



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

BIOSPECIMEN COLLECTION & PROCESSING

Overview

1. Specimen uniformity and quality
2. Site Equipment
3. Procedures
 - Kit Contents
 - Sample Labelling
 - Sample Collection and Processing
 - Shipping Samples
 - Non-Conformance
4. Contact Information

Specimen Uniformity and Quality

GENERAL REMINDERS

Specimen Uniformity and Quality

Most biomarkers are sensitive to *time* and *temperature*

- Standardization of processing across sites is key
- Specimens must be processed within 2 hours of collection
- Reference the *BioSEND Specimen Collection, Processing, and Shipment Manual* as needed
- Do not replace or supplement any kit components without first receiving approval from BioSEND/NINDS

Questions? Email biosend@iu.edu

Site Equipment

Sites will need to supply the following items:

- Gloves
- Alcohol wipes
- Butterfly needles
- Tourniquet
- Gauze pads
- Bandages
- Microcentrifuge tube rack
- Sharps bin and lid
- Crushed ice
- Pipettes and pipette tips
- Centrifuge capable of maintaining 4°C
- -80°C Freezer
- Dry ice

Procedures

MAINTAINING SPECIMEN UNIFORMITY AND QUALITY

Collection Protocol

Visit & Sample Type	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 aliquots, 1500ul	4	4	4	4	4	4	0	0	0

Kit Contents and Ordering

- Kit and supply ordering will be through WebDCU
- Sites will be sent an initial shipment of 24 visit kits

Kit Contents and Ordering

Standard Kit:

- Contains supplies for blood and CSF
- Can be used for any time point



Kit Contents and Ordering

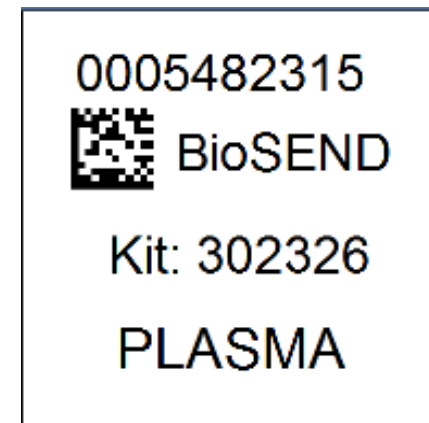
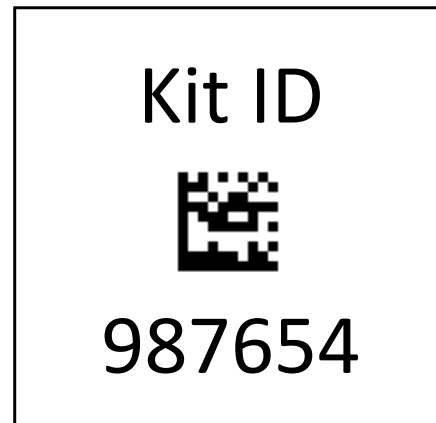
Shipping Kit:



Sample Labelling

Labels are provided by Indiana University

- Please check that all samples are properly labelled to ensure correct identification by IU
- If do not have enough labels to complete a visit, please contact IU *immediately*
- Labelling the tubes during processing prevents sample mix-ups



Sample Labelling

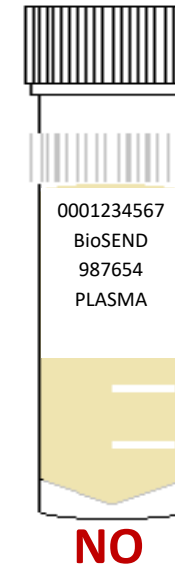
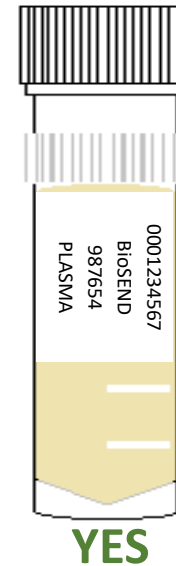
Kit labels are placed on packaging material



Sample Labelling

Please...

