The Biomarkers in the Hyperbaric Oxygen Brain Injury Treatment Trial (BioHOBIT) Study Manual of Procedures

Update: Version 04.2025

April 2025

Section	Change
Document Footer	Updated to "Version (April 2025)"
Section 6.0	Clarification that labeling is "to prevent sample mix-ups and ensure chain-of-custody tracking"

BioSEND

Biospecimen Exchange for Neurological Disorders

National Institute of Neurological Disorders and Stroke Biorepository:

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

Biospecimen Collection, Processing, and Shipment Manual for

The Biomarkers in the Hyperbaric Oxygen Brain Injury Treatment Trial (BioHOBIT)

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:

- Serum
- Plasma
- Buffy Coat (for DNA extraction)
- 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

3.0 BIOSEND INFORMATION

3.1 BioSEND Contacts

Tatiana Foroud, PhD, Principal Investigator

Claire Wegel, Project Manager
Email: cwegel@iu.edu

General BioSEND Contact Information
Phone: 317-278-6158
Email: biosend@iu.edu
Website: www.BioSEND.org

Sample Shipment Address
BioSEND
Indiana University School of Medicine
351 W. 10th Street. TK-217
Indianapolis, IN 46202-5188

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 courier websites to make sure impending weather events (blizzards, hurricanes, etc.) will not impact the shipping or delivery of the samples.

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
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 between December 24th and January 2nd (or the first business day after New Year's Day) Indiana University will be open Monday through Friday for essential operations **ONLY** and will re-open for normal operations on January 2nd. If at all possible, biological specimens for submission to Indiana University should **NOT** be collected and shipped to Indiana University between December 24th and

January 2nd. Should it be necessary to ship blood samples for DNA extraction to Indiana University during this period, please contact the Indiana University staff before December 24th by emailing biosend@iu.edu, so that arrangements can be made to have staff available to process incoming samples. Frozen specimens collected during this period should be held at your site to ship after the first business day in January.

Please see https://biosend.org/holiday-closures 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.

4.1 Protocol Schedule for Biospecimen Submission to BioSEND – BIO-HOBIT

Visit	0hr/ BL	8hr	24hr /D1	Post-HBO (one time between D2-D5)*	D3	D5	D7	D14	D180
Plasma aliquots, 1500ul	4	4	4	4	4	4	4	4	4
Serum aliquots, 1500ul	4	4	4	4	4	4	4	4	4
Buffy Coat	1	1	1	1	1	1	1	1	1
CSF, 1500ul (optional)	4	4	4	4	4	4	0	0	0

***Excluding control subjects**

The initial set of biospecimen should be collected shortly after informed consent or randomization if participant was enrolled under EFIC. The 8 and 24 hour samples may be obtained within 6 - 10 hours and 22 - 26 hours respectively from the initial blood draw. If the initial sample cannot be obtained shortly after informed consent/randomization, it may be obtained as soon as feasible. In situations where the first sample cannot be obtained shortly after informed consent/randomization, the 8 and 24 hour samples should be obtained at 8 (6-10) and 24 (22-26) hours after informed consent/randomization. If feasible, days 3, 5, 7, 14 and 180 samples should be collected at 8 am (+/- 2 hours) to minimize the effects of circadian rhythm on biomarker levels.

During the first 2 weeks of enrollment, biospecimen will be collected only if the subject remains in the hospital. Biospecimen should be obtained from all subjects who are alive at 6 months.

5.0 SPECIMEN COLLECTION KITS, SHIPPING KITS AND SUPPLIES

Research specimen collection kits as well as clinical lab supplies (except dry ice and equipment listed in Section 5.7) will be provided by BioSEND. These materials include blood tubes, boxes for plasma/serum/buffy coat aliquots, as well as partially completed shipping labels to send materials to BioSEND. Barcoded kit labels, collection tube labels, and aliquot tube labels will all be provided by BioSEND. Collection tube labels and aliquot tube labels will be pre-printed with study information specific to the type of sample being drawn. BioSEND will provide a sufficient number of labels 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); any tubes that will remain at the collection site 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

WebDCU notifies BioSEND when kits are required to be shipped, as sites are released to enroll and kits are used. Kit requests are posted to replace expiring kits 14 days prior to expiration.

