National Institute of Neurological Disorders and Stroke Biorepository:

BioSpecimen **E**xchange for **N**eurological **D**isorders, **BioSEND**

Biospecimen Collection, Processing, and Shipment Manual for Clinical Research Consortium for the Study of Cerebellar Ataxia (CRC-SCA) Study

Table of Contents

1.0 PURPOSE

The purpose of this manual is to provide collection site staff (PIs, study coordinators, and the sample collection and processing teams) at various study sites with instructions for collection and submission of biological samples. It includes instructions for biospecimen submission to the BioSpecimen Exchange for Neurological Disorders (BioSEND) located at Indiana University.

This manual includes instructions for the collection, processing, aliquoting and shipping of the following samples:

- Plasma
- Buffy Coat
- Serum
- Whole Blood
- CSF

These procedures are relevant to all study personnel responsible for processing blood specimens to be submitted to BioSEND.

2.0 ABBREVIATIONS

BioSEND	BioSpecimen Exchange for Neurological Disorders
EDTA	Ethylene Diamine Tetra-acetic Acid
IATA	International Air Transport Association
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute
SCA	Spinocerebellar Ataxia

3.0 BioSEND Information

3.1 BioSEND Contacts

Tatiana Foroud, PhD, Principal Investigator
Phone: 317-274-2218

Claire Wegel, Project Manager
Phone: 317-278-6158
Email: cwegel@iu.edu

General BioSEND Contact Information
Fax: 317-278-1100
Email: biosend@iu.edu
Website: www.BioSEND.org

Sample Shipment Mailing Address
BioSEND
Indiana University School of Medicine
351 W. 10th Street, TK-217
Indianapolis, IN 46202-4118

3.2 Hours of Operation

Indiana University business hours are from 8 AM to 5 PM Eastern Time, Monday through Friday.

Frozen samples must be shipped Monday- Wednesday only.

For packaging and shipment details, please refer to Appendix K (Frozen Shipping Instructions)

Check the weather reports and the shipping courier website to make sure impending weather events (blizzards, hurricanes, etc.) will not impact the shipping or delivery of the samples. Couriers often reports anticipated weather delays on their website.

3.3 Holiday Schedules

- Please note that courier services may observe a different set of holidays. Please be sure to verify shipping dates with your courier prior to any holiday.
- **Weekend/holiday deliveries will not be accepted.**

3.4 Holiday Observations

Date	Holiday
January 1	New Year's Day
3rd Monday in January	Martin Luther King, Jr Day
4th Monday in May	Memorial Day
June 19th	Juneteenth (observed)
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

Please note that BioSEND has extended closures surrounding the week of Thanksgiving and the last two weeks of the year. Please contact biosend@iu.edu for details of closures.

Please see https://www.biosend.org/holiday_closures.html for additional information.

4.0 BioSEND Sample Requirements

NINDS approves each study for a specific biospecimen collection protocol. Studies and study sites should make every effort to meet their approved biospecimen collection requirements. The expected number of samples from each site that should be returned to BioSEND are listed in sections 4.1-4.2.

If a sample is not obtained at a particular visit, this should be recorded in the notes section of the **Sample Record and Shipment Notification Form (see Appendix I).** This form is submitted with your sample shipment to BioSEND.

4.1 Protocol Schedule for Biospecimen Submission to BioSEND – CRC-SCA

Visit	All Visits
Serum aliquots, 1ml	6
Plasma aliquots, 1ml	6
Buffy Coat	2
Whole Blood, 3ml	2
CSF aliquots, 1 ml	10

5.0 SPECIMEN COLLECTION KITS, SHIPPING KITS AND SUPPLIES

BioSEND will provide a sufficient number of labels and supplies only for those specimens that are to be shipped back to the BioSEND repository (See the Protocol Schedule for Biospecimen Submission to BioSEND for your site in Sections 4.1-4.2); any tubes that will remain at the collection site or shipped to other repositories should be labeled accordingly. Ensure that all tubes are properly labeled during processing and at the time of shipment according to Section 6.2.

5.1 Kit Supply to Study Sites

Each individual site will be responsible for ordering study kits. We advise sites to proactively confirm kits are on hand ahead of study visits.

Within the kit request module, there is a drop down menu to request kits based on the Principal Investigator at that site. Kits and individual items can be ordered as required through the kit request module.

The link to the kit request module is shown below:

- CRC-SCA: http://kits.iu.edu/biosend/crc-sca

Please allow **TWO weeks** for kit orders to be processed and delivered.

5.2 Specimen Collection Kit General Contents

Collection kits contain the following (for each subject) as designated per your protocol and/or NINDS resource development agreement. Kits provide the necessary supplies to collect samples from a given subject. Do not replace or supplement any of the tubes or kit components provided with your own supplies unless you have received approval from the NINDS/BioSEND Study team to do so. *Please store all kits at room temperature until use.*

BioSEND Supplies

Available upon request from the online kit request module (Section 5.1)

General Items
25 cell cryobox
Cryovial tube (2 ml) with clear cap
Airway bill envelope
Shipping container for dry ice shipment (shipping and Styrofoam® box)
Plastic biohazard bag
Warning label packet
CSF Items
Needle - Spinal Needle Introducer 20G, 0.90 x 32mm
Needle - Whitacre Needle 24G, 0.55 x 90mm
2 Individually Packaged Sterile 50 ml Conical Tube
Conical centrifuge tubes (15 ml)
Lumbar puncture tray (Sprotte® 24G or 22G) (see Lumbar Puncture Tray Components)
Blood Collection Items
Lavender-top EDTA blood collection tube (10 ml), glass
Red-top Serum blood collection tube (10ml), glass
Purple-top EDTA blood collection tube (3 ml), palstic

