

BioSpecimen Exchange for Neurological Disorders (BioSEND)


EPD Training Webinar

BioSEND Training Webinar Overview

1. Study Reminders
2. Site Equipment
3. EPD 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:

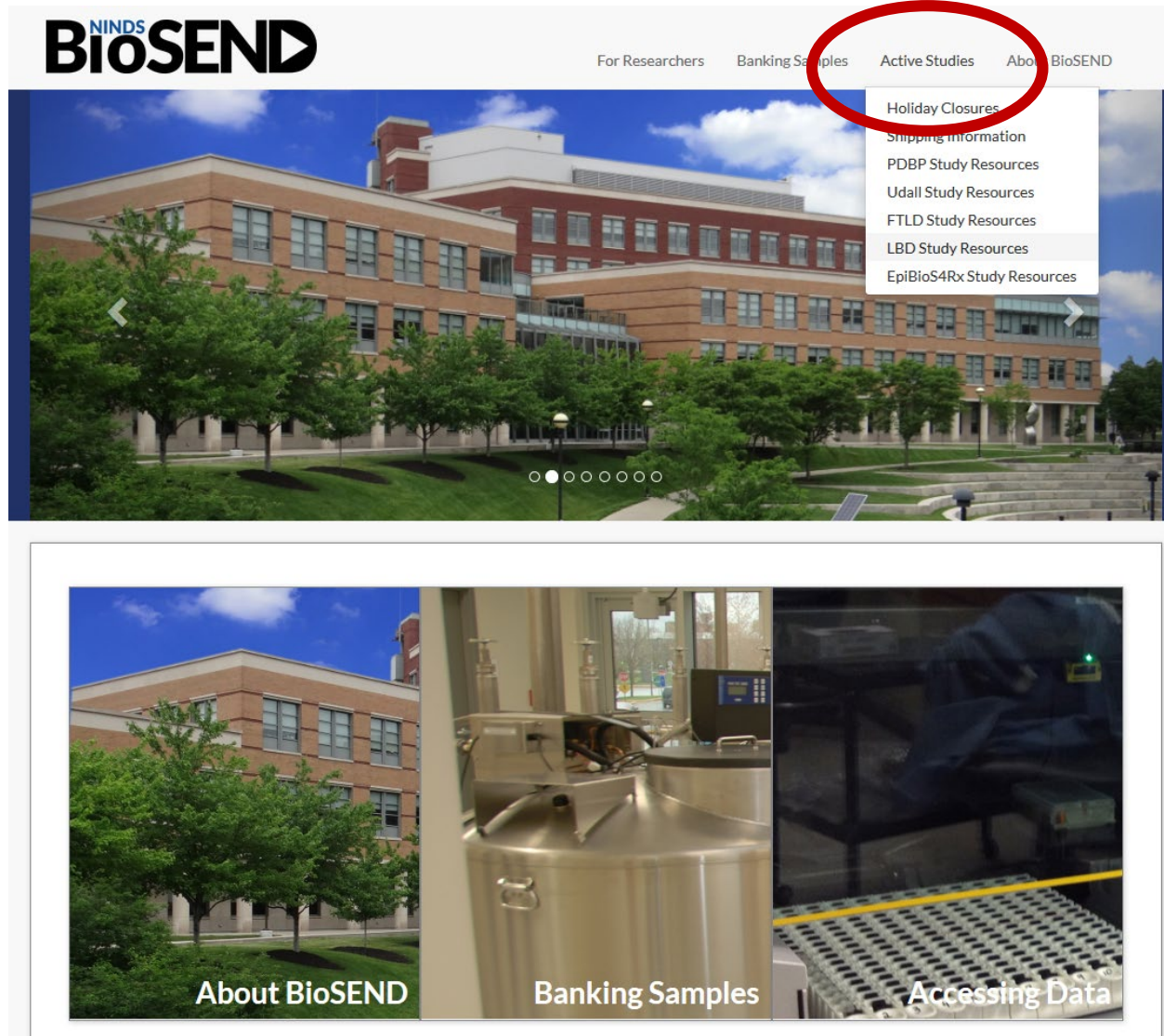
- Personal protective equipment
- Alcohol prep pads
- Butterfly needles and hubs
- Tourniquet
- Gauze pads
- Bandages
- Sharps bin and lid
- Microcentrifuge tube rack
- Test tube rack
- Crushed ice
- Pipettes and pipette tips
- 4°C Centrifuge
- -80°C Freezer
- Dry ice

