# 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

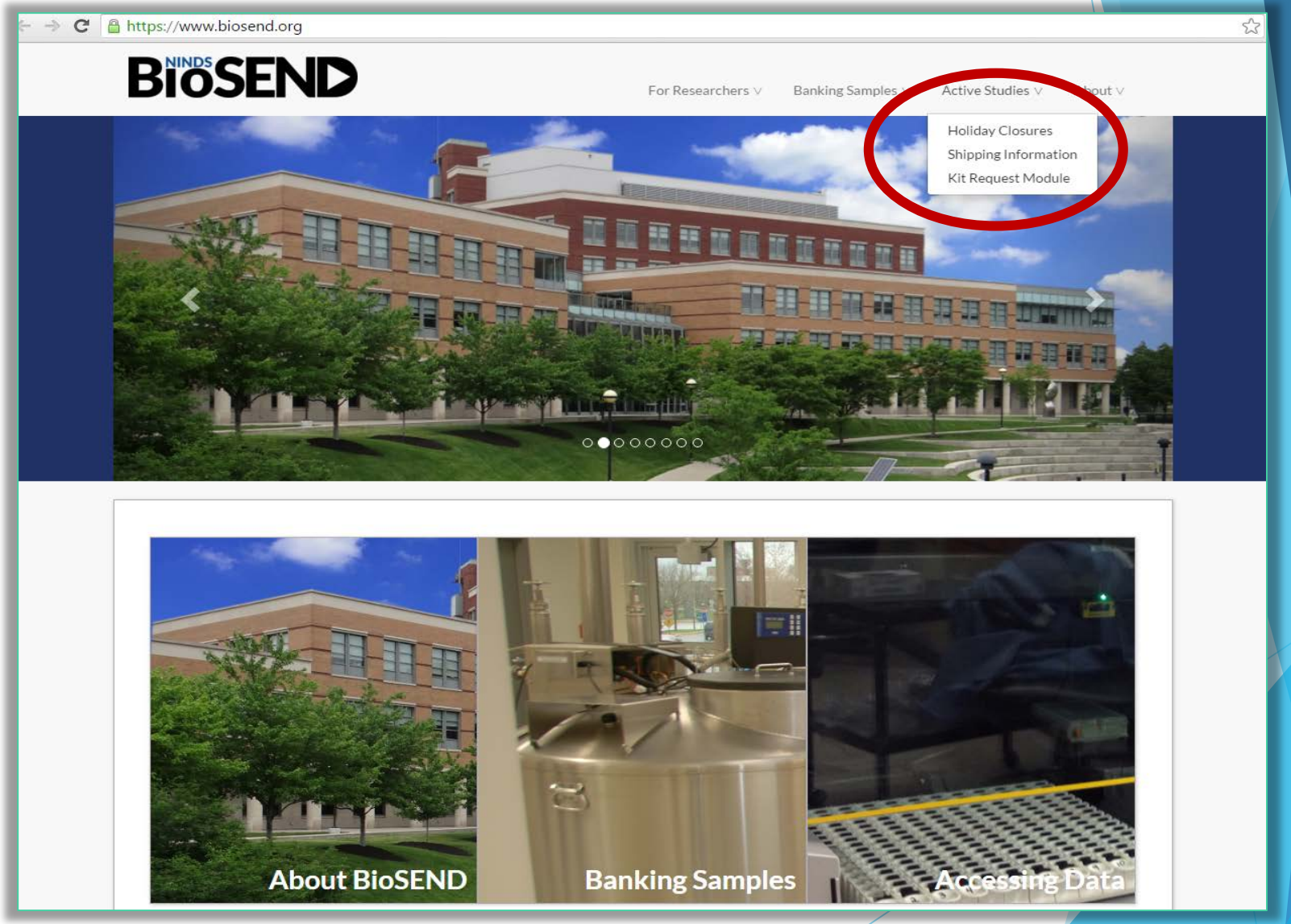
# Equipment

***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

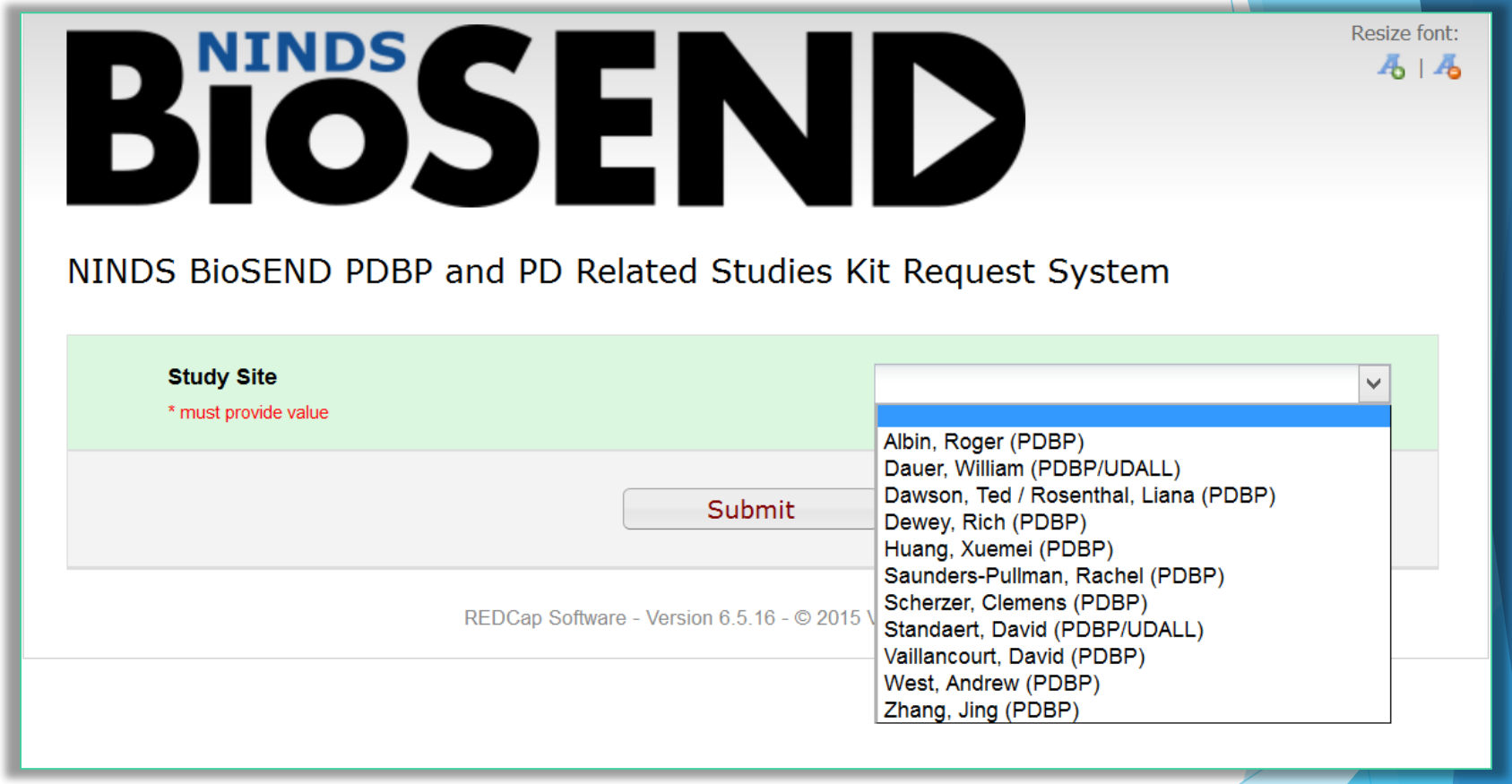
# Requesting Kits

# NINDS BioSEND Website



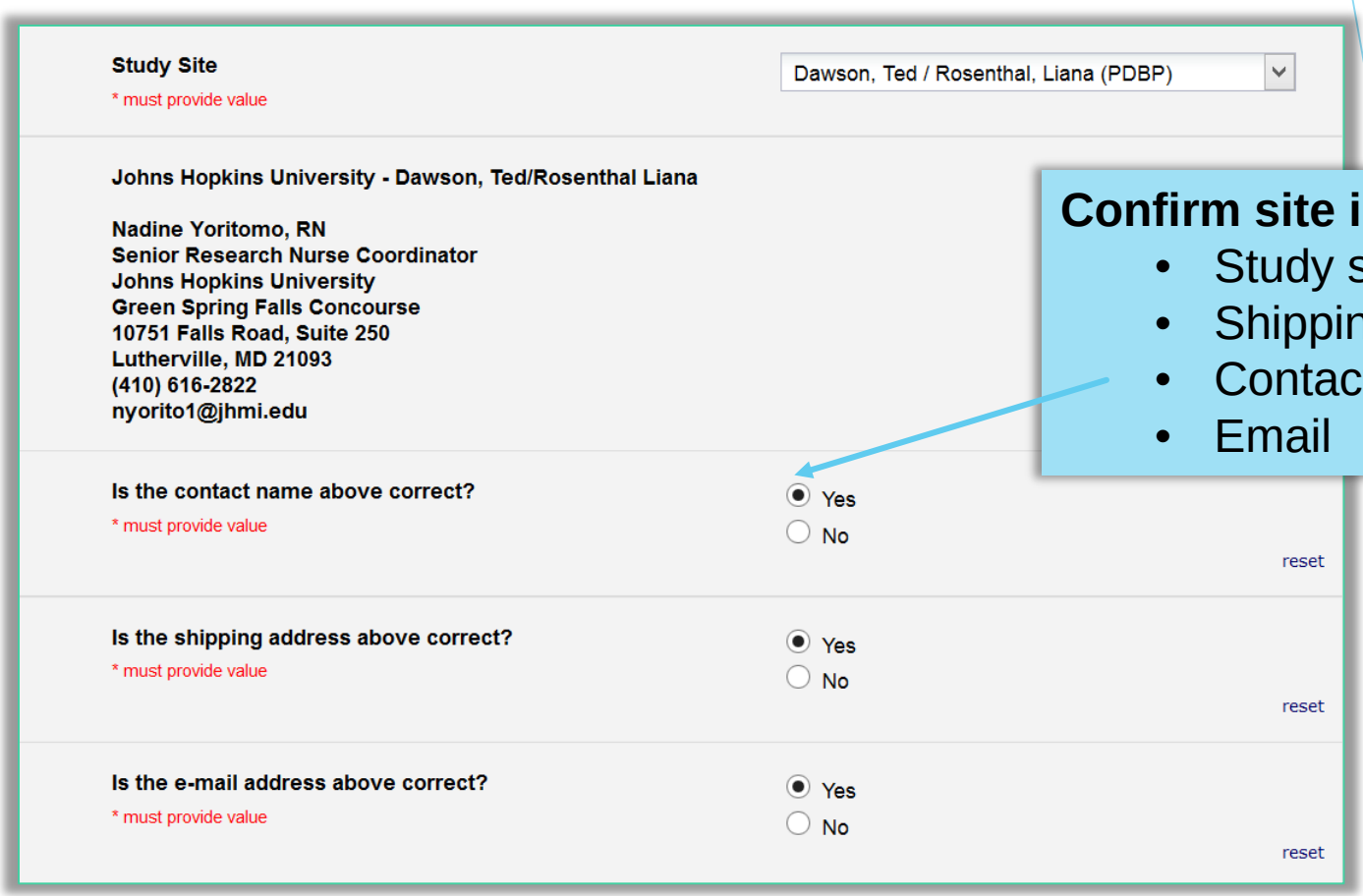

https://www.biosend.org/

# BioSEND Kit Request Module




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