Quantity	Lumbar Puncture Tray Components
1	Sprotte® needle, 24G x 90mm **OR** Sprotte® needle, 22G x 90mm
1	Introducer needle, 1 mm x 30 mm
1	Hypodermic needle, 22G x 1.5”
1	Plastic syringe, (3 ml, luer lock) with 25G x 5/8” needle attached
4	Polypropylene syringe (6 ml, luer lock)
1	Needle stick pad
1	Adhesive bandage
1	Drape, fenestrated, 2 tabs, paper, 18” x 26”
2	Towel, 13.5” x 18”
6	Gauze pad, 2” x 2”
3	Sponge stick applicator
1	Lidocaine 1%, 5 ml
1	Povidone-Iodine Topical Solution, 0.75 oz

5.3 Specimen Collection Kit Contents – CRC-SCA

Quantity	V01 & Follow-Up Visit Kit
2	Serum red-top tube, 10ml (glass)
2	EDTA lavender-top tube, 10ml (glass)
1	EDTA purple-top tube, 3ml (plastic)
15	Siliconized cryovial, 2ml
2	Disposable Pipette, 3ml
1	Biohazard bag w/ absorbent sheet
1	Cryobox, 25-slot
1	Dry ice shipper
1	Case & specimen label set
1	Airway bill envelope
1	Shipping labels (includes dry ice label)

Quantity	CSF Collection Kit
1	LP tray, 24g or 22g (see contents above)
2	Conical tube, 15ml
2	Conical tube, 50ml
1	Disposable Pipette, 3ml
11	Siliconized cryovial, 2ml

Quantity	Supplemental Kit
2	Serum red-top tube, 10ml (glass)
2	EDTA lavender-top tube, 10ml (glass)
2	EDTA purple-top tube, 3ml (plastic)
20	Siliconized cryovial, 2ml
2	Disposable Pipette, 3ml
2	Biohazard bag w/ absorbent sheet
2	Cryobox, 25-slot
2	Conical tube, 15ml
2	Conical tube, 50ml
2	Airway bill envelope
2	Shipping labels (includes dry ice label)

5.4 Site Required Equipment

The following materials and equipment are necessary for the processing of specimens at the collection site and are to be **supplied by the local site**:

- Personal Protective Equipment: lab coat, nitrile/latex gloves, safety glasses
- Tourniquets
- Alcohol Prep Pads
- Gauze Pads
- Bandages
- Butterfly needles and hubs
- Microcentrifuge tube rack
- Test tube rack
- Sharps bin and lid

In order to process samples, project sites must have access to the following equipment:

- Centrifuge capable of ≥ 1500 rcf (1500 x g) with refrigeration to 4°C
- -80°C Freezer

In order to ship specimens, you must provide:

- Dry ice (minimum 10 pounds per shipment)

6.0 SPECIMEN LABELS

Labels must be affixed on all collection and aliquot tubes to ensure unique specimen identity. BioSEND provides labels for all samples being collected and returned to BioSEND. The site is responsible for providing labels for biospecimens that will be retained at the site. If labels are provided but the sample is not collected, please discard the unused labels.

6.1 Types of Labels

Each kit contains all labels required for the return of biospecimens to BioSEND.

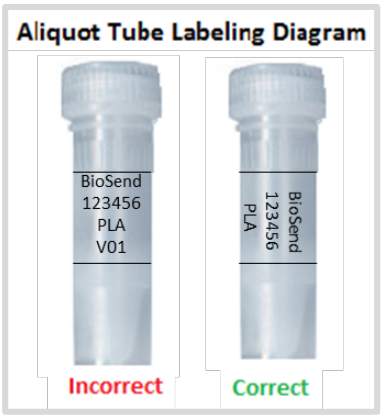
The **Kit Labels** do not indicate a specimen type, but are affixed on BioSEND forms and on specific packing materials. See Appendices K for further instructions.

The **Specimen Labels** are placed on all blood collection and aliquot tubes.

6.2 Affixing Labels

In order to ensure the label adheres properly and remains on the tube, follow these instructions:

- Place blood collection and aliquot labels on **ALL** collection and aliquot tubes **BEFORE** sample collection, sample processing, or freezing. This will help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.
- The blood collection and aliquot tube labels contain a 2D barcode on the left hand side of the label. When turned horizontally, the barcode should be closer to the top (cap end) of the tube.
- Place label **horizontally** on the tube (wrapped around sideways if the tube is upright) and **just below the ridges** of the aliquot tubes (see attached labeling diagram).



- Take a moment to ensure the label is **completely affixed** to each tube. It may be helpful to roll the tube between your fingers after applying the label.

7.0 SPECIMEN COLLECTION AND PROCESSING PROCEDURES

Consistency in sample collection and processing is essential for biomarker studies. All samples are drawn in the same order and then processed in a uniform fashion. **Please read the instructions before collecting any specimens. Have all your supplies and equipment out and prepared prior to drawing blood.**

7.1 Order of Specimen Collection

Blood collection should be performed in the following order:

1. Serum (red top) blood collection for serum
2. EDTA (lavender top) blood collection for plasma and buffy coat
3. EDTA (lavender top) blood collection for Whole Blood

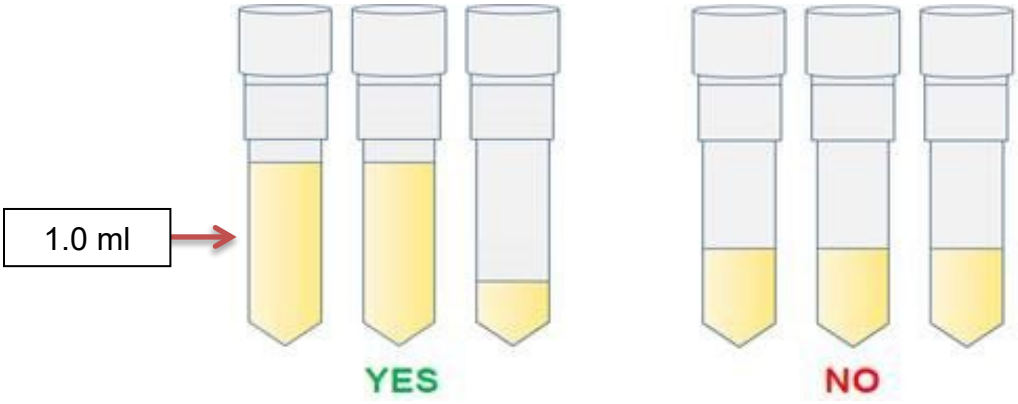
7.2 Blood Collection Protocols

1. Serum (red top) blood collection for serum **(Appendix F)**
2. EDTA (lavender top) blood collection for plasma **(Appendix B)**
3. EDTA (lavender top) blood collection for Buffy Coat **(Appendix C)**
4. EDTA (lavender top) blood collection for Whole Blood **(Appendix D)**

7.4 Filling Aliquot Tubes

In order to ensure that BioSEND receives a sufficient amount of sample for processing and storage, and to avoid cracking of the tubes prior to shipment, each aliquot tube should be filled to the assigned volume (refer to detailed processing instructions for average yield per sample). Over-filled tubes may burst once placed in the freezer, resulting in a loss of that sample.

