



Biospecimen Exchange for Neurological Disorders

## **Microbiome-gut-brain dysfunction in prodromal and symptomatic Lewy body diseases (GutBrainLBD)**

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BIOSPECIMEN COLLECTION & PROCESSING

# Overview

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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

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GENERAL REMINDERS

# Specimen Standardization and Quality

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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](mailto:biosend@iu.edu)

# Site Consumables and Equipment

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*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

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MAINTAINING SPECIMEN UNIFORMITY AND QUALITY

# Biospecimen Collection Protocol

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	BL
<b>Whole Blood, 3ml</b>	X
<b>Buffy coat</b> (2 aliquots)	X
<b>Plasma</b> (6 x 1.5ml)	X
<b>Urine</b> (2 x 10ml)	X
<b>Stool</b>	X

*Note that BioSEND will provide an additional collection tube and cryovials for plasma and buffy coat to be retained at site. Those samples should be labeled and stored according to local site protocol.*

# Kit Contents and Ordering

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- 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
  - Contains collection, processing, and shipping supplies specific to each visit
  - Includes barcoded labels
  - The supplies/labels in each study visit kit are intended for that visit only



# Kit Contents and Ordering – REDCap Survey

- <http://kits.iu.edu/biosend/gutbrainlbd>

Order kits online through the Kit Request Module for:

- Blood & Stool kits
- Shipping Kits
- Supplemental Kit
- Extra Supplies

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

**BioSEND**  
Biospecimen Exchange for Neurological Disorders  
**NINDS Biosend GutBrainLBD Kit Request System**

Study Site

Mind Research Network ▼

\* must provide value

**Mind Research Network**  
Sephira Ryman  
1101 Yale Blvd NE  
Albuquerque, NM 87106  
sryman@mrn.org  
505-363-4662

# Kit Contents and Ordering: Confirm Site Info

GutBrainLBD Kit Request Module

<b>Study Site</b> <small>* must provide value</small>	Indiana Univers	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		
Verify contact information and update if needed		
<b>Is the contact name above correct?</b> <small>* must provide value</small>	<input type="radio"/> Yes <input checked="" type="radio"/> No	reset
<b>New Contact Name</b> <small>* must provide value</small>	Claire Wegel	
<b>Is the shipping address above correct?</b> <small>* must provide value</small>	<input type="radio"/> Yes <input type="radio"/> No	reset
<b>Is the e-mail address above correct?</b> <small>* must provide value</small>	<input type="radio"/> Yes <input type="radio"/> No	reset
<b>Is the phone number above correct?</b> <small>* must provide value</small>	<input type="radio"/> Yes <input type="radio"/> No	reset

