



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

BIOSPECIMEN COLLECTION & PROCESSING

Overview

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

Specimen Uniformity and Quality

GENERAL REMINDERS

Specimen Standardization 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 Biomarker 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 Consumables and 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

Biospecimen Collection Protocol - Blood

Collection Tube	Drawn At	Specimen Type	Aliquot Volume	Total Number of Aliquots	Cryovial Cap Color	Shipping Temperature
1 EDTA (plastic) Blood Collection Tubes, 10ml	Baseline	Plasma	1.5ml	3	Purple	Frozen
	Baseline	Buffy Coat	~750ul	1	Clear	Frozen
1 SERUM (plastic) Blood Collection Tubes, 10ml	Baseline	Serum	1.5ml	3	Red	Frozen

Biospecimen Collection Protocol - Tissue

Collection Tube	Drawn At	Specimen Type	Aliquot Volume	Total Number of Aliquots	Shipping Temperature
2 Prefilled Formalin Tubes, Green-top, 30ml 2	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 Fresh Frozen (no preservative), 30ml	Baseline	Membrane	1 section	1	Frozen
	Baseline	Dura	1 section	1	Frozen
Individually wrapped conical tube, 15ml	Baseline	Subdural Hematoma Fluid	15ml	1	Frozen

Kit Contents and Ordering

- All sites will be sent a Supplemental Kit with their first kit shipment
 - Contains extra blood collection tubes and processing supplies
 - May be used to replace items in study visit kits
- Study Visit Kits should be ordered as soon as visits are planned
 - Includes barcoded labels, collection, processing, and shipping supplies
 - The contents of a kit are not tied to a specific subject or time point but each kit should only be used for 1 subject

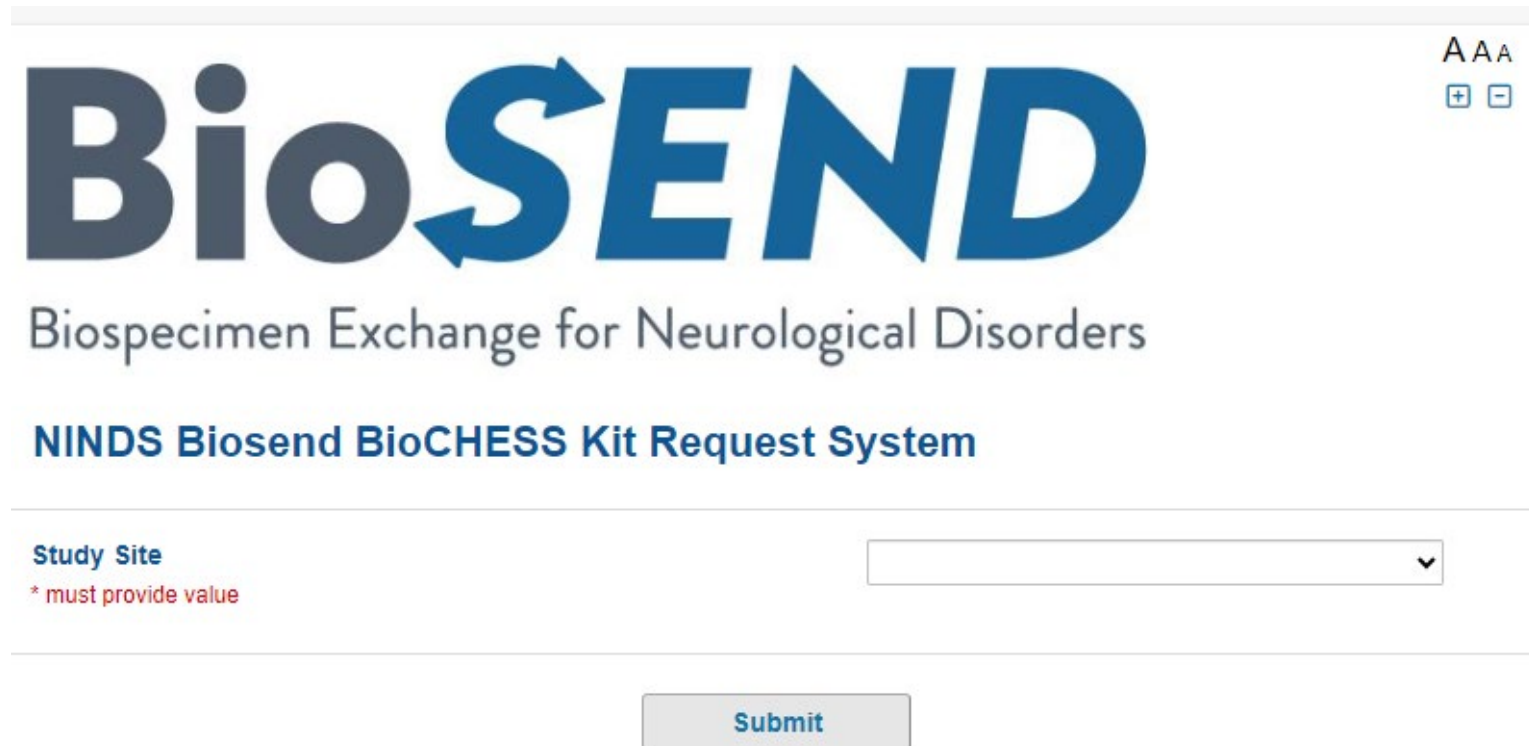
Kit Contents and Ordering – REDCap Survey

