# **NINDS Biorepository**

**BioSpecimen Exchange for Neurological Disorders, BioSEND** 

Training Webinar

Presented by: Scott Kaiser

### **Webinar Overview**

- 1. Site Equipment
- 2. BioSEND
  - Kit Contents and Ordering
  - Sample Labelling
  - Sample Collection and Processing
  - Shipping Samples
  - Quality Control
- 3. 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

### Equipment

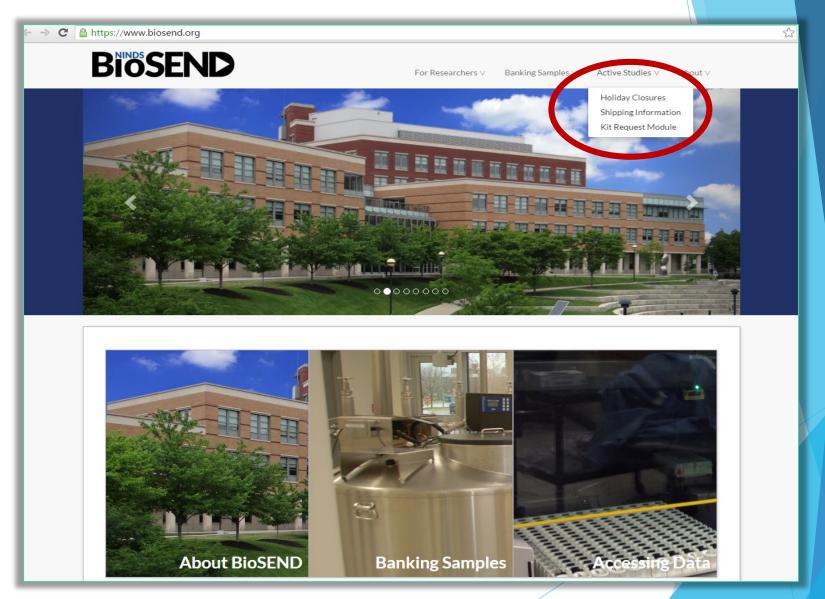
#### The following items are to be supplied by the site:

- Personal protective equipment
- Alcohol prep pads
- Butterfly needles and hubs
- Tourniquet
- Gauze pads
- Bandages
- Sharps bin and lid

- Microcentrifuge tube rack
- Test tub rack
- Crushed ice
- Pipettes and pipette tips
- 4°C Centrifuge
- -80°C Freezer
- Dry ice