Please generate as many standard-sized aliquots as possible, with any remaining volume used to create a single residual aliquot.



8.0 Packaging and Shipping Instructions

ALL study personnel responsible for shipping should be certified in biospecimen shipping. If not available at your University, training and certification is available through the CITI training site (Course titled "Shipping and Transport of Regulated Biological Materials" at https://www.citiprogram.org/).

8.1 Sample Record and Shipment Notification Form

The Specimen Collection and Processing Form should be completed for all samples submitted to BioSEND. Please see Appendix I for further instructions.

8.2 Shipping Instructions

All samples for CRC-SCA are shipped frozen. Reference Appendix K for frozen shipping instructions.

8.3 Shipping Address

All samples are shipped to the BioSEND laboratory:

BioSEND
Indiana University School of Medicine
351 W. 10th Street, TK-217
Indianapolis, IN 46202-4118

9.0 Data Queries and Reconciliation

Appendix I must be completed the day that samples are collected to capture information related to sample collection and processing. This form includes information that will be used to reconcile sample collection and receipt, as well as information essential to future analyses.

The USF data collection team will be collaborating with BioSEND to reconcile information captured in the database compared to samples received and logged at BioSEND. Information that appears incorrect in the USF database will be queried through the standard system. Additional discrepancies that may be unrelated to data entry will be resolved with the Principal Investigator in a separate follow up communication. If applicable, a non-conformance report will be provided to sites.

Data discrepancies with samples shipped and received at BioSEND may result from:

- Missing samples
- Incorrect samples collected and shipped
- Damaged or incorrectly prepared samples
- Unlabeled or mislabeled samples
- Discrepant information documented on the BioSEND Sample Forms to information entered into the USF database.
- Samples frozen and stored longer than three months at the site

10.0 APPENDICES

Appendix B: Whole Blood Collection for Isolation of Plasma

Appendix C: Whole Blood Collection for Isolation of Buffy Coat

Appendix D: Whole Blood Collection for Banking

Appendix F: Whole Blood Collection for Isolation of Serum

Appendix G: Cerebrospinal Fluid Collection

Appendix I: Sample Record and Shipment Notification Form

Appendix K: Frozen Shipping Instructions

Appendix O: Low Fat Diet Menu Suggestions

Appendix Q: UPS ShipExec™ Thin Client Instructions

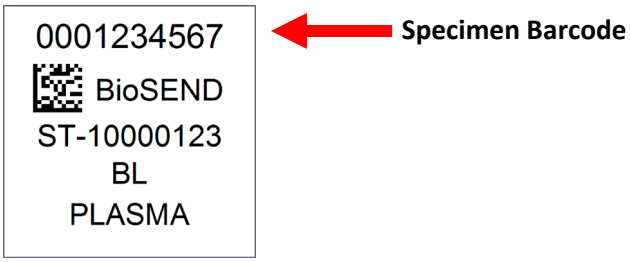
Appendix B – Whole Blood Collection for Isolation of Plasma

Whole Blood Collection for Isolation of Plasma: 10 ml Purple-Top EDTA (glass) tube(s) and cryovials are provided by BioSEND for the collection of plasma.

1. **CRITICAL STEP: Store empty Purple-Top EDTA tubes at room temperature 64°F – 77°F (18°C to 25°C) prior to use.**

2. Place pre-printed collection and aliquot "**PLASMA**" label on 10 ml purple-top EDTA tube(s) and on six of the 2 ml cryovial tubes. The six labeled cryovials will be shipped to BioSEND. Any remaining cryovials can be retained by the site and labeled per site standards. Labels for aliquots kept by the site are not provided by BioSEND.

3. Please ensure that aliquots for BioSEND are kept in numerical order (by specimen barcode) throughout the aliquoting and shipping process.



4. Pre-chill the labeled cryovials on wet ice for at least 5 minutes.

5. Set centrifuge to 4°C to pre-chill before use. Time needed to pre-chill the centrifuge to 4°C will depend on your centrifuge model.

6. Using a blood collection set and a holder, collect blood into the **purple top 10 ml EDTA (glass) tube(s)** using your institution's recommended procedure for standard venipuncture technique.

The following techniques shall be used to prevent possible backflow:

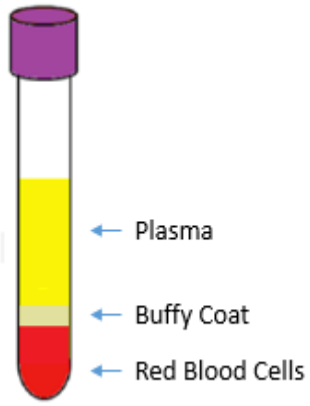
a. Place donor's arm in a downward position.
b. Hold tube in a vertical position, below the donor's arm during blood collection.
c. Release tourniquet as soon as blood starts to flow into the tube.
d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

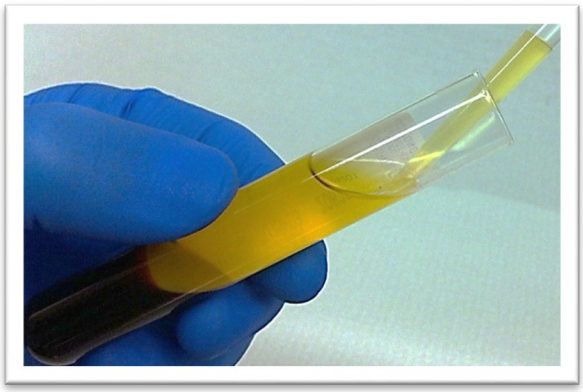
7. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube vacuum is designed to draw 10 ml of blood into the tube.

