u>
 - For plasma and CSF aliquots, please freeze samples upright in the cryobox provided



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. EDTA 6 ml (purple top) blood collection for DNA
- 2. EDTA **10 ml (lavender top)** blood collection for plasma and buffy coat

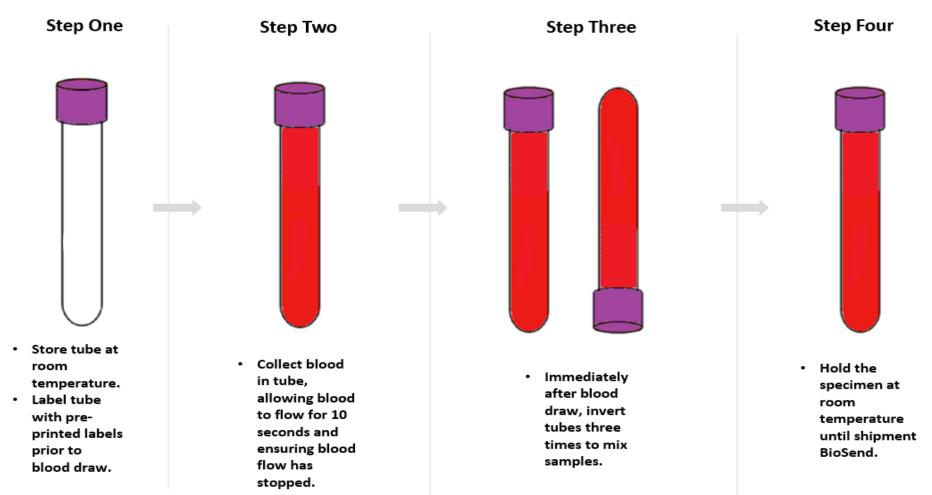






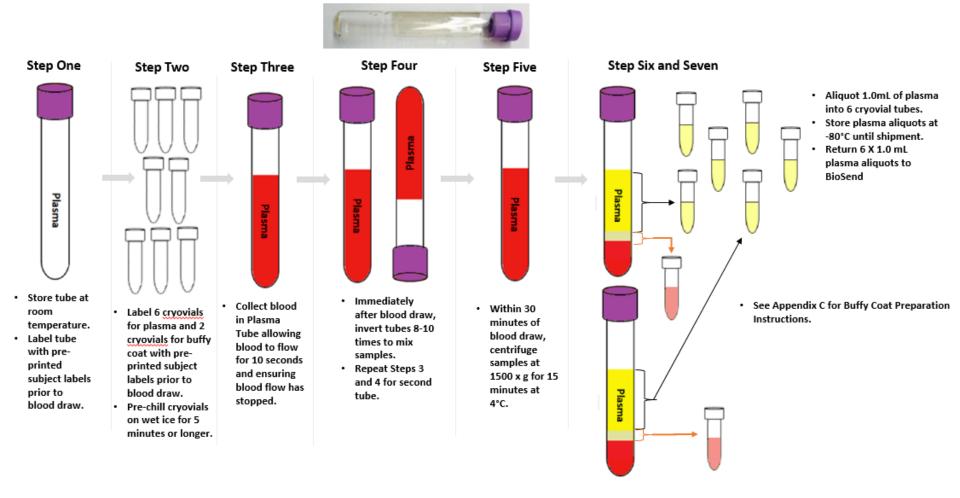
DNA Preparation (6 ml Lavender Top Tube)





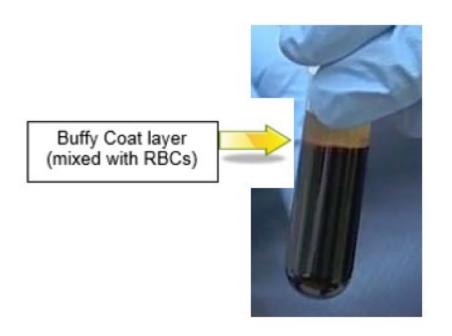


Plasma Preparation (10ml Lavender Top Tube)



BIOSEND

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.

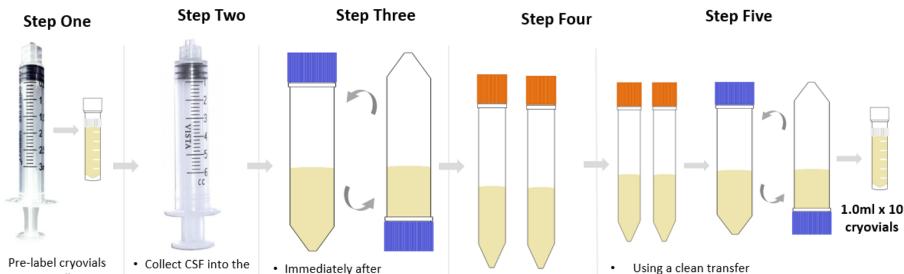


Lumbar Puncture Procedure

- 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



CSF Collection and Preparation (15-20 ml total)



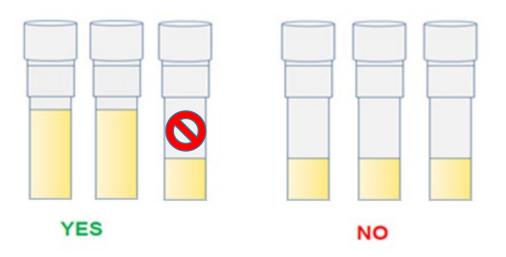
- Pre-label cryovials prior to collection.
- Collect CSF into the 3ml luer lock syringe or by gravitational pull.
- · Dispense 1-2 ml into cryovial.
- · Send to local lab for testing.