- Label all collection and aliquot tubes before cooling, collecting, processing, or freezing samples
- Label only 1 visit's tubes at a time to avoid mix-ups
- Wrap the label around the tube horizontally - label position is important for all tube types
- Make sure the label is completely adhered by rolling between your fingers



Sample Collection and Processing

Blood Tube Draw Order

7ml Serum



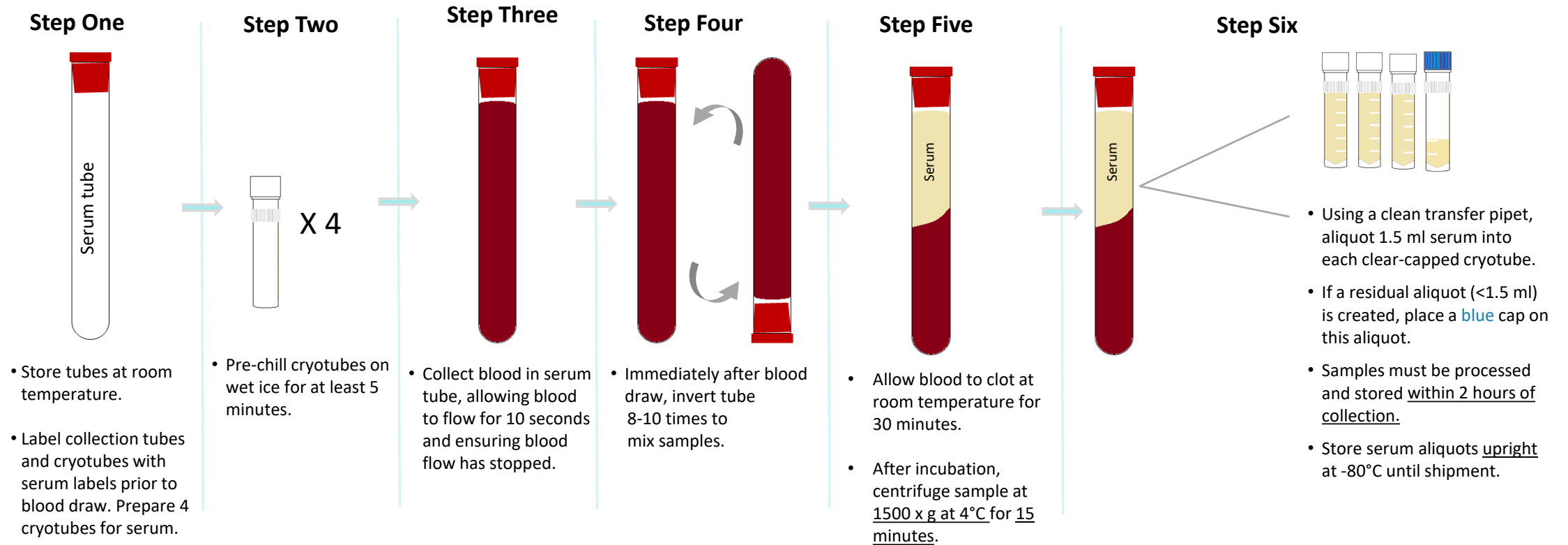
1

7ml EDTA

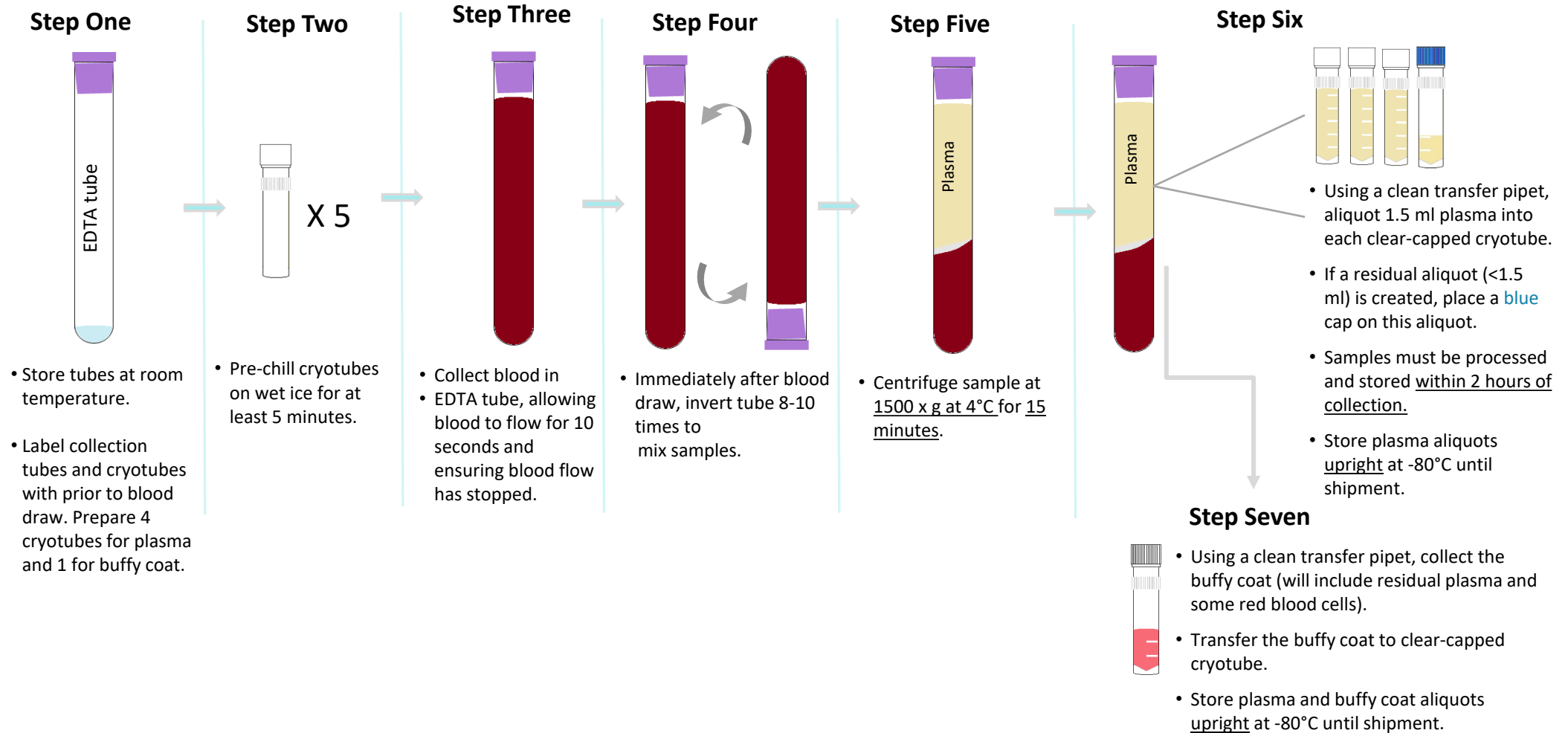


2