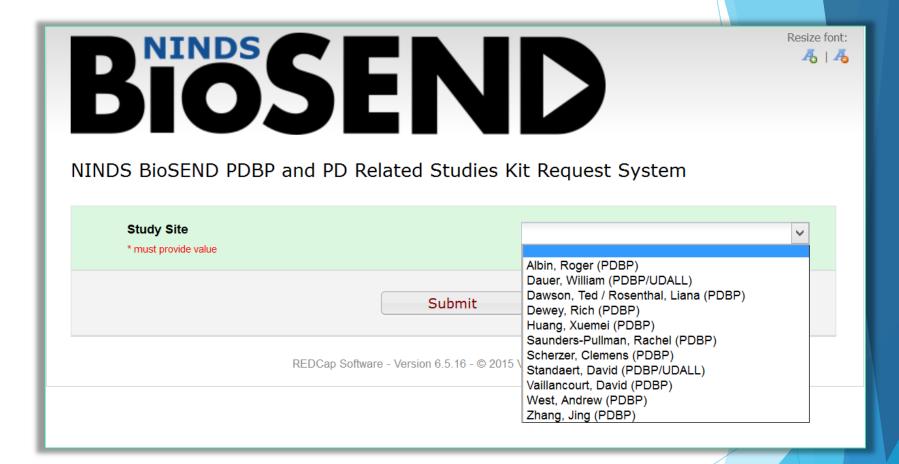
# Requesting Kits

### NINDS BioSEND Website



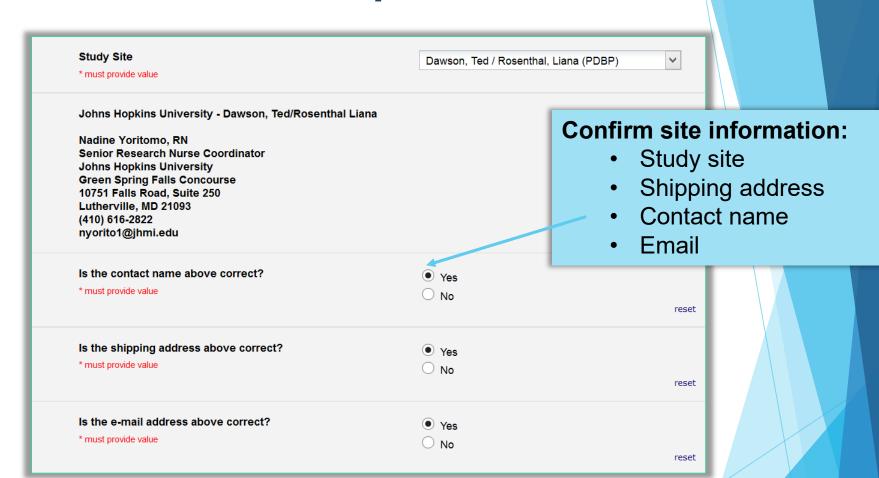
https://www.biosend.org/

### BioSEND Kit Request Module

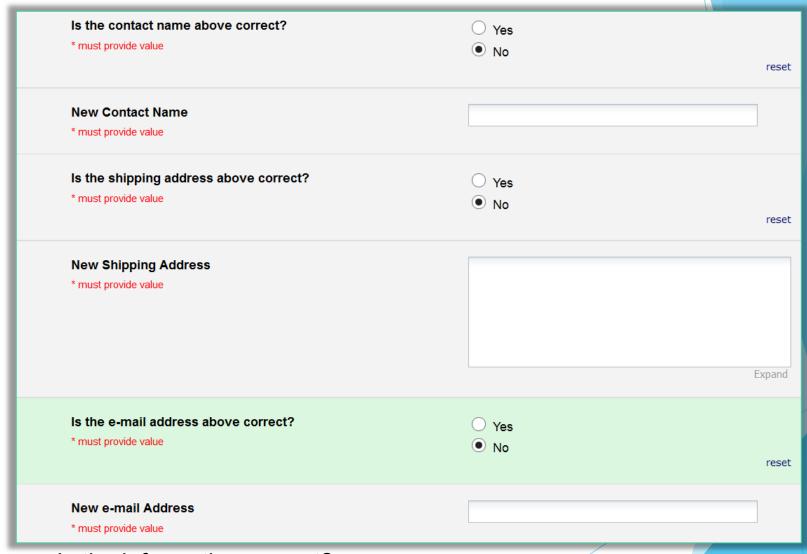


- http://kits.iu.edu/biosend/pd
- Choose your site PI from the drop-down list.

## **BioSEND Kit Request Module**



### **BioSEND Kit Request Module**



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

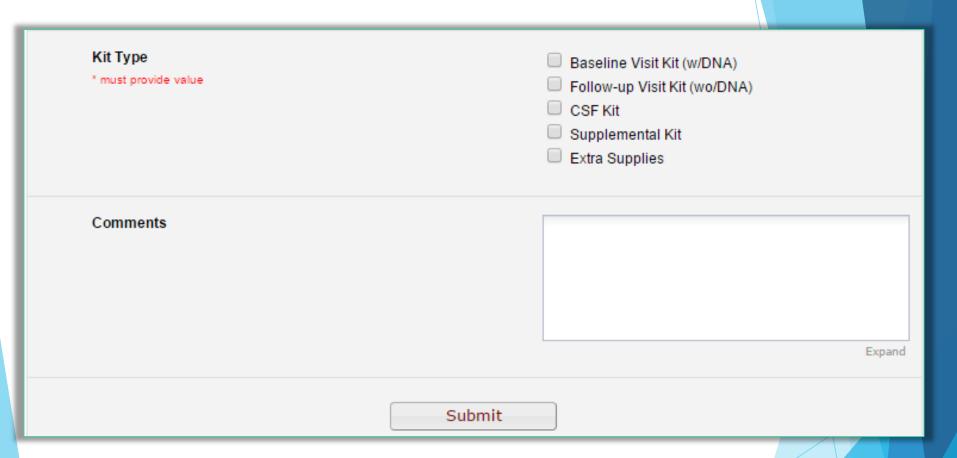
# BioSEND Kit Request Module: Type by Site

#### **Example A**

Kit Type * must provide value	Baseline Visit Kit (w/DNA) Supplemental Kit Extra Supplies
Example B	
Kit Type * must provide value	Baseline Visit Kit (w/DNA) Follow-up Visit Kit (wo/DNA) CSF Kit Supplemental Kit Extra Supplies

- Each PDBP site uses slightly different kit components
- Kits differ based on baseline and longitudinal visits
  - Typical difference is the collection of a blood tube for DNA only at the baseline visit

## BioSEND Kit Request Module: Kit Type



- Choose your kit type
- Only kit types used by your site will show up as a selection

# BioSEND Kit Request Module: Baseline Kit

Kit Type **Please allow two weeks for shipment**  * must provide value	Baseline Visit Kit (w/DNA) Follow-up Visit Kit (wo/DNA) CSF Kit Supplemental Kit Extra Supplies Labels Only Please specify in comments if you need kits before the standard two week shipment time.
Baseline Kit Quantity * must provide value	
Comments	Expand

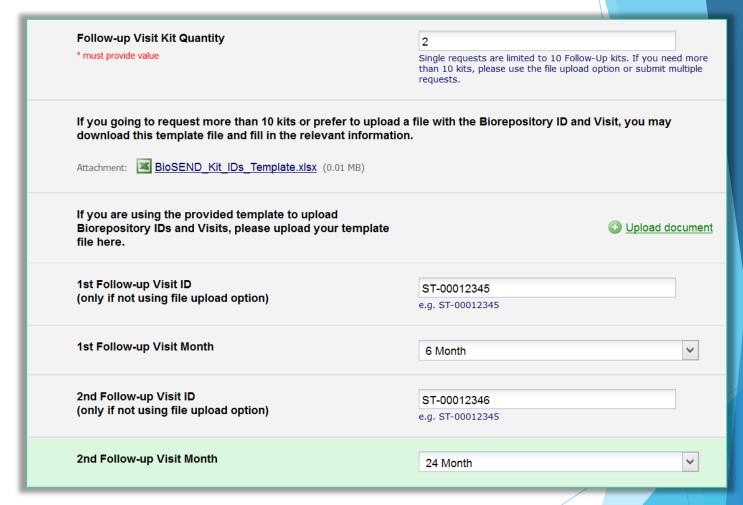
#### Each PDBP Baseline Collection Kit Contains:

- 3 Lavender-top EDTA tube (10 ml)
- 3 Red-top serum tube (10 ml)
- 2 Purple-top EDTA tubes (6 ml)
- 4 PAXgene® tubes (2.5 ml)
- 36 Siliconized cryovials, sterile (2ml)
- 1 Cryobox
- 1 Warning label packet (incl. dry ice label)
- 2 Shipping instruction sheets
- 1 FedEx® Overpack
- 2 FedEx® return Airbills
- 1 Shipping container for dry ice shipments
- 1 Shipping kit for ambient shipments
- 2 Biohazard bag
- 2 100 ml absorbent sheet
- 2 6-tube bubble pouch
- 30 Cryohold pre-printed labels

- BioSEND creates ST numbers for baseline kits
- Enter quantity
- List of kit contents shown below

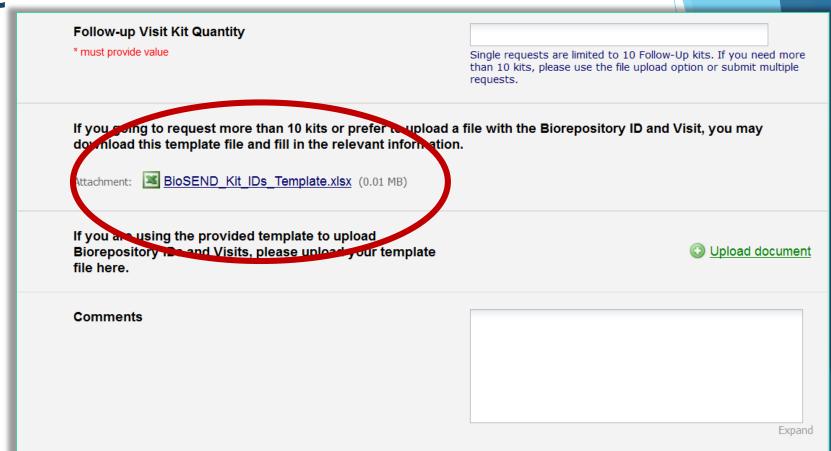
## BioSEND Kit Request Module: Follow-up

Kit



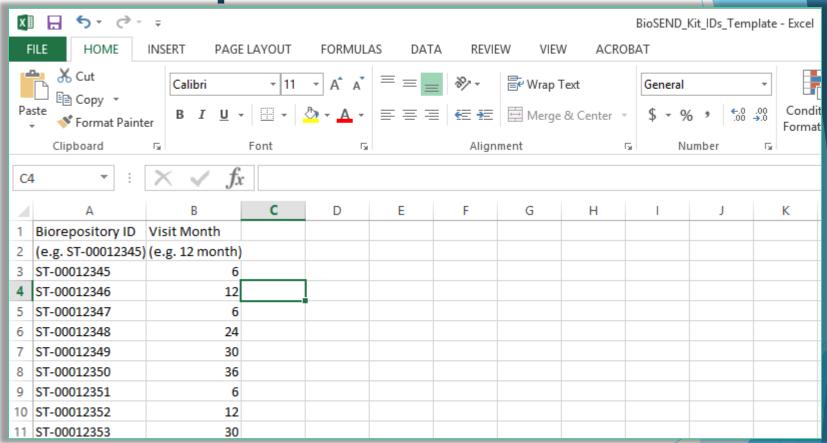
- Up to 10 kits:
  - Enter the PDBP Subject ID ST Number (Example: ST00012345)
  - Enter the follow up visit month

# BioSEND Kit Request Module: Follow-up Kit



More than 10 kits – download template

# BioSEND Kit Request Module: Follow-up Kit

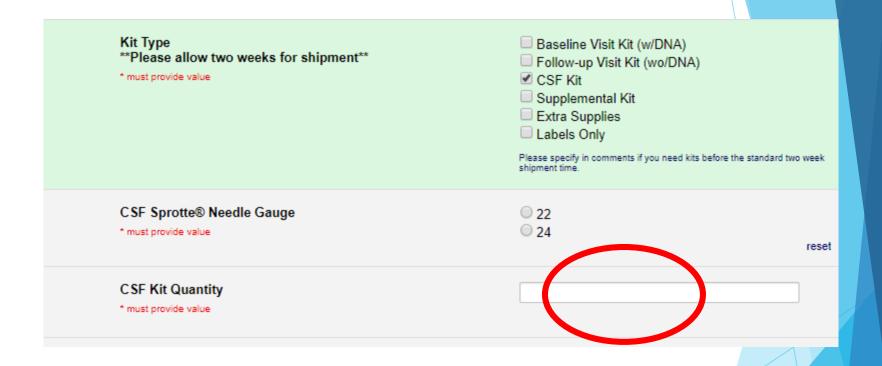


Complete template with ST numbers & visit types

# BioSEND Kit Request Module: Follow-up Kit

### Follow-up Visit Kit Quantity \* must provide value Single requests are limited to 10 Follow-Up kits. If you need more than 10 kits, please use the file upload option or submit multiple requests. If you going to request more than 10 kits or prefer to upload a file with the Biorepository ID and Visit, you may download this template file and fill in the relevant information. ■ BioSEND Kit IDs Template.xlsx (0.01 MB) Attachment: If you are using the provided template to upload Upload document Biorepository IDs and Visits, please upload your template file here. Comments Expand