Kits are always sent in multiples of 8. The initial shipment of kits is 24. A resupply of 8 kits is sent when the kit inventory reaches 16.

5.2 Specimen Collection Kit General Contents

Collection kits contain the following as designated per the BioHOBIT protocol. Do not replace or supplement any of the tubes or kit components provided with your own supplies unless you have received approval from the BioHOBIT Study team to do so. *Please store all kits at room temperature until use.* Note that "supplemental" kits will be provided should you require additional supplies from those contained in the visit specific kits.

Individual Supplies*

25-slot cryobox
Siliconized cryotube (2ml) with clear cap
Siliconized cryotube (2ml) with blue cap
15ml conical tube
50ml conical tube
Return airbill
Shipping container for dry ice shipment (shipping and Styrofoam box)
Biohazard bag with absorbent sheet
EDTA (Purple-Top) Blood Collection Tube (7 ml)
Serum (Red-Top) Blood Collection Tube (7 ml)
Disposable graduated transfer pipette, 3ml
Shipping label packet

***Available upon request**

Standard Collection Kit

Quantity	Supply Components
1	EDTA (Purple-Top) Blood Collection Tube (7 ml)
1	Serum (Red-Top) Blood Collection Tube (7 ml)
11	Siliconized cryotube (2ml) with clear cap
4	Siliconized cryotube (2ml) with blue cap
1	15ml conical tube
3	Disposable graduated transfer pipette, 3ml
1	Collection tube & cryotube label set

Standard Shipping Kit

Quantity	Supply Components
4	Biohazard bag with absorbent sheet
4	25-slot cryobox
1	Shipping label packet (dry ice, UN3373, & fragile)
1	Airway bill envelope
1	Insulated shipper

Supplemental Kit

Quantity	Supply Components
5	EDTA (Purple-Top) Blood Collection Tube (7 ml)
5	Serum Determination (Red-Top) Blood Collection Tube (7 ml)
2	Plastic biohazard bag with absorbent sheet
2	25-slot cryobox
10	Siliconized cryotube (2ml) with clear cap
10	Siliconized cryotube (2ml) with blue cap
5	50ml conical tube
5	15ml conical tube
5	Disposable graduated transfer pipette, 3ml
2	Airway bill envelope
2	Warning label packet

We realize there may be instances where additional supplies are needed; therefore, one supplemental kit will be provided with the initial kit shipment for each site

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
- Wet ice

In order to process samples consistently across all projects and ensure the highest quality samples possible, 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:

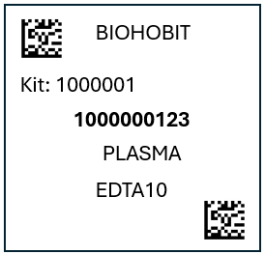
- Dry ice (approximately 10 pounds per shipment)

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

The **Kit Labels** link all specimens collected from a single participant at one visit. There will be a different Kit Number for each visit a subject has. These labels are used on packaging materials; see Appendix K for further instructions.

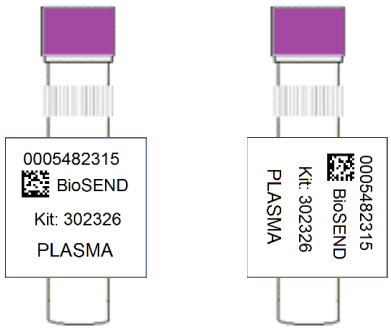


The **Specimen Labels** are placed on all blood collection and aliquot tubes. Each Specimen Label has a unique barcode that is tied to the Kit. The labels indicate the specimen type, as well as the collection tube for that specimen. For example, the label to the right would be placed on a plasma aliquot 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 collection and aliquot labels on **ALL** collection tubes and cryotubes **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.
- Place label **horizontally** on the tube (wrapped around sideways if the tube is upright) and **just below the ridges** of the cryotube.



