

Chronic Subdural Hematoma Treatment with Embolization Versus Surgery Study (BioCHESS)

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| Section | Change |
|---------------------|--|
| Document Footer | Updated to “Version (April 2025)” |
| Throughout Document | Minor changes made to phrasing and wording. No alterations to procedures were made. |

National Institute of Neurological Disorders and Stroke Biorepository:



Biospecimen Collection, Processing, and Shipment Manual for
**Chronic Subdural Hematoma Treatment with Embolization
Versus Surgery Study (BioCHESS)**

Contents

| | | |
|------|--|----|
| 1.0 | Purpose | 5 |
| 2.0 | Abbreviations | 5 |
| 3.0 | BioSEND Information..... | 6 |
| 4.0 | Protocol Schedule for Biospecimen Submission to BioSEND – BioCHESS | 8 |
| 5.0 | Specimen Collection Kits and Supplies | 9 |
| 6.0 | Specimen Labels | 13 |
| 7.0 | Specimen Collection and Processing Procedures | 15 |
| 8.0 | Packaging and Shipping Instructions | 17 |
| 9.0 | Data Queries and Reconciliation | 18 |
| 10.0 | Appendices: | |
| | Appendix B: Whole Blood Collection for Plasma and Buffy Coat | |
| | Appendix F: Whole Blood Collection for Isolation of Serum | |
| | Appendix I: Specimen Collection and Processing Form | |
| | Appendix K: Frozen Shipping Instructions | |
| | Appendix L: Ambient Shipping Instructions | |
| | Appendix Q: UPS ShipExec™ Thin Client Instructions | |
| | Appendix V: Tissue Collection Instructions | |

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
- Dura (in Formalin, *RNA Later*[™], and fresh frozen (no preservative))
- Membrane (in Formalin, *RNA Later*[™], and fresh frozen (no preservative))
- Subdural Hematoma Fluid

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 |
| SDH | Subdural Hematoma |

3.0 BioSEND Information

3.1 BioSEND Contacts

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General BioSEND Contact Information

Phone: 317-278-6158

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. Ambient samples may be shipping Monday – Thursday.

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

For up-to-date holiday closure information and instructions, please visit <https://biosend.org/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 |
| June 19 th | Juneteenth (observed) |
| 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 |

Please note that BioSEND has extended closures surrounding the week of Thanksgiving and the last two weeks of the year. BioSEND will post notice of these closures on biosend.org and send email reminders in advance. Please contact biosend@iu.edu if you are unsure whether the day you plan to ship is permissible.

4.0 Protocol Schedule for Biospecimen Submission to BioSEND – BioCHES

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.

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

| Collection Tube | Collection Visit | Specimen Type | Aliquot Volume | Total Number of Aliquots | Shipping Temperature |
|---|------------------|-------------------------|----------------|--------------------------|----------------------|
| 1 EDTA (plastic) Blood Collection Tube, 10ml | Baseline | Plasma | 1.5ml | 3 | Frozen |
| | Baseline | Buffy Coat | ~750ul | 1 | Frozen |
| 1 Serum (plastic) Blood Collection Tube, 10ml | Baseline | Serum | 1.5ml | 3 | Frozen |
| 1 Individually wrapped Conical, 15ml | Baseline | Subdural Hematoma Fluid | 15ml | 1 tube | Frozen |
| 2 Prefilled Formalin Tubes, Green-top, 30ml | Baseline | Membrane | 1 section | 1 | Ambient |
| | Baseline | Dura | 1 section | 1 | Ambient |
| 2 Prefilled <i>RNA Later</i> TM Tubes, Green-top, 30ml | Baseline | Membrane | 1 section | 1 | Ambient |
| | Baseline | Dura | 1 section | 1 | Ambient |
| 2 Green-top Tubes, 30ml, Fresh frozen (no preservative) | Baseline | Membrane | 1 section | 1 | Frozen |
| | Baseline | Dura | 1 section | 1 | Frozen |

5.0 Specimen Collection 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. Any tubes that will remain at the collection site or shipped to other repositories should be labeled accordingly.

Links to each study's kit request module, sample submission forms, and other information can be found at <https://biosend.org/coordinate-studies/active-studies>.