<https://redcap.link/biochesskitrequest>

Order kits online through the Kit Request Module for:

- Blood kits
- Shipping kits
- Extra Supplies

Please provide as much notice as possible when ordering kits and/or supplies.



The screenshot shows the NINDS Biosend BioCHESS Kit Request System interface. At the top, the logo "BioSEND" is displayed in large blue letters, with the "S" stylized as a circular arrow. Below the logo, the text "Biospecimen Exchange for Neurological Disorders" is shown in a smaller font. Underneath that, the title "NINDS Biosend BioCHESS Kit Request System" is displayed in a bold blue font. In the top right corner, there are accessibility icons for text size (AAA) and a plus/minus icon. Below the title, there is a form field labeled "Study Site" in blue text, with a red asterisk and the text "* must provide value" below it. To the right of the label is a dropdown menu with a downward arrow. At the bottom of the form, there is a grey "Submit" button.

BioSEND
Biospecimen Exchange for Neurological Disorders
NINDS Biosend BioCHESS Kit Request System

Study Site

* must provide value

Submit

Kit Contents and Ordering: Confirm Site Info

BioCHESS Kit Request Module

Study Site * must provide value	Indiana University	Select your site from the drop-down list
Indiana University School of Medicine Carolyn Dunifon Dept. of Medical & Molecular Genetics 351 West 10th Street, TK-318 Indianapolis, IN 46202-3002 (317) 274-5751 cdunifon@iu.edu		
Is the contact name above correct? * must provide value		Verify contact information and update if needed
		☐ Yes ☒ No reset
New Contact Name * must provide value		Claire Wegel
Is the shipping address above correct? * must provide value		☐ Yes ☐ No reset
Is the e-mail address above correct? * must provide value		☐ Yes ☐ No reset
Is the phone number above correct? * must provide value		☐ Yes ☐ No reset

Kit Contents and Ordering: Kit Types

BioCHESS Kit Request Module

- Kits are not specific to a subject or time point. After collection, sites will indicate the subject and time point to which BioSEND should link the samples.
- Standard collection kit for plasma, buffy coat, serum, and tissue contains supplies for one subject-visit.
- Shipping Kits are ordered independently of Blood Kits and contain supplies for one subject-visit.

Kit Type

****Please allow two weeks for shipment****

*** must provide value**



Blood and Tissue Collection Kit



Frozen Shipping Kit



Ambient Shipping Kit



Extra Supplies

Please specify in comments if you need kits before the standard two week shipment time.

Kit Contents and Ordering: Kit Breakdown

BioCHESS Kit Request Module

Comments

Expand

Each Blood Collection Kit contains:

- 1 - EDTA tube, 10ml (plastic)
- 1 - Serum tube, 10ml (plastic)
- 3 - Cryovial (Sarstedt®) with purple cap, 2ml
- 3 - Cryovial (Sarstedt®) with red cap, 2ml
- 1 - Cryovial (Sarstedt®) with clear cap, 2ml
- 1 - Individually wrapped conical, 15ml
- 2 - Prefilled Formalin tube, 30ml
- 2 - Prefilled *RNA Later*TM tube, 30ml
- 2 - Green top tube, 30ml
- 2 - Disposable pipette, 3ml
- 2 - Resealable tube pouch
- 1 - Cryobox, 25 slot
- 1 - Specimen/Case label set-- Kit numbers