EPD Biospecimen Collection Protocol

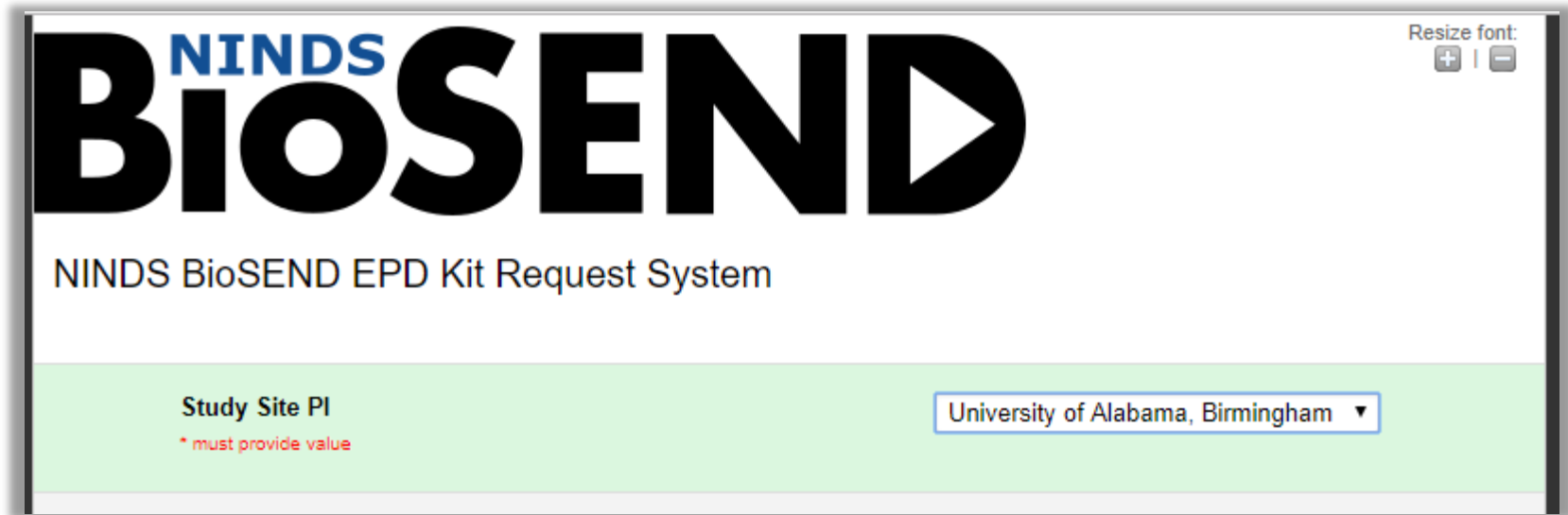
	BL
Plasma (6 x 1ml)	X
RNA (2 x 2.5ml)	X
Buffy coat (2 aliquots)	X

Requesting Kits

NINDS BioSEND Website



BioSEND Kit Request Module



The screenshot shows the NINDS BioSEND EPD Kit Request System interface. At the top, the NINDS BioSEND logo is displayed in large, bold letters. Below the logo, the text "NINDS BioSEND EPD Kit Request System" is visible. In the top right corner, there is a "Resize font:" option with plus and minus icons. The main form area has a light green background. On the left, the label "Study Site PI" is shown with a red asterisk and the text "* must provide value" below it. On the right, there is a drop-down menu with the text "University of Alabama, Birmingham" and a downward arrow.

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

BioSEND Kit Request Module

UAB

The University of Alabama at Birmingham SC 360K
1720 7th Ave South
Birmingham, AL 35294

Is the contact name above correct?

* must provide value

☒ Yes
☐ No

reset

Is the shipping address above correct?

* must provide value

☒ Yes
☐ No

reset

Is the e-mail address above correct?

* must provide value

☒ Yes
☐ No

reset

Confirm site information:

- Study site
- Shipping address
- Contact name
- Email

BioSEND Kit Request Module

Is the contact name above correct? <small>* must provide value</small>	<input type="radio"/> Yes <input checked="" type="radio"/> No	reset
New Contact Name <small>* must provide value</small>	<input type="text"/>	
Is the shipping address above correct? <small>* must provide value</small>	<input type="radio"/> Yes <input checked="" type="radio"/> No	reset
New Shipping Address <small>* must provide value</small>	<div><input type="text"/></div> <div>Expand</div>	
Is the e-mail address above correct? <small>* must provide value</small>	<input type="radio"/> Yes <input checked="" type="radio"/> No	reset
New e-mail Address <small>* must provide value</small>	<input type="text"/>	