5.1 Ordering Kits and Supplies

Study sites are responsible for ordering study kits. We advise sites to proactively confirm kits are on hand ahead of study visits.

Kits and individual items can be ordered as required through the BioSEND kit request module:

- **BioCHESS** <https://redcap.link/biochesskitrequest>

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

5.2 Kit Contents

Collection kits contain the following (for each subject) and provide the necessary supplies to collect samples from a given subject visit. 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.

Frozen shipping kits contain the supplies to ship up one subject visit worth of samples (that is, one cryobox, two 30-ml tubes (no preservative), and one 15ml conical may be shipped in a single shipper). Ambient Shipping kits will be provided to ship membrane and dura tissue samples in formalin and *RNALater*TM to BioSEND.

| Quantity | Blood and Tissue Collection Kit |
|----------|--|
| 1 | EDTA purple-top tube, 10ml (plastic) |
| 1 | Serum red-top tube, 10ml (plastic) |
| 1 | 15ml individually wrapped conical |
| 2 | Prefilled Formalin Vial green-top, 30ml |
| 2 | Prefilled <i>RNALater</i> TM Tube green-top, 30ml |
| 2 | Empty Centrifuge Tube green-top, 30ml |
| 3 | Cryovial (2ml) with purple cap |
| 3 | Cryovial (2ml) with red cap |
| 1 | Cryovial (2ml) with clear cap |
| 2 | Disposable pipette, 3ml |
| 2 | Resealable tube pouch |
| 1 | Cryobox, 25-slot |
| 1 | Kit & specimen label set |

| Quantity | Frozen Shipping Kit |
|----------|--|
| 1 | Large Plastic Biohazard bag w/ absorbent sheet |
| 1 | UPS Airbill Sleeve |
| 1 | Shipping box/Styrofoam container |
| 1 | UN3373 label |
| 1 | Fragile label |
| 1 | Dry ice label |

| Quantity | Ambient Shipping Kit |
|----------|--|
| 1 | Large Plastic Biohazard bag w/ absorbent sheet |
| 1 | UPS Airbill Sleeve |
| 1 | Ambient Shipper (ThermoSafe) |
| 1 | UPS Clinpak |
| 1 | Cold Pack |

| | |
|---|-----------------------|
| 1 | List of Contents Card |
| 1 | UN3373 label |
| 1 | Fragile label |

5.3 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

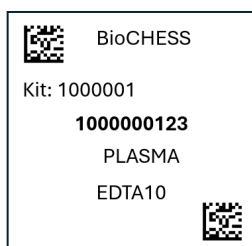
Labels must be affixed on all collection and aliquot tubes to prevent sample mix-ups and ensure chain-of-custody tracking. BioSEND provides labels for all samples being collected and returned to BioSEND. The site is responsible for providing labels for biospecimens that will be retained at the site. 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 Appendix K for further instructions.

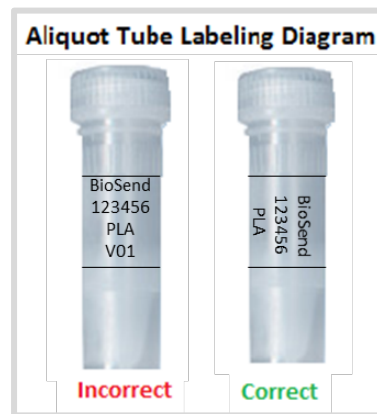


The **Specimen Labels** are placed on specimen tubes shipped to BioSEND. Each Specimen Label has a unique barcode that is tied to the Kit. The specimen type and collection tube will be noted on the tube. For example, the label to the left would be placed on a plasma aliquot generated from a 10ml EDTA tube.

6.2 Affixing Labels

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

- Place blood specimen 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 specimen 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 Blood Collection Protocols

EDTA can contaminate subsequent tubes if collected first, so they should be collected after other tube types. ***Blood collection tubes should be collected in the following order:***

1. Serum
2. EDTA (plasma)

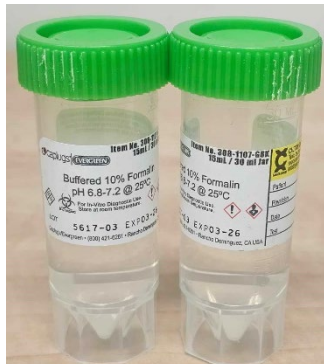
For detailed instructions on collection and processing the specific sample types, see the Appendices referenced below.

