



Biospecimen Exchange for Neurological Disorders

Intermediate-Size Expanded Access Trial of Autologous Hybrid
TREG/Th2 Cell Therapy (RAPA-501) of Amyotrophic Lateral Sclerosis

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 1500 RCF (x g)
- -80°C Freezer
- Dry ice

Procedures

MAINTAINING SPECIMEN UNIFORMITY AND QUALITY

Biospecimen Collection Protocol

Sample Type & Volume	Screening/BL Day 0	Apheresis	Cycle 1 Day 1 (D35) ± 14	Cycle 2 Day 1 (D77) ± 14	Cycle 3 Day 1 (D119) ± 14	Cycle 4 Day 1 (D161) ± 14	Follow-up Visit (D190) ± 14
Serum aliquots, 1.5ml	3	3	3	3	3	3	3

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
 - 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

<https://redcap.link/rapakit>
s

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.



Biospecimen Exchange for Neurological Disorders

Protocol: RAPA-501-ALS-EAP (Intermediate-Size Expanded Access Trial of Autologous Hybrid TREG/Th2 Cell Therapy (RAPA-501) of Amyotrophic Lateral Sclerosis)

Please order kits and supplies below. Email biosend@iu.edu with any questions.

Study Site

* must provide value

Kit Contents and Ordering: Confirm Site Info

RAPA-501-EAP Kit Request Module

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

Kit Contents and Ordering: Kit Types

RAPA-501-EAP 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.
- Shipping Kits are ordered independently of Blood Kits
- A single Shipping Kit may be used to send samples for up to 18 visits

Kit Type **Please allow two weeks for shipment** * must provide value	<input checked="" type="checkbox"/> Blood Collection Kit <input type="checkbox"/> Shipping Kit <input type="checkbox"/> Extra Supplies
Blood Collection Kit Quantity * must provide value	<input type="text"/>
Comments	<input type="text"/> <small>Expand</small>

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

Kit Contents and Ordering: Kit Breakdown

RAPA-501-EAP Kit Request Module

Comments	<div data-bbox="1778 319 2333 496" style="border: 1px solid #ccc; height: 124px;"></div> <p data-bbox="2321 504 2402 525" style="text-align: right; font-size: small;">Expand</p>
<p data-bbox="996 582 1243 611">Blood Collection Kit</p> <p data-bbox="996 691 1365 719">Blood Collection Kit Contents:</p> <ul data-bbox="996 729 1533 891" style="list-style-type: none"><li data-bbox="996 729 1447 758">1 - Red-top Serum tube (10ml) plastic<li data-bbox="996 768 1467 796">3 - Red-capped cryovial (Sarstedt), 2ml<li data-bbox="996 806 1429 835">1 - Disposable transfer pipette (3ml)<li data-bbox="996 859 1533 888">10 - Cryohold specimen labels-- Kit numbers	

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



Kit Contents and Ordering: Blood & Shipping Kits

Blood Kit (frozen):



Shipping Kit:



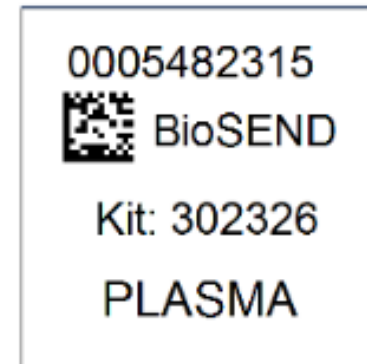
Kit and Supply Ordering

- 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

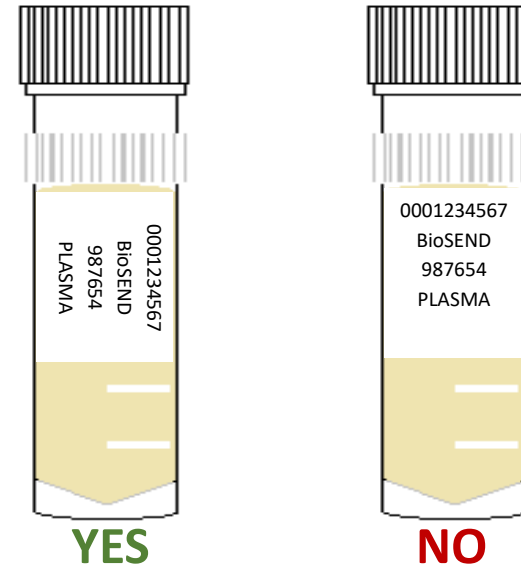
- Please check that all samples are properly labelled to ensure correct identification by IU
- If do not have enough labels to complete a visit, please contact IU *immediately*
- Labelling the tubes during processing prevents sample mix-ups