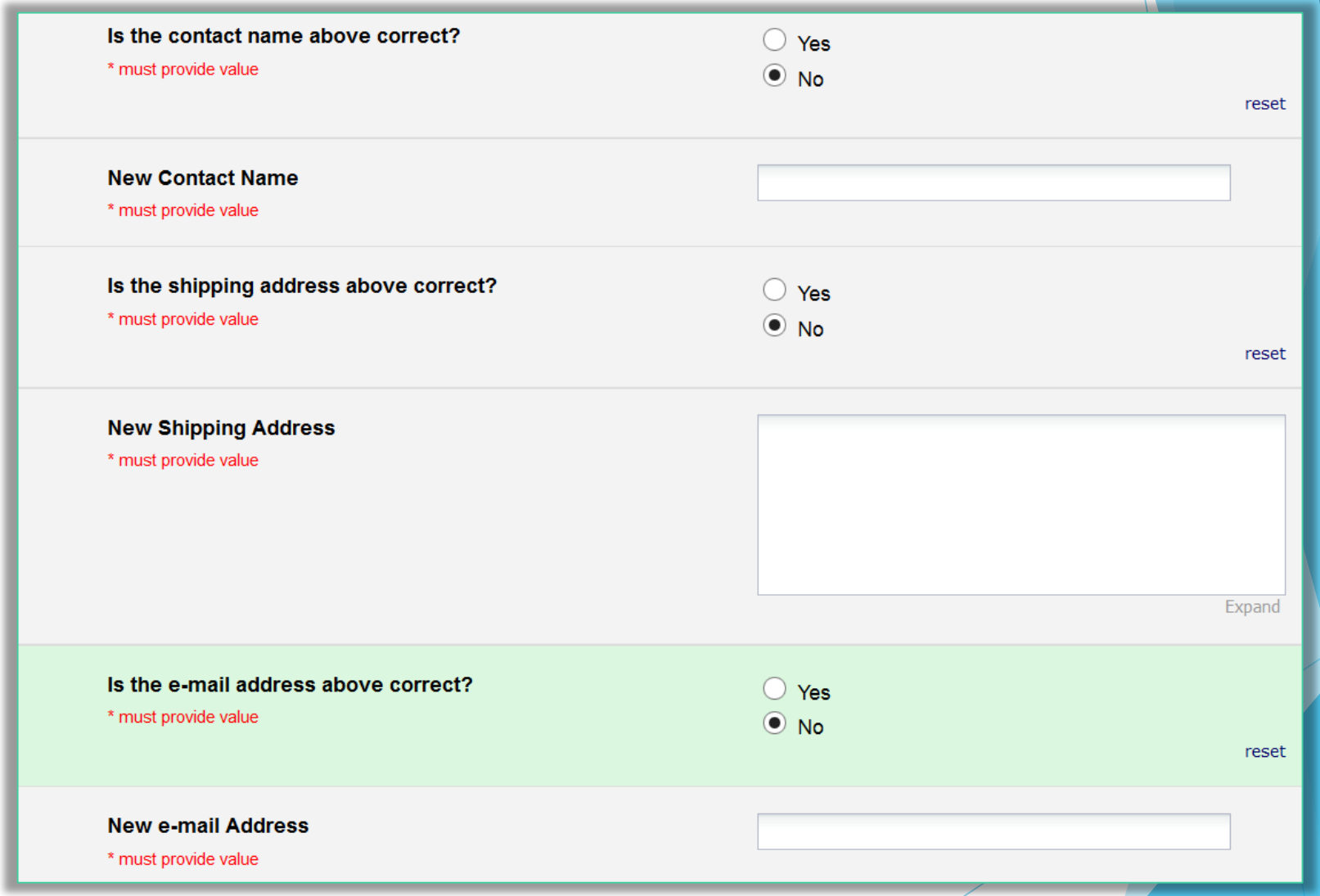
# BioSEND Kit Request Module




**Confirm site information:**

- Study site
- Shipping address
- Contact name
- Email

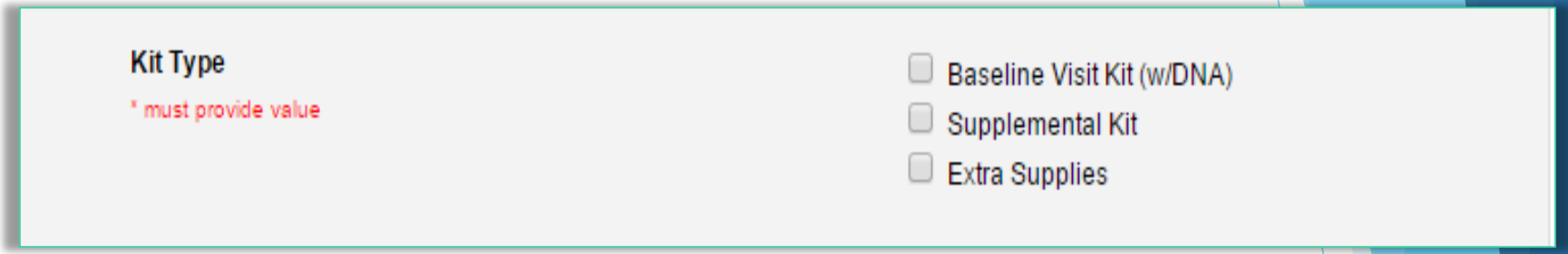
# BioSEND Kit Request Module




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

# BioSEND Kit Request Module: Type by Site

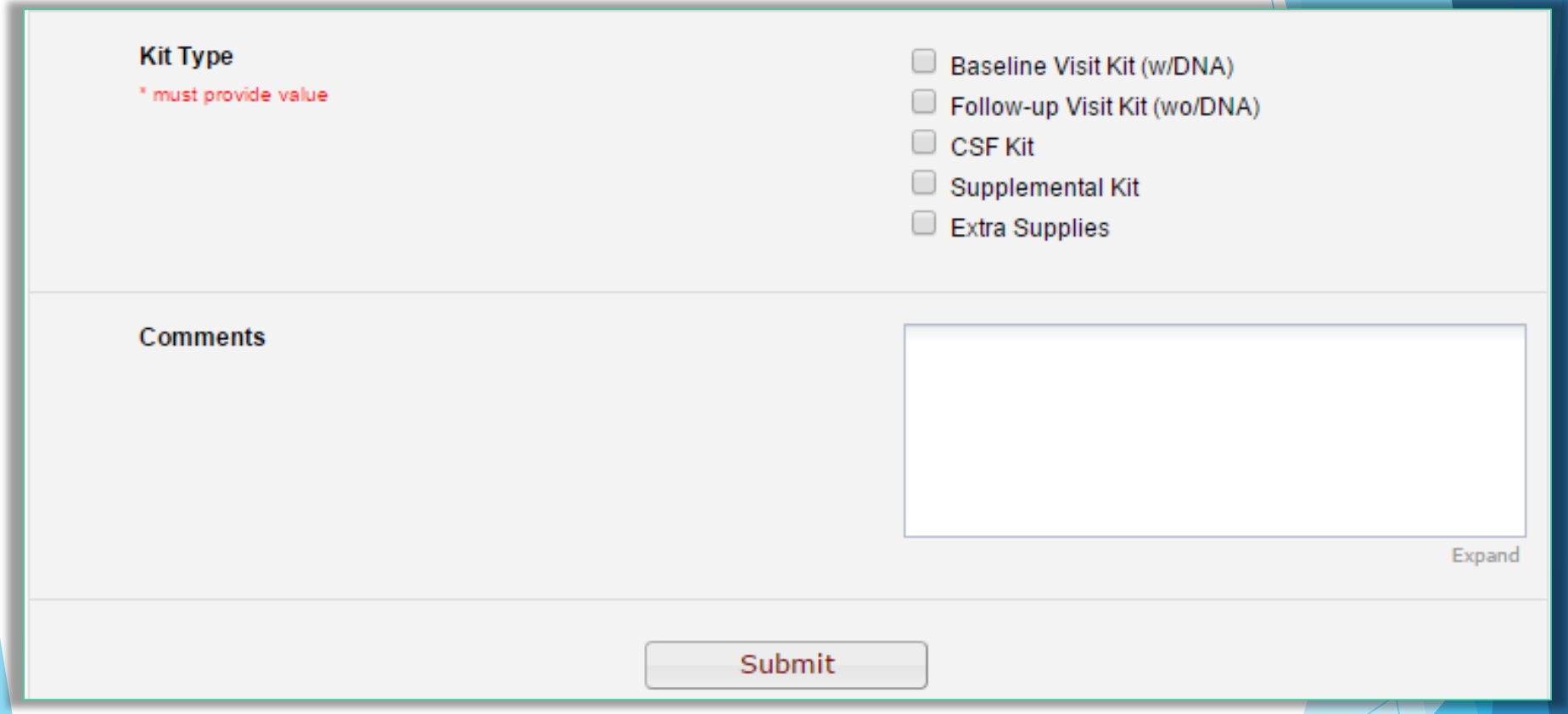
**Example A**




**Example B**

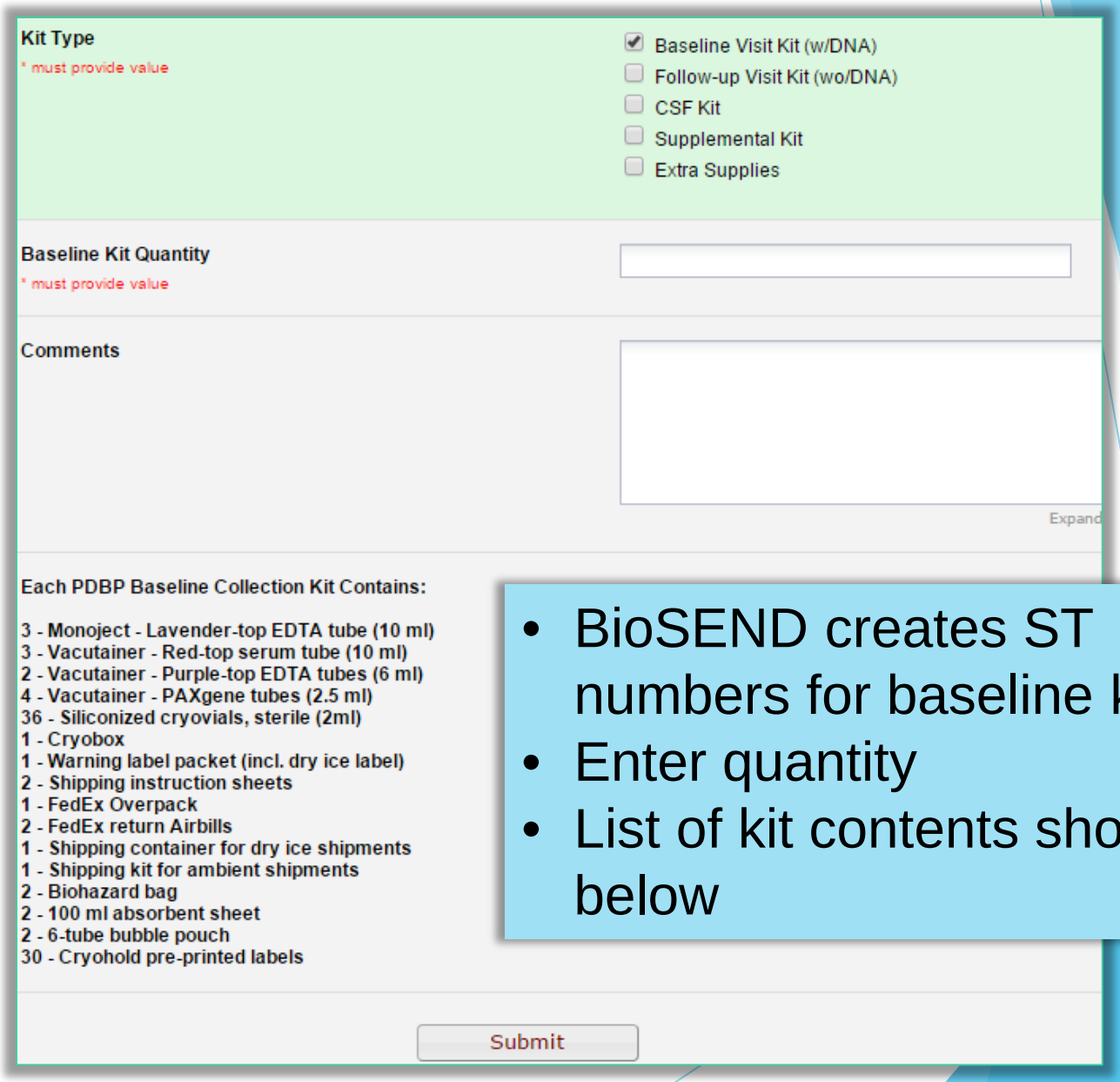