# Kit Contents and Ordering: Kit Types

## GutBrainLBD 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.

**Kit Type**  
**\*\*Please allow two weeks for shipment\*\***  
**\* must provide value**

☒ Specimen Collection Kit

☐ Ambient (Stool) Shipping Kit

☐ Frozen (Blood/Urine) Shipping Kit

☐ Supplemental Kit

☐ Extra Supplies

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

**Specimen Collection Kit Quantity**  
**\* must provide value**

**Comments**

Expand

# Kit Contents and Ordering: Kit Breakdown

GutBrainLBD Kit Request Module

**Comments**

Expand

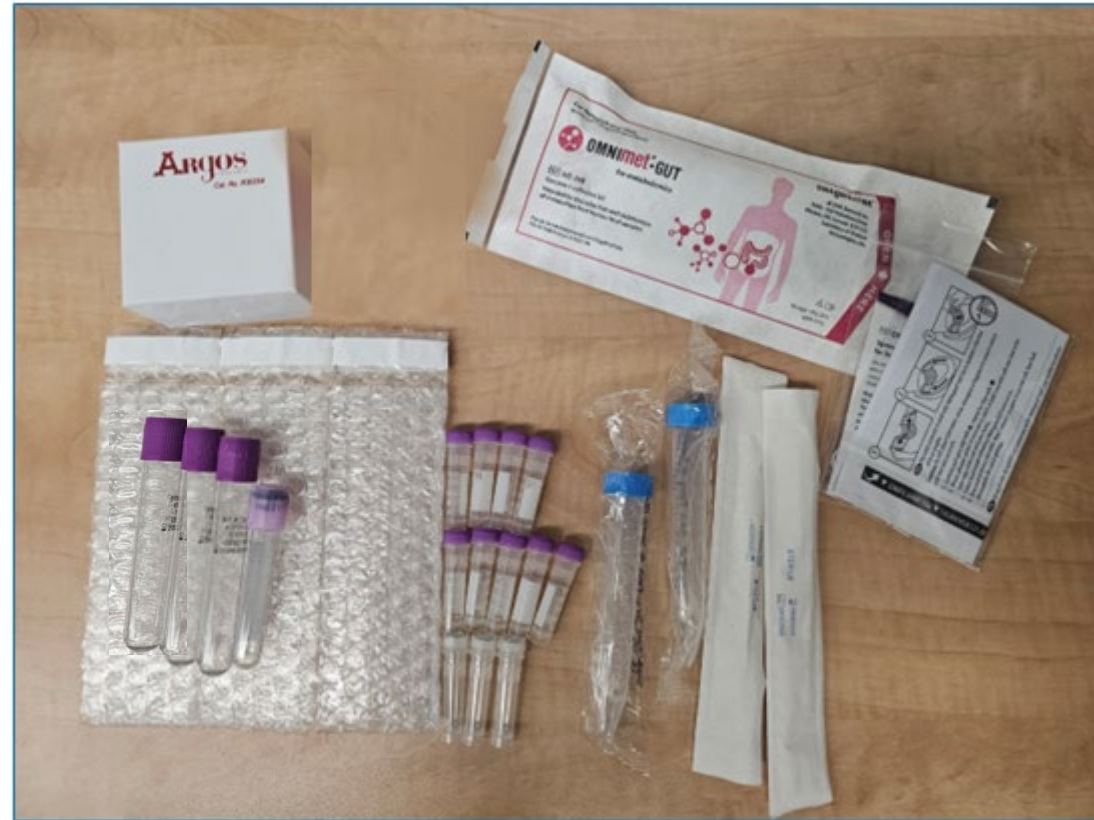
**Specimen Collection Kit contains:**

- 9 - Cryovial (Sarstedt®) with purple cap, 2ml
- 3 - Cryovial (Sarstedt®) with clear cap, 2ml
- 3 - EDTA (plastic) tube, 10ml
- 1 - EDTA (plastic) tube, 3ml
- 4 - Bubble-tube sleeve
- 1 - Urine Cup
- 2 - Individually Wrapped Conical Tube (15mL)
- 2 - Disposable pipet, 3ml
- 1 - Cryobox, 25 cell
- 1 - ME-200 stool kit
- 1 - OMR-200 stool kit
- 1 - Toilet accessory
- 1 - Spoon accessory
- 1 - Label set (Kit & Specimen labels)

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

# Kit Contents and Ordering: Collection Kit

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# Kit Contents and Ordering: Shipping Kits

Frozen Shipping Kit:



Ambient Shipping Kit:



# Collection Volumes

Total blood and urine volumes

Sample Type	Amount
Whole Blood for Plasma and Buffy Coat	20 ml
Whole Blood for Banking	3 ml
Urine	20 ml

# Kit and Supply Ordering

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- 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

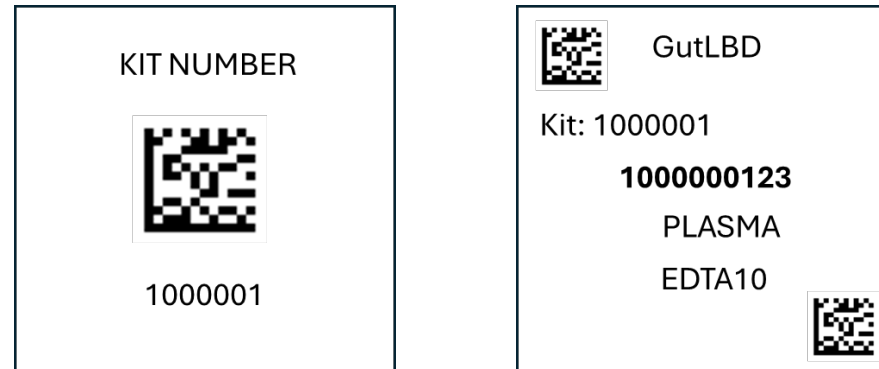


# Sample Labelling: Example Labels

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## 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