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



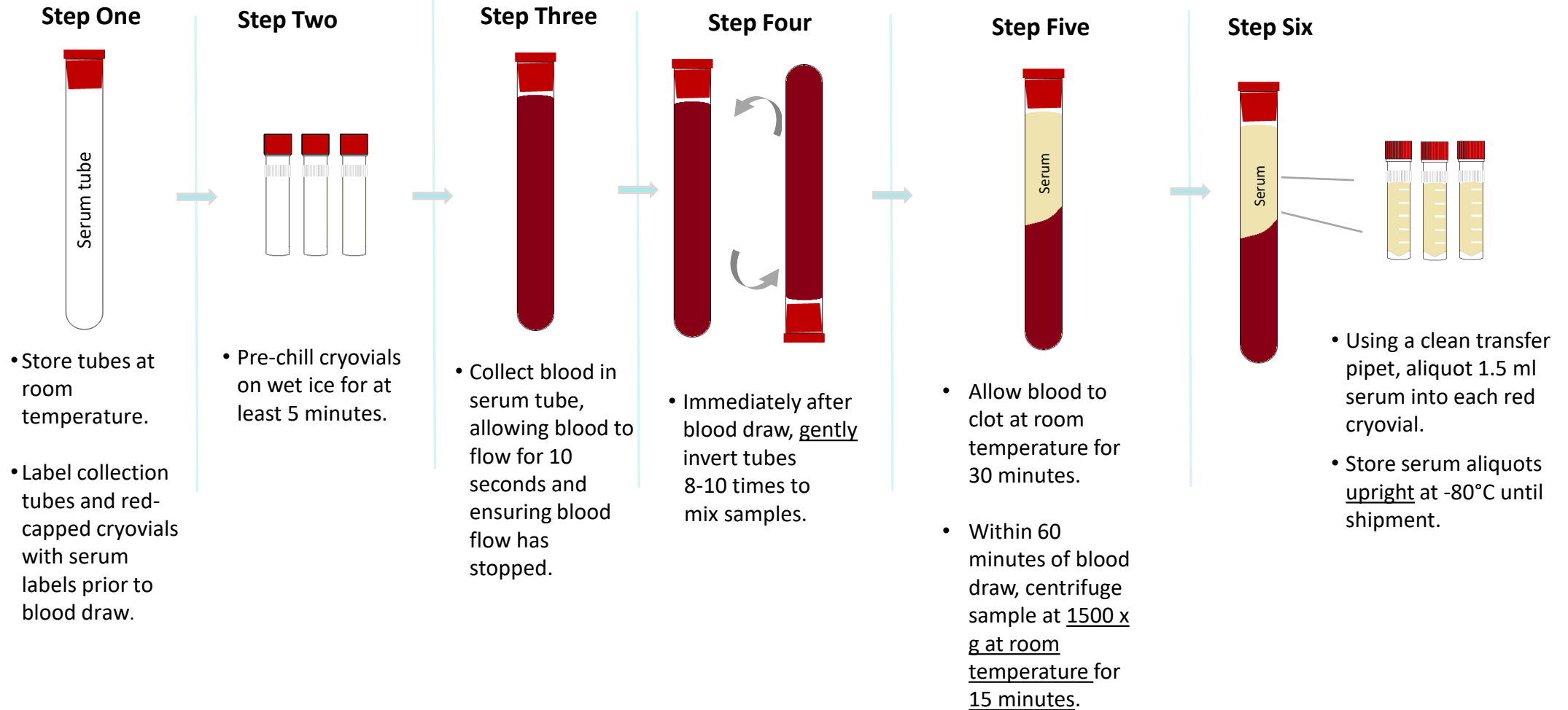
Sample Collection and Processing

*One 10ml plastic serum tube is
provided for the collection and
processing of serum*

1 x 10ml Serum
tubes



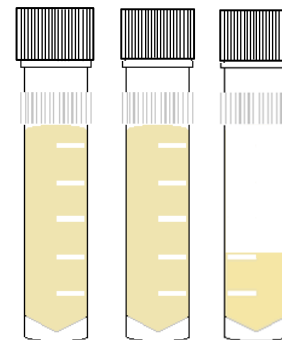
Sample Collection and Processing: Serum



Sample Collection and Processing: Aliquots

Filling biomarker serum aliquots:

- 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: 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 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: Issue #2 continued

Troubleshooting Blood Collection

Issue #2: Hemolyzed serum 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

Direct Link:

<https://redcap.link/RAPASampleForm>

First page captures basic subject and visit information

BioSEND

Returning?

