BioSpecimen Exchange for Neurological Disorders (BioSEND)

DLB Training Webinar



BioSEND Training Webinar Overview

- Study Reminders
- 2. Site Equipment
- 3. DLB Biospecimen Collection Protocol
- 4. Study Visit Protocol
- 5. Kits & Samples
 - Requesting Kits
 - Labels
 - Sample Collection & Processing
 - Sample Shipment
- 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

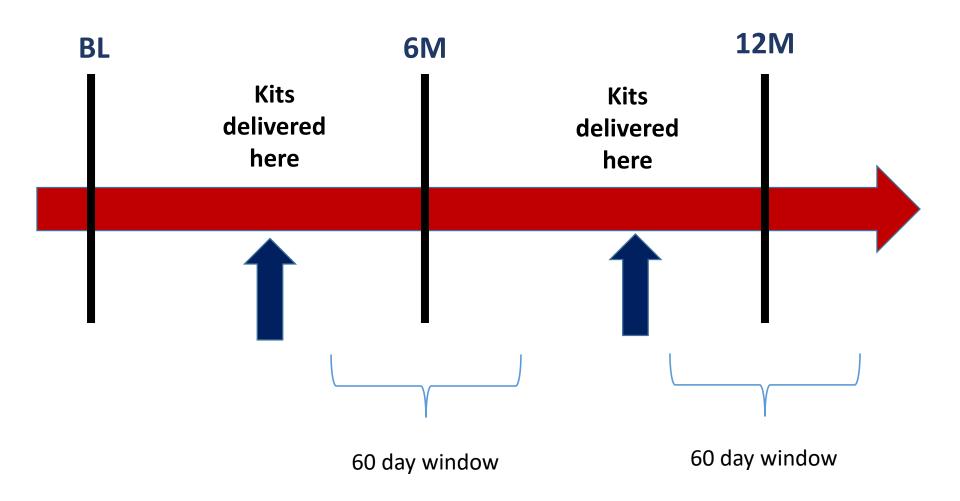


DLB Biospecimen Collection Protocol

	BL	6M	12M	24M	36M	48M
DNA (6ml)	X					
Whole Blood (6ml)	X	X	X	X	X	X
Plasma (6 x 1ml)	X	X	X	X	X	X
Serum (6 x 1ml)	X	X	X	X	X	X
RNA (2 x 2.5ml)	X	X	X	X	X	X
CSF (10 x 1ml)	X		X	X	X	X



Study Visit Protocol





Study Visit Protocol

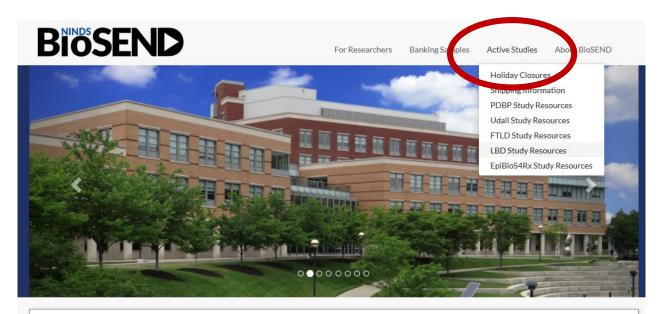
- After subject completes baseline visit, BioSEND sets up automated kit sending schedule for subject's subsequent visits
- Schedule gives 2 month window around the longitudinal study visit target (1 month on either side)
- BioSEND will send kits prior to start of study window
 - Reduces effort for study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