Incorrect **Correct**

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 (purple top) blood collection for plasma and buffy coat

7.2 Blood Collection Protocols

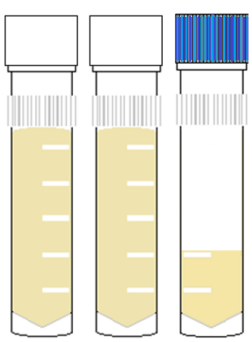
1. Serum (red top) blood collection for serum **(Appendix F)**
2. EDTA (purple top) blood collection for plasma and buffy coat **(Appendix B)**

7.3 Cerebrospinal Fluid Processing Protocol

1. Cerebrospinal Fluid Processing **(Appendix G)**

7.4 Filling Aliquot Tubes (Plasma, Serum, and CSF)

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 site is supplied with sufficient collection tubes to provide the specimen volume described in the Protocol Schedules for Biospecimen Submission (see Section 4).



YES

NO

Please note: For serum, plasma, and CSF aliquots, please fill as many clear-capped cryotubes to 1.5ml as possible. If any remaining sample is left, aliquot this residual volume into a blue-caped cryovial.

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 Send shipment notification to BioSEND

- Emailed to BioSEND@iu.edu at the time the samples are being shipped
- Please include CRF from WebDCU and tracking number

8.2 Shipping Instructions

Reference Appendix K for frozen shipping instructions

*****Important Note*** Include samples for only one subject per shipping container.**

For frozen shipments, include no more than two packing envelopes per shipping container in order to have room for a sufficient amount of dry ice to keep samples frozen up to 24 hours.

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 46202-5188

9.0 Data Queries and Reconciliation

The FITBIR 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 FITBIR 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 shipping manifests compared to information entered into the FITBIR database.
- Samples frozen and stored longer than three months at the site

10.0 APPENDICES

Appendix B: Whole Blood Collection for Isolation of Plasma and Buffy Coat

Appendix F: Whole Blood Collection for Isolation of Serum

Appendix G: Cerebrospinal Fluid Processing

Appendix K: Frozen Shipping Instructions

Appendix Q: UPS ShipExec™ Thin Client Instructions

Appendix B – Whole Blood Collection for Isolation of Plasma and Buffy Coat

1. Store empty 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**" labels on 7 ml lavender-top EDTA tube and on 4 of the 2 ml cryotube tubes. One cryotube will have a blue sticker on top, which is for a residual plasma aliquot (if created). Place "**BUFFY COAT**" label on one of the 2ml cryotubes.

3. Pre-chill the labeled cryotubes 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. Using a blood collection set and a holder, collect blood into the lavender top 10 ml EDTA tube using your institution's recommended procedure for standard venipuncture technique.

The following techniques can be used to prevent possible backflow:

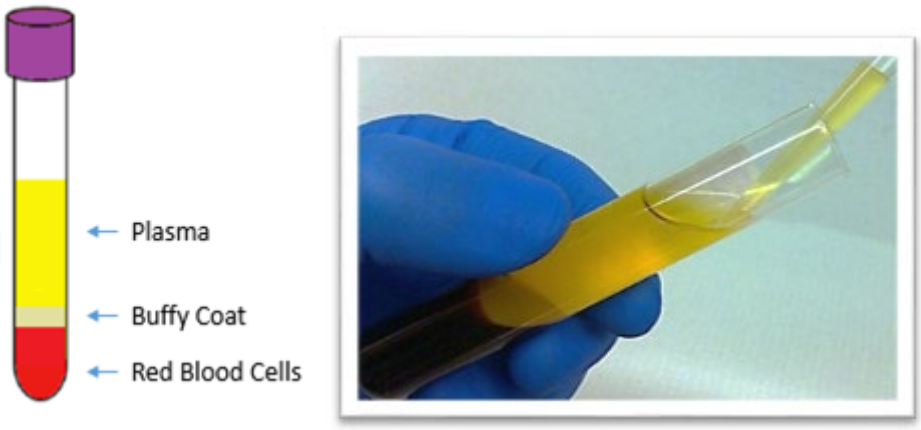
a. Place donor's arm in a downward position.
b. Hold tube in a vertical position, below the donor's arm during blood collection.
c. Release tourniquet as soon as blood starts to flow into the tube.
d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

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 7 ml of blood into the tube.