- Each PDBP site uses slightly different kit components
- Kits differ based on baseline and longitudinal visits
  - Typical difference is the collection of a blood tube for DNA only at the baseline visit

# BioSEND Kit Request Module: Kit Type



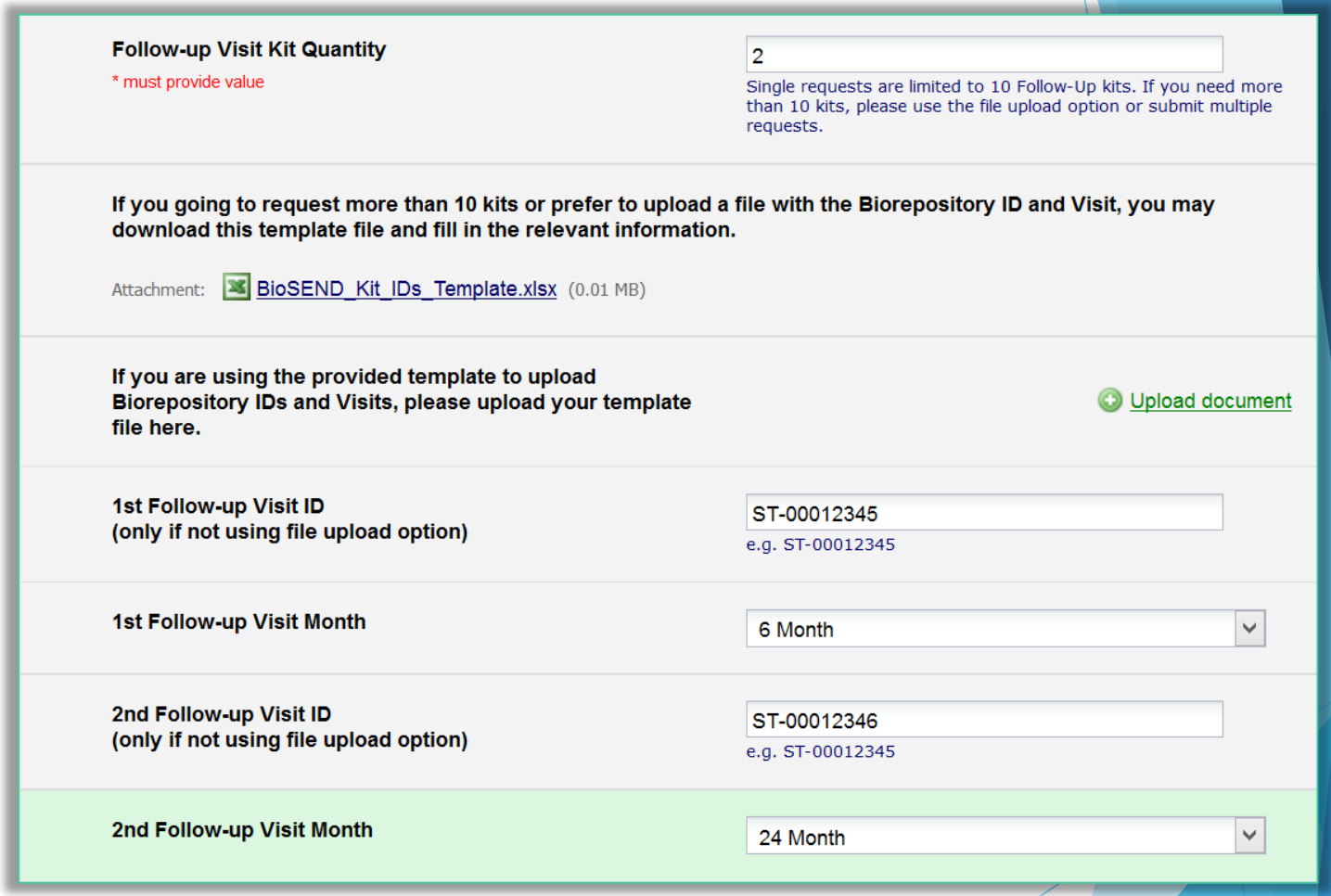

- Choose your kit type
- Only kit types used by your site will show up as a selection