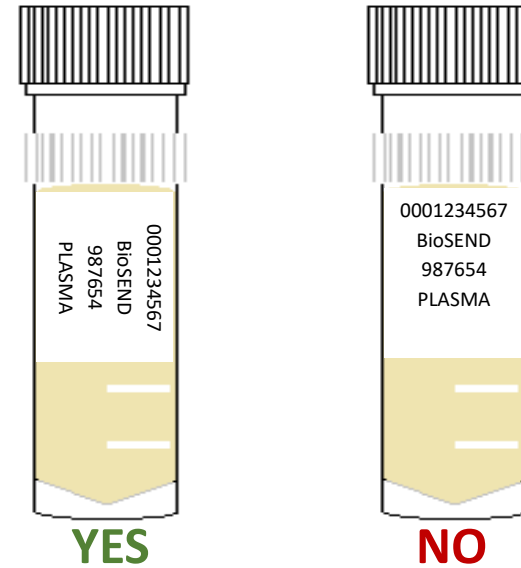


# Sample Labelling: Label Placement

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Please...

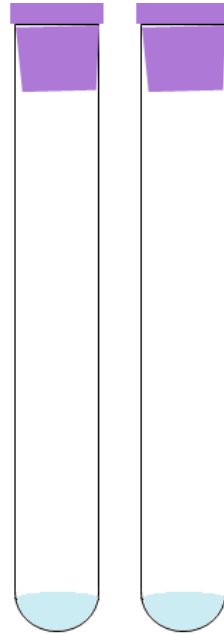
- Label all collection and aliquot tubes before cooling, collecting, processing, or freezing samples
- Label only 1 subject's tubes at a time to avoid mix-ups
- Wrap the label around the tube horizontally - label position is important for all tube types
- Make sure the label is completely adhered by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