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

8. 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. **Samples must be processed and stored within 2 hours of collection.**

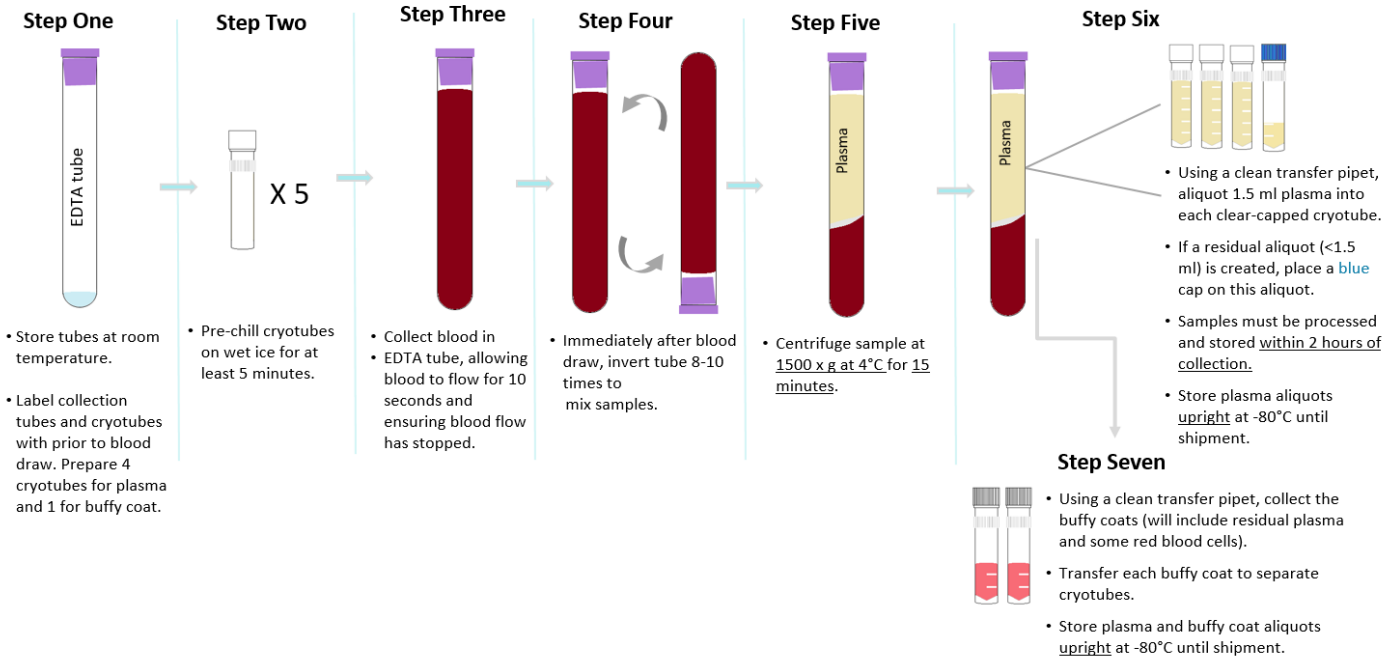
9. Remove the plasma by tilting the tube and placing the pipette tip along the lower side of the wall. **Use caution not to touch the buffy coat or packed red blood cells at the bottom of the tube so that the plasma is not contaminated** (see below). Using a disposable tipped micropipette, transfer plasma into the pre-labeled cryotubes. Aliquot 1.5 ml per cryotube.



10. Place the labeled cryotubes 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.

11. 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

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

2. Place provided "**SERUM**" collection tube labels on the serum red-top tube; place the serum cryotube labels on 4 of the 2ml cryotubes. One cryotube will have a blue sticker on top, which is for a residual plasma aliquot (if created).

3. Pre-chill labeled cryotubes 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 7 ml red-top serum tube using your institution's recommended procedure for standard venipuncture technique.

The following techniques can 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 7 ml of blood into the tube.

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

8. Allow blood to clot at room temperature for at least 30 minutes.

9. After clotting period, centrifuge tube for 15 minutes at 1500 RCF (x g) at 4°C. It is critical that the tube be centrifuged at the appropriate speed and temperature to ensure proper serum separation. **Samples must be processed and stored within 2 hours of collection**.