- Whole Blood Collection for Plasma and Buffy Coat (**Appendix B**)
- Whole Blood Collection for Isolation of Serum (**Appendix F**)

7.2 Tissue Collection Protocols

- Tissue Collection Instructions (**Appendix V**)

Tissue samples for Dura and Membrane will be collected in the following order priority (1) Formalin, (2) *RNALater*[™], and (3) fresh frozen (empty / no preservative) 30ml conical tube. Dura and Membrane samples in Formalin and *RNALater*[™] will be shipped ambient to BioSEND on the same day of collection. Fresh frozen Dura and Membrane samples in the empty / no preservative 30ml conical will be shipped frozen to BioSEND. Subdural Hematoma Fluid (SDH fluid) will be drawn and placed in a 15ml sterile conical and shipped frozen to BioSEND.



7.3 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. Each kit includes the appropriate collection tubes to provide the specimen volume described in the Protocol Schedules for Biospecimen Submission ([see Section 4](#)). BioSEND provides only the supplies and labels for specimens intended for the BioSEND repository.

Please generate as many standard-sized (1.5ml) plasma aliquots as possible. Buffy Coats will generally be between 0.5ml-1ml in volume. Please fill as many aliquot tubes as possible to the standard volume, using any remaining sample to generate a single “residual” aliquot (i.e., less than standard volume).

To assist in the preparation and aliquoting of samples, colored caps are used for the aliquot tubes. The chart below summarizes the association between cap color and type of aliquot.

| Cap Color | Specimen Type |
|-----------|---------------|
| Purple | Plasma |
| Clear | Buffy Coat |
| Red | Serum |

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/>).

We encourage all studies to use our custom UPS shipping interface to create shipping waybills and schedule package pickups. More information can be found at <https://biosend.org/shipping-resources>.

8.1 Specimen Collection and Processing Form

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

8.2 Shipping Instructions

Blood samples, SDH Fluid, and fresh frozen (empty tube / no preservative) Dura and Membrane samples are shipped frozen. Reference Appendix K for frozen shipping instructions and Appendix Q for generating airway bills and scheduling pick-ups.

Dura and Membrane samples in Formalin and *RNALater*TM will be shipped ambient to BioSEND on the same day of collection. Reference Appendix L for ambient shipping instructions and Appendix V for Tissue 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.

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

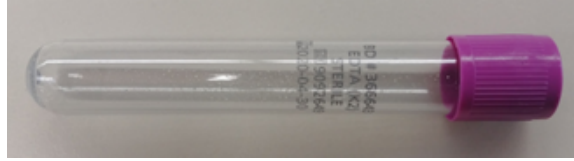
- Missing samples
- Incorrect samples collected and shipped
- Damaged or incorrectly prepared samples
- Unlabeled or mislabeled samples
- Samples frozen and stored longer than three months at the site

10.0 Appendices

- Appendix B: Whole Blood Collection for Plasma and Buffy Coat
- Appendix F: Whole Blood Collection for Isolation of Serum
- Appendix I: Specimen Collection and Processing Form
- Appendix K: Frozen Shipping Instructions
- Appendix L: Ambient Shipping Instructions
- Appendix Q: UPS ShipExec™ Thin Client Instructions
- Appendix V: Tissue Collection Instructions

Appendix B – Whole Blood Collection for Plasma and Buffy Coat

One 10 ml EDTA (plastic) tube is provided in each Blood Collection Kit for the collection and generation of plasma and buffy coat



1. Store empty EDTA (plastic) tube at room temperature 64°F – 77°F (18°C to 25°C) prior to use.
2. Place “PLASMA” specimen labels on the 10 ml EDTA tube and on the three purple-capped 2 ml cryovial tubes. Place “BUFFY COAT” specimen labels on the a clear-capped 2ml cryovial tubes.
3. Pre-chill the labeled cryovials on wet ice for at least 5 minutes.
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. Collect whole blood into the tubes using your institution’s recommended procedure for standard venipuncture technique.
 - a. Subject is required to lay down for 30 minutes after IV catheter is placed before the blood draw occurs.
 - b. Collect blood directly from the IV catheter using a Luer adapter.
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 vacuum is designed to draw 10 ml of blood into the tube.
7. Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tube 8 – 10 times. **Do not shake the tubes!**
8. 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.
9. 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**