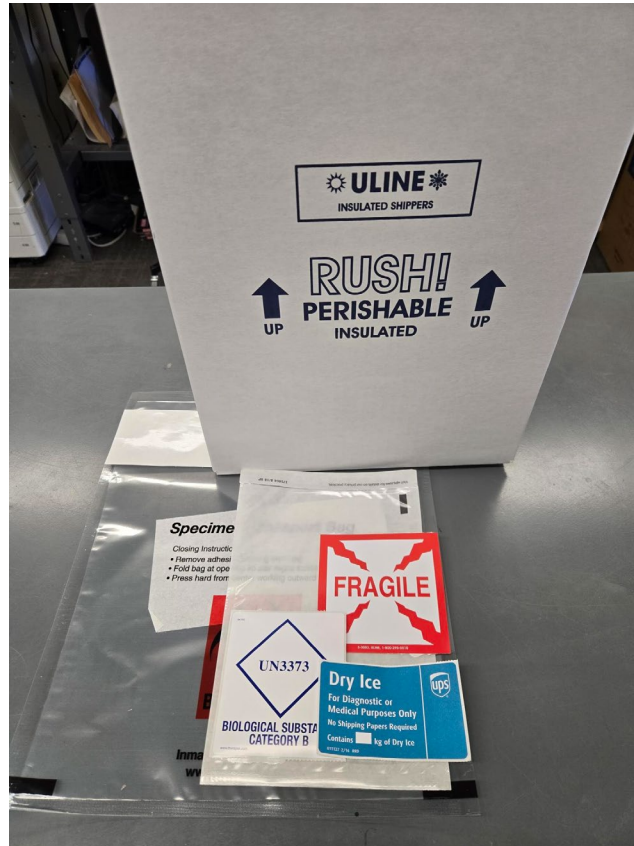
Kit contents of selected kit will appear at the bottom of the page

Submit

Kit Contents and Ordering: Blood Kits



Kit Contents and Ordering: Shipping kits



Frozen shipping kit



Ambient shipping kit

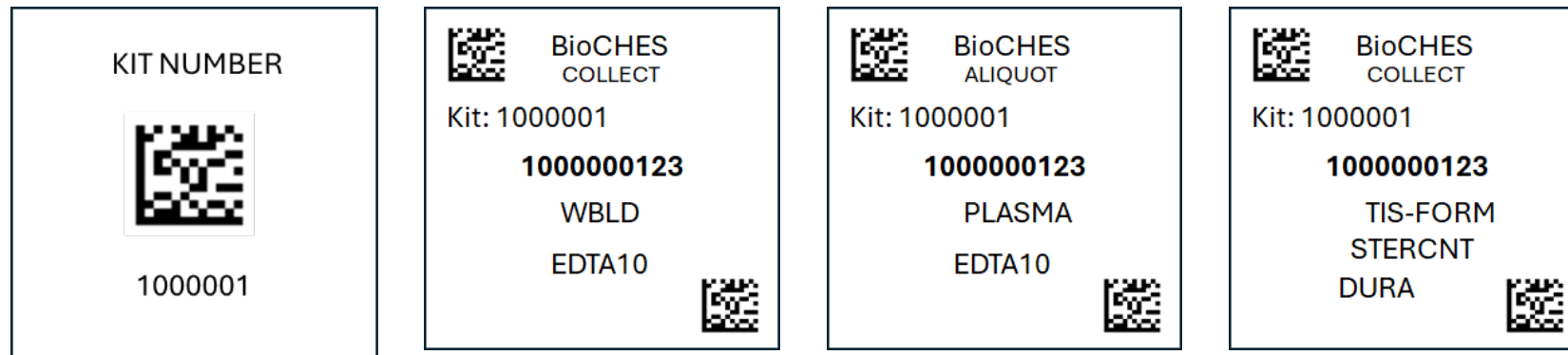
Kit and Supply Ordering

- Click “Submit” to send order to BioSEND; staff will confirm receipt of your order
- Please allow two week turn-around time for kit shipments
- If urgent request needed, please note date needed by in comments and email BioSEND. We cannot guarantee urgent orders, but we will do our best to accommodate.
- BioSEND will send confirmation of shipment and tracking number when supplies ship

Sample Labelling: Example Labels

Labels are provided by Indiana University

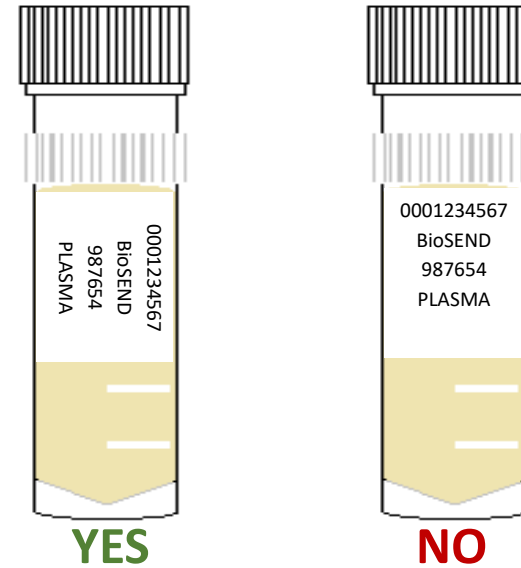
- Please check that all samples are properly labelled to ensure correct identification by IU
- If do not have enough labels to complete a visit, please contact IU *immediately*
- Labelling the tubes during processing prevents sample mix-ups



Sample Labelling: Label Placement

Please...