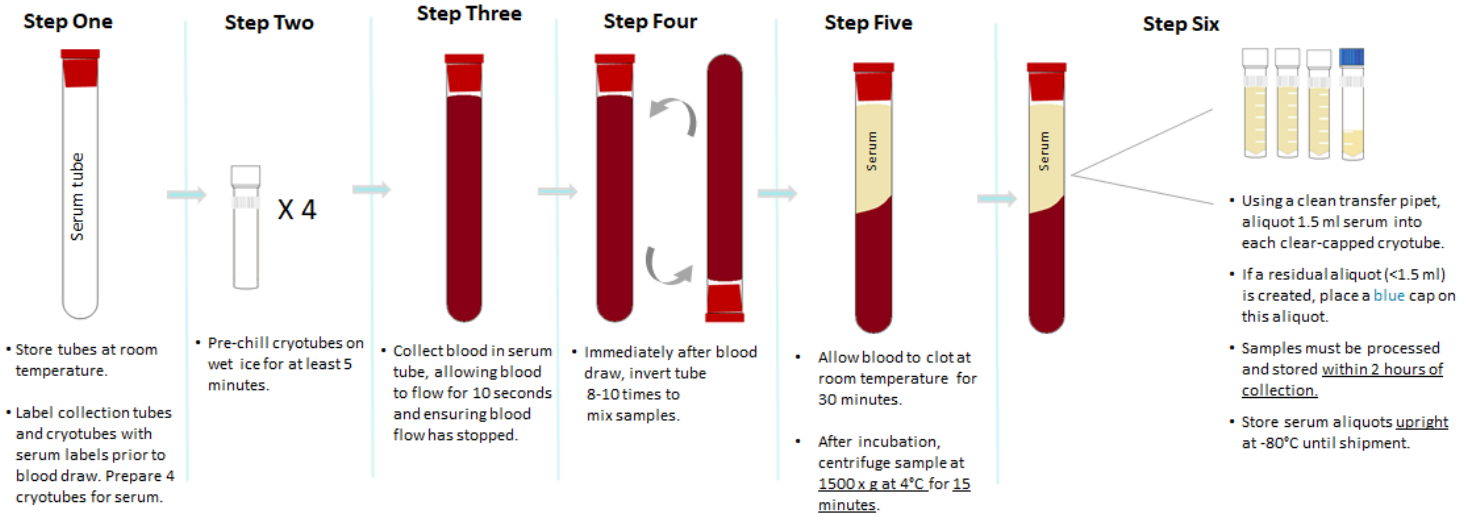
10. Remove the serum by tilting the tube and placing the pipette tip along the lower side of the tube wall. Use caution to pipet only the serum layer and not the red blood cell layer.

Using a disposable tipped micropipette, transfer serum into the pre-labeled cryotubes. Aliquot 1.5 ml per clear-capped cryotube. If any sample remains after creating the 1.5 ml aliquots, aliquot the remaining sample into a blue-capped cryotube (to create a residual sample).

11. Place the labeled cryotubes in the provided cryotube box. Transfer to -80°C Freezer as soon as possible. Ensure the aliquots 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.

Sample Collection and Processing: Serum



Appendix G — Cerebrospinal Fluid Collection

1. CSF Collection and Processing Procedures

1.1 General Guidelines

The decision to place an External Ventricular Drainage (EVD) is a local clinical decision and is not affected by a patient's participation in Bio-HOBIT. Similarly, indications and procedures for CSF drainage (continuous vs. intermittent drainage) is a local clinical decision and not prescribed in the HOBIT or Bio-HOBIT protocol.

CSF collected for research purposes is fluid that would otherwise be discarded.

- Procedures for inserting the EVD and for collecting fluid from the system are also governed by local Neuro ICU protocols.

- Published guidelines from the American Association of Neuroscience Nurses are available (Am Assc Neurosci Nurses (2011) Care for the patient undergoing intracranial pressure monitoring/external ventricular drainage or lumbar drainage. Glenview (IL) 37 p. [164 Refs]). Link to PDF

- A video demonstrating CSF collection is available here: https://vimeo.com/user120054989/CSFfromEVD
  DISCLOSURE: This tutorial is to assist trained personnel in CSF collection from an EVD.
  - Each site may differ in procedure. Check your local Neuro ICU protocol.
  - Also, this video shows betadine for cleaning the port in a sterile fashion; at som institutions, this may have been changed to chlorhexidine.