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

BioSEND Kit Request Module: Kit Type

Kit Type

****Please allow two weeks for shipment****

* must provide value

- ☐ Baseline Visit Kit
- ☐ Supplemental Kit
- ☐ Extra Supplies

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

BioSEND Kit Request Module: Baseline Kit

Kit Type **Please allow two weeks for shipment** <small>* must provide value</small>	<input checked="" type="checkbox"/> Baseline Visit Kit <input type="checkbox"/> Supplemental Kit <input type="checkbox"/> Extra Supplies <small>Please specify in comments if you need kits before the standard two week shipment time.</small>
Baseline Kit Quantity <small>* must provide value</small>	<input type="text"/>

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

BioSEND Kit Request Module: Supplemental Kit

Kit Type **Please allow two weeks for shipment** <small>* must provide value</small>	<input type="checkbox"/> Baseline Visit Kit <input checked="" type="checkbox"/> Supplemental Kit <input type="checkbox"/> Extra Supplies <small>Please specify in comments if you need kits before the standard two week shipment time.</small>
Supplemental Kit Quantity <small>* must provide value</small>	<input type="text"/>
Comments	<div><input type="text"/></div> <div>Expand</div>
Each Supplemental Kit Contains: 2 100 ml absorbent sheets 2 4-tube bubble pouches 2 Cryoboxes 15 Siliconized sterile cryogenic vials (2 ml) 2 Biohazard bags 2 PAXgene® tubes (2.5 ml) 2 Lavender-top EDTA tubes (10 ml) 2 Warning label packets	
<div>Submit</div>	

- Contains a variety of extra kit pieces

BioSEND Kit Request Module: Extra Supplies

Kit Type **Please allow two weeks for shipment** <small>* must provide value</small>	<input type="checkbox"/> Baseline Visit Kit <input type="checkbox"/> Supplemental Kit <input checked="" type="checkbox"/> Extra Supplies <small>Please specify in comments if you need kits before the standard two week shipment time.</small>
6-Tube Bubble Pouch	<input type="radio"/> 2 <input type="radio"/> 4 reset
Cryobox	<input type="radio"/> 2 <input type="radio"/> 4
Siliconized Sterile Cryogenic Vial (2 ml)	<input type="radio"/> 10 <input type="radio"/> 20
FedEx® Return Airbill	<input type="radio"/> 2 <input type="radio"/> 4 reset
Screw-Top Centrifuge Tubes (15ml)	<input type="radio"/> 2 <input type="radio"/> 4 reset
Shipping Container for Dry Ice Shipments	<input type="radio"/> 2 <input type="radio"/> 4 reset

- Allows you to choose specific supplies and particular quantities

BioSEND Kit Request Module: Multiple Orders

Kit Type

****Please allow two weeks for shipment****

* must provide value

☒ Baseline Visit Kit

☒ Supplemental Kit

☒ Extra Supplies

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

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

BioSEND Kit Request Module: Submit

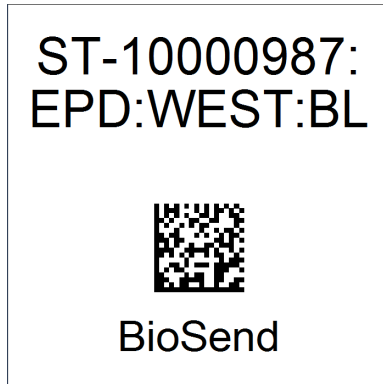
- Click “Submit” to turn in your request.
- The BioSEND staff will notify you that your request has been received and address any issues.

A screenshot of the NINDS BioSEND EPD Kit Request System web form. The form is titled "NINDS BioSEND" and "NINDS BioSEND EPD Kit Request System". It contains several sections for data entry: "Study Site PI" with a dropdown menu showing "University of Alabama, Birmingham"; "UAB" with address information; three confirmation questions ("Is the contact name above correct?", "Is the shipping address above correct?", "Is the e-mail address above correct?") each with "Yes" and "No" radio buttons; "Kit Type" with checkboxes for "Baseline Visit Kit", "Supplemental Kit", and "Extra Supplies"; and a "Comments" section with a text area. The "Submit" button at the bottom is circled in red. A "Reset font" link is in the top right corner. The "Submit" button is a grey rectangle with the word "Submit" in black text.