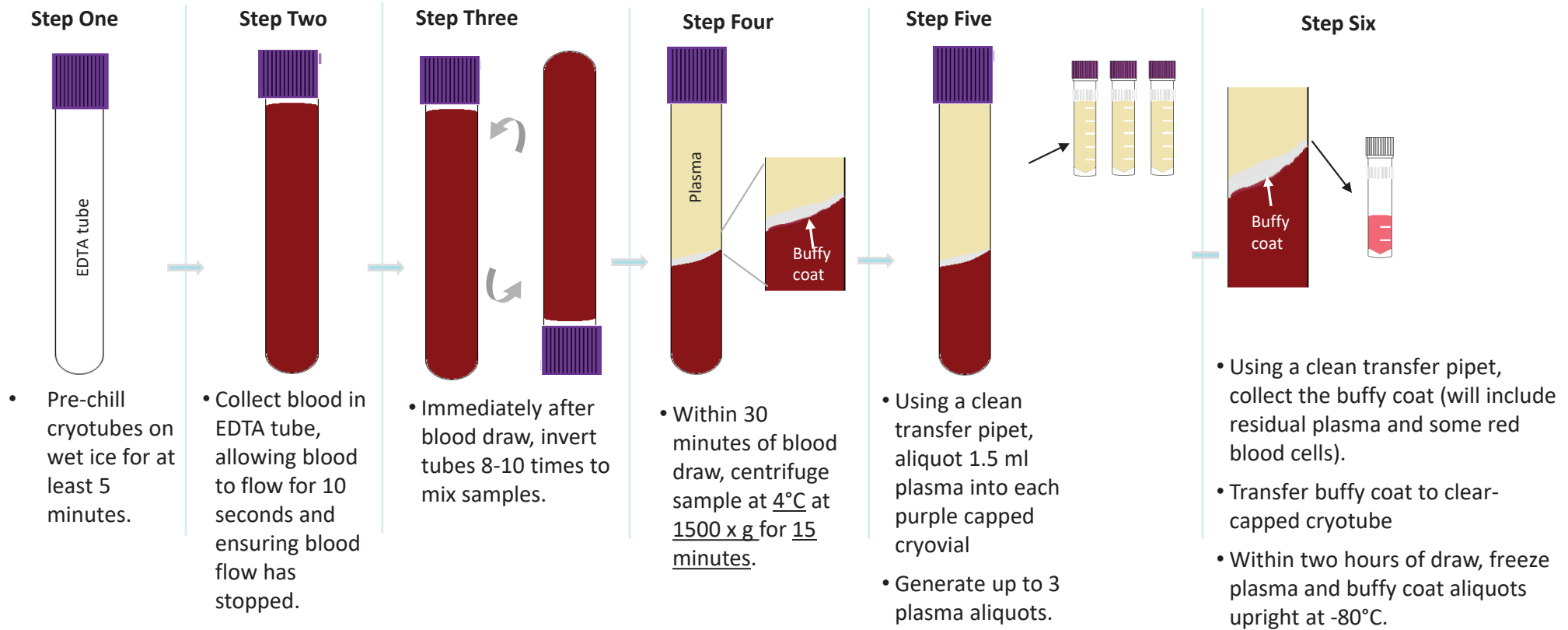
tube so that the plasma is not contaminated (see below). Using a disposable tipped micropipette, transfer plasma into the purple-capped cryovials. Aliquot 1.5 ml per cryovial. If you cannot obtain 3 plasma aliquots, please note “low volume draw” on the Sample Record and Shipment Notification form (Appendix I) under “Notification of Problems”. Each 10 ml EDTA tube should yield approximately 4-5 ml of plasma.

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



11. After plasma and buffy coat has been aliquoted into cryovials, **discard** the 10ml EDTA collection tubes. Do not send these tubes to BioSEND.
12. Complete the Specimen Collection and Processing form (Appendix I).
13. 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.
14. Ship the frozen plasma aliquots to BioSEND according to Appendix K – Frozen Shipping Instructions.