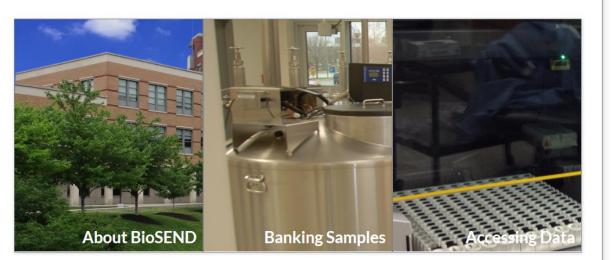


Requesting Kits



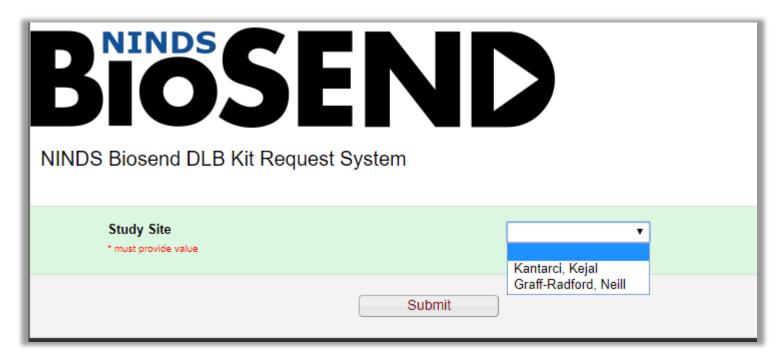
NINDS BioSEND Website







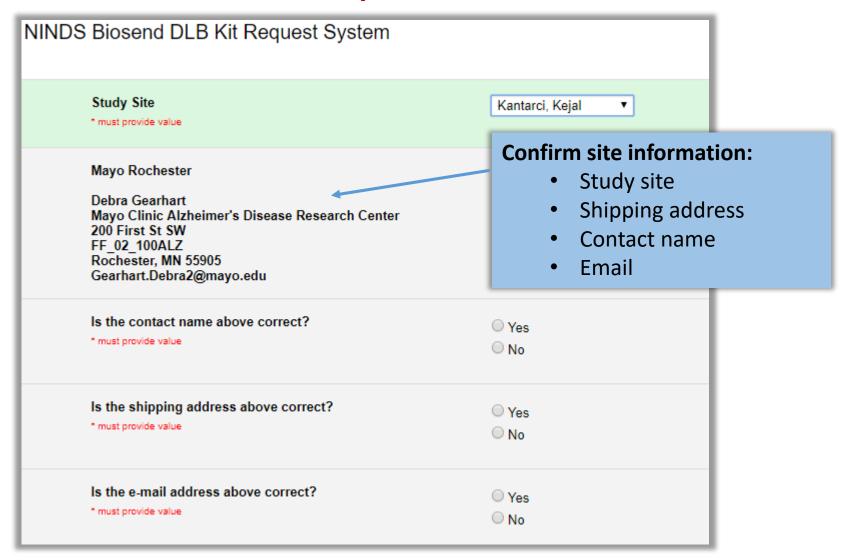
BioSEND Kit Request Module



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

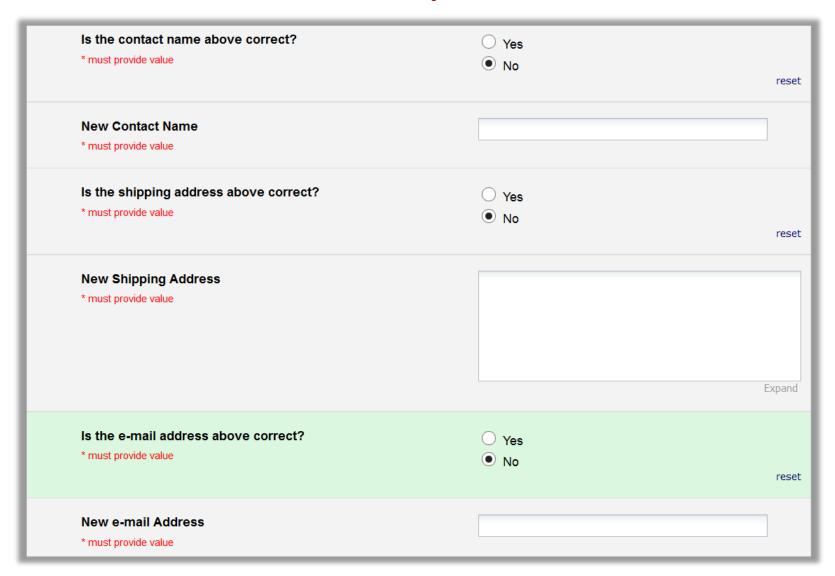


BioSEND Kit Request Module





BioSEND Kit Request Module





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