Labels

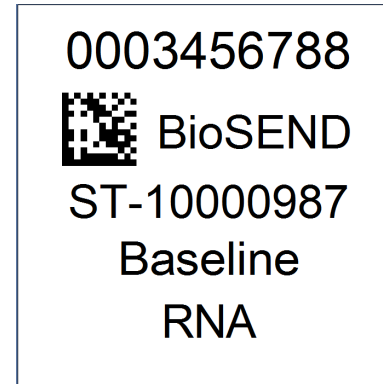
Types of Labels

Case Label



Identify study and PI

Specimen Label



Identify individual biospecimens

Case Label



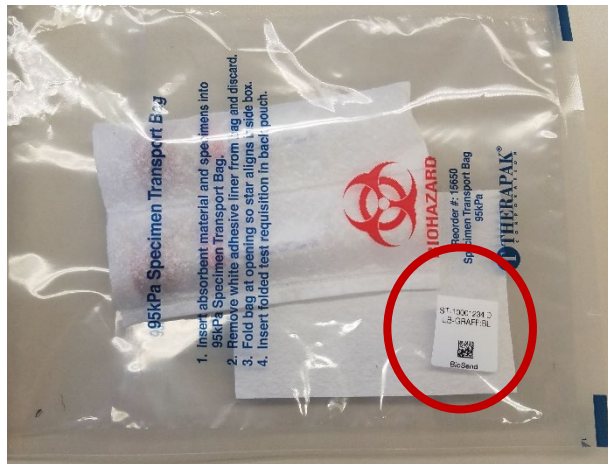
← Subject Number
← Study – PI
← Visit

← 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

0003456788



BioSEND

ST-10000987

Baseline

RNA



Biospecimen Number



Repository = BioSEND



Subject Number



Visit Type



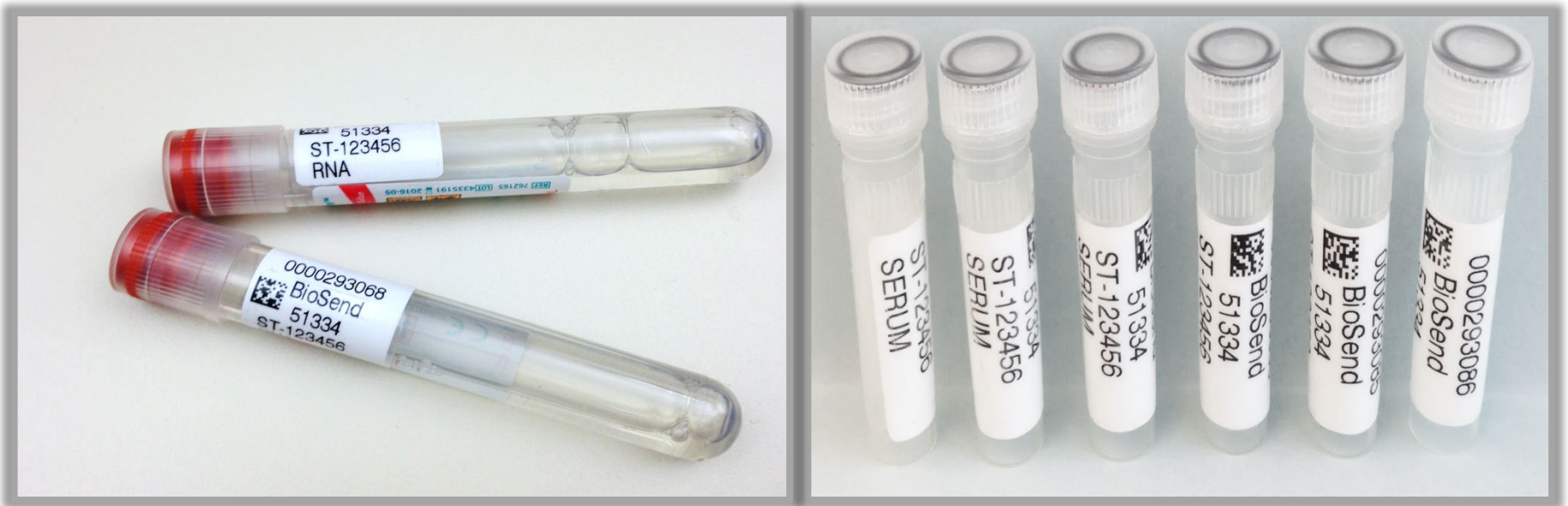
Specimen Type

Collection and Aliquot Tube Label

Collection and Aliquot Tube Labels are placed on:

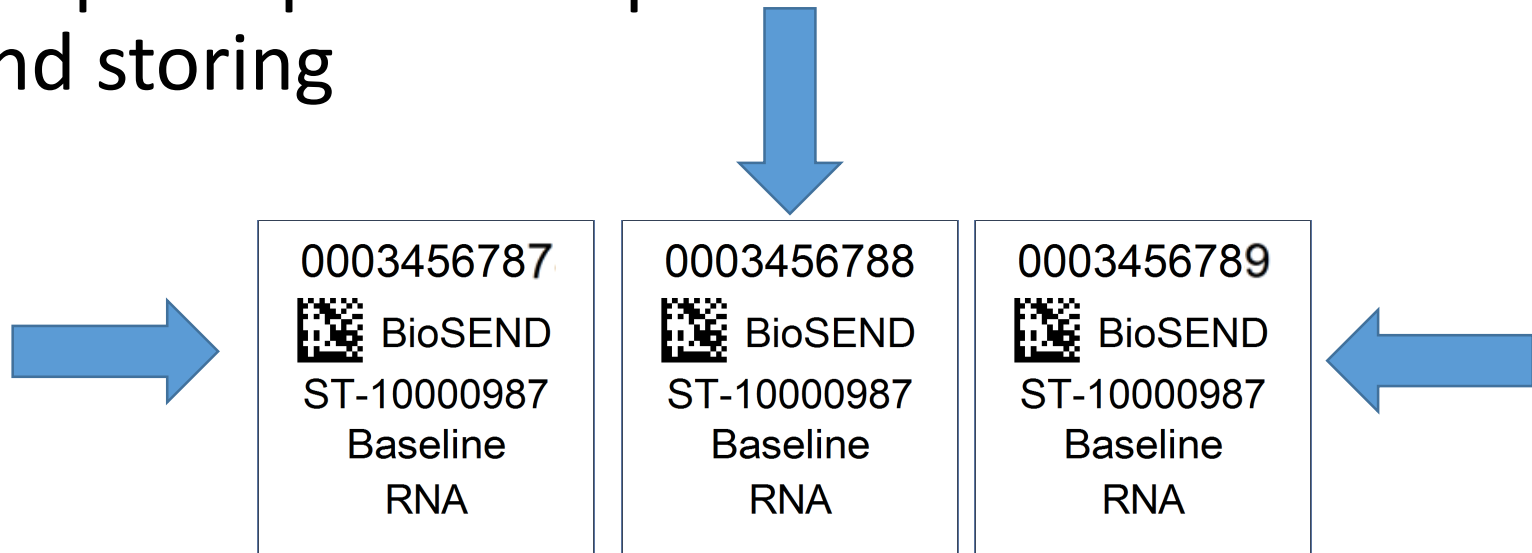
- All collection tubes
- All aliquot tubes (Cryovials)

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



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 fasting for 8 hours or more. If fasting is not feasible, follow suggested low-fat diet.
- G force \neq 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 and buffy coat aliquots, please freeze samples upright in the cryobox provided

Calculating Centrifugation Speed

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

Centrifuge Calculator

Rotational Speed and centrifugal force

To calculate the relative centrifugal force at the given rotor speed and given rotor radius, please enter the values in the appropriate fields and press the Calculate RCF key.

The Calculate Speed key provides information on the required rotational speed at the given relative centrifugal force and the given rotor radius.

Please specify centrifuge and rotor or enter rotor's radius directly

5810/5810 R

Rotors for 5810/5810 R

- 30-place fixed-angle rotor for 1.5-2.0 ml tubes (F-45-30-11)
- 30-place Aerosol-tight fixed-angle rotor for 1.5-2.0 ml tubes (FA-45-30-11)
- 6 x 85 ml High-speed fixed-angle rotor (F-34-6-38)

= Radius 10 cm

Relative centrifugal force (RCF)

RCF 1500 x g

Speed

Speed 3663 rpm

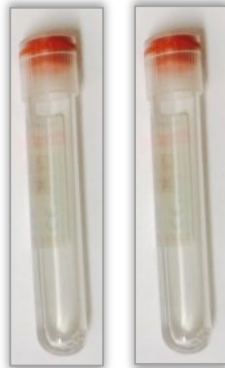
Calculate Speed >>

<< Calculate RCF

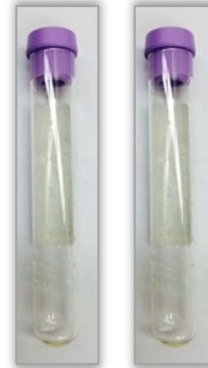
****The 3663 rpm speed was calculated using a hypothetical radius of 10 cm and a RCF of 1500 x g.***