Sample Collection and Processing: Plasma & Buffy Coat



Appendix F – Whole Blood Collection for Isolation of Serum

One 10 ml Serum (plastic) tube is provided in each Blood Collection Kit for the collection and generation of serum

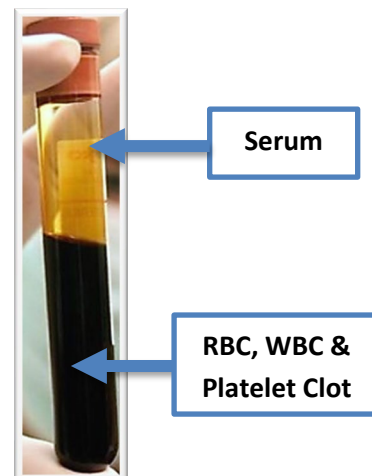
1. Store empty serum determination (red-top) tube at room temperature 64°F – 77°F (18°C to 25°C) prior to use.
2. Place pre-printed specimen labels noted as “SERUM” on the serum determination red-top tube and on three of the 2 ml red-capped cryovials prior to blood draw. Three cryovials will be shipped 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. Collect whole blood into the tubes using your institution’s recommended procedure for standard venipuncture technique.
 - a. Subject is required to lay down for 30 minutes after IV catheter is placed before the blood draw occurs.
 - b. Collect blood directly from the IV catheter using a Luer adapter.
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. Immediately after blood collection, **gently** invert/mix (180 degree turns) the serum determination tube 8-10 times. **Do not shake the tubes!**
8. 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 3 x 1.5 ml aliquots to BioSEND.

10. Complete the Specimen Collection and Processing Form (Appendix I).

11. Place the labeled cryovials in the 25 slot cryovial box. **Transfer to -80°C Freezer within two hours, ensuring cryovials are frozen upright.** Store all samples upright at -80°C until shipped to BioSEND on dry ice.

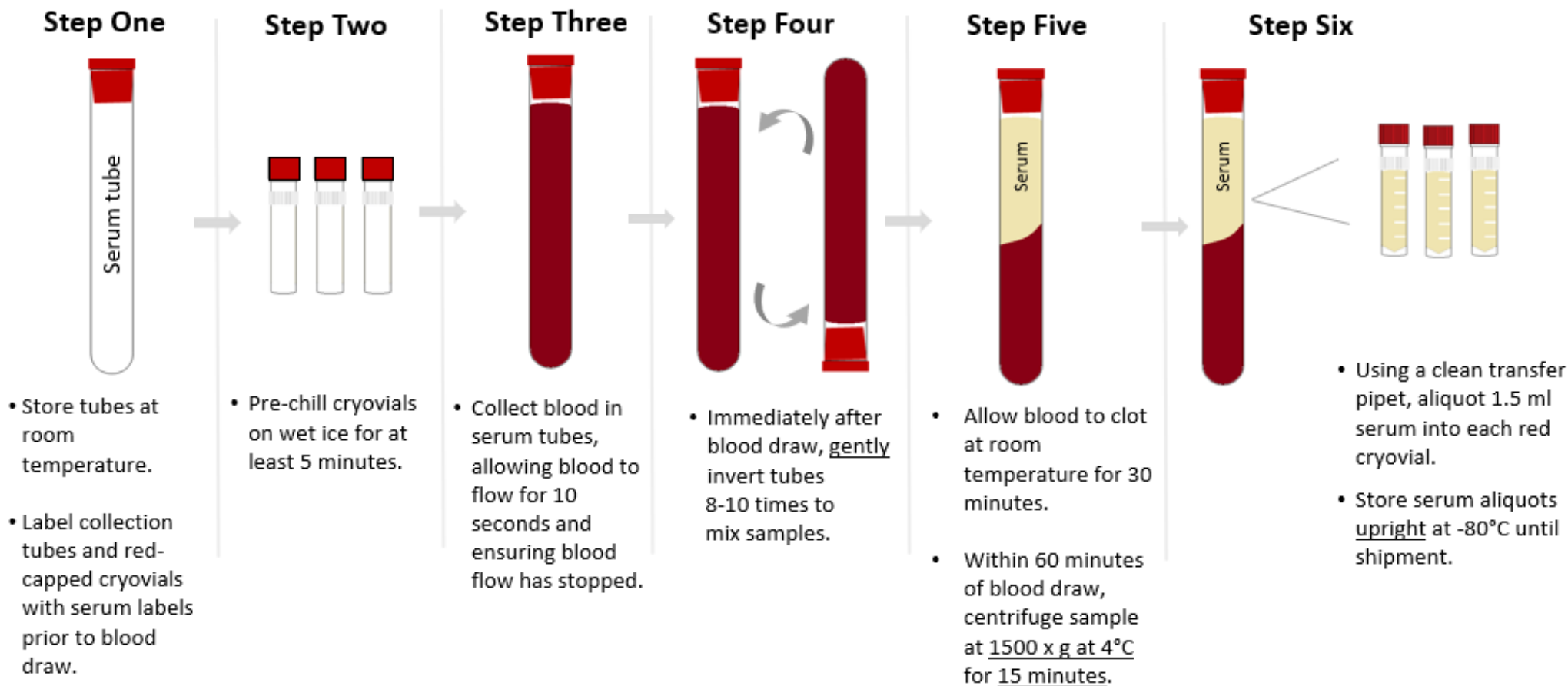
12. Ship the frozen serum aliquots to BioSEND according to Appendix K – Frozen Shipping Instructions.