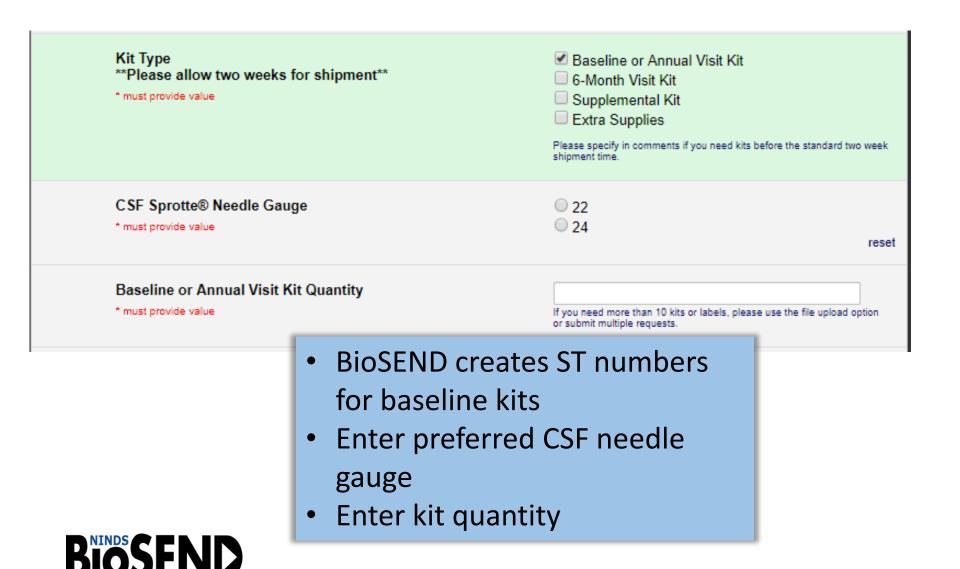
BioSEND Kit Request Module: Kit Type



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

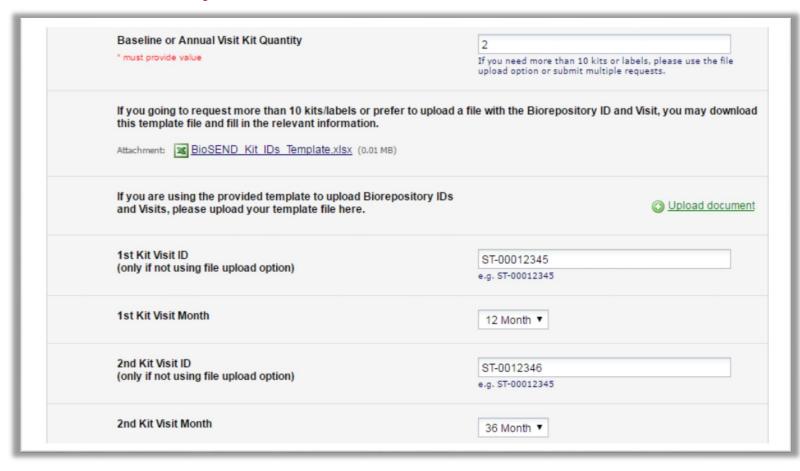


BioSEND Kit Request Module: Baseline or Annual Kit



BioSEND Kit Request Module: Annual Visits

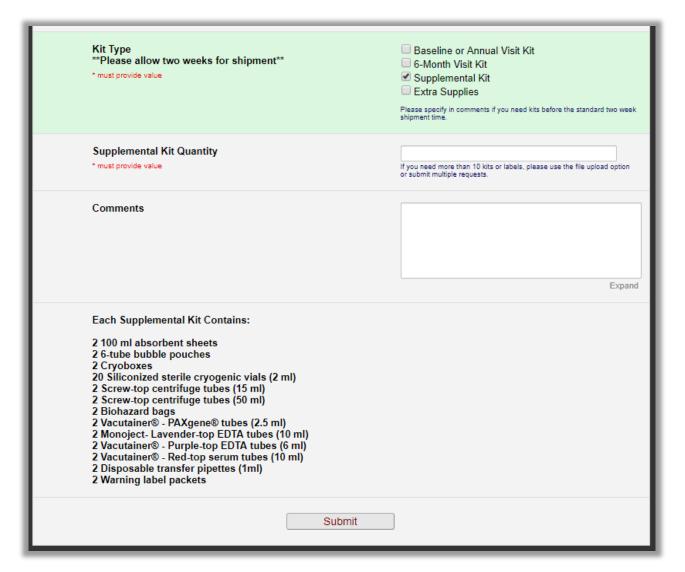
Only use this if the subject will be seen ahead of a study visit window