### BioSEND Kit Request Module: CSF Kit



Enter quantity

### BioSEND Kit Request Module: Supplemental

Kit Type  **Please allow two weeks for shipment**  * must provide value	■ Baseline Visit Kit (w/DNA) ■ Follow-up Visit Kit (wo/DNA) ■ CSF Kit ■ Supplemental Kit ■ Extra Supplies ■ Labels Only  Please specify in comments if you need kits before the standard two week shipment time.
Comments	Expand
Each Supplemental Kit Contains:  2 100 ml absorbent sheets 2 6-tube bubble pouches 2 Cryoboxes 10 Siliconized sterile cryogenic vials (2 ml) 2 Screw-top centrifuge tubes (15 ml) 2 Screw-top centrifuge tubes (50 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 tubes (10 ml) 2 Warning label packets	
	Submit

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 Visit Kit (w/DNA) ■ Follow-up Visit Kit (wo/DNA) ■ CSF Kit ■ Supplemental Kit ■ Extra Supplies ■ Labels Only  Please specify in comments if you need kits before the standard two week shipment time.
6-Tube Bubble Pouch	<ul> <li>Allows you to choose specific supplies and</li> </ul>
Cryobox	particular quantities
Siliconized Sterile Cryogenic Vial (2 ml)	① 10 ② 20 reset
FedEx® return Airbill	© 2 © 4 reset
Lumbar Puncture Trays with Lidocaine	© 2 © 4 reset
Needles - Introducer	○ 5 reset

# BioSEND Kit Request Module: Multiple Orders

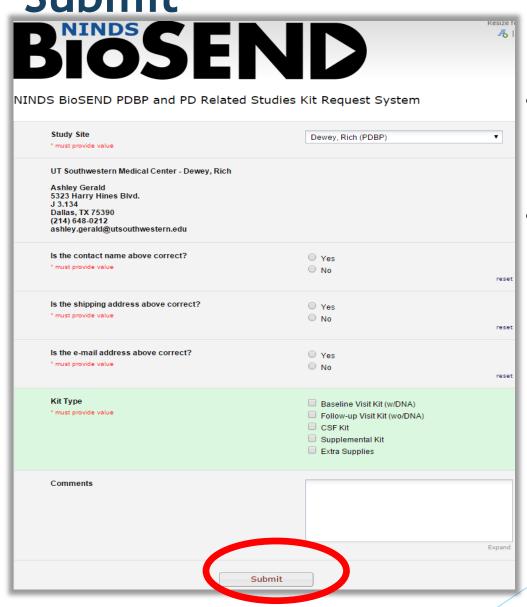
#### Kit Type

\* must provide value

- ✓ Baseline Visit Kit (w/DNA)
- ✓ Follow-up Visit Kit (wo/DNA)
- ✓ CSF Kit
- ✓ Supplemental Kit
- Extra Supplies

 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.