Serum

RBC, WBC &
Platelet Clot

Serum Preparation –10 ml Serum (Red Top) Tube



Appendix I – Specimen Collection and Processing Form

A Specimen Collection and Processing Form must be completed for each subject-visit submitted to BioSEND. This form includes a Frozen Shipping Manifest and an Ambient Shipping Manifest that should be completed in advance of shipping to BioSEND. These forms should also be physically included in the frozen and ambient shippers. The form can be completed via REDCap by following the bellow link:

For links to your specimen processing form, to download the most recent version of of this manual, and access recordings of BioSEND trainings, please visit <https://biosend.org/coordinate-studies/active-studies> and choose your study from the drop-down list.

- **Link to Specimen Collection and Processing Form:** <https://redcap.link/BioCHESSSampleForm>

Please note that there is a Save & Return option at the bottom of the survey. This may be used if, for example, you are ready to complete the Collection and Processing portion of the form, but not yet ready to complete the Frozen Shipping Manifest.

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.

BioCHESS Ambient Shipping Manifest

Please verify/update the information below. When you click the "Submit" button below, a PDF copy of the Ambient Shipping Manifest will be emailed to you for Subject [subj_id]. Please print a copy of that document and include it in the shipping container with Kit #[kit_num].

Because no samples were fixed in formalin, all specimens should be shipped frozen. Click SUBMIT to skip this form.

Please verify that the caps on the formalin tubes are screwed on tightly to prevent leakage in transit.

Study Site:

BioCHESS Subject ID:

IU Kit Number:

Dura shipping in formalin

Membrane shipping in formalin

Dura shipped in RNALater

Membrane shipped in RNALater

Date of collection:

Time of collection:

(24-hour clock)

Shipping Information - Please complete.

Date of shipment:

Did/will you use the IU UPS interface to generate the shipping label?

- Yes
 No

Which shipping service did you use?

- UPS
 FedEx
 World Courier
 Other

What is the shipment tracking number?

BioCHESS Frozen Shipping Manifest

Please verify/update the information below. When you click the "Submit" button below, a PDF copy of the Frozen Shipping Manifest will be emailed to you for Subject [subj_id].

Please print a copy of that document and include it in the Kit #[kit_num] shipping container.

Study Site:

BioCHESS Subject ID:

IU Kit Number:

Tissue

Date of tissue collection:

Dura shipped fresh frozen (no preservative)

Membrane shipped fresh frozen (no preservative)

SDH Fluid

Volume of SDH fluid shipped

Serum

Date of blood collection:

Number of SERUM aliquots shipped:

PLASMA EDTA

Number of PLASMA EDTA aliquots shipped:

Number of BUFFY COAT aliquots shipped:

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 edition 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