- Label all collection and aliquot tubes before cooling, collecting, processing, or freezing samples
- Label only 1 subject'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



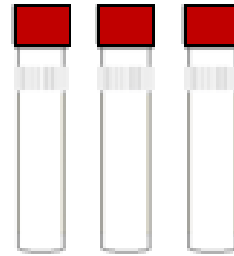
Collection Volumes

Total blood volumes

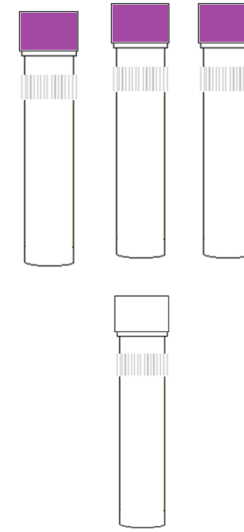
Sample Type	Amount
Whole Blood for Plasma and Buffy Coat	10 ml
Whole Blood for Serum	10 ml

Sample Collection and Processing - Blood

Supplies provided for the collection of serum, plasma and buffy coat

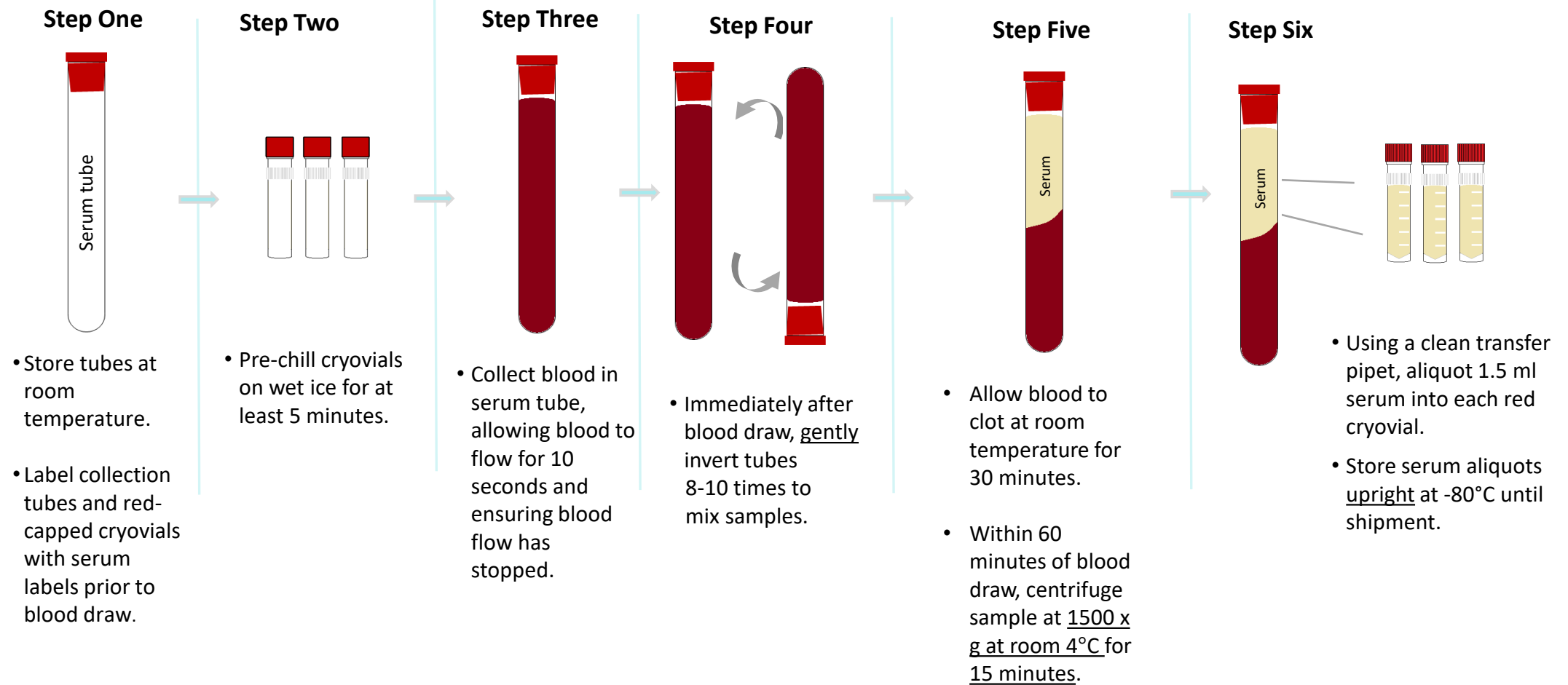


1 x 10ml Serum
(plastic) collection
tubes

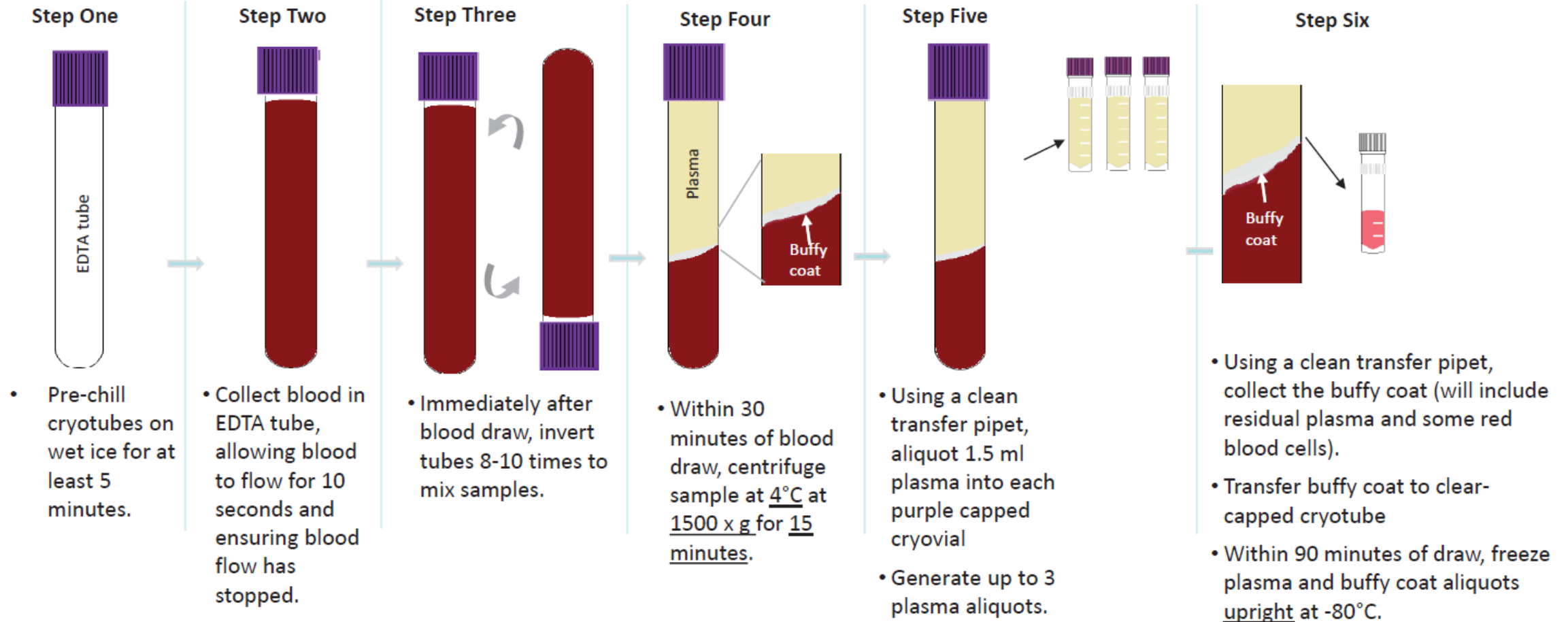


1 x 10ml EDTA
(plastic) collection
tubes

Sample Collection and Processing: Serum