Order of Specimen Collection

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



**PAXgene
(RNA)**

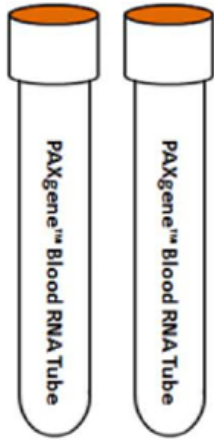


**EDTA
(Plasma)**

PAXgene™ Preparation (2.5ml Tube)

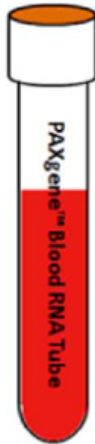


Step One



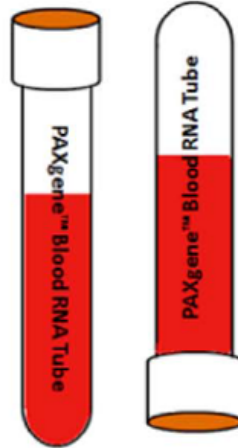
- Store tubes at room temperature.
- Label tubes with pre-printed subject labels prior to blood draw.

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.



Plasma Preparation (10ml Lavender Top Tube)

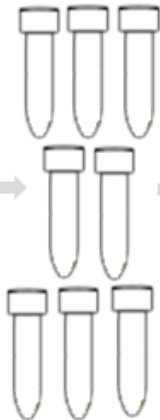


Step One



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

Step Two



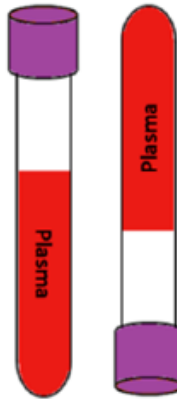
- Label 6 cryovials for plasma and 2 cryovials for buffy coat with pre-printed subject labels prior to blood draw.
- Pre-chill cryovials on wet ice for 5 minutes or longer.

Step Three



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

Step Four



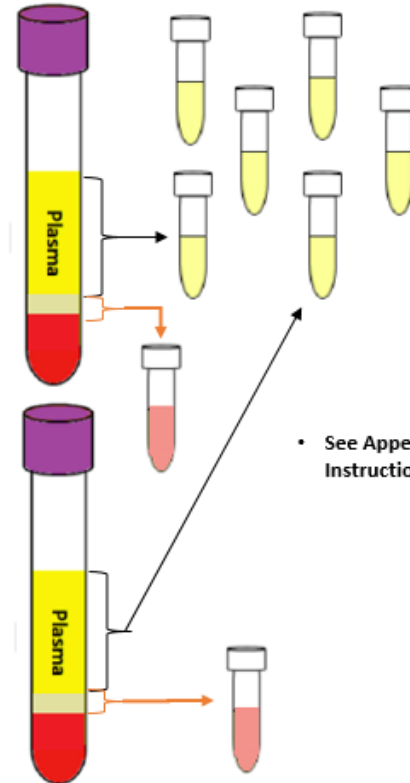
- Immediately after blood draw, invert tubes 8-10 times to mix samples.
- Repeat Steps 3 and 4 for second tube.

Step Five



- Within 30 minutes of blood draw, centrifuge samples at 1500 x g for 15 minutes at 4°C.