# BioSEND Kit Request Module: Baseline Kit



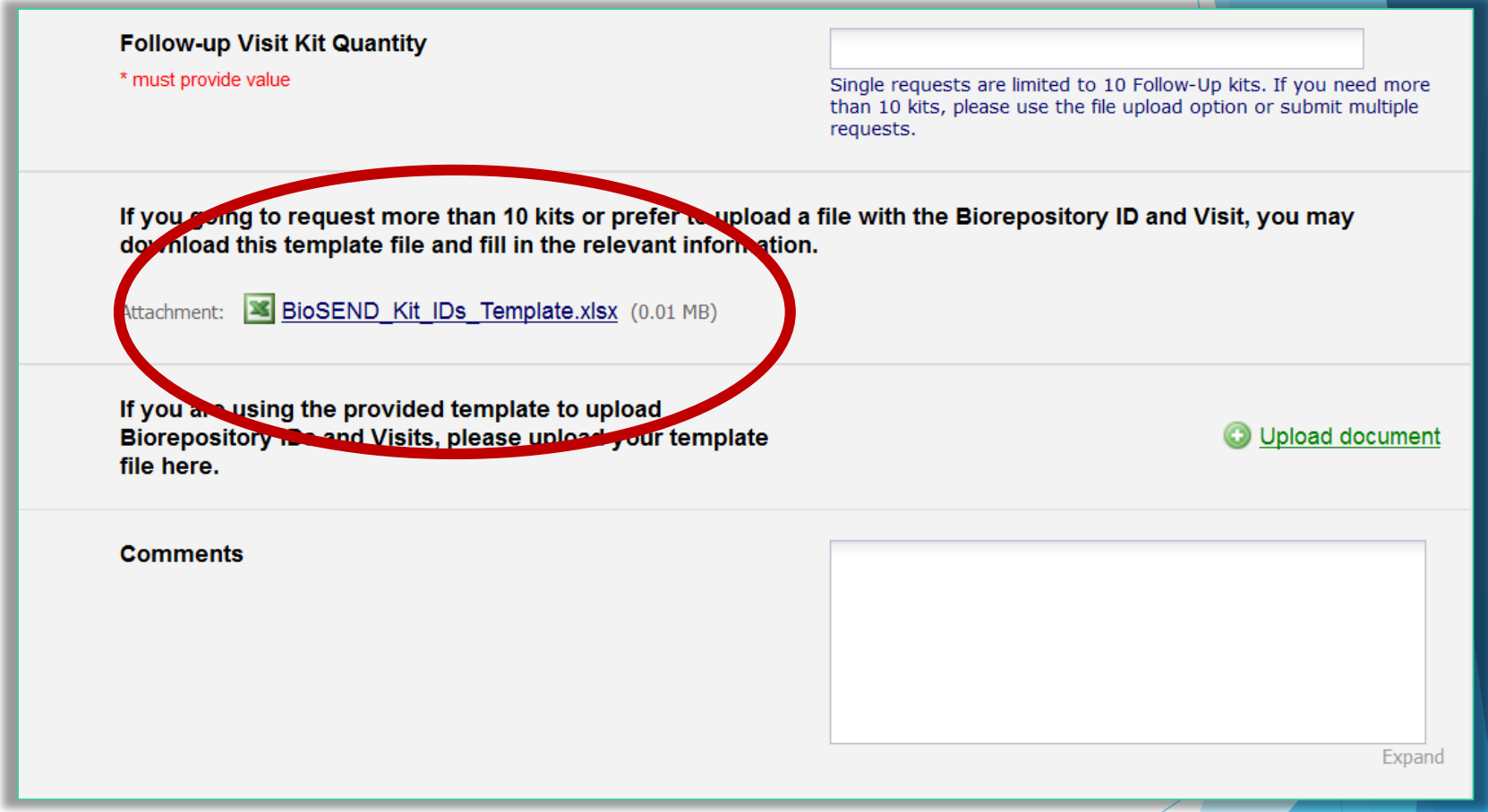

- BioSEND creates ST numbers for baseline kits
- Enter quantity
- List of kit contents shown below

# BioSEND Kit Request Module: Follow-up Kit



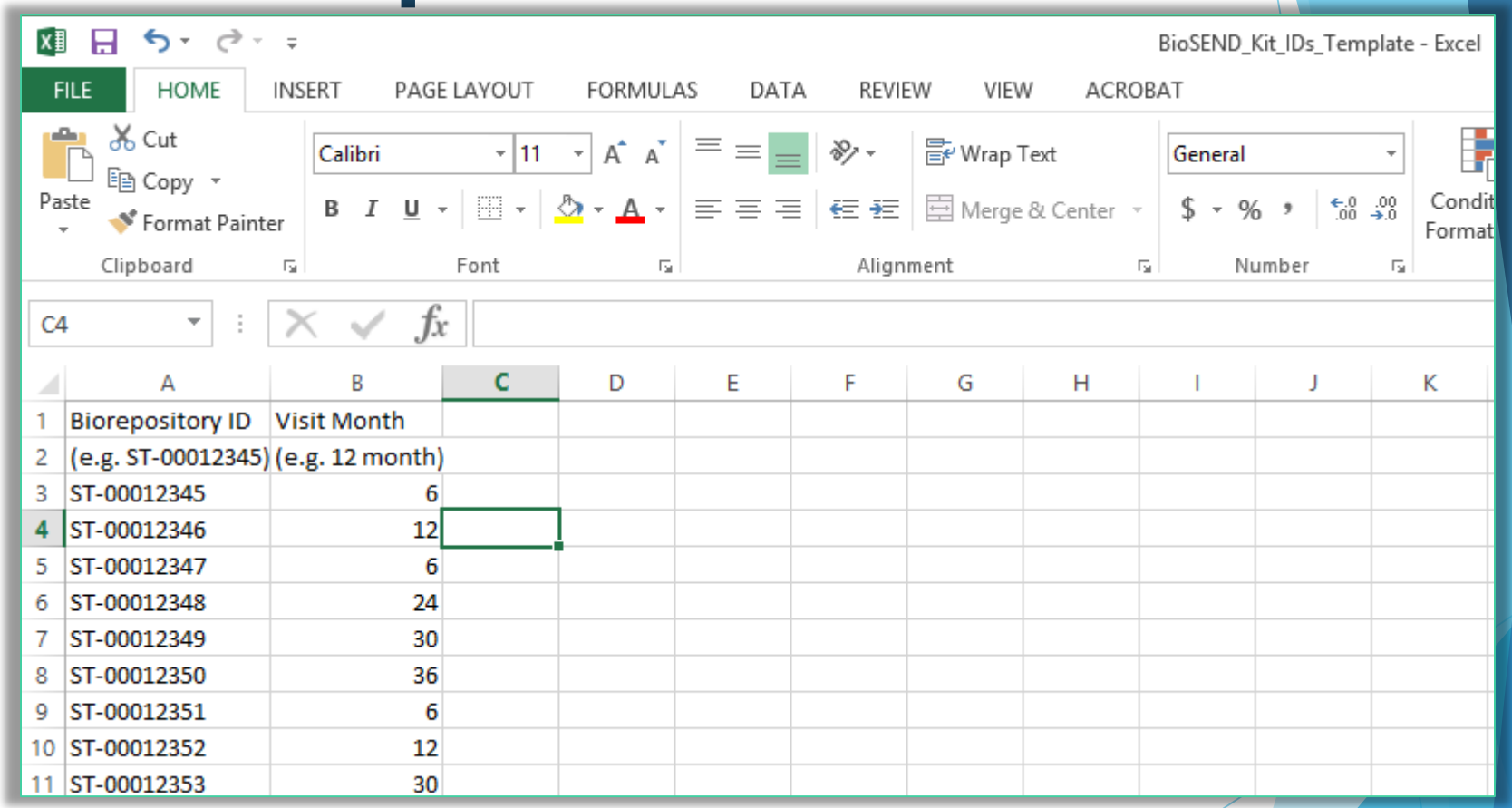

- Up to 10 kits:
  - Enter the PDBP Subject ID - ST Number (Example: ST00012345)
  - Enter the follow up visit month

# BioSEND Kit Request Module: Follow-up Kit




- More than 10 kits – download template

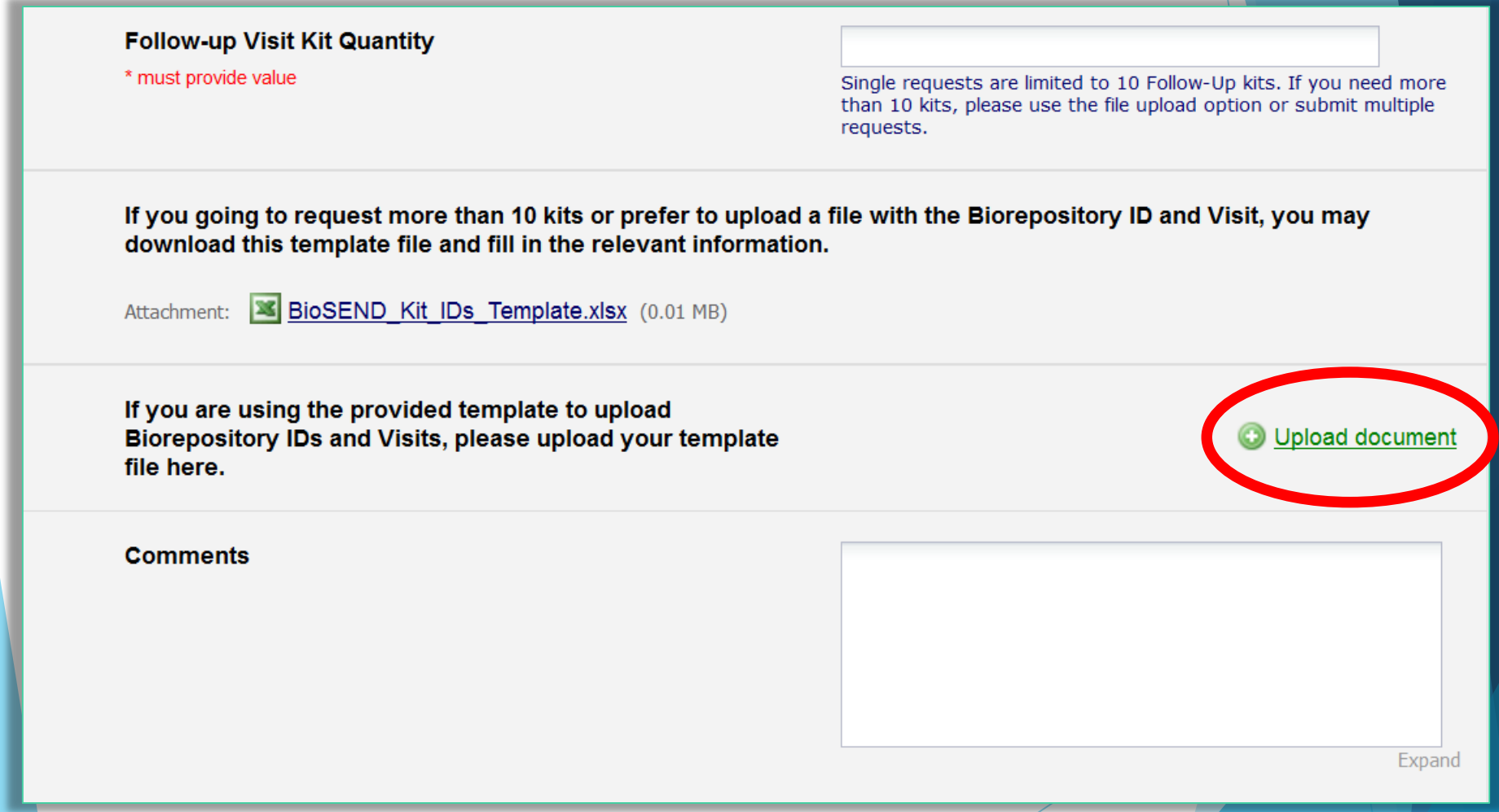
# BioSEND Kit Request Module: Follow-up Kit