# Sample Collection and Processing

*Blood Draw Order*

2 x 10ml EDTA (glass)  
collection tubes



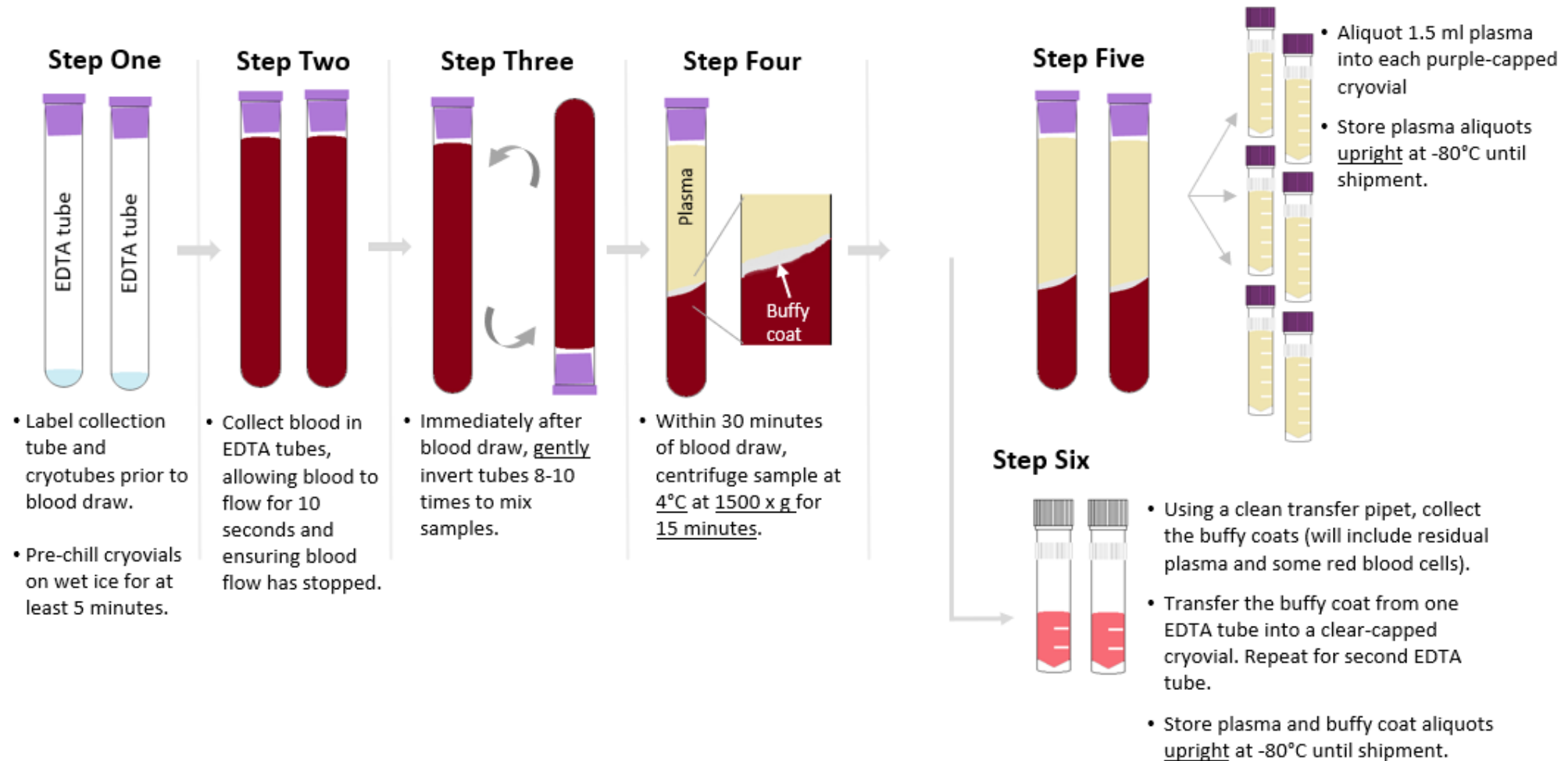
**1**

1 x 3ml EDTA for WBLD



**2**

# Sample Collection and Processing: Plasma & Buffy Coat



# Sample Collection and Processing: Whole Blood

## Step One



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

## Step Two



- Collect blood into 3ml EDTA tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

## Step Three



- Immediately after blood draw, invert tube 8-10 times to mix sample.

## Step Four



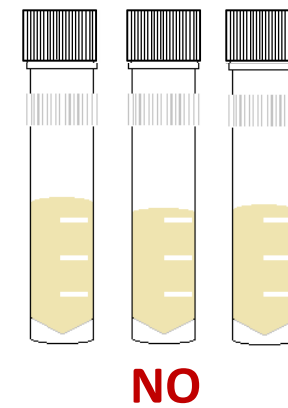
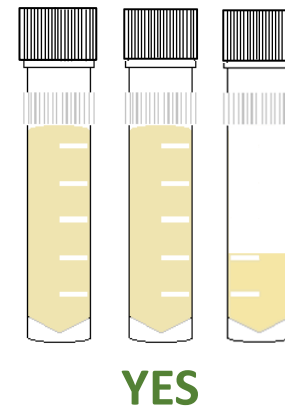
- Transfer to -80°C. Store upright and keep frozen until shipment to BioSEND.