8. **CRITICAL STEP: Immediately after blood collection, gently invert/mix (180 degree turns) the Lavender-Top EDTA tube(s) 8 – 10 times. Do not shake the tubes!**

9. Within 30 minutes of blood collection, centrifuge balanced tubes for 15 minutes at 1500 RCF (x g) at 4°C. **It is critical that the tubes be centrifuged at the appropriate speed and temperature to ensure proper plasma separation (see worksheet in Appendix H to calculate RPM in your particular rotor).**

10. Remove the plasma by tilting the tube and placing the pipette tip along the lower side of the wall. **Use caution not to touch the buffy coat or packed red blood cells at the bottom of the tube so that the plasma is not contaminated** (see below). Using a disposable tipped micropipette, transfer plasma into the pre-labeled cryovials. Aliquot 1.0 ml per cryovial. Send 6 1.0 ml aliquots to BioSEND. If you cannot obtain the requested number of aliquots, please note "low volume draw" on the Sample Record and Shipment Notification form (Appendix I) under "Notification of Problems". Each 10 ml EDTA tube should yield, on average, 4 ml of plasma.





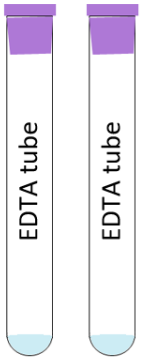
11. Complete the **Sample Record and Shipment Notification form (Appendix I).**

12. Place the labeled cryovials in the 25 slot cryobox. Place the cryobox UPRIGHT on dry ice. Transfer to **-80°C freezer as soon as possible, within 2 hours of blood draw**. Store all samples at **-80°C until shipped** to BioSEND on dry ice.

13. Ship the frozen plasma aliquots to BioSEND according to **Appendix K – Frozen Shipping Instructions.**

Plasma Preparation –10 ml EDTA (Purple Top) Tube

Step One



- Store tubes at room temperature.
- Label tubes with preprinted plasma labels prior to blood draw.

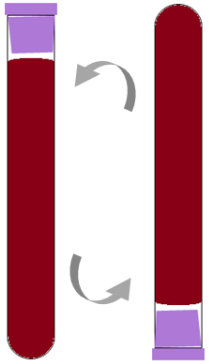
Step Two

- Label 6 cryotubes with plasma aliquot labels.
- Pre-chill cryotubes on wet ice for at least 5 minutes

Step Three

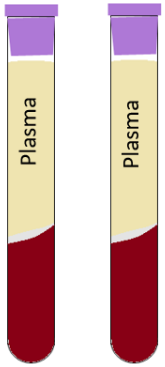
- Collect blood in EDTA tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Four



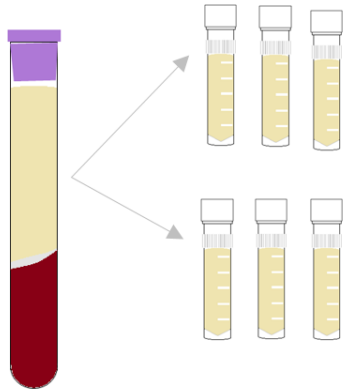
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Five



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

Step Six



- Using a clean transfer pipet, aliquot 1.0 ml plasma into each cryotube.
- Store plasma aliquots upright at -80°C until shipment.

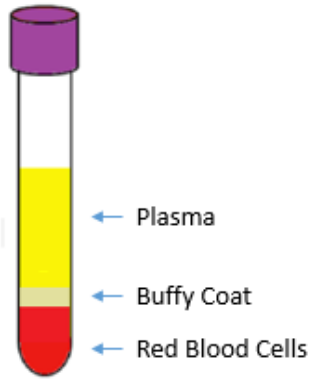
Appendix C –Whole Blood Collection for Isolation of Buffy Coat

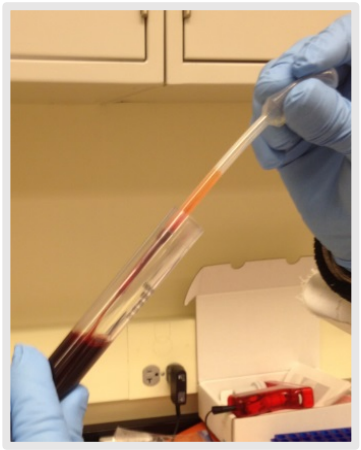
Whole Blood Collection for Isolation of Buffy Coat: 10 ml Lavender-Top EDTA tube(s) and cryovials are provided by BioSEND for the collection of the buffy coat.

1. **CRITICAL STEP: Store Lavender-Top EDTA tubes at room temperature 64^{0}F - 77^{0}F (18°C to 25°C) before use.**

2. Place pre-printed Collection and Aliquot "**Buffy Coat**" label onto cryovials with clear cap.

3. After plasma has been removed from the EDTA lavender-top tube (see Appendix B), aliquot buffy coat layer (see figure below) into labeled cryovial with clear cap using a disposable graduated micropipette. All of the buffy coat from a single 10 ml lavender-top EDTA tube will be placed into one cryovial. The buffy coat aliquot is expected to have a reddish color from the red blood cells.





4. Complete the **Sample Record and Shipment Notification form (Appendix I).**

5. Place the labeled cryovial in the 25-slot cryovial box with the plasma cryovials and place on dry ice. Transfer to a **-80°C Freezer when possible**. Store all samples UPRIGHT at **-80°C until shipped** to BioSEND on dry ice.