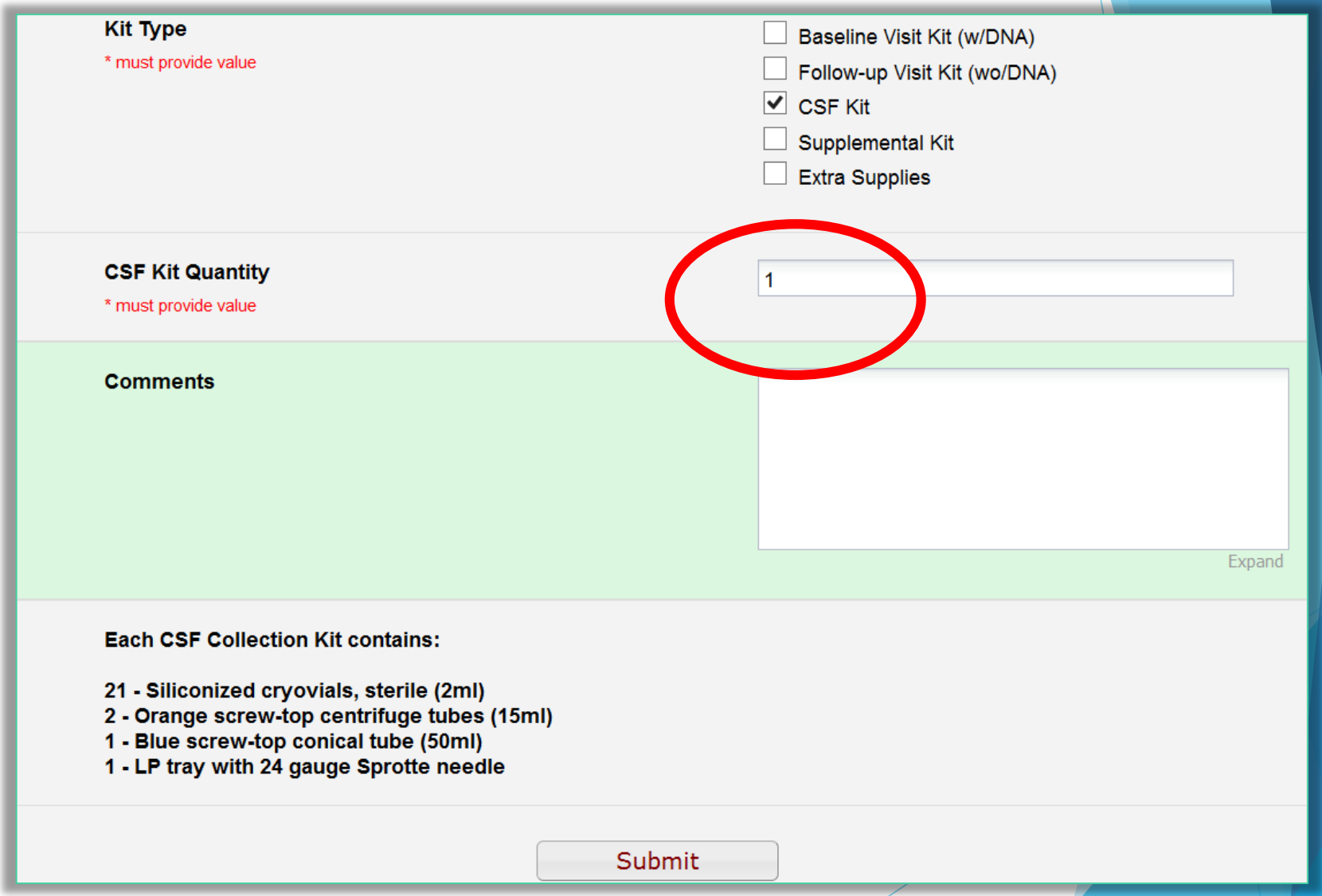

| | A | B | C | D | E | F | G | H | I | J | K |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Biorepository ID | Visit Month | | | | | | | | | |
| 2 | (e.g. ST-00012345) | (e.g. 12 month) | | | | | | | | | |
| 3 | ST-00012345 | 6 | | | | | | | | | |
| 4 | ST-00012346 | 12 | | | | | | | | | |
| 5 | ST-00012347 | 6 | | | | | | | | | |
| 6 | ST-00012348 | 24 | | | | | | | | | |
| 7 | ST-00012349 | 30 | | | | | | | | | |
| 8 | ST-00012350 | 36 | | | | | | | | | |
| 9 | ST-00012351 | 6 | | | | | | | | | |
| 10 | ST-00012352 | 12 | | | | | | | | | |
| 11 | ST-00012353 | 30 | | | | | | | | | |