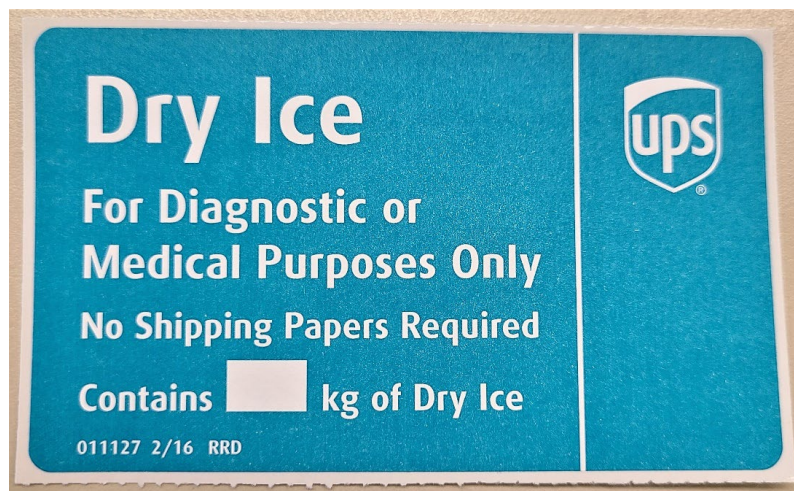
1. Generate airway bill and schedule courier pick-up, as needed.
 - For instructions on generating airway bills and scheduling using the UPS ShipExec™ Thin Client system, see Appendix Q.
2. Record the tracking number onto the Frozen Shipping Manifest portion of the Specimen Collection and Processing Form (Appendix I).
3. Make a copy of the Frozen Shipping Manifest to include in the shipment.
4. Place all frozen labeled cryotubes in the cryobox. Only include specimens from one subject in each cryobox.
5. Frozen shipping kits contain the supplies to ship up one subject visit worth of samples (that is, one cryobox, two 30-ml tubes (no preservative), and one 15ml conical may be shipped in a single shipper).



6. Place the cryobox containing labeled cryotubes, 15ml conical containing subdural hematoma fluid, two 30ml conicals (no preservative) containing dura and membrane sections into a large, 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 Kit Label to the outside of the biohazard bag.
7. Place approximately 2-3 inches of dry ice in the bottom of the Styrofoam® shipping container.
8. Place the large biohazard bag containing the cryobox, 15ml conical, and two 30ml conicals into the provided Styrofoam® shipping container on top of the dry ice.
9. Please ensure that the cryobox is placed so that the cryovials are upright in the shipping container (as pictured).



10. Fully cover the large biohazard bag with approximately 2 inches of dry ice.
11. The inner Styrofoam® shipping container must contain a minimum of 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.
12. Replace the lid on the Styrofoam® container. Place the completed Frozen Shipping Manifest from the Sample Collection and Processing 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.
13. 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.
14. Complete the UPS Dry Ice Label (blue) with the following information:
 - Net weight of dry ice in kg (this amount must match the amount recorded on the airway bill)
 - Do not cover any part of this label with other stickers, including pre-printed address labels.



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 of shipment by completing the Specimen Collection and Processing Form and Frozen Shipping Manifest in REDCap (see Appendix I for details). Submission of this form will send an automatic notification of your shipment to the BioSEND team.**
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 L – Ambient Shipping Instructions

IMPORTANT!

Ambient samples must be shipped Monday – Thursday 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

Ambient whole blood and tissue samples are Category B UN3373 and as such must be triple packaged and compliant with IATA Packing Instructions. *See the latest edition 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 should 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

BioSEND Packaging and Shipment Instructions – Ambient Shipments

1. Place refrigerant pack in the freezer 24 hours before shipment.
2. 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.
3. Record the tracking number onto the Ambient Shipping Manifest portion of the Specimen Collection and Processing Form (Appendix I).
4. Make a copy of the Ambient Shipping Manifest to include in the shipment.
5. Place the four 30ml conicals containing formalin-fixed and RNALater dura and membrane tissue samples in the biohazard bag with the absorbent material
6. Remove as much air as possible from the biohazard bag, and seal the bag according to the directions on the bag. Place kit label on outside of biohazard bag.



7. Place the specimen into the Styrofoam cooler, and then place the refrigerant pack on top of the specimen.
8. Place a copy of the Sample Collection and Processing form and a completed IATA List of Contents sheet (provided in kit) on top of the cooler and close the cardboard box. Please do NOT tape the cardboard box closed.
9. Close shipping box and place within the provided overpack and seal.
10. Affix airway bill to the sealed overpack. Place the UN3373 and Fragile stickers on the outside of the overpack.
11. Specimens should be sent to the address below. Ambient shipments should be sent Monday through Thursday.