# Sample Collection and Processing: Aliquots

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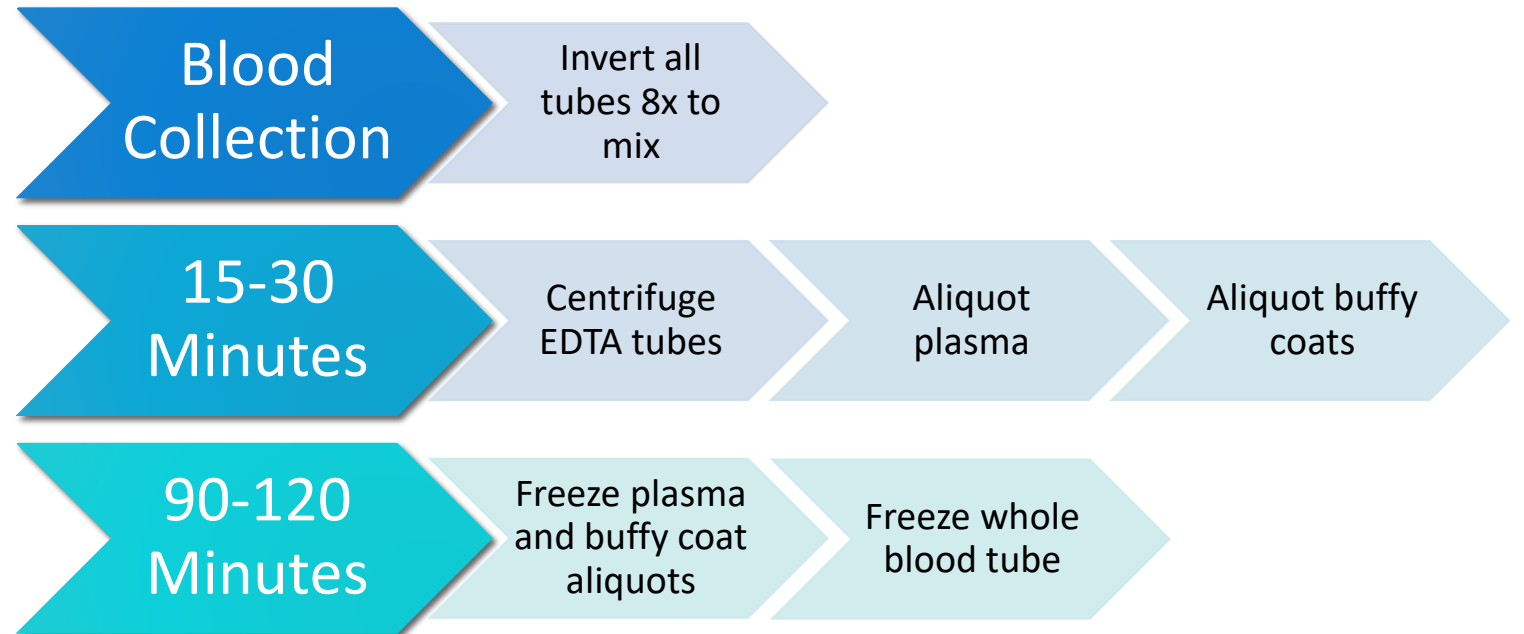
Filling biomarker plasma aliquots:

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



# Sample Collection and Processing: Timeline

Timeline for blood processing



# Sample Collection and Processing: Issue #1

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## 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

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## 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](http://www.bd.com/vacutainer/pdfs/techtalk/TechTalk_Jan2004_VS7167.pdf)

# Sample Collection and Processing: Issue #2 continued

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## 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