- Complete template with ST numbers & visit types

# BioSEND Kit Request Module: Follow-up Kit

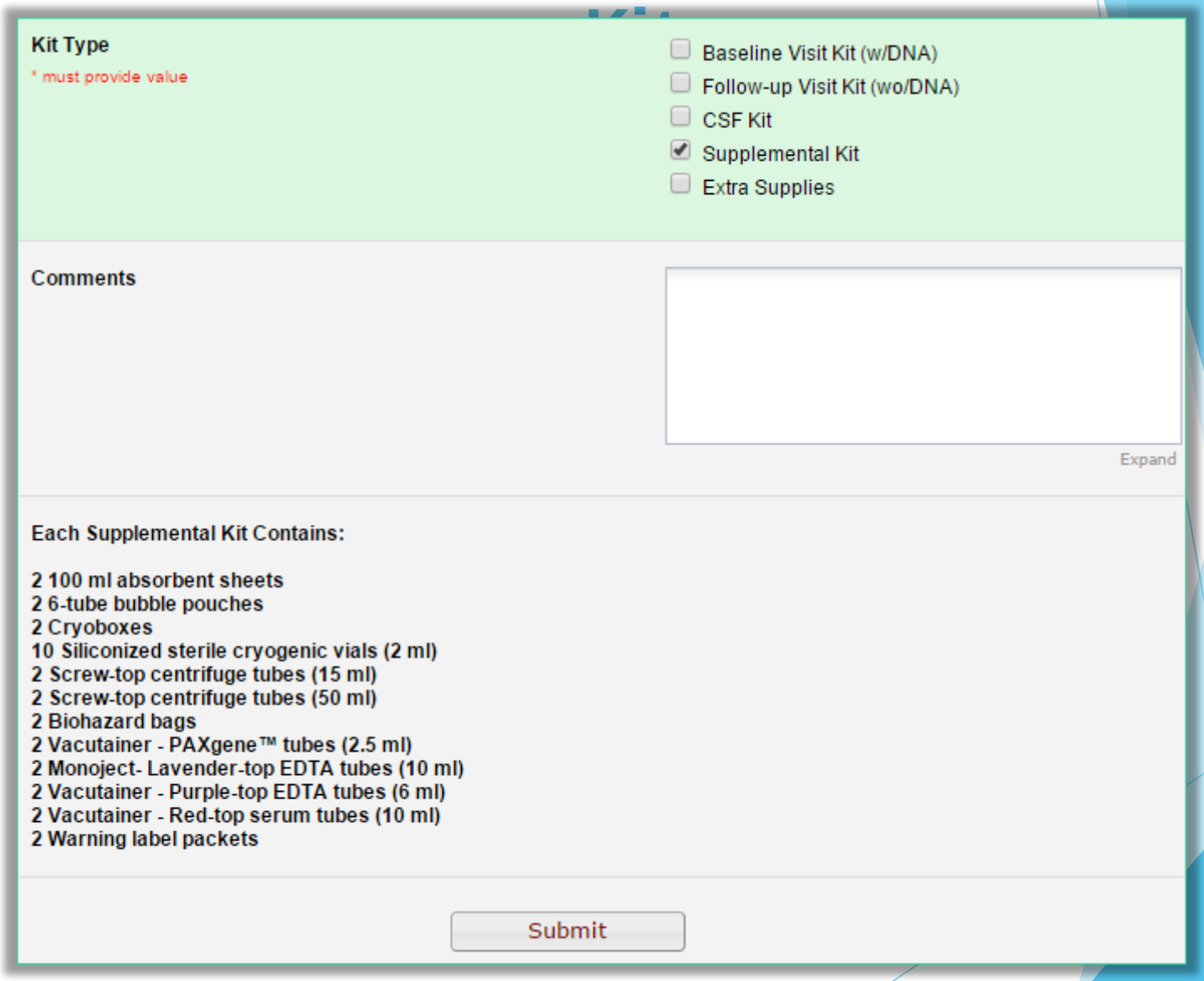


# BioSEND Kit Request Module: CSF Kit



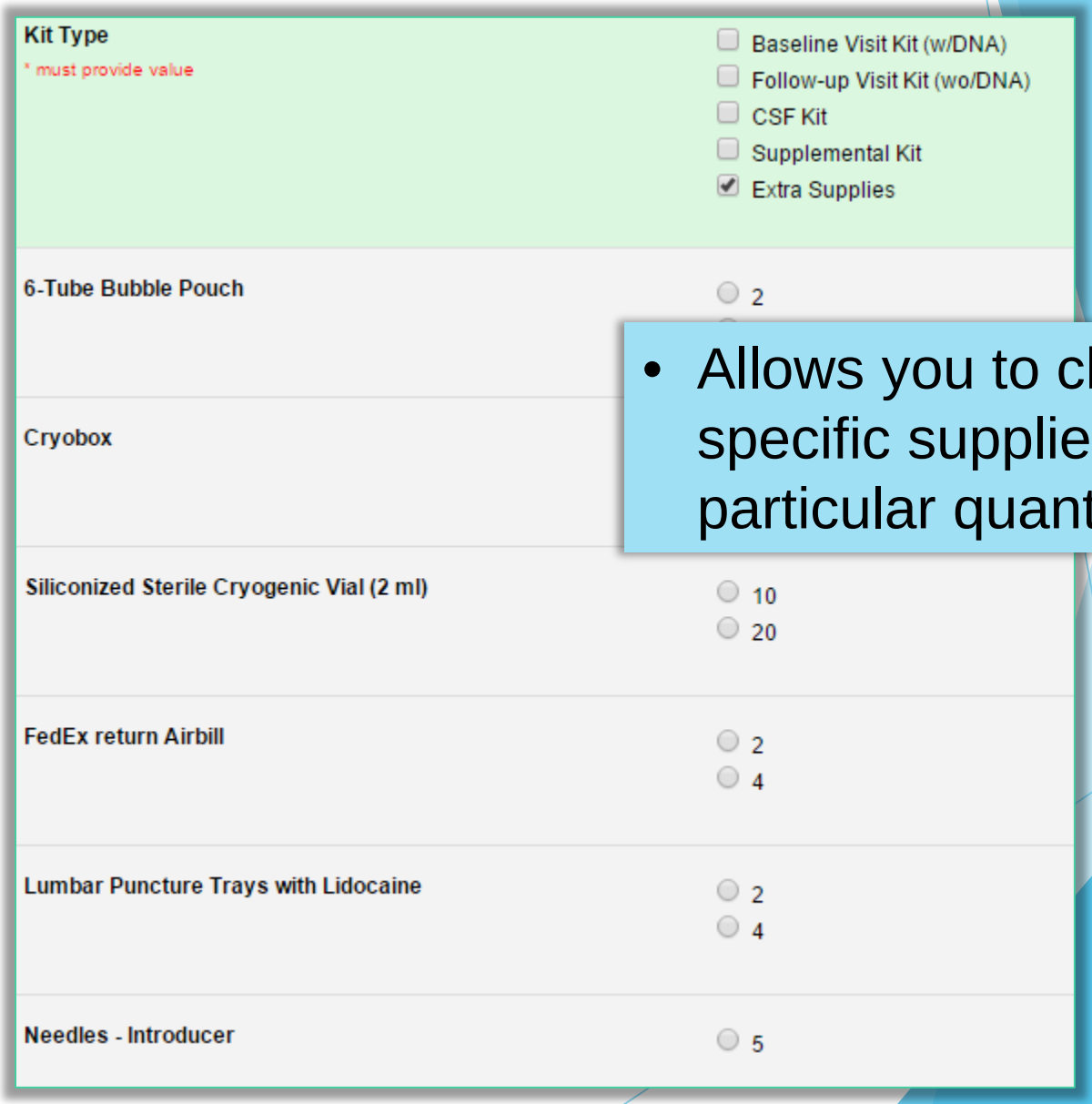

- Enter quantity

# BioSEND Kit Request Module: Supplemental Kit




- Contains a variety of extra kit pieces

# BioSEND Kit Request Module: Extra Supplies

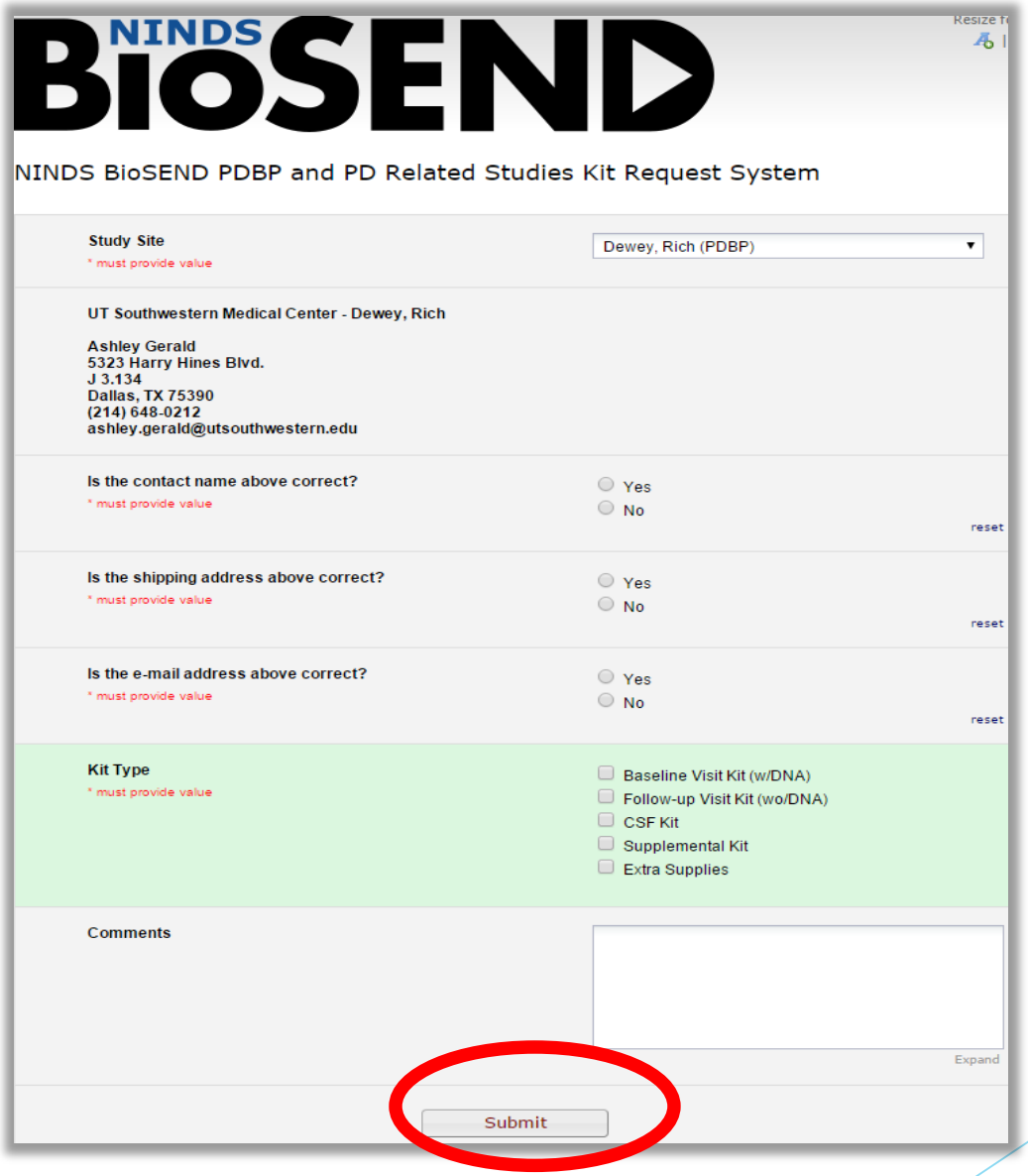



- Allows you to choose specific supplies and particular quantities

# 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


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 lid of frozen shippers (pre-labeled)*




# Collection and Aliquot Tube Label


**Biospecimen Number**

**Study = BioSEND**

**Case Number**

**Subject Number**

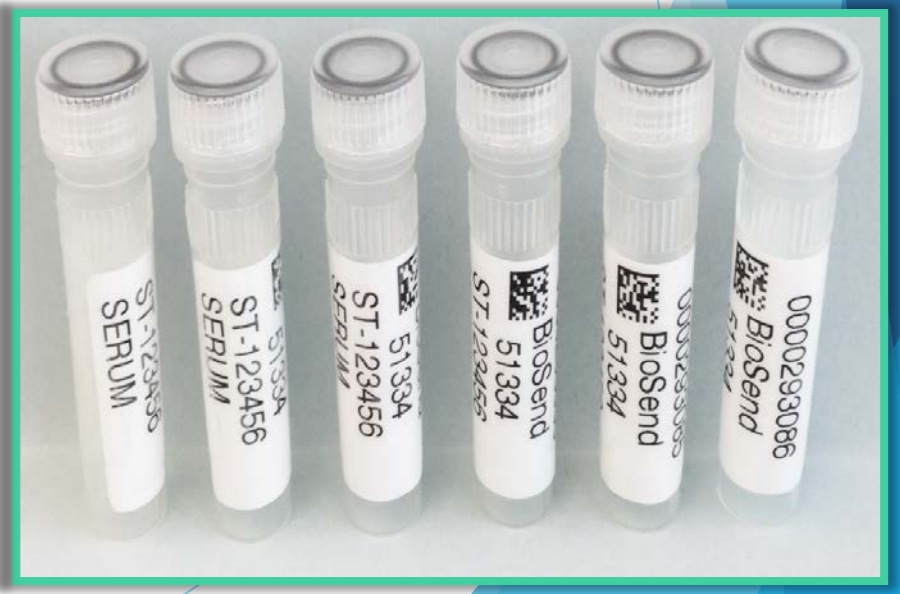
**Specimen Type**

# 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

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 *fasting for 8 hours* or more. If fasting is not feasible, follow suggested low-fat 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:

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

**R being the radius of rotation measured in millimeters*

$$(RPM/1000)^2 = 1500/(1.12 \times 100mm)$$

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

$$RPM/1000 = \sqrt{(1500/1.12 \times 100)}$$

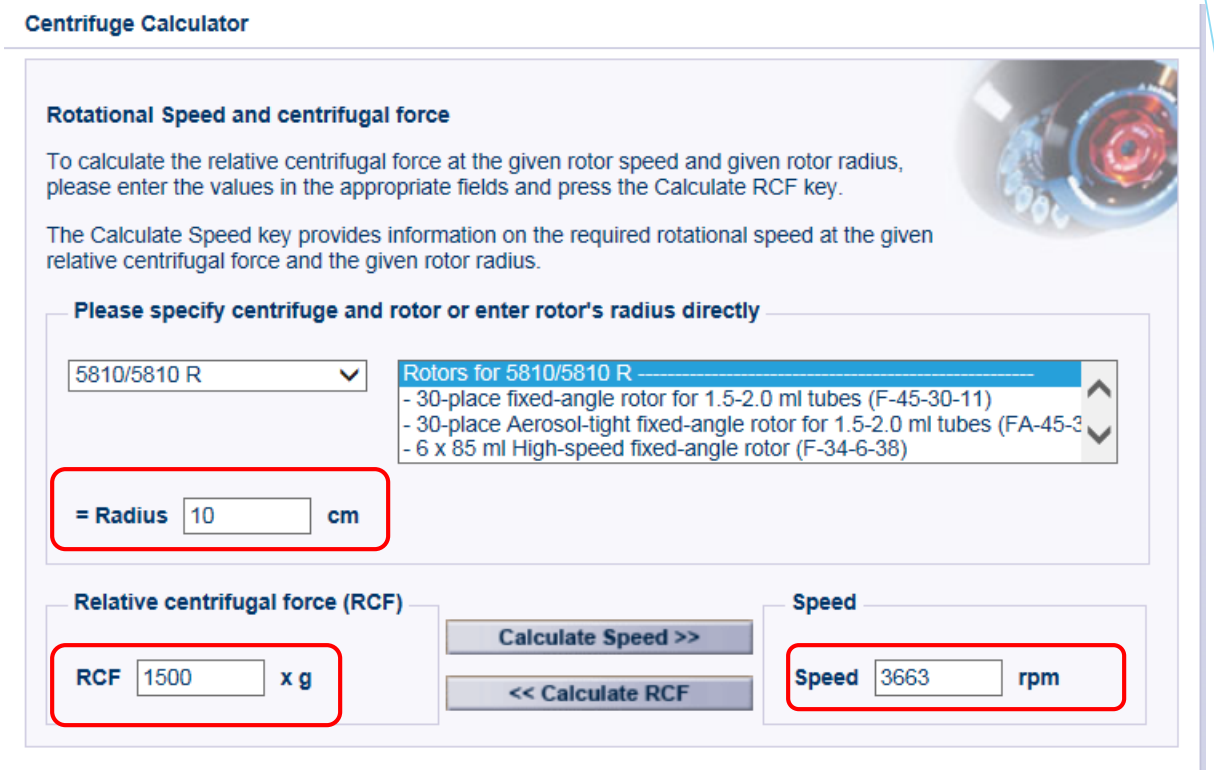
$$RPM = 1000 \times \sqrt{(15/1.12)}$$

$$= 3660$$

***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***

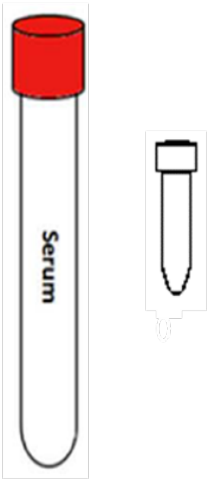
# Order of Specimen Collection

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

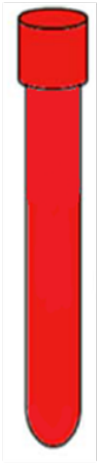
## Serum Preparation (10 ml Red Top Tube)

### Step One




- Store tubes at room temperature.
- Label tubes with pre-printed subject labels prior to blood draw.
- Label cryovial tubes with preprinted labels.
- Pre-chill cryovials on wet ice for 5 minutes or longer.

### Step Two



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

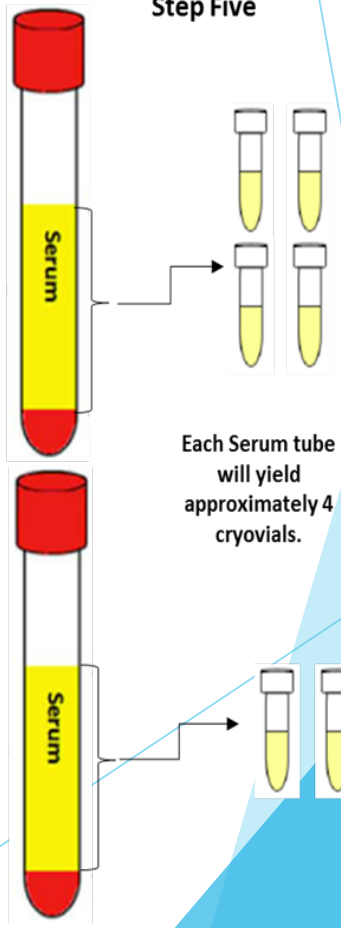
### Step Three

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

### Step Four

- Allow blood to clot for 30 minutes.
- Within 60 minutes of blood draw, centrifuge samples at 1500 x g for 15 minutes at 4°C.

### Step Five



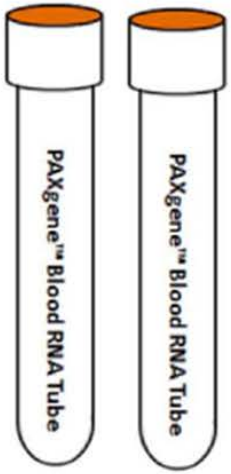

Each Serum tube will yield approximately 4 cryovials.

- Aliquot 1.0 mL into each cryovial tube.
- Store serum aliquots at -80°C until shipment.
- Return 4-6 1.0 mL aliquots to BioSEND.

# PAXgene diagram

## PAXgene™ Preparation

### Step One




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

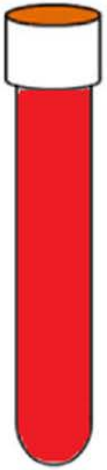
### Step Two

- 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 additional tubes.

### Step Four



- Incubate tubes upright at room temperature for 24 hours before freezing samples.

### Step Five


- After 24 hours incubation at room temperature, store tubes upright in a -80°C in a wire rack until shipment.

# DNA

## DNA Preparation (6 ml Lavender Top Tube)

### Step One

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

### Step Two

- Collect blood in tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

### Step Three

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

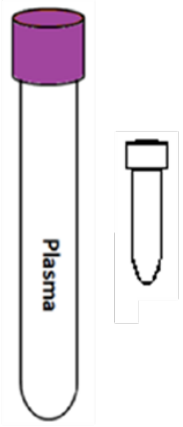
### Step Four

- Hold the specimen at room temperature until shipment BioSend.

# Plasma

## Plasma Preparation (10 ml Lavender Top Tube)

### Step One

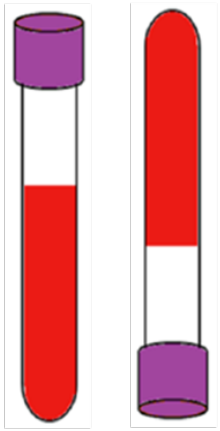



- Store tubes at room temperature.
- Label tubes with pre-printed subject labels prior to blood draw.
- Label cryovial tubes with preprinted labels.
- Pre-chill cryovials on wet ice for 5 minutes or longer.

### Step Two

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

### Step Three

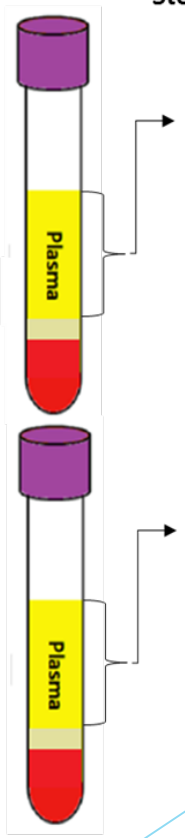


- Immediately after blood draw, invert tubes 8-10 times to mix samples.

### Step Four

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

### Step Five




Each EDTA tube will yield approximately 5 cryovials.

- Aliquot 1.0 ml into each cryovial tube.
- Store plasma aliquots at -80°C until shipment.
- Return 6 1.0 mL aliquots to BioSEND.

# Whole blood

## Whole Blood (6 ml Lavender Top Tube)

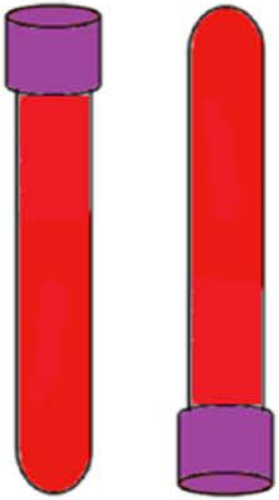
### Step One

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

### Step Two

- Collect blood in tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

### Step Three



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

### Step Four

- Transfer to -80°C freezer. Store upright and keep frozen until shipment BioSend.

# 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

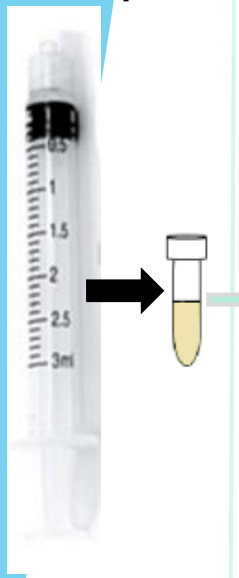
# LP Procedure

- 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

## Step One

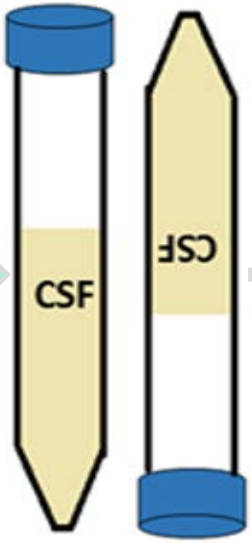


- Collect CSF into the 3 mL luer lock syringe or by gravitational pull.
- Dispense 1-2 mL in a cryovial.
- Send to local lab for testing.

## Step Two

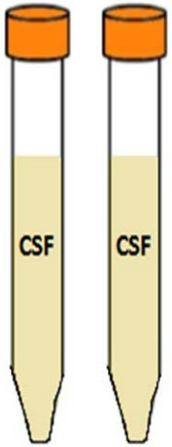
- Collect CSF into 6 mL luer lock syringe or by gravitational pull.
- Collect approved volume into 50 mL conical tube.

## Step Three




- Immediately after collection, invert 50 mL conical tube 3-4 times to mix sample.

## Step Four

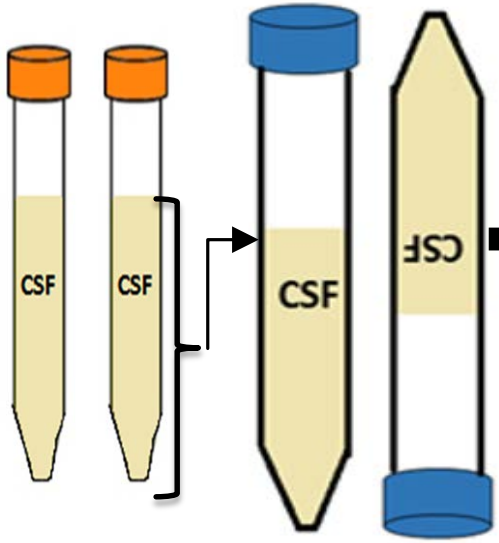



- Transfer CSF into 2 15 ml conical tubes.
- Within 15 minutes of collection, centrifuge samples at room temperature at 2000 x g for 10 minutes.

## Step Five

- Label tubes with pre-printed subject labels prior to collection .