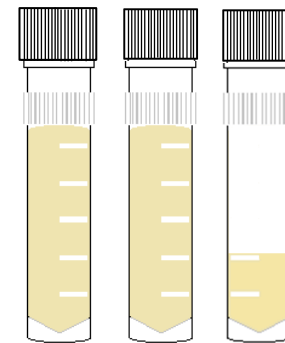
Sample Collection and Processing: Plasma & Buffy Coat



Sample Collection and Processing: Aliquots

Filling biomarker plasma and serum aliquots:

- Fill as many cryovials as possible to 1.5 ml (plasma and serum)
- Over-filled vials may burst in freezer!
- Ship ALL material to IU, even if final vial is less than standard volume



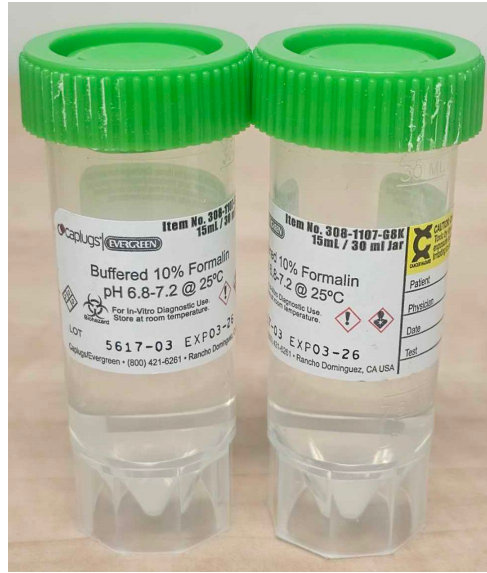
YES



NO

Sample Collection and Processing

Supplies provided for the collection of SDH Fluid, dura and membrane tissue in Formalin, RNALater, and Fresh Frozen (no preservative)



2 x 30ml tubes with pre-filled Formalin



2 x 30ml tubes with RNALater™



2 x 30ml tubes Fresh-frozen samples (no preservative)