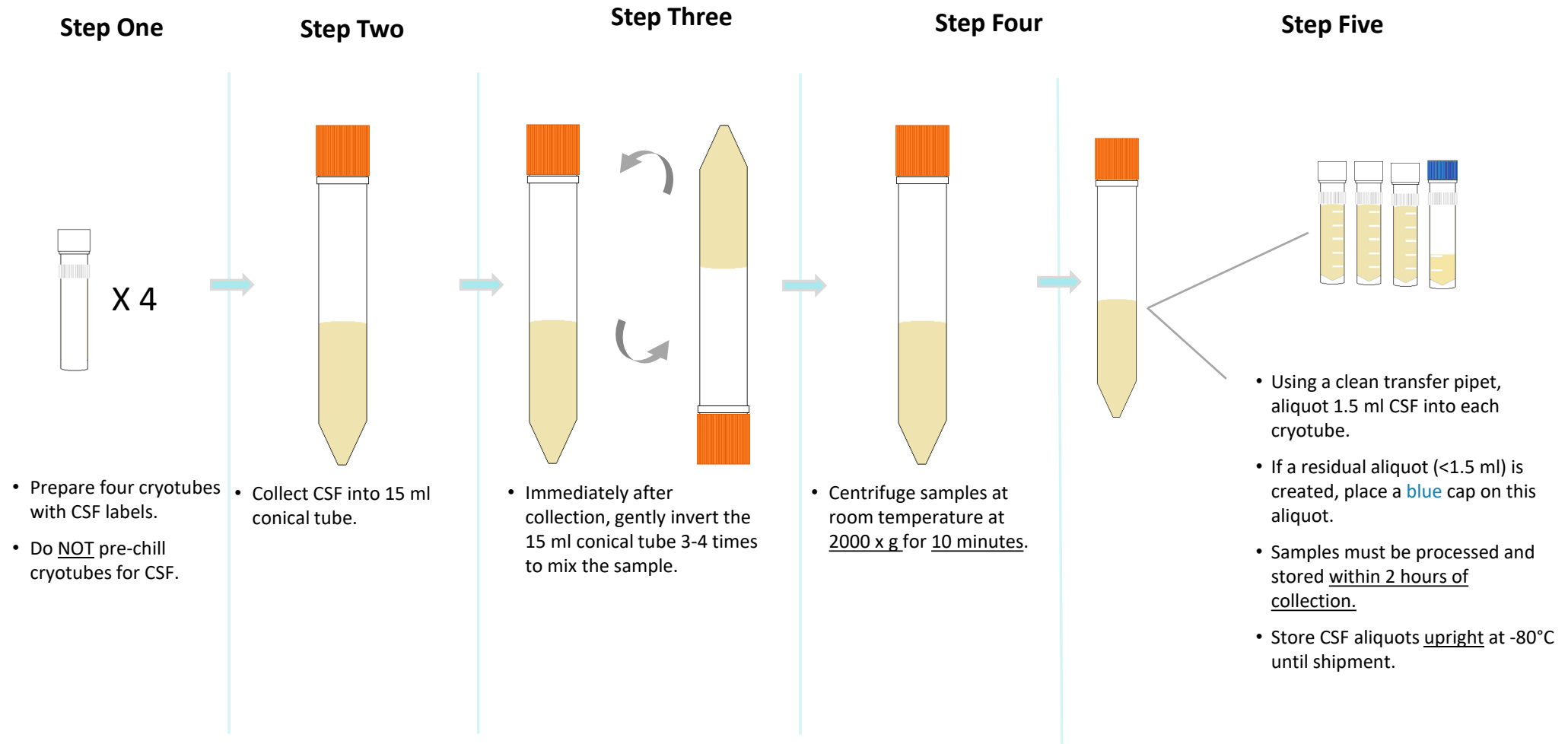
Sample Collection and Processing: Serum



Sample Collection and Processing: Plasma & Buffy Coat



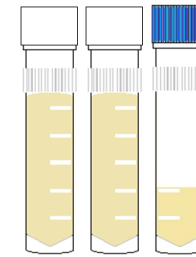
Sample Processing: CSF



Sample Collection and Processing: Aliquots

Filling biomarker serum, plasma, and CSF aliquots:

- Ensure that you are using the correct vials
 - Clear cap = CSF and buffy coat
 - Blue cap = residuals
- Fill as many cryovials as possible to 1.5 ml
- Over-filled vials may burst in freezer!
- Ship ALL material to IU, even if final vial is less than standard volume



YES



NO

Sample Collection and Processing

Troubleshooting Blood Collection

Issue #1: Tube with little/no vacuum

- Always check expiration date on the tube before beginning blood draw and discard expired tubes
- Store tubes at “room temperature” – extreme temperature can affect vacuum
- Keep extra collection tubes from supplemental kit nearby during blood draw to replace “bad” tubes
- If this is a frequent occurrence, report tube type and lot number to IU.