- Up to 10 kits can be requested:
 - Enter the DLB Subject ID ST Number
 - (Example: ST-00012345)
 - Enter the follow up visit month



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 or Annual Visit Kit ■ 6-Month Visit Kit ■ Supplemental Kit ■ Extra Supplies Please specify in comments if you need kits before the standard two week shipment time.
6-Tube Bubble Pouch	© 2 © 4 reset
Cryobox	© 2 © 4
Siliconized Sterile Cryogenic Vial (2 ml)	 Allows you to choose specific supplies and
FedEx® return Airbill	particular quantities reset
Lumbar Puncture Trays with Lidocaine	© 2 © 4 reset
Needles - Introducer	○ 5 reset



BioSEND Kit Request Module: Multiple Orders

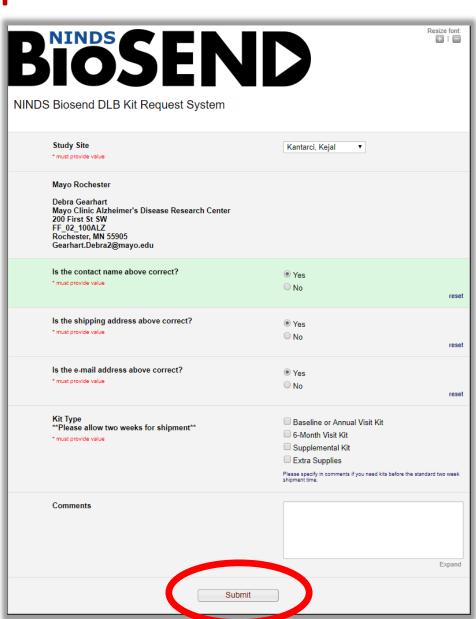


 You can order more than one type of kit in a single kit request



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

ST-10001234: DLB-GRAFF:BL



Identify study and PI

Specimen Label

0003456124



ST-10001234 BL

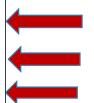
RNA

Identify individual biospecimens



Case Label

ST-10001234: DLB-GRAFF:BL



Subject Number

Study – Pl Visit



BioSend



Biorepository Name



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











Collection and Aliquot Tube Label

Specimen Type

Biospecimen Number 0003456124 **BioSEND Study = BioSEND DLB Subject Number** ST-10001234 BL **Visit Type** RNA



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



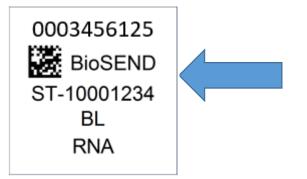


Aliquot Labels

 Keep samples in sequential order when labeling and storing











Sample Collection & Processing

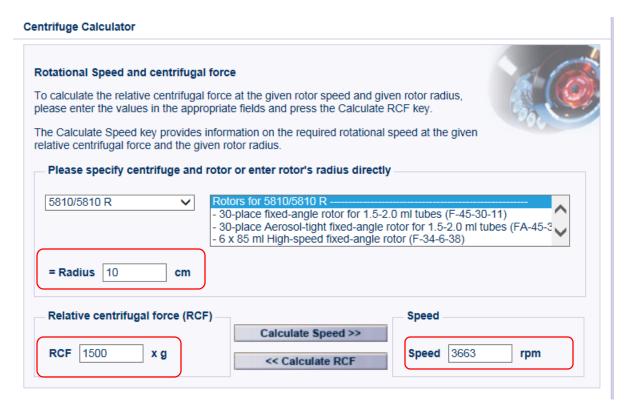
Reminders:

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



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 (only at BL visit)
- 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)