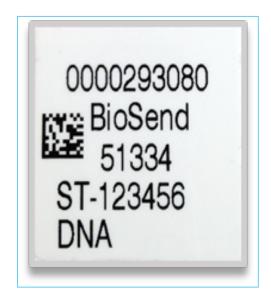
# Labels

### Types of Labels

Case Label



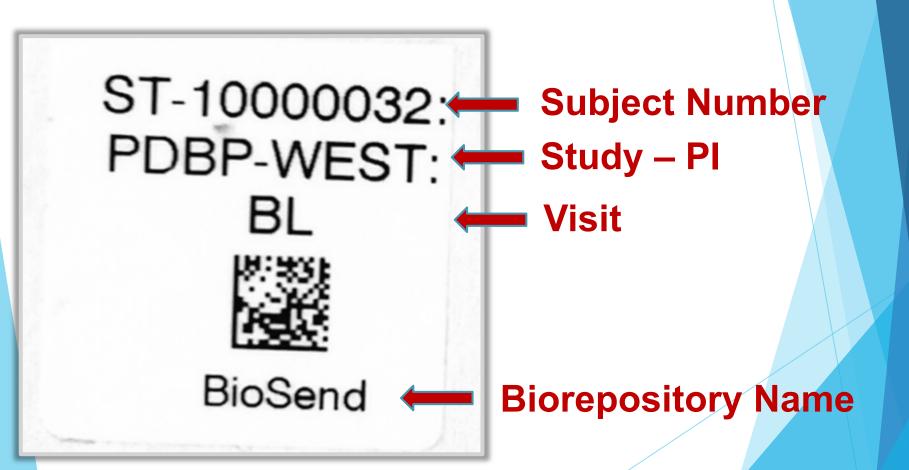
Specimen Label



Identify Study and PI

Identify Individual biospecimens

# Case Label



### **Case Labels**

#### Case labels are placed:

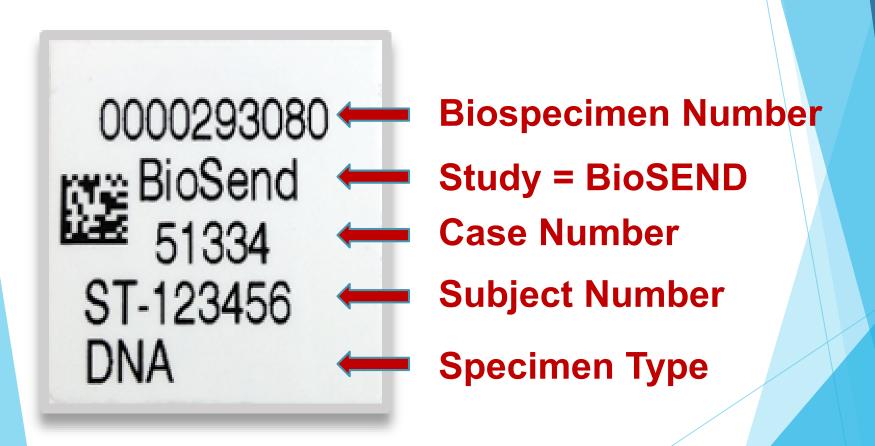
- On the plastic biohazard bag of the cryovial transport box
- On the plastic biohazard bag for the PAXgene® tubes
- On the lid of the shipping canisters.
- On lid of frozen shippers (pre-labeled)







## Collection and Aliquot Tube Label

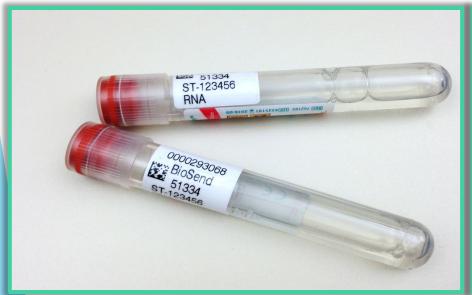


### Collection and Aliquot Tube Label

Collection and Aliquot Tube Labels are placed on:

- All collection tubes
- All aliquot tubes (Cryovials)

Be sure that the tube has the correct speciment type on the label





### Aliquot labels

Keep samples in sequential order when labeling and storing

0000293081

BioSend 51334

ST-123456

Plasma

0000293082

BioSend 51334

ST-123456

Plasma

0000293083

BioSend 51334

ST-123456

Plasma



### 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 lowfat 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

### **Calculating Centrifugation Speed**

http://clinfield.com/2012/07/how-to-convert-centrifuge-rpm-to-rcf-or-g-force/

The force exerted on a particle in a centrifuge is a simple function of the rotation speed of the centrifuge and the radius of rotation. The actual equation is:

RCF or G-force = 
$$1.12 \times R \times (RPM/1000)^2$$

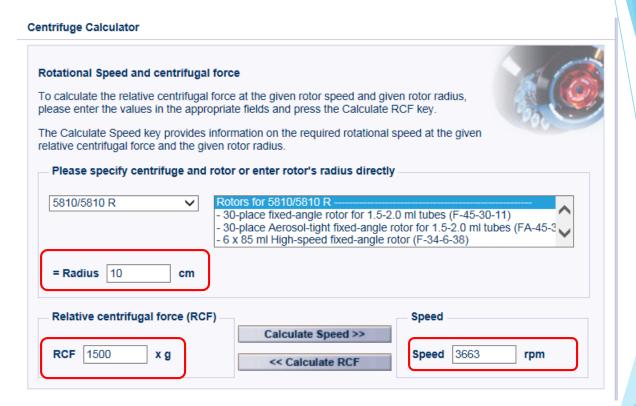
\*R being the radius of rotation measured in millimeters

$$(RPM/1000)^2 = 1500/(1.12 \times 100 \text{mm})$$
  
 $\sqrt{(RPM/1000)^2} = \sqrt{1500/(1.12 \times 100 \text{mm})}$   
 $RPM/1000 = \sqrt{(1500/1.12 \times 100)}$   
 $RPM = 1000 \times \sqrt{(15/1.12)}$   
 $= 3660$ 

\*Please refer to Appendix F in the BioSEND Biologics Manual for further information regarding the centrifugation of samples

### Calculating Centrifugation Speed

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

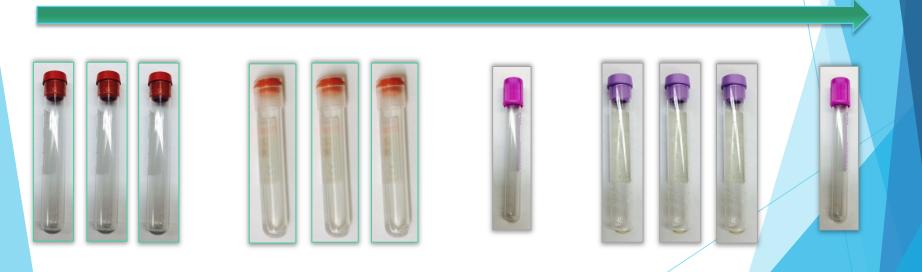


\*The 3663 rpm speed was calculated using a hypothetical radius of 10 cm and a RCF of 1500 x g.