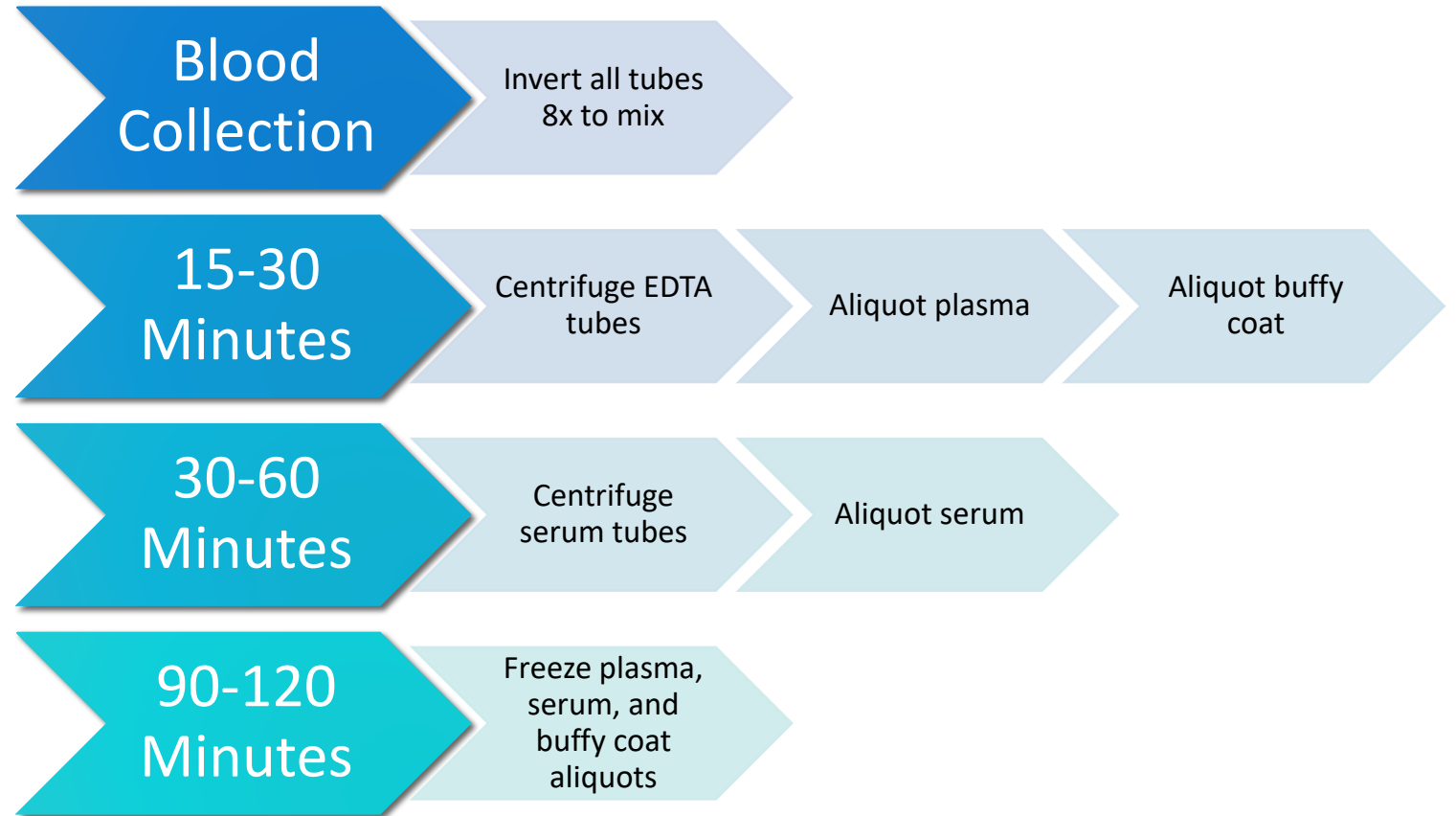
1 x 15ml tube for SDH Fluid

Sample Collection and Processing: Dura, Membrane, and SDH Fluid

- Please maintain the sterile field and avoid puncturing the brain
- Dissect and cut Dura and Membrane sample into three sections.
- Collect 15ml of SDH Fluid and place in sterile conical
- Tissue samples for Dura and Membrane will be collected in the following order priority (1) Formalin, (2) *RNALater*TM, and (3) fresh frozen (no preservative).
- Fully submerge Dura and Membrane tissue in Formalin and *RNALater*TM
- Tighten caps on all tubes to avoid leaking in transit

Sample Collection and Processing: Timeline

Timeline for blood processing



Sample Collection and Processing: Issue #1

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 vacutainer 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: Issue #2

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, basalic, and cephalic veins from antecubital region of arm
Prolonged tourniquet use	Tourniquet should be released after no more than 1 min, excessive fist clenching should be avoided
Not allowing alcohol to dry on skin before venipuncture	Without touching, allow the venipuncture site to air dry
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: Issue #2 continued

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

Sample Collection and Processing Form

Specimen Collection and Processing Form

<https://redcap.link/BioCHESSSampleForm>

First page captures basic subject and visit information



Biospecimen Exchange for Neurological Disorders Protocol: BioCHESS

Please complete the Specimen Collection and Processing Form, below.