6. Ship the frozen buffy coat aliquot to BioSEND according to **Appendix K – Frozen Shipping Instructions.**

Buffy Coat Preparation –10 ml EDTA (Purple Top) Tube

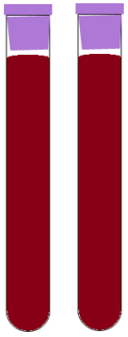
Step One

- Store tubes at room temperature.
- Label tubes with preprinted plasma labels prior to blood draw.

Step Two

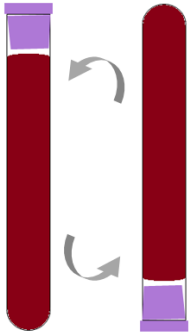
- Label 2 cryotubes with buffy coat aliquot labels.
- Pre-chill cryotubes on wet ice for at least 5 minutes.

Step Three



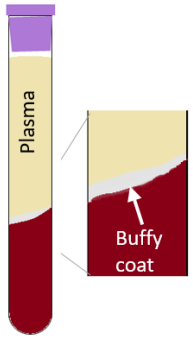
- Collect blood in EDTA tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Four



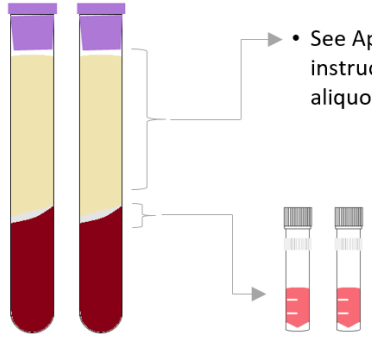
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Five



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

Step Six

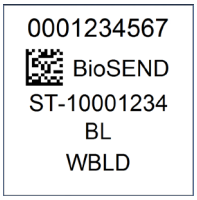


- See Appendix B for instruction on aliquoting plasma

- Using a clean transfer pipet, collect the buffy coat (may contain residual plasma and RBCs).
- Transfer the buffy coat from one EDTA into a cryotube. Transfer the buffy coat from the second EDTA into a second cryotube.
- Store buffy coat aliquots upright at -80°C until shipment.

Appendix D – Whole Blood Collection (No Processing)

Two 3ml Purple-Top EDTA Tube are provided by BioSEND for Whole Blood collection (to be shipped to BioSEND FROZEN; no processing required).



1. Store empty Whole Blood EDTA tubes at room temperature, 64°F - 77°F (18°C to 25°C) before use.
2. Place pre-printed specimen label (WBLD) on the **two 3ml purple top EDTA tube** prior to blood draw.
3. Using a blood collection set and a holder, collect whole blood into the tubes using your institution's recommended procedure for standard venipuncture technique.

   The following techniques shall be used to prevent possible backflow:

   a. Place donor's arm in a downward position.
   b. Hold tube in a vertical position, below the donor's arm during blood collection.
   c. Release tourniquet as soon as blood starts to flow into tube.
   d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

4. **Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tubes 8-10 times. Do not shake the tube!**
5. Complete the Sample Record and Shipment Notification form (Appendix I).
6. Place the Purple-Top EDTAs in a **WIRE** or **PLASTIC** rack. Do **NOT** use a Styrofoam rack. This will cause the Purple-Top EDTA tube to crack when frozen. Place the Purple-Top EDTA tubes immediately to a **-80°C Freezer.**
7. Ship the whole blood tube to BioSEND according to **Appendix K - Frozen Shipping Instructions.**

WBLD Preparation – 2 x 3 ml K2 EDTA (Purple Top) Tube

Step One

- Store tubes at room temperature.
- Label tubes with preprinted WBLD label prior to blood draw.

Step Two

- Collect blood into both 3ml EDTA tubes, 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

- Transfer to -80◦C. Store upright and keep frozen until shipment to BioSEND.

Appendix F – Whole Blood Collection for Isolation of Serum

Whole Blood Collection for Isolation of Serum: 10 ml red-top serum (glass) tubes and cryovials are provided by BioSEND for the collection of serum.

1. **CRITICAL STEP: Store empty serum determination (red-top) tubes at room temperature 64°F – 77°F (18°C to 25°C) prior to use.**

2. Place pre-printed specimen labels noted as "**SERUM**" on the serum determination red-top tubes and on six of the 2 ml cryovials prior to blood draw. Six cryovials will be shipped to BioSEND; the remaining cryovials will be retained by the site and labeled accordingly.

3. Pre-chill labeled cryovials on wet ice for at least 5 minutes or longer.

4. Set centrifuge to 4°C to pre-chill before use. Time needed to pre-chill the centrifuge to 4°C will depend on your centrifuge model.

5. Using a blood collection set and a holder, collect blood into the **10 ml red-top serum (glass) tubes** using your institution's recommended procedure for standard venipuncture technique.

   The following techniques shall be used to prevent possible backflow:
   a. Place donor's arm in a downward position
   b. Hold tube in a vertical position, below the donor's arm during blood collection
   c. Release tourniquet as soon as blood starts to flow into tube.
   d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

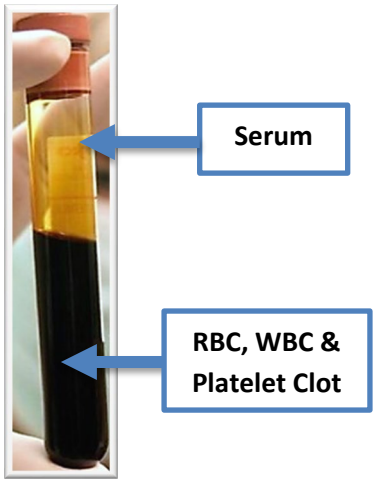
6. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.

7. **CRITICAL STEP: Immediately after blood collection, gently invert/mix (180 degree turns) the serum determination tube 8-10 times. Do not shake the tubes!**

8. **CRITICAL STEP: Allow blood to clot at room temperature for at least 30 minutes.**

   - Within 30 to 60 minutes from blood collection, centrifuge balanced tubes for 15 minutes at 1500 RCF (x g) at 4°C. **It is critical that the tubes be centrifuged at the appropriate speed and temperature to ensure proper serum separation.**

9. Remove the serum by tilting the tube and placing the pipette tip along the lower side of the tube wall. **Use caution to pipet only the serum layer and not the red blood cell layer.** Using a disposable tipped micropipette, transfer serum into the pre-labeled cryovials. Aliquot 1.0 ml per cryovial. Send 6 1.0 ml aliquots to BioSEND. Each 10 ml Serum tube should yield, on average, 4.5 ml of serum.