- 5ml luer lock syringe or by gravitational pull
- Transfer sample into 50 ml conical tube.
- collection, gently invert the 50 ml conical tube 3-4 times to mix the
- sample.
- · Transfer CSF into two 15 ml conical tubes.
- · Within 15 minutes of collection, centrifuge samples at room temperature at 2000 x g for 10 minutes.
- 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 upright at -80°C until shipment to BioSEND.



Plasma, Buffy Coat & CSF Aliquots

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





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



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



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)

Sample Shipment



Ambient Sample Shipment

- 6 ml purple (EDTA) whole blood for DNA collection tube
 - Collected at time of one CSF collection
- 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



Ambient Shipper Components

Styrofoam shipper with outer cardboard box

IATA 'Contents' Sheet



Biohazard bag with absorbent material

Cool pack (place in fridge prior to collection/shipment)

Collection tube will be in blood kit



Labeling Ambient Sample Shipments

- Cardboard box will be labeled with UN3373 sticker.
- Place the box and a copy of the LBD 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.





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.



Frozen Samples

- All other samples are shipped frozen
 - Plasma, Buffy Coat & CSF
- 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





Packaging and Shipping Frozen Samples



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



Place biohazard bags upright in box



FILL dry ice to top of box



Packaging and Shipping Frozen Samples

- You will use approximately 10lbs (4.5kg) of dry ice per standard shipper.
- Please do not ship more than 2 subjects worth of samples in a single shipper.



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



Shipping Frozen Samples

- Hold packaged samples in a -80°C freezer until pickup.
- Samples should be received at BioSEND within 2 weeks of collection.



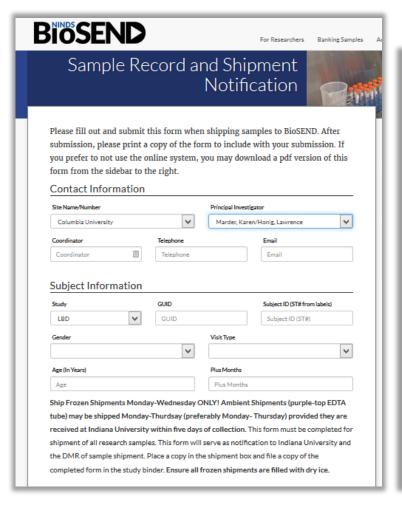


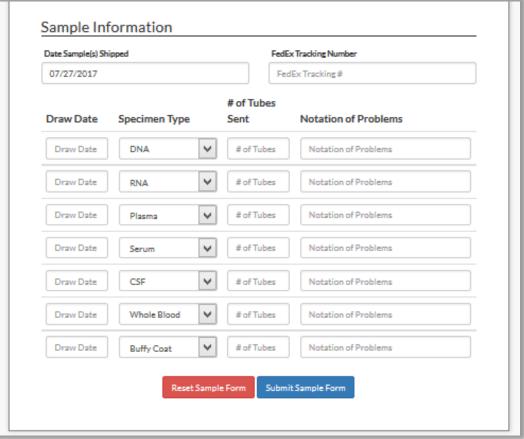
Sample Shipment Notification Form

Sample Record and Shipment Notification						
Study:	SCA					
Site Name:			Prin	cipal Investigator:		
Coordinator:		Т	elephone:		Email:	
Please list only ONE subject per Sample Record Summary and Shipment Notification Form						
			٦			
Study ID:			Subject ID (ST# from pre-printer	d labels):	
Gender:				V	isit 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 Sa	mple(s) Shipped:			FedEx Tracking	Number:	
In the table below, please indicate the date of specimen collection and number of tubes/aliquots submitted.						
			ted by Submit	er/Site		
		Number of Tubes/ Aliquots sent to				
Dates of Draw	Specimen Type	BioSEND		Nota	tion of Prob	lems
	DNA		1			
	RNA		1			
	Buffy Coat Plasma					
	Serum		1			
	CSF		1			
	Whole Blood					
			1			
Contact Information: Indiana University; Email: biosend@iu.edu Ph: 317-278-0594						



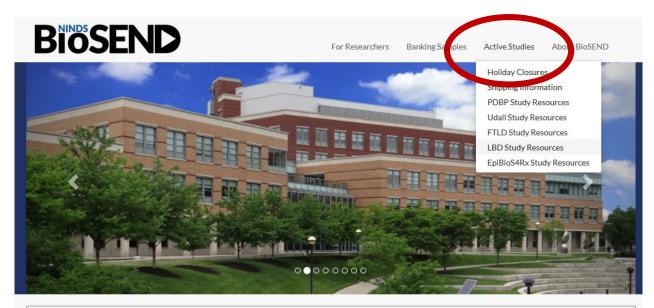
Sample Shipment Notification Form, Online







NINDS BioSEND Website







Holiday Closures

Date	Holiday
January 1	New Year's Day
3 rd Monday in January	Martin Luther King, Jr Day
4 th Monday in May	Memorial Day
July 4	Independence Day (observed)
1 st Monday in September	Labor Day
4 th Thursday in November	Thanksgiving
4 th Friday in November	Friday after Thanksgiving
December 25	Christmas Day



BioSEND Contact Information

• Questions?

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

Email: biosend@iu.edu



Questions?