Sample Collection and Processing

Troubleshooting Blood Collection

Issue #2: Hemolyzed serum and/or plasma caused by incorrect collection

Cause: Blood Collection Methods	Corrective Action
Improper venipuncture site	Draw from median cubital, basilic, and cephalic veins from antecubital region of arm
Prolonged tourniquet use	Tourniquet should be released after no more than 1 min, excessive fist clenching should be avoided
Not allowing alcohol to dry on skin before venipuncture	Without touching, allow the venipuncture site to air dry
Use of too large/small bore needle resulting in excess force applied to blood	Avoid using too small/large needle. Needle size dependent on the subject's physical characteristics & amount of blood to be drawn. Most commonly used sizes are 19 – 23.
Pulling/pushing plunger too fast while drawing/transferring blood	Avoid drawing the syringe plunger too forcefully when collecting blood
Ensure all blood collection assemblies are fitted securely, to avoid frothing	

For more information, visit: http://www.bd.com/vacutainer/pdfs/techtalk/TechTalk_Jan2004_VS7167.pdf

Sample Collection and Processing

Troubleshooting Blood Collection

Issue #2: Hemolyzed serum and/or plasma caused by incorrect processing

Cause: Sample Processing Methods	Corrective Actions
Vigorous mixing/shaking	Gently invert blood collection tube when mixing additive with specimen, follow guidelines in Biologics Manual regarding number of times to invert each type of tube
Not allowing serum to clot for recommended time	Serum tubes without clot activator should be allowed to clot for 60 min in a vertical position
Exposure to excessive heat or cold	Keep samples at ambient temperature until processing
Prolonged contact of serum/plasma with cells	Do not store uncentrifuged samples beyond recommended time

For more information, visit: http://www.bd.com/vacutainer/pdfs/techtalk/TechTalk_Jan2004_VS7167.pdf

Shipping Samples: Frozen

Packing and Shipping Frozen Samples

- All BioHOBIT samples ship frozen
- Ship frozen samples on dry ice
- Frozen samples should be shipped **only** Monday through Wednesday
- Always fill carton to **top** with dry ice
- Do not pack shipment until the day of pickup



Shipping Samples: Frozen

Packing and Shipping Frozen Samples

- Shippers use approx. 10lbs of dry ice
- Place layer of dry ice in between cryoboxes



Class 9 Dry Ice Label should not be covered with other stickers and must be completed, or FedEx will reject/return your package!

Shipping Samples

Packing and Shipping Frozen Samples

Shipper's Declaration not Required.

Dry Ice amount must be in kilograms.

Note: 2 lbs. = 1 kg.

Airwaybills / airmails must have the following:
1. Dry Ice; 9; UN 1845
2. $\frac{\text{Number}}{\text{(Number pkgs)}} \times \frac{\text{wt}}{\text{(wt)}} \text{ Kg}$

Net weight of dry ice in kg

Dry Ice kg.

Your name & address

Shipper's Name and Address

UN 1845

Consignee Name and Address

IU information and address

9

06426 1/01 FRD

The form is a diamond-shaped label with a black and white striped pattern. It contains fields for shipper and consignee information, a large '9' in the center, and the UN 1845 hazard code. Red arrows point to specific fields with explanatory text.

Shipping Samples

FedEx

- Complete "From" portion of FedEx airbill

MUR 1

FedEx Package
Express US Airbill

FedEx Tracking Number: 8094 7376 7946

Form ID No. 0215

Sender's Copy

1 From Please print and press hard.

Date _____ Sender's FedEx Account Number _____

Sender's Name _____ Phone () _____

Company _____

Address _____ Dept./Floor/Suite/Room _____

City _____ State _____ ZIP _____

2 Your Internal Billing Reference First 24 characters will appear on invoice. 46-846-95

3 To

Recipient's Name: NINDS BIOSEND Phone (317) 278-6158

Company: INDIANA UNIV SCHOOL OF MED.