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

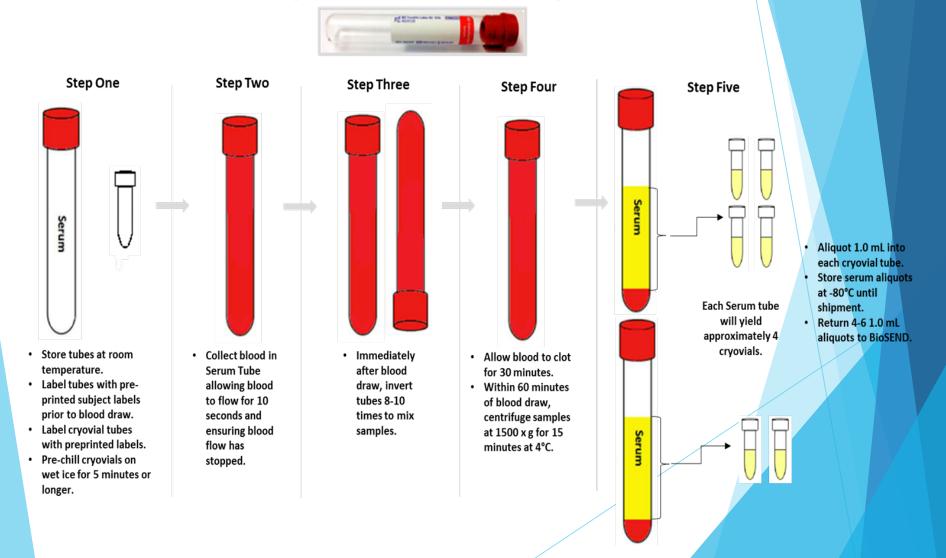
### Order of Specimen Collection

- 1. Serum (red top) blood collection for serum (if applicable)
- 2. PAXgene® tube for RNA
- 3. EDTA 6 ml (purple top) blood collection for DNA (usually only at baseline)
- 4. EDTA 10 ml (lavender top) blood collection for plasma
- 5. EDTA 6 ml (purple top) blood collection for whole blood



### Serum Diagram

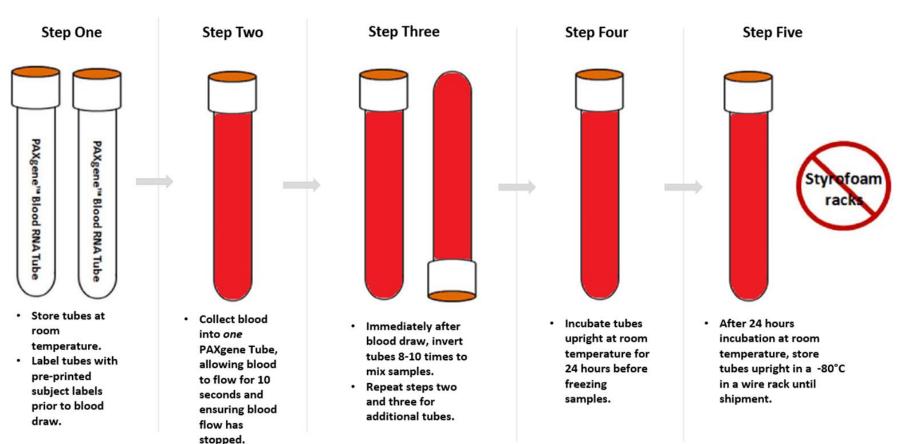
### **Serum Preparation (10 ml Red Top Tube)**