Returning?

AAA



Page 1 of 4

Study Site

Email address of staff member completing this form

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

BioCHESS Subject ID:

Subject's sex (used for DNA quality control)

IU Kit Number

7 characters remaining

Next Page >>

Save & Return Later

Sample Collection and Processing Form

Second page captures processing information

AA_A

+

-

BioSEND

Biospecimen Exchange for Neurological Disorders

Page 2 of 4

Tissue Collection

Date of tissue collection

31

Today

M-D-Y

Time of tissue collection

Now

MM

If unknown, provide time of surgical procedure start.

Use 24 Hour clock.

Was Dura collected in Pre-filled formalin?

Yes

No

reset

Was Dura collected in Pre-filled RNALater?

Yes

No

reset

Was Dura collected for Fresh Frozen (no preservative)?

Yes

No

reset

Sample Collection and Processing Form

PDF form of responses will be emailed to you. Print a copy of the Frozen Shipping Manifest and include with shipment.

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:

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?

Sample Collection and Processing Form

PDF form of responses will be emailed to you. Print a copy of the Ambient Shipping Manifest and include with shipment.

Sample Collection and Processing Form

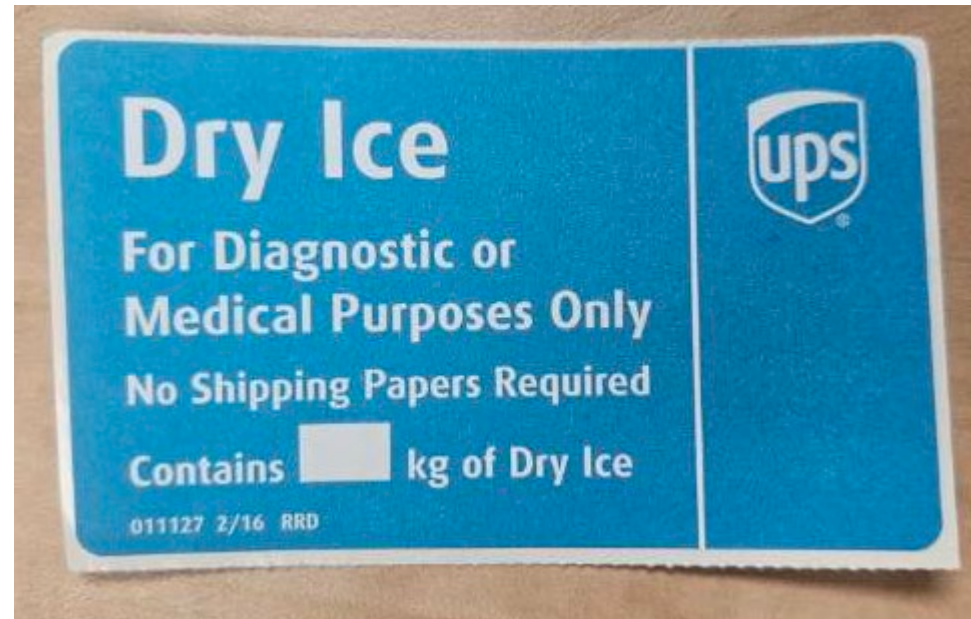
Submission of the Frozen and Ambient Shipping Manifest portion of the form serves as shipment notification to BioSEND

- Must be completed prior to shipment
- If samples are shipped and not received, BioSEND will follow-up with courier. It is recommended that sites also track shipment to ensure safe delivery

Shipping Samples

Packing and Shipping Frozen
Samples

UPS Dry Ice label should not be covered with other stickers
and must be completed, or UPS will reject/return your
package!



Shipping Frozen Samples: Tips

Packing and Shipping Frozen Samples

- Serum, Plasma, Buffy coat, Subdural Hematoma Fluid, and fresh frozen (no preservative) Dura / Membrane sections will ship frozen on dry ice
- Please verify that all caps are screwed on tightly to prevent leakage in transit.
- 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

Do not ship more than 1 biohazard bags in a single shipper

- Allows room for dry ice to keep samples frozen in transit
- Minimizes loss in the rare but unfortunate event of courier issues



Shipping Ambient Samples: Tips

Packing and Shipping Ambient Samples

- Dura / Membrane sections in Formalin and *RNALater*[™] will ship Ambient
- Ambient samples should be shipped same day as collection.
- Ambient samples collected on Friday can be refrigerated or left at room temperature and shipped the following Monday.
- Please verify that all caps are screwed on tightly to prevent leakage in transit