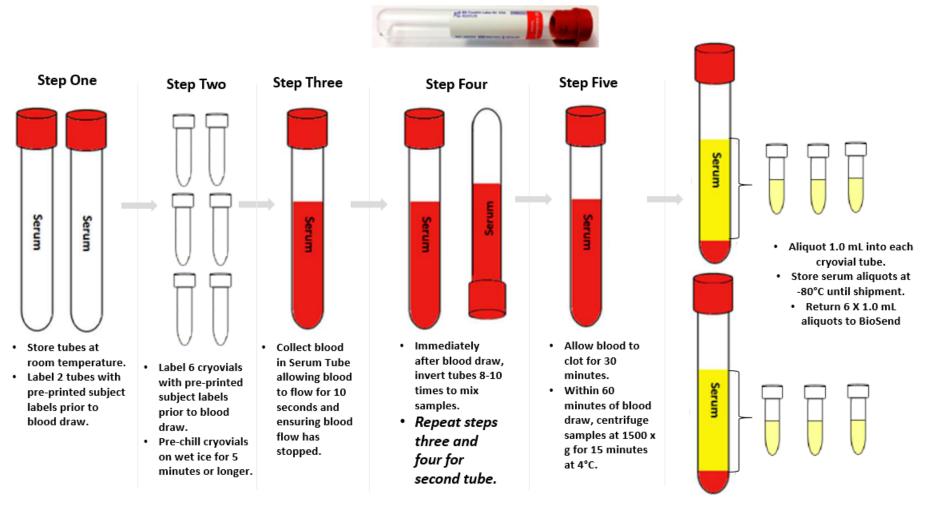
EDTA (Plasma)



EDTA (WB)



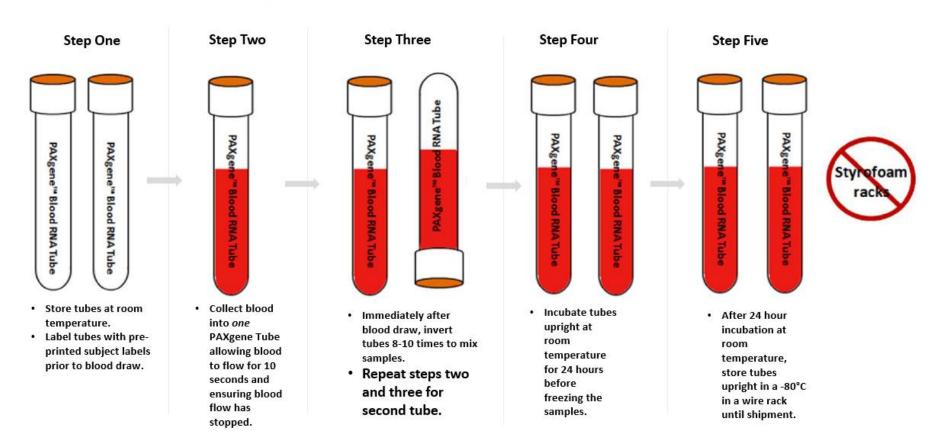
Serum Preparation (10ml Red Top Tube)





PAXgene[™] Preparation (2.5ml Tube)