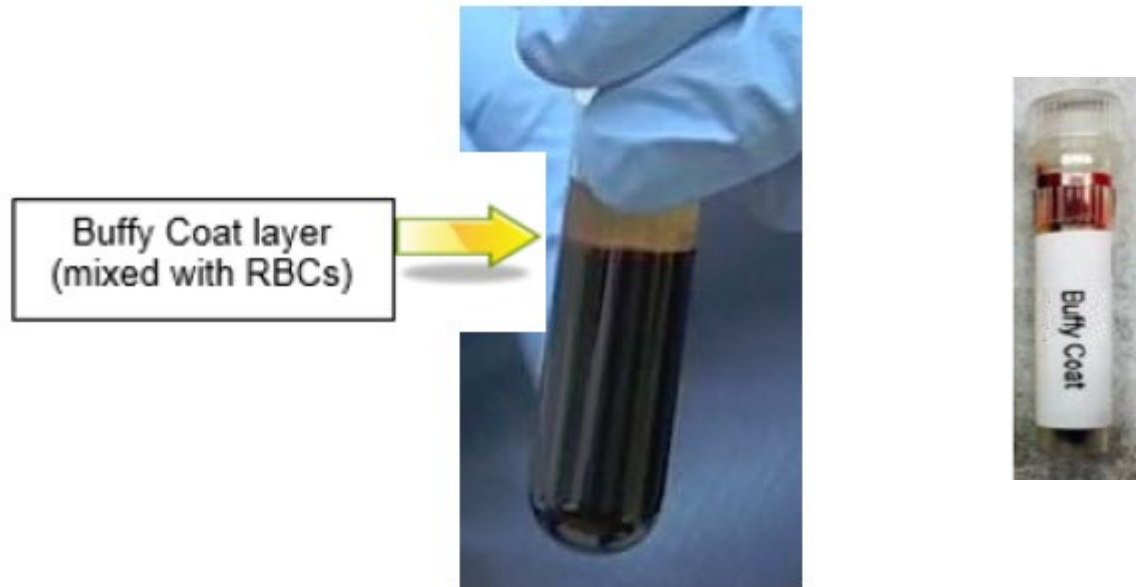
Step Six and Seven



- 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

- See Appendix C for Buffy Coat Preparation Instructions.

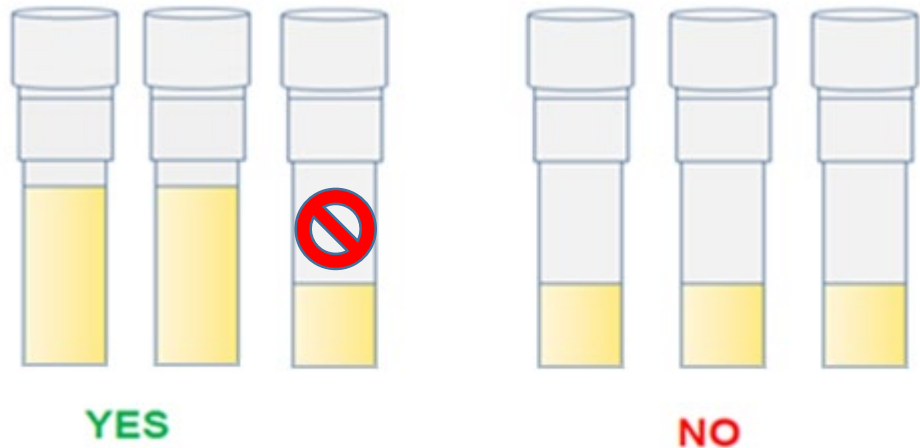
Buffy Coat Collection



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.

Plasma and Buffy Coat Aliquots

- Fill cryovials to 1ml
- Over-filled vials may burst in freezer
- Ship material to BioSEND
 - 6 Plasma aliquots
 - 2 Buffy Coat
- 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 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) 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

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)

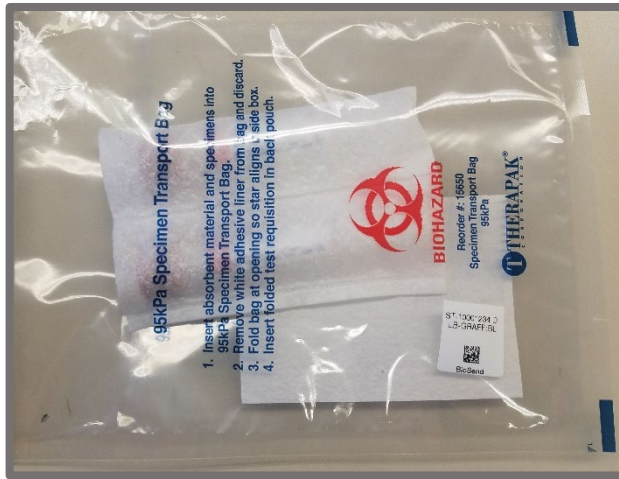
Sample Shipment

Frozen Samples

- All other samples are shipped frozen
 - Plasma, buffy coat 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