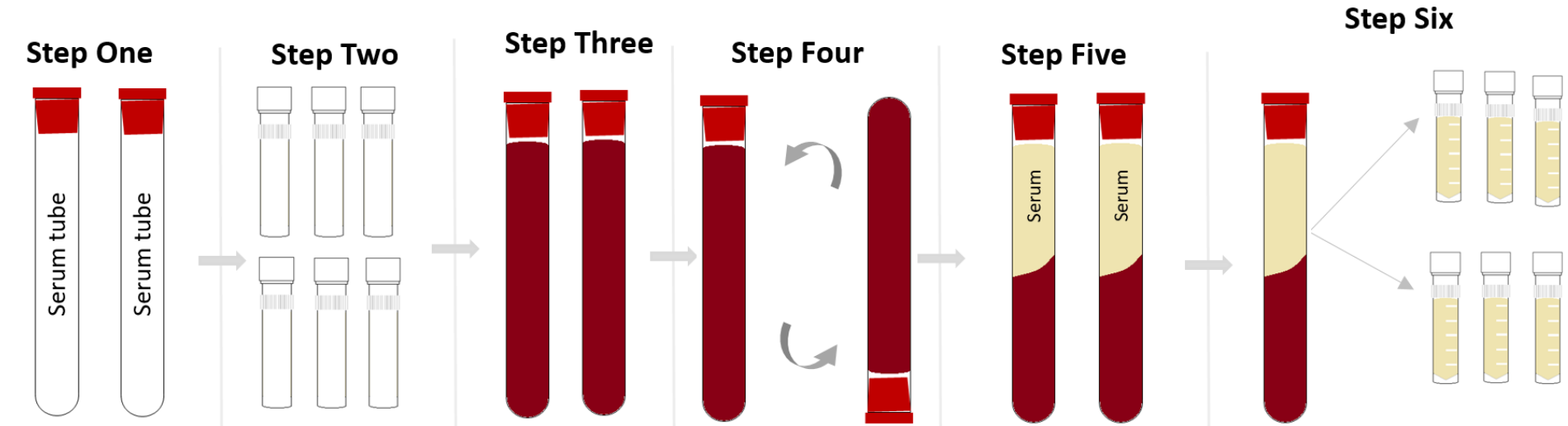


10. Complete the **Sample Record and Shipment Notification form (Appendix I).**

11. Place the labeled cryovials in the 25 slot cryovial box. Place the cryobox UPRIGHT on dry ice. Transfer to **-80°C Freezer as soon as possible**. Store all samples UPRIGHT at **-80°C until shipped** to BioSEND on dry ice.

12. Ship the frozen serum aliquots to BioSEND according to **Appendix K – Frozen Shipping Instructions.**

Serum Preparation –10 ml Serum (Red Top) Tube



Step One

- Store tubes at room temperature.
- Label tubes with preprinted serum labels prior to blood draw.

Step Two

- Label 6 cryotubes with serum aliquot labels.
- Pre-chill cryotubes on wet ice for at least 5 minutes

Step Three

- Collect blood in serum tubes, 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.

Step Five

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

Step Six

- Using a clean transfer pipet, aliquot 1.0 ml serum into each cryotube.
- Store serum aliquots upright at -80°C until shipment.

Appendix I – Sample Record and Shipment Notification Form

A Sample Record and Shipment Notification Form must be completed for each subject-visit submitted to BioSEND. This form should be completed in advance of shipping to BioSEND also be physically included in the shipper. The form can be completed via REDCap by following the bellow link:

- **Link to Sample Collection and Processing Form:**
  http://kits.iu.edu/biosend/CRCSCASampleForm

It is preferred that you complete the form online via the REDCap link above. However, a copy of the printed form is available on the following pages, should you need a back-up option. Please note that if you do not complete the form online, you will need to email a copy of the form directly to biosend@iu.edu prior to shipment.

CRC-SCA Sample Record and Shipment Notification Form

Please verify/update the information below. When you click the "Submit" button below, a PDF copy of the Sample Record and Shipment Notification Form will be emailed to you.

Please print a copy of that document and include it in the shipping container.

Study Site:

- ◯ Columbia University
- ◯ Emory University
- ◯ Houston Methodist Hospital
- ◯ Johns Hopkins University
- ◯ Massachusetts General Hospital (Harvard Medical School)
- ◯ Northwestern University
- ◯ University California-Los Angeles
- ◯ University California-San Francisco
- ◯ University of Chicago
- ◯ University of Florida
- ◯ University of Michigan
- ◯ University of Minnesota
- ◯ University of Montreal Hospital (CRCHUM)
- ◯ University of Pennsylvania
- ◯ University of South Florida
- ◯ University of Texas Southwestern
- ◯ University of Washington

Email address of staff member completing this form

_____

Note: A copy of the completed sample form will be sent to this address.

Local ID:

_____
(Formatted like IU-01-001)

IU Kit Number:

_____
(6 digit number on BioSEND labels)

Sex (used for DNA quality control)

- ◯ Female
- ◯ Male
- ◯ Other - provide details

Other - provide details

_____

Visit:

- ◯ Screening
- ◯ PRN
- ◯ Baseline
- ◯ 6 months
- ◯ 12 months
- ◯ 18 months
- ◯ 24 months
- ◯ 36 months
- ◯ 48 months
- ◯ 60 months
- ◯ 72 months
- ◯ 84 months
- ◯ 96 months
- ◯ 108 months
- ◯ 120 months
- ◯ 132 months
- ◯ 144 months
- ◯ 156 months
- ◯ 168 months
- ◯ 192 months

Date of blood collection:

_____

Was CSF collected at this visit?