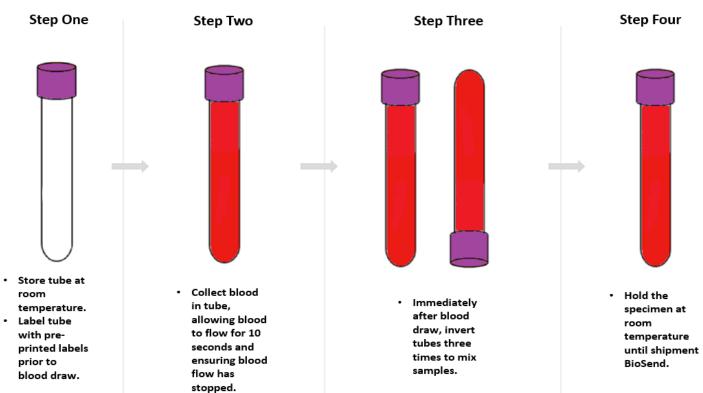






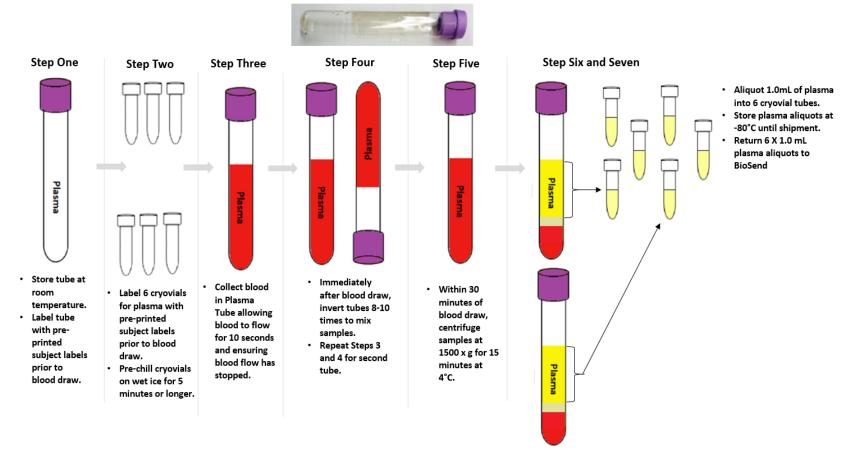
DNA Preparation (6 ml Lavender Top Tube)







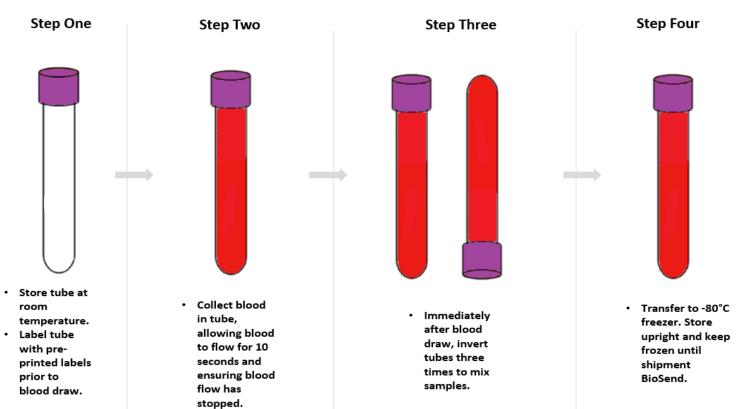
Plasma Preparation (10ml Lavender Top Tube)





Whole Blood (6 ml Lavender Top Tube)





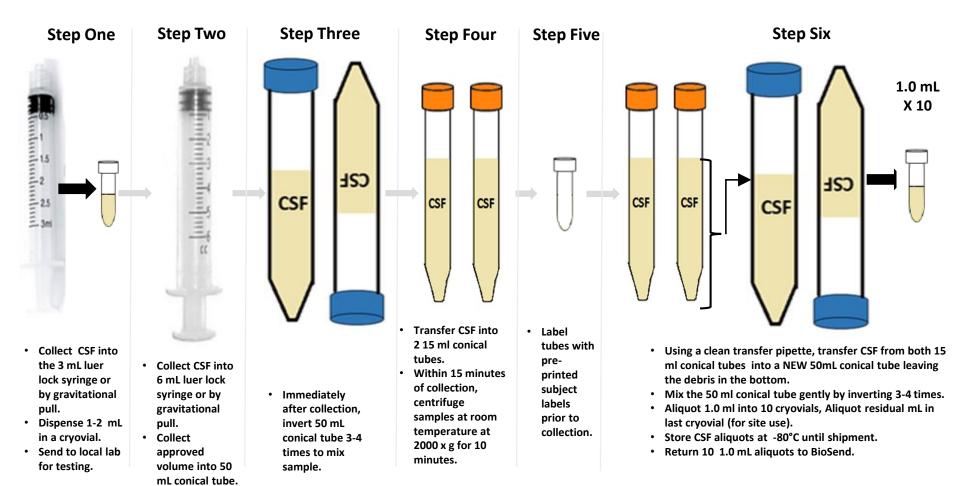


Lumbar Puncture Procedure

- 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



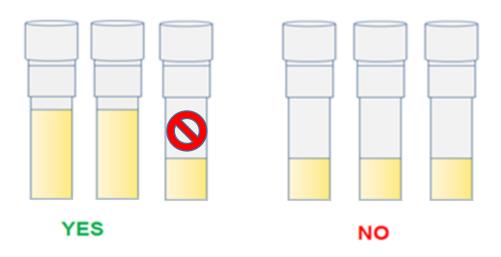
CSF Preparation Processing





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
- 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 collection 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 BL 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



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.