Shipping Samples – UPS: <https://kits.iu.edu/UPS>

The screenshot shows the 'Shipping Form' for the Indiana University School of Medicine. The form is divided into two main sections: 'Ship From' and 'Shipment Information'. The 'Ship From' section contains fields for Code, Company, Contact, Address 1, Address 2, Address 3, City, State/Province, Postal Code, and Country/Territory. The 'Shipment Information' section contains fields for Study Group, Weight, Dry Ice Weight, and Description of Return. A 'Pickup Request' button is located between the two sections. At the bottom right, there are 'Reset' and 'Ship' buttons. Annotations with arrows point to various parts of the form: 'Search for address' points to the search icon in the 'Ship From' section; 'Choose Study' points to the 'Study Group' dropdown; 'Enter weight' points to the 'Weight' and 'Dry Ice Weight' fields; 'Schedule Pickup' points to the 'Pickup Request' button; and 'Click "Ship"' points to the 'Ship' button.

INDIANA UNIVERSITY
SCHOOL OF MEDICINE

and Affiliated Biorepositories

Ship From

Search for address

Code

Company

Contact

Address 1

Address 2

Address 3

City

State/Province

Postal Code

Country/Territory

Shipment Information

Choose Study

Study Group

Weight

Dry Ice Weight

Description of Return

Enter weight

Pickup Request

Schedule Pickup

Click "Ship"

Reset Ship

Shipping Samples via UPS

IU UPS ShipExec Shipping Portal

- Print out UPS air waybill
- Ensure all elements (barcode, return address, etc.) printed clearly
- Fold and insert UPS air waybill into clear plastic sleeve on package

JOHN SMITH INDIANA UNIVERSITY 410 WEST 10TH STREET INDIANAPOLIS IN 46202	2 LBS	1 OF 1
RS		
SHIP TO: SCHOOL OF MEDICINE 317-278-2694 INDIANA UNIVERSITY TK 217 351 W 10TH ST INDIANAPOLIS IN 46202		
	IN 461 9-01	
		
UPS NEXT DAY AIR		1
TRACKING #: 1Z 976 R8W 84 3985 8595		
 SAMPLE		
BILLING: P/P DESC: Biological Specimens RETURN SERVICE		
Reference No.1: 4087277		
XOL 20.03.09 NV45 83.0A 12/2019		 TM

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
June 19	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

Please also consider weather when shipping. UPS will post service updates on their webpage. Reach out to BioSEND if you are unsure if it is safe to ship.

Non-Conformance

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

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



Non-Conformance Reporting con't

Most common non-conformance issues:

- Shipment notification not sent
- Samples shipped for weekend/holiday delivery
- Sample form incomplete/inaccurate
- Low volume
- Unlabeled or mislabeled tube(s)
- Sample hemolysis



Non-Conformance and Inventory Reporting

Most common non-conformance issues:

- BioSEND will notify sites directly of any issues upon receipt
- BioSEND will email sites a monthly inventory report of all samples received from that site to date
- ***If you are experiencing issues, please reach out to us for help! It is much easier to prevent an issue before sample collection & shipment than trying to fix it after the fact***

BioSEND.org

On the website, you can:

- Access your study's kit request module and sample submission form
- Download the most recent version of the Manual of Procedures
- View a recording of this training
- Find information about holiday closures
- Access shipping resources

Study Resources

KIT REQUEST MODULE

Please follow the below link to access the Kit Request Module. This link will direct you to a REDCap database where study coordinators and staff may request kits, individual supplies, and/or labels. Please allow a total of two weeks for kit requests to be fulfilled.

[Kit Request System →](#)

SPECIMEN COLLECTION AND PROCESSING FORM

Please use the below link to access the collection and processing form for this protocol. This form must be completed prior to shipment. We also ask that all shipments include a physical copy of the shipping manifest portion of the form.

[Specimen Collection and Processing Form →](#)

MANUAL OF PROCEDURES

The below downloadable manual was created specifically for the DxCTEII study. Please feel free to explore the manual through the hyperlinked 'Table of Contents'. Questions concerning any part of the manual may be directed to biosend@iu.edu for further clarification.

[Manual of Procedures ↓](#)

TRAINING SLIDES

These slides correspond to the BioSEND DxCTEII protocol training. Training is available upon request by contacting biosend@iu.edu.

[Training Slides ↓](#)

SAMPLE SHIPPING

BioSEND can receive samples Monday-Friday, excluding holidays. Frozen samples should be shipped M-W. Ambient samples may be shipped on Th.

[Generate UPS airbill or schedule pickup →](#)
[Check holiday closures →](#)
[What do I do for Friday blood draws →](#)

Contacts

Indiana University

General Questions/Shipment Notifications:

biosend@iu.edu

317-278-6158

Request kits:

<https://redcap.link/biochesskitrequest>

Request sample form:

<https://redcap.link/BioCHESSSampleForm>