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

12. Notify BioSEND of shipment by completing the Specimen Collection and Processing Form and Ambient Shipping Manifest in REDCap (see Appendix I for details). Submission of this form will send an automatic notification of your shipment to the BioSEND team.

13. Use courier tracking number 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.

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. Schedule 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.

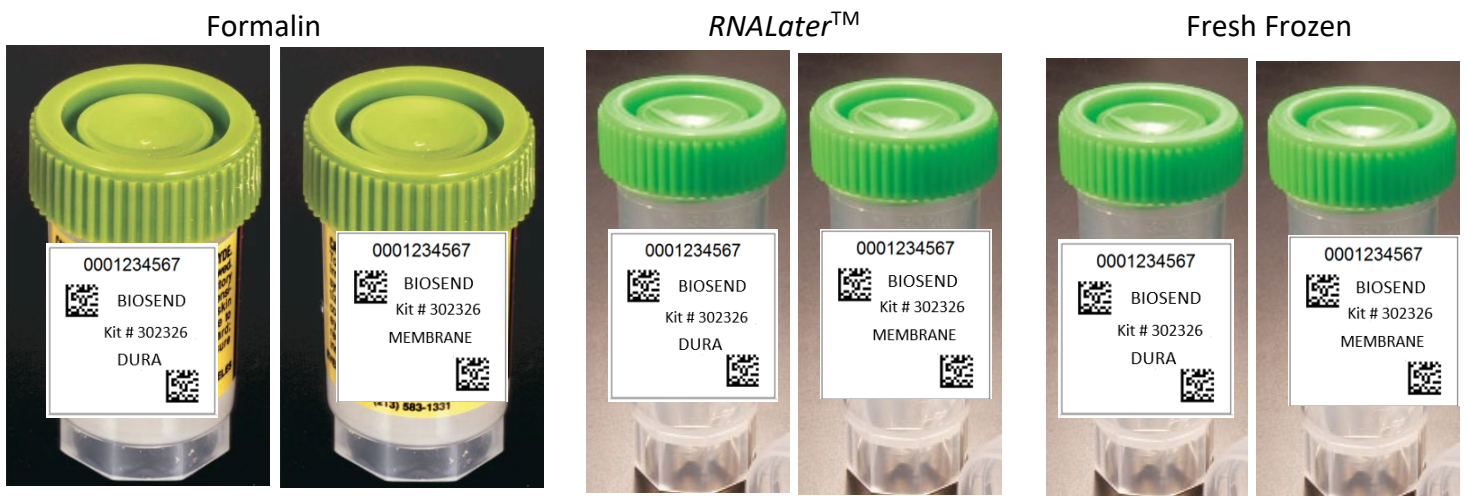
Appendix V — Tissue Collection Instructions

Please use best practice and facility SOPs for collecting and sectioning tissue samples.

1. Dura and Membrane Collection

- a. Once the Dura and Membrane samples have been surgically retrieved, split each sample into three sections.
 - i. One section should be placed in the prefilled formalin and shipped to IU ambient. Be sure to submerge the section in the formalin to ensure the fixative penetrates the tissue.
 - ii. One section should be placed in the prefilled *RNA Later*TM tube and shipped to IU ambient. Be sure to submerge the section in the *RNA Later*TM to ensure the fixative penetrates the tissue.
 - iii. One section should be placed in the green-top tube with no preservative and shipped to IU frozen. Alternatively, the section can be placed in the container and frozen at -80.
 - iv. Make sure all caps are secured tightly to prevent leakage.
- b. **If either tissue sample is too small to section into three pieces, please prioritize in the following order:**
 1. Prefilled Formalin Tube, green-top, 30ml
 2. Prefilled RNA Later Tube, green-top, 30ml
 3. Empty / No preservative Green-top Tube, 30ml (Fresh Frozen)

Reference Appendix K for Frozen Shipping Instructions and Appendix L for Ambient Shipping Instructions



2. **Subdural Hematoma Fluid** - Collect Subdural Hematoma Fluid into sterile 15ml individually wrapped conical and freeze upright in -80 freezer. Do not collect more than 14ml of fluid in the tube, as overfilled tubes may leak or burst when freezing.