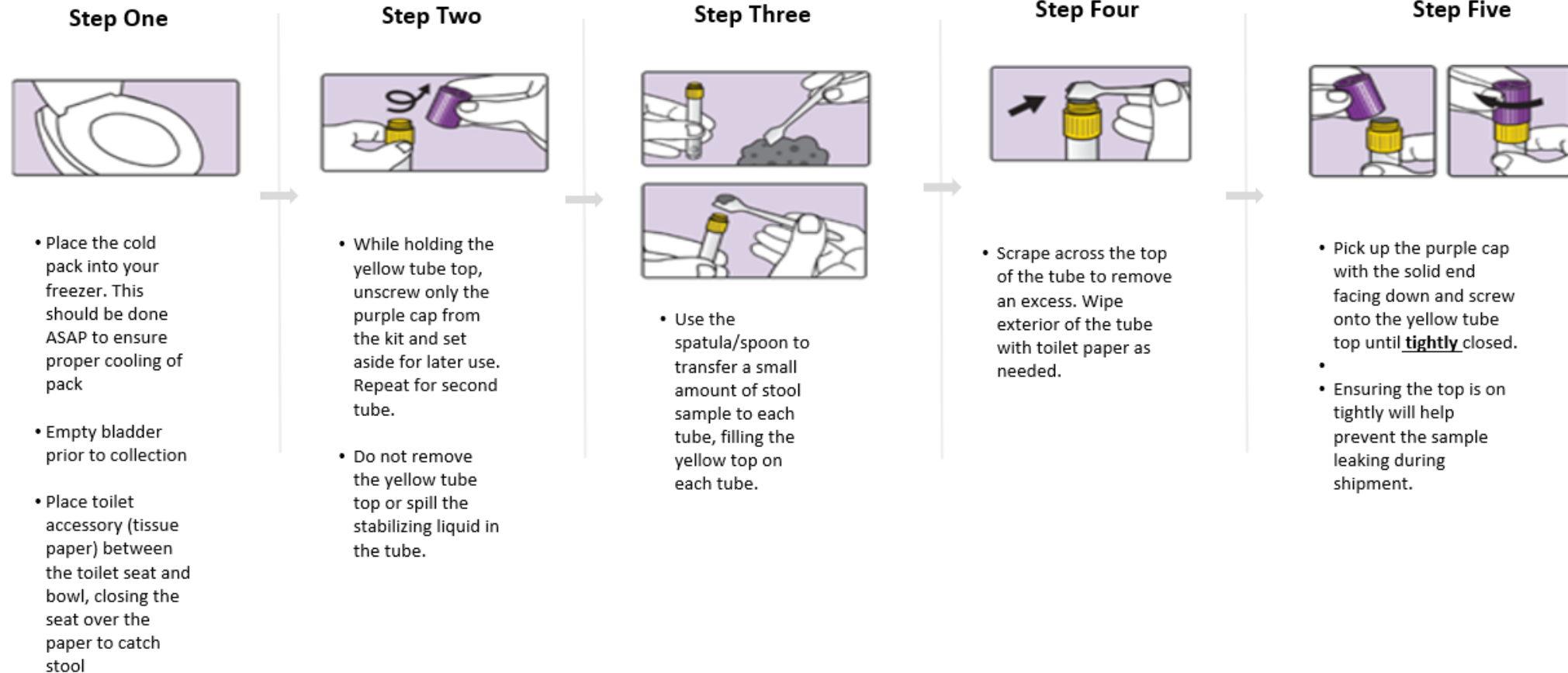
For more information, visit: [http://www.bd.com/vacutainer/pdfs/techtalk/TechTalk\\_Jan2004\\_VS7167.pdf](http://www.bd.com/vacutainer/pdfs/techtalk/TechTalk_Jan2004_VS7167.pdf)

# Urine Sample Collection

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2. Ask study subject to collect a urine specimen in the site provided toilet hat. Urine should be collected midstream and should remain as sterile as possible.
3. Label two 15 ml conical tubes with pre-printed “URINE” labels.
4. Transfer 10mL urine from the toilet hat into each of the two 15 mL conical tubes.
5. Within 60 minutes of collection, freeze and store samples **upright** at  $-80^{\circ}\text{C}$  until shipment.

# At-Home Stool Collection – Participant Instructions



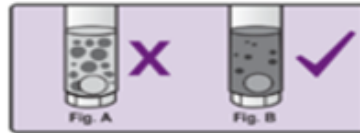
# At-Home Stool Collection – Participant Instructions

## Step Six



- Shake the sealed tube as hard and fast as possible in a back-and-forth motion for a minimum of 30 seconds

## Step Seven



- Ensure the stool sample is mixed with the stabilizing liquid in the tube. Not all the particles will dissolve, but they should be small.
- If large particles remain, continue to shake the tube until they are broken up.

## Step Eight



- Place tubes into the provided biohazard bag and seal
- Place biohazard bag and cold pack in the shipper and replace the Styrofoam lid. Do **not** seal the box yet.

## Step Nine

- Record Date and Time of stool collection on the Sample Form.
- Fold sample form and place it on top of the closed Styrofoam box.
- Seal box using provided seal tape

## Step Ten



- Discard of spoon/spatula and toilet accessory in trash
- Place sealed package in mailbox or take to nearest USPS shipping location. The package can remain at **room temperature** until it is picked up.