### PAXgene diagram

### PAXgene<sup>™</sup> Preparation

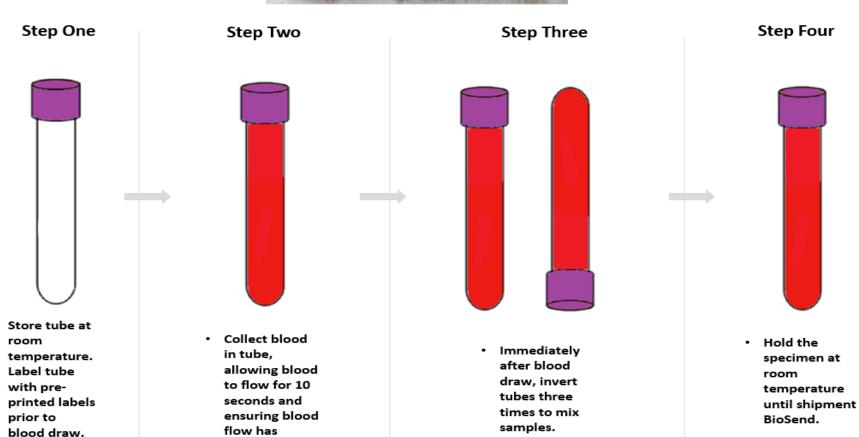




### DNA

### **DNA Preparation (6 ml Lavender Top Tube)**

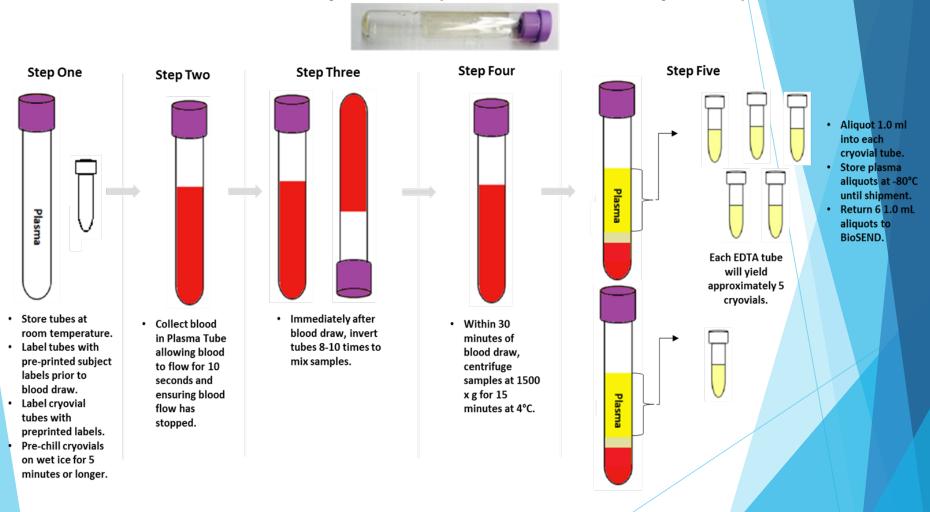




stopped.

### Plasma

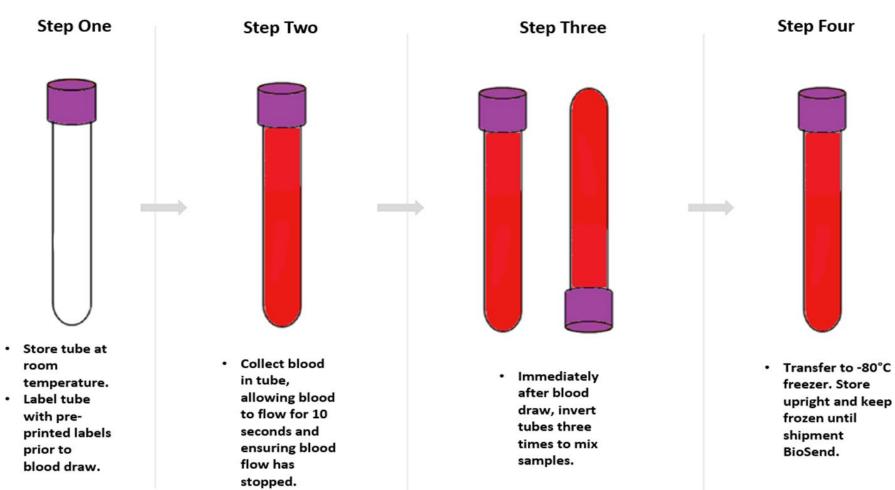
#### Plasma Preparation (10 ml Lavender Top Tube)



### Whole blood

#### Whole Blood (6 ml Lavender Top Tube)





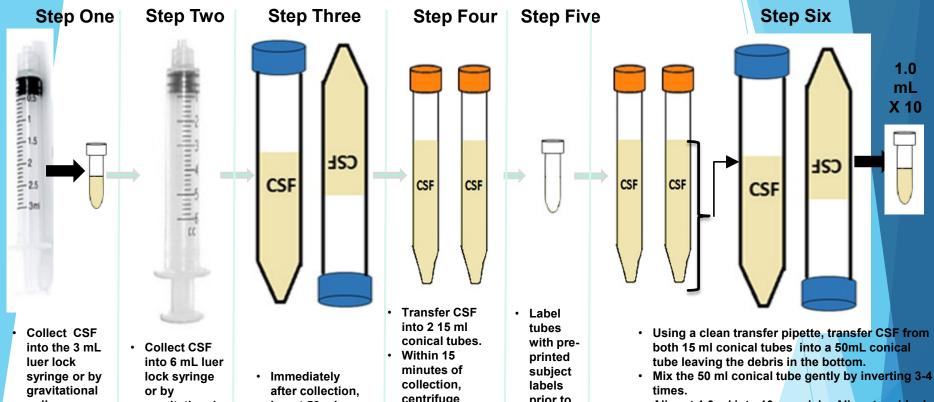
#### LP Procedure

- 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

#### LP Procedure

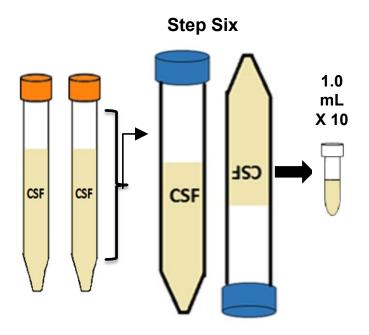
- Collect first 1-2 ccs, place in cryovial
  - Send within 4 hours of collection to local lab for routine analysis (protein, cell count, glucose)
- Collect additional CSF, up to 15-20 ccs total, including the amount used for local labs, and transfer to 50 mL conical
  - Immediately mix
  - Transfer CSF to two 15 mL conical tubes
  - Spin at 2000x g for 10 minutes at ROOM TEMPERATURE
  - Immediately aliquot approx. 1.0 mL into aliquot tubes
  - Place labeled cryovials in cryobox
  - Freeze at -80°C immediately

#### **CSF Preparation Processing**



- pull. Dispense 1-2
- mL in a cryovial.
- Send to local lab for testing.
- gravitational pull.
- Collect approved volume into 50 mL conical tube.
- invert 50 mL conical tube 3-4 times to mix sample.
- centrifuge samples at room temperature at 2000 x q for 10 minutes.
- prior to collection
- 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.