- ◯ Yes
- ◯ No

Date of CSF collection:

_____

SERUM

Number of SERUM aliquots shipped:

_____
(6 x 1ml aliquots expected)

PLASMA EDTA

Number of PLASMA EDTA aliquots shipped:

_____
(6 x 1ml aliquots expected)

Number of BUFFY COAT aliquots shipped:

_____
(2 aliquots expected)

WHOLE BLOOD EDTA

Number of WHOLE BLOOD tubes shipped:

_____
(2 x 3ml tubes expected)

CSF

Number of CSF aliquots shipped:

_____
(10 x 1ml aliquots expected)

NOTES

Please record any issues with collection/processing:

_____

Shipping Information - Please complete.

Frozen shipments should be sent Monday-Wednesday only. Please check for holiday closures and weather issues prior to shipping. Contact us at biosend@iu.edu if you are unsure whether or not it is safe to ship.

Date of shipment:

_____

Did/will you use the IU UPS interface (ShipExec©) to generate the shipping label?

◯ Yes
◯ No

Which shipping service did you use?

◯ UPS
◯ FedEx
◯ World Courier
◯ Other

What is the shipment tracking number?

_____

Appendix K – Frozen Shipping Instructions

IMPORTANT!

Frozen samples must be shipped Monday – Wednesday only, using Next Day Air delivery

Please be aware of holidays and inclement weather and plan your shipments accordingly. Reach out to biosend@iu.edu if you have any questions

Specimens being shipped to BioSEND are Category B UN3373 specimens and as such must be triple packaged and compliant with IATA Packing Instructions. *See the latest eEdition of the IATA regulations for complete documentation.*

Triple packaging consists of a primary receptacle(s), a secondary packaging, and a rigid outer packaging. The primary receptacles must be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured, or leak their contents into the secondary packaging. Secondary packaging must be secured in outer packaging with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging.

IATA Packing and Labeling Guidelines

- The primary receptacle (cryovials or blood collection tubes) must be leak proof and must not contain more than 1 L total.
- The secondary packaging (plastic canister or biohazard bag) must be leak proof and if multiple blood tubes are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent direct contact with adjacent blood tubes.
- Absorbent material must be placed between the primary receptacle (cryovials or blood collection tubes) and the secondary packaging. The absorbent material must be of sufficient quantity to absorb the entire contents of the specimens being shipped. Examples of absorbent material are paper towels, absorbent pads, cotton balls, or cellulose wadding.
- A shipping manifest listing the specimens being shipped must be included between the secondary and outer packaging.
- The outer shipping container must display the following labels:
  - ✓ Sender's name and address
  - ✓ Recipient's name and address
  - ✓ Responsible persons (shipper and recipient)
  - ✓ The words "Biological Substance, Category B"
  - ✓ UN3373
  - ✓ Class 9 label including UN 1845, and net weight of dry ice contained

BioSEND Packaging and Shipment Instructions – Frozen Shipments

1. Generate airway bill and schedule courier pick-up, as needed.

    - For instructions on generating airway bills and scheduling using the UPS ShipExec™ Thin Client system, see Appendix Q.

2. Record the tracking number onto the Sample Record and Shipment Notification form (Appendix I).

3. Make a copy of the Sample Record and Shipment Notification form.

4. Place all frozen labeled cryotubes in the cryobox. Only include specimens from one subject in each cryobox.

5. Place the cryobox in a clear plastic biohazard bag (do NOT remove the absorbent material found in the bag), and seal the biohazard bag according to the instructions on the bag. Affix a Case Label to the outside of the biohazard bag.

6. Place approximately 2-3 inches of dry ice in the bottom of the Styrofoam® shipping container.

7. If your protocol is collecting frozen whole blood, DNA, or RNA, place labeled tubes in bubble sleeves and seal.

8. Place the tubes in a clear plastic biohazard bag (do NOT remove the absorbent material found in the bag), and seal the biohazard bag according to the instructions on the bag. Affix a Case Label to the outside of the biohazard bag.

9. Place the biohazard bag containing the cryobox into the provided Styrofoam® shipping container on top of the dry ice. Please ensure that the cryobox is placed so that the cryovials are upright in the shipping container (as pictured).

10. Fully cover the cryobox with approximately 2 inches of dry ice. Do not include more than 2 subjects' worth of samples in a single shipper.

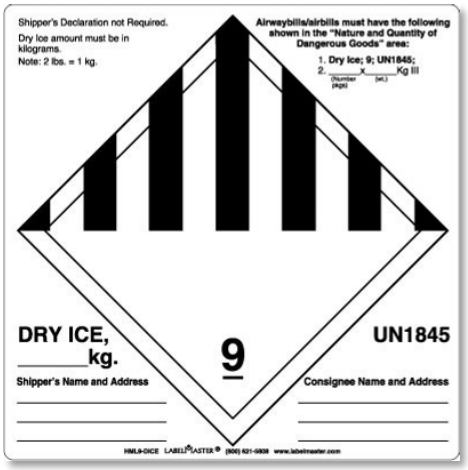
11. If including additional biohazard bags in package, include a layer of dry ice (approximately 2 inches) between each biohazard bag.

12. The inner Styrofoam® shipping container must contain approximately 10 lbs (or 4.5 kg) of dry ice. The dry ice should entirely fill the inner box to ensure the frozen state of the specimens.

13. Replace the lid on the Styrofoam® container. Place the completed Sample Record and Shipment Notification form in the package on top of the Styrofoam® lid for each patient specimen, and close and seal the outer cardboard shipping carton with packing tape.

14. Print a copy of your UPS® airway bill generated through the UPS ShipExec™ Thin Client system (see Appendix Q). Place airway bill into the provided airway bill envelope and affix envelope to package.

15. Complete the Class 9 UN 1845 Dry Ice Label (black and white diamond) with the following information:
    - Your name and return address
    - Net weight of dry ice in kg (this amount must match the amount recorded on the airway bill)
    - Consignee name and address:



BioSEND
IU School of Medicine
351 W. 10th Street
TK-217
Indianapolis, IN 46202

- Do not cover any part of this label with other stickers, including pre-printed address labels.