- The collection of CSF from the EVD system is performed by trained Neuro ICU nurses or physicians; however, trained research personnel may be granted permission at your institution (check local hospital protocols).

- At most centers, collection of 0.5 – 1 mL of CSF is routinely done daily, to monitor for infection. CSF for research purposes will in most cases be collected at the same time as the daily routine accession of the system.
  - If insufficient CSF is produced, priority will be given for fluid required for patient care.

- An effort should be made to collect the first CSF available at the time of insertion of the EVD. Up to 5 mL should be collected.

1.2 CSF Collection

Steps for Whole CSF Collection

1. CSF is collected daily from the buretrol. If a bag change occurs in the morning, allow at least 2 hours before collection from buretrol. Collect CSF no longer than 2 hours (+1 hour) after the am bag change.

2. **Fresh fluid** is collected as follows:
   a. Fluid is collected **using sterile technique** directly from the buretrol.
   b. Up to 5 mL is collected (although in most cases it will be less) and transferred to single polypropylene conical centrifuge tube.
   c. Fluid is allowed to drain into buretrol by gravity (**never aspirated**).

3. Cell contamination of ventricular CSF is a significant confound. To minimize, CSF is centrifuged.

4. Transfer fluid within 30 minutes of collection to the laboratory and centrifuge at 2000 RCF (x g) for 10 minutes.

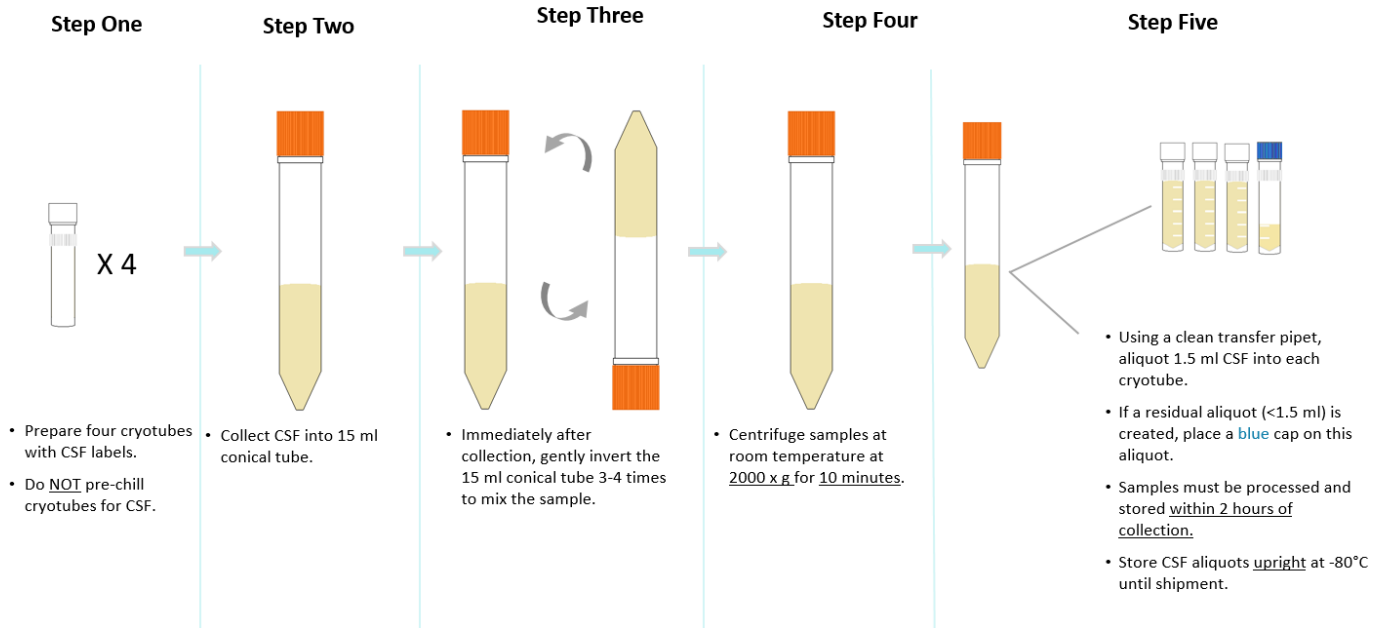
5. Aliquot supernatant into 1.5 ml polypropylene cryovials (provided) using a micropipette with disposable tip.
   a. Up to 4 aliquots are prepared, each containing to 1 .5 mL.
   b. Place a BLUE cap on residual aliqout (<1.5ml), if generated.