# Stool Collection – Prepare Supplies for Participant

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Ensure all supplies are ready for the participant to take home

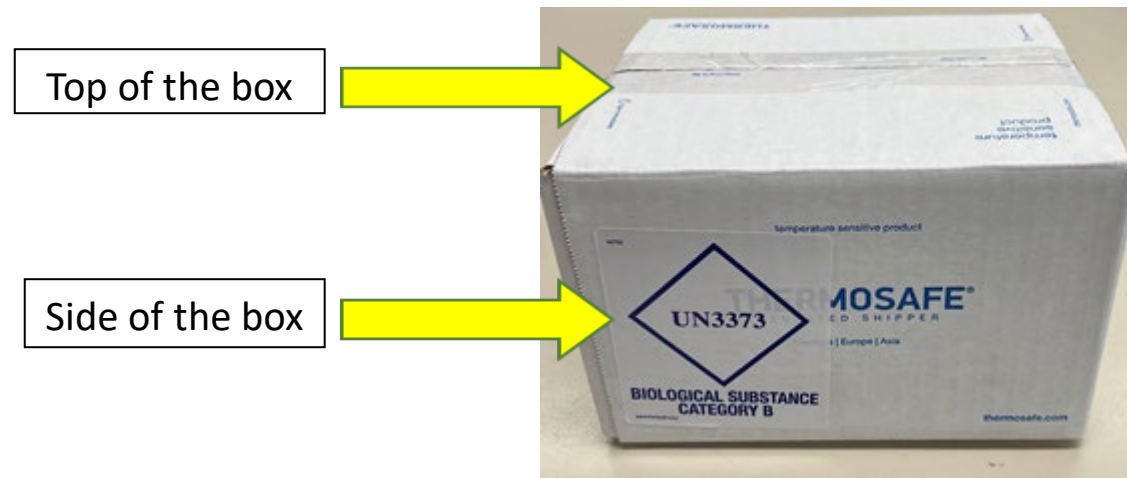
- Label the stool collection tubes prior to giving them to participant
- The participant should take home the stool collection tubes, spoon/spatula accessory, toilet accessory, seal tape, cold pack (for shipper), pre-labeled shipper, and partially completed sample form
- Cold pack can be placed in the participant's freezer prior to shipping



# Stool Collection – Prepare Supplies for Participant

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- Place the UN3373 and "E" Hazardous Materials in Exempt Quantity labels to the side of the ambient/stool shipper
- Place biohazard and fragile labels on box, taking care to not overlap labels



# Stool Collection – Prepare Supplies for Participant

- Apply the BioSEND USPS Express shipping label (1) and the USPS postage sticker (2) to the bottom of the ambient shipper
- The USPS postage sticker must be placed above the barcode (3) and must not be covered it up





# Stool Collection – Prepare Supplies

1. Print a copy of the Stool Sample and Shipment Notification Form
2. Complete top portions of the sample form
3. Email partially completed form to [biosend@iu.edu](mailto:biosend@iu.edu)
4. Include partially completed form in supplies to take home with participant, noting the remaining fields on the form for them to complete

## Appendix T — Stool Sample and Shipment Notification Form

To: BioSEND Email: <a href="mailto:biosend@iu.edu">biosend@iu.edu</a>	
<b>FOR STUDY STAFF TO COMPLETE:</b>	Tracking number: _____
Site Contact: _____	Date: _____
Phone: _____	Email: _____
<b>FOR STUDY STAFF TO COMPLETE:</b>	
Study: GutBrainLBD	<div style="border: 1px dashed black; padding: 20px; text-align: center;">KIT BARCODE</div>
Clinical ID: _____	
Sex:    M       F	
<b>FOR STUDY PARTICIPANT TO COMPLETE:</b>	
<i>Stool Collection:</i>	
Date Collected: _____	[MM/DD/YY]    Time of Collection: _____ (24-hour clock)
Date last ate: _____	[MM/DD/YY]    Time last ate: _____ (24-hour clock)

# Shipping Stool Samples

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Stool samples should be shipped **the same day** of collection

- Participants can either place the package in their mailbox or take it to a local USPS shipping location

# Sample Collection and Processing Form

Specimen Collection and Processing Form

Direct Link:

<https://redcap.link/GutBrainLBDSampleForm>

First page captures basic subject and visit information



Biospecimen Exchange for Neurological Disorders

Please complete the Specimen Collection and Processing Form, below.