#### **CSF Preparation Processing**

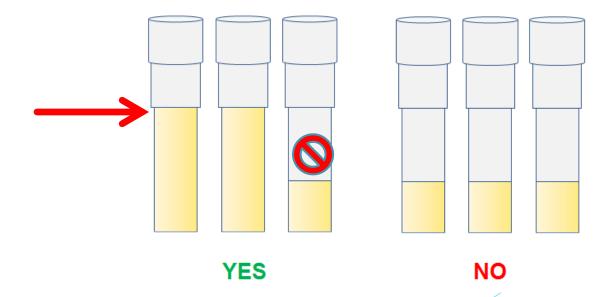


- Using a clean transfer pipette, transfer CSF from both 15 ml conical tubes into a 50mL conical tube leaving the debris in the bottom.
- Mix the 50 ml conical tube gently by inverting 3-4 times.
- Aliquot 1.0 ml into 10 cryovials, Aliquot residual mL in last cryovial (for site use).
- Store CSF aliquots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

Combine the CSF in two-15 ml conical tubes into a single 50 ml conical tube to ensure complete mixing and reduce possible batch effects in CSF aliquots generated from different conical tubes

### Serum, Plasma and CSF Aliquots

- Fill cryovials to 1ml (bottom of ridged section)
- 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 temps 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)

# **Ambient Sample Shipment**

- 6 ml purple (EDTA) whole blood for DNA collection tube
  - Typically collected at baseline 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 Sample

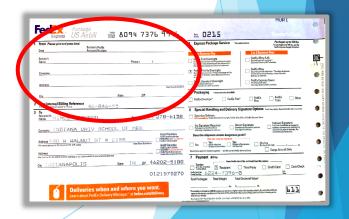


- Insert tube into the tube sleeve.
- Insert the sleeve into the canister.
- Seal the canister tightly.
- Place case label on canister.
- Wrap the canister in the enclosed bubble wrap.
- Place canister into the cardboard box.

# Labeling Ambient Sample Shipments

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

# Frozen Samples

- All other samples are shipped frozen
  - ▶ Plasma, serum, CSF, PAXgene® and whole blood
- 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



### Packing 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 must be received at BioSEND within 2 weeks of collection.



# **Bulk Shipping**

- Bulk shipping is available, with NINDS approval
  - Samples from 2+ subjects can be shipped together
- Appropriate for high volume sites



# Shipping Frozen Samples

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



# Sample Shipment Notification Form

#### Sample Record and Shipment Notification

Site Name/Number:		Principal Investigator:		
Coordinator:	Tel	lephone:	Email:	
	Please list only ONE subject per Sample	Record Summary and Shipme	nt Notific	ation Form
GUID:		Subject ID (ST# from pre-printed	i lables):	
Gender:		VI	sit Type:	
Age In Years:		Date Sample(s)	Shipped:	
Plus Months:		FedEx Tracking	Number:	
Subject Indicator:		Subject's Diagnosis:		

Instructions: Ship Frozen Shipments Monday- Wednesday ONLYI Ambient Shipments (purple-top EDTA tube) may be shipped Monday- Friday (preferably Monday- Thursday) 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 filled with dry les.

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 (Site fills this in)	Volume of Draw (mL) (Site does NOT need to fill in any longer)	Notation of Problems	
	DNA				
	RNA				
	Plasma				
	Serum				
	CSF				
	WB				

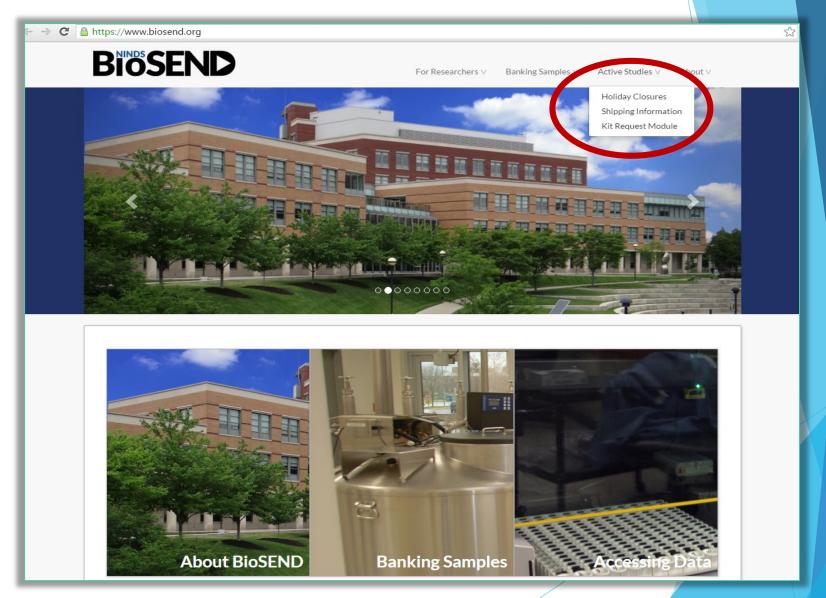
Contact Information: Indiana University, Email: biosend@iu.edu Ph: 317-278-0495
Data Management Resource (DMR); Email: PDBP-OPS@mail.nlh.gov

### **BioSEND Website**

Basic information now but will be expanding to include more information

https://www.biosend.org

## NINDS BioSEND Website



https://www.biosend.org/

# **Holiday Closures**

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

### **BioSEND Contact Information**

Questions?

Please contact: Scott Kaiser

Phone: 317-278-0495

► Email: <u>biosend@iu.edu</u>