## Step Six

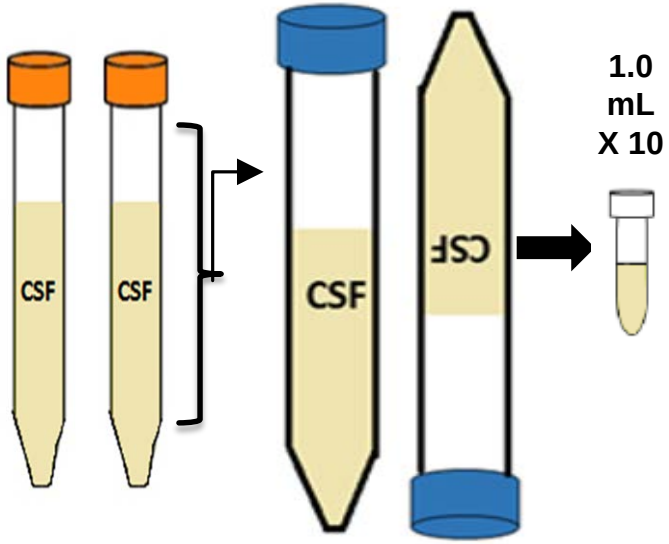



1.0 mL X 10

- 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.

# CSF Preparation Processing

**Step Six**

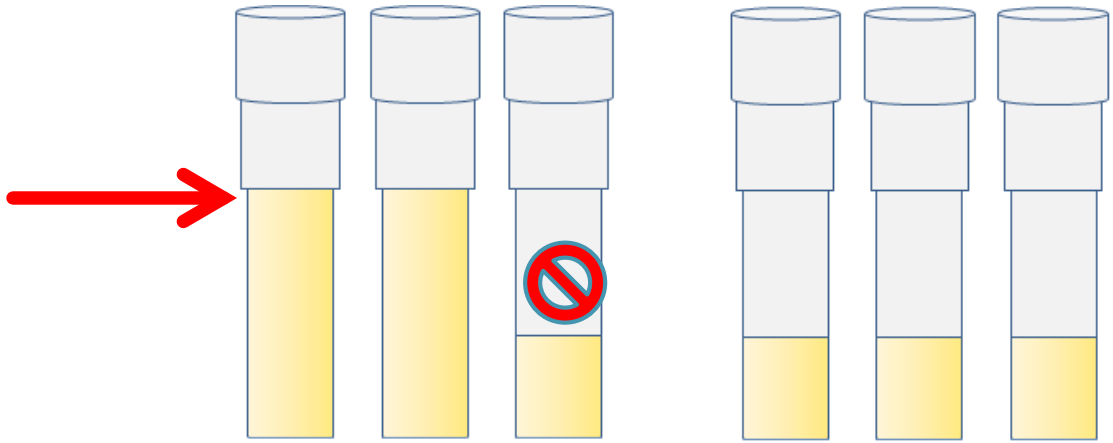



- **Using a clean transfer pipette, transfer CSF from both 15 ml conical tubes into a 50mL conical tube leaving the debris in the bottom.**
- **Mix the 50 ml conical tube gently by inverting 3-4 times.**
- **Aliquot 1.0 ml into 10 cryovials, Aliquot residual mL in last cryovial (for site use).**
- **Store 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: Vacutainer 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 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)

# Ambient Sample Shipment

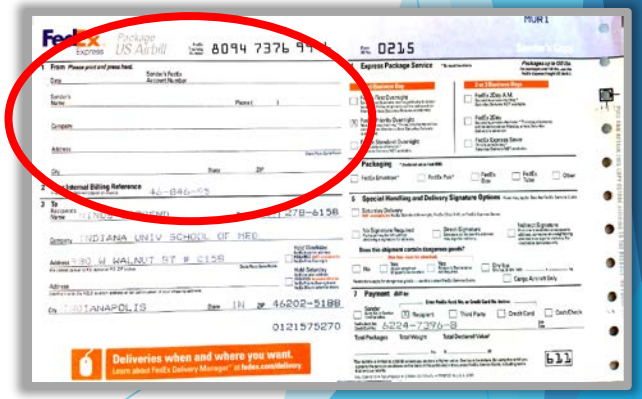
- 6 ml purple (EDTA) whole blood for DNA collection tube
  - Typically collected at baseline visit
- **Monday – Thursday only via FedEx Priority Overnight**
- Schedule FedEx pickup
- Email Sample Record and Shipment Notification Form including the FedEx tracking number **AHEAD OF SHIPMENT to BioSEND**

# 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.

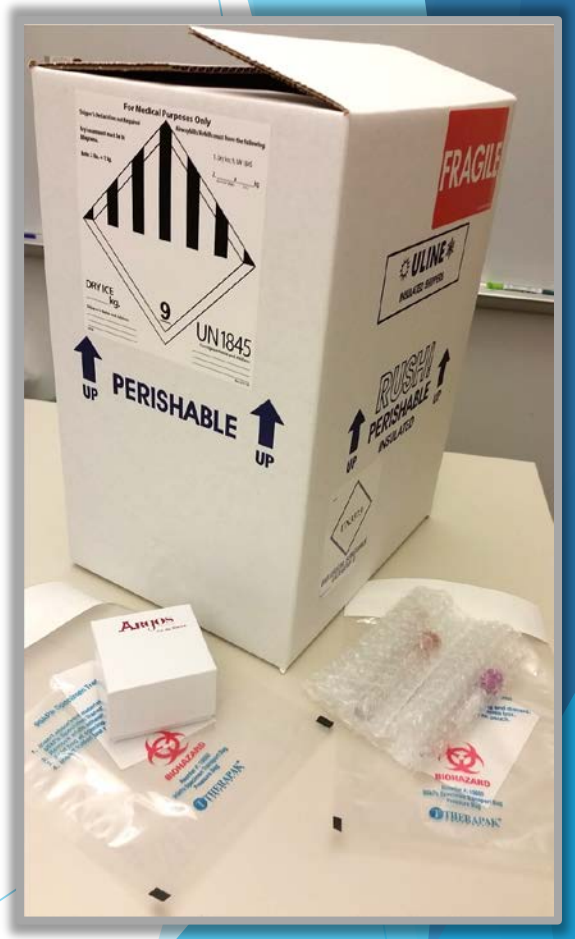



# Shipping Ambient Samples

- 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: _____ 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: _____ Date Sample(s) Shipped: _____

Plus Months: _____ FedEx Tracking Number: _____

Subject Indicator: _____ Subject's Diagnosis: _____

**Instructions: Ship Frozen Shipments Monday- Wednesday ONLY! 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 ice.**

**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 | | | |

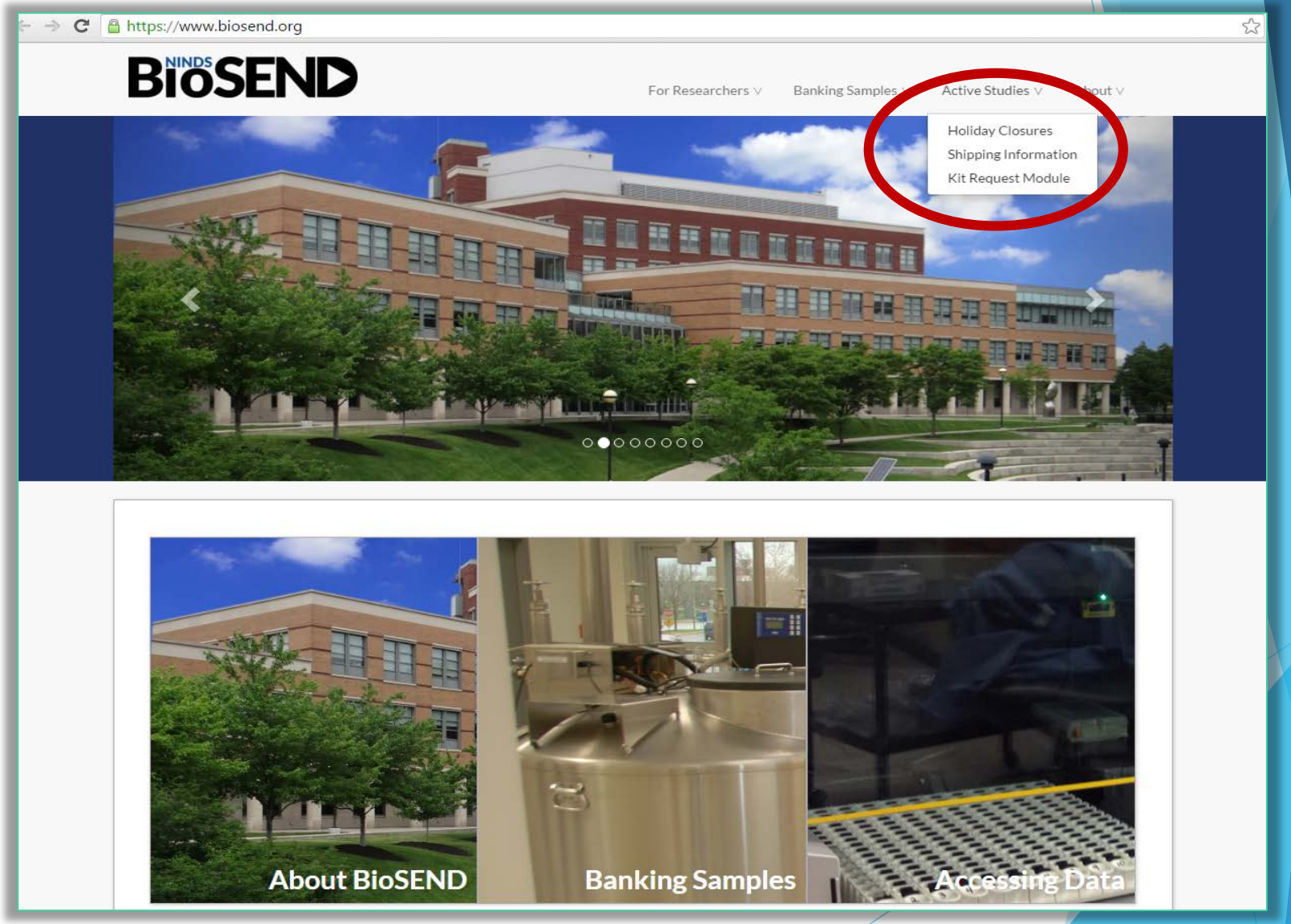
**Contact Information: Indiana University; Email:** biosend@iu.edu **Ph:** 317-278-0495
**Data Management Resource (DMR); Email:** PDBP-OPS@mail.nih.gov

# 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 |
| 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 |

# BioSEND Contact Information

- Questions?

  Please contact: Scott Kaiser or Mallory Wills

  - Phone:  317-278-0495 and 317-274-5740
  - Email: biosend@iu.edu