[Returning?](#)

A A A



Page 1 of 3

Study

GutBrainLBD ▼

Study Site

Mind Research Network ▼

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.*

Clinical ID:

Sex (used for DNA quality control)

Visit

BL ▼

IU Kit Number






6 characters remaining

Next Page >>

Save & Return Later

# Sample Collection and Processing Form

Second page captures processing information

Blood Collection and Processing	
Date of venipuncture blood collection	<input type="text"/>  Today M-D-Y
Time of venipuncture blood collection	<input type="text"/>  Now H:M Use 24 Hour clock
Date participant last ate	<input type="text"/>  Today M-D-Y
Time participant last ate	<input type="text"/>  Now H:M
2. PLASMA and BUFFY COAT (Purple-top EDTA tubes, 10 mL)	
Was blood collected and processed for PLASMA EDTA?	<div><div>Yes</div><div>No</div><div>reset</div></div>
Time of PLASMA EDTA tube centrifugation	<input type="text"/>  Now H:M Use 24 Hour clock
Duration of PLASMA EDTA tube centrifugation	<input type="text" value="15"/> minutes
Rate of PLASMA EDTA tube centrifugation	<input type="text" value="1500"/> x g

# 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.

## GutBrainLBD Frozen Shipping Manifest

Page 1

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 \_\_\_\_.

Please print a copy of that document and include it in the Kit # \_\_\_\_ shipping container.

Study ☐ GutBrainLBD

Study Site: ☐ Mind Research Network

Clinical ID: \_\_\_\_\_

Visit: ☐ BL

IU Kit Number: \_\_\_\_\_

Date of blood collection: \_\_\_\_\_

### Shipping Information - Please complete.

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

Date of shipment: \_\_\_\_\_

Did/will you use the IU UPS interface 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

Submission of the 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 Frozen Samples: Tips

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## Packing and Shipping Frozen Samples

- Plasma, buffy coats and urine all 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

## Packing and Shipping Frozen Samples

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

Shipper's Declaration not Required.

Dry Ice amount must be in kilograms.

Note: 2 lbs. = 1 kg.

Airwaybills / airbills must have the following:

1. Dry Ice; 9; UN 1845
2.  $\frac{\text{Number}}{\text{(Number pks)}} \times \frac{\text{wt}}{\text{(wt)}} \text{ Kg}$

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 RRD




# Shipping Samples: Frozen

Do not ship more than 4 biohazard bags in a single shipper (equivalent to two subject-visits).

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



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

**INDIANA UNIVERSITY**  
SCHOOL OF MEDICINE

and Affiliated Biorepositories

Search for address

↓

Code

Company

Contact

Address 1

Address 2

Address 3

City

State/Province

Postal Code

Country/Territory

Ship From

Clear

Icahn School of Medicine - Mt. Sinai

Kenny Persaud

1425 Madison (Icahn Building)

Icahn L4 39

New York

NY

10029

United States

Shipment Information

Study Group

Weight

Dry Ice Weight

Description of Return

Pickup Request

SSBC

20

LB

10

LB

Biologic Specimens

Choose Study

↓

Enter weight

↑

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 <sup>rd</sup> Monday in January	Martin Luther King, Jr Day
4 <sup>th</sup> Monday in May	Memorial Day
June 19	Juneteenth (observed)
July 4	Independence Day (observed)
1 <sup>st</sup> Monday in September	Labor Day
4 <sup>th</sup> Thursday in November	Thanksgiving
4 <sup>th</sup> 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

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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

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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

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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](mailto: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](mailto: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

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## Indiana University

General Questions/Shipment Notifications:

[biosend@iu.edu](mailto:biosend@iu.edu)

317-278-6158

Request kits:

<http://kits.iu.edu/biosend/gutbrainlbd>