Address: 850 W WALNUT ST # C15B Dept./Floor/Suite/Room _____
We cannot deliver to P.O. boxes or P.O. ZIP codes.

Address: _____
Use this line for the HOLD location address or for continuation of your shipping address.

City: INDIANAPOLIS State: IN ZIP: 46202-5188

0121575270

4 Express Package Service * To most locations.

Next Business Day

FedEx First Overnight
Earliest next business morning delivery to select locations. Friday shipments will be delivered on Monday unless Saturday Delivery is selected.

FedEx Priority Overnight
Next business morning*. Friday shipments will be delivered on Monday unless Saturday Delivery is selected.

FedEx Standard Overnight
Next business afternoon*. Saturday Delivery NOT available.

2 or 3 Business Days

FedEx 2Day A.M.
Second business morning*. Saturday Delivery NOT available.

FedEx 2Day
Second business afternoon*. Thursday shipments will be delivered on Monday unless Saturday Delivery is selected.

FedEx Express Saver
Third business day*. Saturday Delivery NOT available.

5 Packaging * Declared value limit \$500.

FedEx Envelope* FedEx Pak* FedEx Box FedEx Tube Other

6 Special Handling and Delivery Signature Options Fees may apply. See the FedEx Service Guide.

Saturday Delivery
NOT available for FedEx Standard Overnight, FedEx 2Day A.M., or FedEx Express Saver.

No Signature Required
Package may be left without obtaining a signature for delivery.

Direct Signature
Someone at Recipient's address may sign for delivery.

Indirect Signature
If no one is available at recipient's address, someone at a neighboring address may sign for delivery. For residential deliveries only.

Does this shipment contain dangerous goods?
One box must be checked.

No Yes (see attached Shipper's Declaration) Yes Shipper's Declaration not required Dry Ice, I UN 1845 _____ x _____ kg

Restrictions apply for dangerous goods — see the current FedEx Service Guide. Cargo Aircraft Only

7 Payment Bill to: Enter FedEx Acct. No. or Credit Card No. below.

Sender Acct. No. in Section 1 will be billed. Recipient Third Party Credit Card Cash/Check

FedEx Acct. No. 6224-7396-8

Total Packages _____ Total Weight _____ lbs. Total Declared Value¹ _____ \$

Deliveries when and where you want.
Learn about FedEx Delivery ManagerSM at fedex.com/delivery.

677

¹Our liability is limited to US\$100 unless you declare a higher value. See back for details. By using this airbill you agree to the service conditions on the back of this airbill and in the current FedEx Service Guide, including terms that limit our liability.
Rev. Date 5/15 • Part #162134 • ©1984-2015 FedEx • PRINTED IN U.S.A. SPN

Shipping Samples: Closures

Date	Holiday
January 1	New Year's Day
3 rd Monday in January	Martin Luther King, Jr Day
4 th Monday in May	Memorial Day
July 4	Independence Day (observed)
1 st Monday in September	Labor Day
4 th Thursday in November	Thanksgiving
4 th Friday in November	Friday after Thanksgiving
December 25	Christmas

IU will be closed for shipments the week of Thanksgiving (11/23-11/27)

Non-Conformance

Non-conformance to standard procedures may reduce the utility of the biospecimens:

- Not processing serum/plasma within 2 hours of collection allows for breakdown of certain proteins and small molecules
- Over/under centrifuging changes plasma, serum, CSF composition



Non-Conformance Reporting

Most common non-conformance issues:

- Samples shipped for weekend/holiday delivery
- Sample form incomplete/inaccurate
- Unlabeled or mislabeled tube(s)
- Sample hemolysis



Contacts

Indiana University

General Questions:

biosend@iu.edu

Biorepository Project Manager:

Claire Wegel

cwegel@iu.edu

Tel: 317.278.6158

Biorepository Clinical Research Coordinator:

Carolyn Dunifon

cdunifon@iu.edu

Tel: 317.274.5751