6. Place cryovials in 81-grid cryovial box and freeze samples immediately in -80^{o}C freezer.

7. The following are noted on the applicable CRF (Form 521a, 521b, or 521c):
   a. Appearance of fluid (clear, cloudy, bloody)
   b. Date and Time of collection
   c. Time of centrifugation and freezing

8. Collect CSF during the first 5 days of enrollment
   a. Whenever possible, collect the BioHOBIT blood sample at the same time as collecting the CSF sample. This will provide a paired blood sample for some of the CSF samples.

Sample Processing: CSF



Step One

- Prepare four cryotubes with CSF labels.
- Do NOT pre-chill cryotubes for CSF.

Step Two

- Collect CSF into 15 ml conical tube.

Step Three

- Immediately after collection, gently invert the 15 ml conical tube 3-4 times to mix the sample.

Step Four

- Centrifuge samples at room temperature at 2000 x g for 10 minutes.

Step Five

- Using a clean transfer pipet, aliquot 1.5 ml CSF into each cryotube.
- If a residual aliquot (<1.5 ml) is created, place a blue cap on this aliquot.
- Samples must be processed and stored within 2 hours of collection.
- Store CSF aliquots upright at -80°C until shipment.

Appendix K – Frozen Shipping Instructions

IMPORTANT!

FROZEN SAMPLES MUST BE SHIPPED MONDAY THROUGH WEDNESDAY ONLY USING PRIORITY OVERNIGHT DELIVERY

Please be aware of holidays and inclement weather, and plan your shipments accordingly.

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 Appenxi Q.

2. Place all frozen labeled aliquots of plasma, serum, CSF, and buffy coat in the cryobox. Only include specimens from one subject in each cryobox.

3. Place the cryobox in the clear plastic biohazard bag. Leave the absorbent sheet in the biohazard bag and seal according to the instructions on the biohazard bag. Affix a Kit Label of those kits included in cryobox to the outside of the biohazard bag.

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

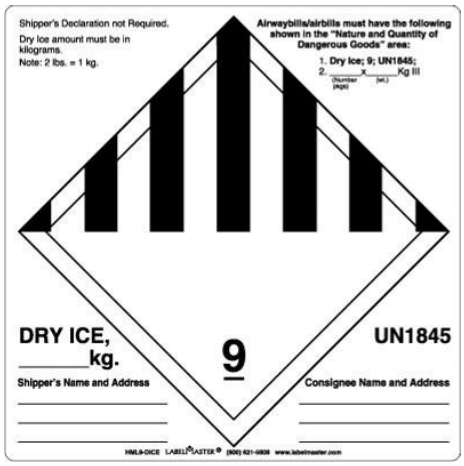
5. Place the biohazard bag containing the cryobox into the provided Styrofoam® shipping container on top of the dry ice. Please ensure that the cryobox is placed so that the cryovials are upright in the shipping container (as pictured).

6. Fully cover the cryobox with approximately 2 inches of dry ice. Please do not include more than two subjects' cases in a single box.

7. 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 and be placed on top of the cryoboxes to ensure the frozen state of the specimens.

8. Replace the lid on the Styrofoam® container. Close and seal the outer cardboard shipping carton with packing tape.

9. Complete the Class 9 UN 1845 Dry Ice Label 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 airbill)
- 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.

10. Apply all provided warning labels (UN3373, Dry Ice Label and Fragile Label) as well as the completed UPS® return airbill to the outside of package, taking care not to overlap labels.

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

12. 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

13. Use courier tracking 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 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.