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


Sample Shipment Notification Form, Online

NINDS

BioSEND

For Researchers Banking Samples About

Sample Record and Shipment Notification

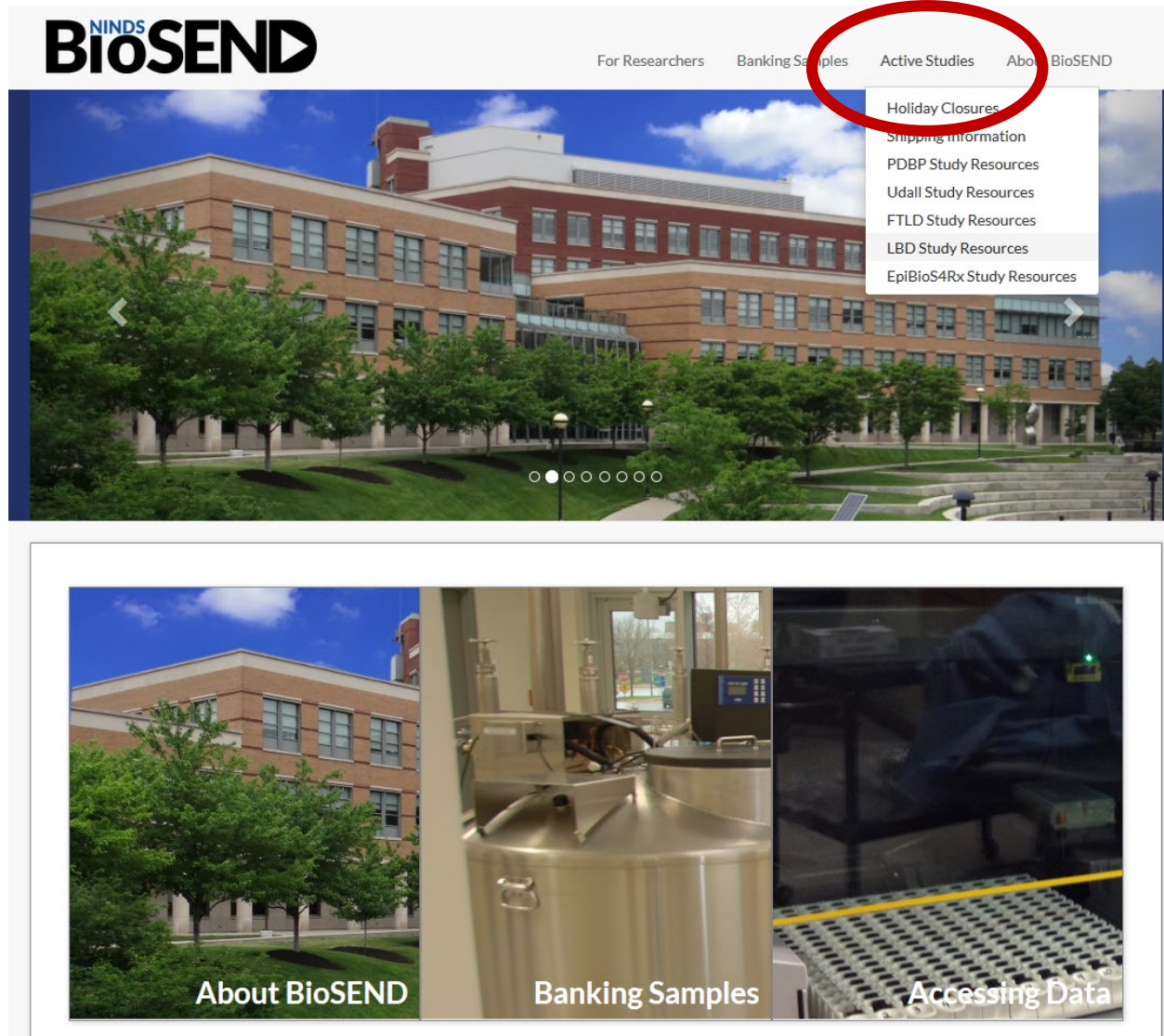


Sample Information

Date Sample(s) Shipped	FedEx Tracking Number
<input type="text" value="07/27/2017"/>	<input type="text" value="FedEx Tracking #"/>

Draw Date	Specimen Type	# of Tubes Sent	Notation of Problems
<input type="text" value="Draw Date"/>	<input type="text" value="DNA"/>	<input type="text" value="# of Tubes"/>	<input type="text" value="Notation of Problems"/>
<input type="text" value="Draw Date"/>	<input type="text" value="RNA"/>	<input type="text" value="# of Tubes"/>	<input type="text" value="Notation of Problems"/>
<input type="text" value="Draw Date"/>	<input type="text" value="Plasma"/>	<input type="text" value="# of Tubes"/>	<input type="text" value="Notation of Problems"/>
<input type="text" value="Draw Date"/>	<input type="text" value="Serum"/>	<input type="text" value="# of Tubes"/>	<input type="text" value="Notation of Problems"/>
<input type="text" value="Draw Date"/>	<input type="text" value="CSF"/>	<input type="text" value="# of Tubes"/>	<input type="text" value="Notation of Problems"/>
<input type="text" value="Draw Date"/>	<input type="text" value="Whole Blood"/>	<input type="text" value="# of Tubes"/>	<input type="text" value="Notation of Problems"/>
<input type="text" value="Draw Date"/>	<input type="text" value="Buffy Coat"/>	<input type="text" value="# of Tubes"/>	<input type="text" value="Notation of Problems"/>

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: Scott Kaiser (sckaiser@iu.edu)

- Email: biosend@iu.edu

Questions?