IMPORTANT!
Complete the required fields on your airway bill and Class 9 Dry Ice labels, or courier may reject or return your package.

16. Apply all provided warning labels (UN3373, Dry Ice Label and Fragile Label), taking care not to overlap labels with each other or with airway bill.

17. Hold packaged samples in -80°C freezer until time of courier pick-up/drop-off.

18. Specimens should be sent to the address below. Frozen shipments should be sent Monday through Wednesday only to avoid shipping delays on Thursday or Friday.

BioSEND
IU School of Medicine
351 W. 10th Street
TK-217
Indianapolis, IN 46202

19. **Notify BioSEND by email (biosend@iu.edu) that a shipment has been sent and attach the Sample Record and Shipment Notification form to your email. Do not ship until you've contacted and notified BioSEND staff about the shipment in advance.**

20. Use courier tracking system to ensure the delivery occurs as scheduled and is received by BioSEND.

In addition to tracking and reconciliation of samples, the condition and amount of samples received are tracked by BioSEND for each sample type. Investigators and clinical coordinators for each project are responsible for ensuring that the requested amounts of each fluid are collected to the best of their ability and that samples are packed with sufficient amounts of dry ice to avoid thawing in the shipment process.

Appendix O – Low Fat Diet Menu Suggestions

Foods to avoid prior to blood collection:

Avoid: *All fats and nuts such as:*

- Butter
- Cream
- Bacon fat
- Lard
- All oils
- All margarine
- All nuts
- Peanut butter
- Coconut
- Whole seeds such as pumpkin and sunflower

Avoid: *All milk and dairy products such as:*

- All whole milk products
- All cheese
- All products containing cheese
- Sour cream
- All ice cream
- Milk chocolate

Avoid: *High fat prepared foods and foods naturally high in fat:*

All red meats or meats containing fat such as pork and:

- Fatty meats such as:
  - Luncheon meats
  - Organ meats
  - Bacon
- Fatty fish such as:
  - Salmon
  - Mackerel

- Salad dressing and mayonnaise
- Buttered, au gratin, creamed, or fried vegetables

- Fried foods
- Gravies and sauces

- Fried snacks such as:
  - Chips
  - Crackers
  - French Fries
- Baked goods and frosting

Appendix Q - UPS ShipExec™ Thin Client Instructions

***** The shipment label in ShipExec should not be created until the day of shipment *****

1) Log in to the UPS ShipExec™ Thin Client website: https://kits.iu.edu/UPS or https://kits.iu.edu/ups.
    a. To request an account, complete the following survey: https://redcap.uits.iu.edu/surveys/?s=88TTWY3KAF
2) Find the "Shipping" dropdown menu in the top left corner of the screen and click on "Shipping and Rating".
3) Once the Indiana University page loads, look for the "Study Group" dropdown menu under "Shipment Information" on the right side of the screen. Choose your study from the dropdown menu.
4) After selecting your study, click on the magnifying glass icon on the left side of the screen under "Ship From".
5) An address book and filters will populate the screen. On the right side of the screen, a list of all the site addresses within the study you selected should populate.
    a. Filter the list down more by looking to the left side of the screen and searching for their address by filling in the "Company", "Contact", or "Address 1" fields. Click on the Search button when ready.
    b. Once you have found your site address, click on the "Select" button to the left of the address.
6) Make sure your address populated in the fields under "Ship From" on the main page.
    a. If you accidentally selected the wrong address, click on the "Reset" button on the bottom right of the screen. After the page reloads and clears the information, select your study again from the "Study Group" menu and click on the magnifying glass icon again to search for your correct address.
    b. To change the address for your site and study group, please complete the following survey: https://redcap.uits.iu.edu/surveys/?s=88TTWY3KAF
7) Enter the total weight of your package in the "Weight" field on the right side of screen under the name of your study.
    a. Leave the "Dry Ice Weight" field empty or enter "0" if shipping an ambient sample.
8) Enter the weight of the dry ice for frozen shipments in the "Dry Ice Weight" field.
    a. The "Dry Ice Weight" field can *never* be higher than the "Weight" field.
    b. **(Steps 9-10 can be skipped if you do not need to schedule a pickup)**
9) After entering the weights, click on the blue "Pickup Request" button.
10) When the Create Pickup Request box pops up, enter information into all the fields provided.
    a. Enter the "Earliest Time Ready" and "Latest Time Ready" in 24-hour format.
        i. Scheulde pickup at a minimum 1 hour *before* the "Earliest Time Ready"
    b. Choose a name and phone number that is the best contact if the UPS driver has question related to picking up your package
    c. Entering the "Room Number" and "Floor" will help the UPS driver locate your package
        i. The "Floor" field only allows numerical characters while the "Room Number" field is free text.
    d. Click "Save" when done.
11) Once you are certain that all the correct information has been entered, click the "Ship" button in the bottom right corner of the screen.
12) If no red error messages pop up at the top of your screen after clicking on "Ship", then you should have 2 downloaded PDF files: Shipment Receipt & UPS Package Label

   a. Shipment Receipt will list a “Pickup No.” that references your specific package if there is ever an issue with UPS picking up your package

13) Print out the UPS airway bill to any printer at your location.
   a. Fold the UPS airway bill and slide it inside the plastic UPS sleeve.
   b. Peel the back off the plastic UPS sleeve and stick the sleeve to your package, making sure it is laying as flat as possible along the surface of the package.
14) Place your package in the spot designated in your pickup request, or wherever your daily UPS pickups occur.
15) If you need to reprint your airway bill or void your shipment, click on “History” at the top of the main screen.
   a. If your shipment does not automatically pop up, enter the date of shipment and then click “Search”.
   b. To reprint your airway bill, click on the printer icon to the far left under “Action”
   c. To void your shipment, click on the “X” icon to the far left under “Action”
      i. If you created an airway bill that you no longer need, you must void the shipment to ensure your study will not be charged for the shipment.