Labeling Ambient Sample Shipments

- Apply the UN3373 label to the outside of the cardboard box.
- Place the box and a copy of the DLB 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, 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





Packaging and Shipping Frozen Samples











Pack bags, place upright & side-by-side

FILL dry ice to top of box



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.



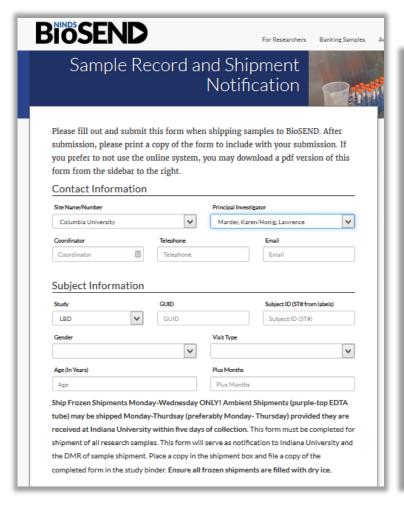


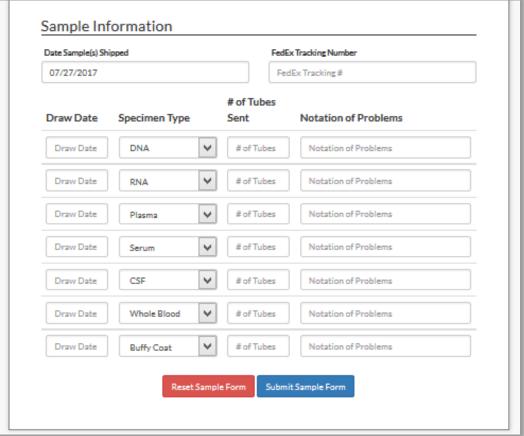
Sample Shipment Notification Form

Study:			_							
Site Name:				Pr	incipal Inve	stigator:				
Coordinator:			Tele	ephone:			Email:			
	Please list only O	NE subject per S	Sample F	Record Su	ımmary and	d Shipmen	t Notifica	tion Form		
GUID:				Subject ID	(ST# from p	pre-printed	labels):			
Gender:						Vis	it Type:			
Age in Years:						Plus I	Months:			
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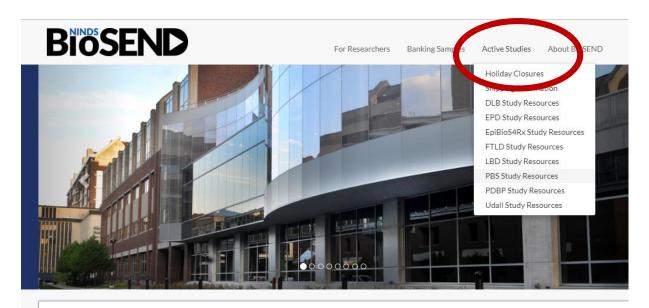
Sample Shipment Notification Form, Online







NINDS BioSEND Website







Longitudinal Imaging Biomarkers of Disease Progression in DLB Active Study Page



Welcome DLB Study staff, coordinators, and PI's. This section encompasses study specific tools and resources for your reference. If you have any questions, comments, or new ideas please contact biosend@iu.edu or by phone directly at (317)278-0594.

Specimen Table

Cohort	Population	Genomic DNA	RNA	Plasma	Serum	CSF	Whole Blood
DLB	Dementia with Lewy Body	✓	✓	✓	✓	✓	✓

Study Resources

Kit Request Module

Study Specific Sample Notification Forms

DLB Manual of Procedures

DLB Training Slides

DLB Site Listing

Downloads

Sample Shipment Form (pdf) Sample Shipment Form (xlsx) DLB Manual of Procedures DLB Training Webinar

Additional Resources

Online Sample Form

Contact Us

biosend@iu.edu 317-278-0594



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?