AAA



Biospecimen Exchange for Neurological Disorders

Protocol: RAPA-501-ALS-EAP (Intermediate-Size Expanded Access Trial of Autologous Hybrid TREG/Th2 Cell Therapy (RAPA-501) of Amyotrophic Lateral Sclerosis)

Please complete the Specimen Collection and Processing Form, below.

Page 1 of 2

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.

RAPA ALS Subject ID:

ex. 501-EAP-01-003

Subject's biological sex (used for DNA quality control)

Visit

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
1. SERUM (red-top tubes, 10 mL)	
Was blood collected and processed for SERUM?	<input checked="" type="button" value="Yes"/> <input type="button" value="No"/> reset
Time of SERUM tube centrifugation	<input type="text"/>  Now H:M Use 24 Hour clock
Duration of SERUM tube centrifugation	<input type="text" value="15"/> minutes
Rate of SERUM tube centrifugation	<input type="text" value="1500"/> x g
Temperature of SERUM tube centrifugation	<input type="text" value="4"/> degrees Celsius
Total volume of SERUM collected	<input type="text"/> mL
Number of SERUM aliquots created for BioSEND	<input type="text"/> Each aliquot should be 1.5 mL
Time SERUM aliquots were placed in freezer	<input type="text"/>  Now H:M Use 24 Hour clock

RAPA ALS 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: Mass General Hospital

RAPA ALS Subject ID: _____
(ex. 501-EAP-01-003)

Visit: BL
 D35
 D77
 D119
 D161
 D190

IU Kit Number: _____

Date of blood collection: _____

SERUM

Number of SERUM aliquots shipped: _____

WHOLE BLOOD

Number of Whole Blood tubes shipped: _____

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 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

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

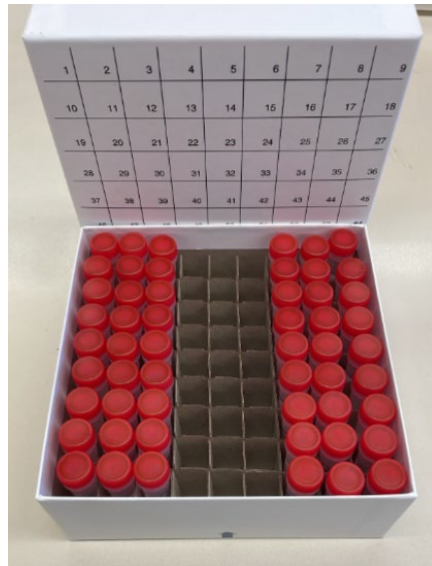
Sample Collection and Processing Form

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 Samples: Frozen

Serum aliquots will be stored in an 81-slot cryobox as they are collected. Once a cryobox is filled completely (i.e., contains 18 visits), the cryobox should be shipped to BioSEND. Specimens should be shipped on at least a quarterly basis (i.e., every 3 months). If a cryobox is not filled completely at the end of the quarter, the partial cryobox should still be shipped to BioSEND.



Shipping Frozen Samples: Tips

Packing and Shipping Frozen Samples

- Serum ship frozen 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



Class 9 Dry Ice Label should not be covered with other stickers and must be completed, or UPS 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 / airbills must have the following:

- Dry Ice; 9; UN 1845
- $\frac{\text{Number}}{\text{(Number pkgs)}} \times \frac{\text{wt}}{\text{(wt)}} \text{ Kg}$

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

06426 1/01 FRD

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

The screenshot shows the shipping interface for Indiana University School of Medicine. The header includes the IU logo and the text "INDIANA UNIVERSITY SCHOOL OF MEDICINE". Below the header, the text "and Affiliated Biorepositories" is displayed. The interface is divided into two main sections: "Ship From" and "Shipment Information".

Ship From Section:

- Search for address: A search bar with a magnifying glass icon and a "Clear" button.
- Code: A text input field.
- Company: Icahn School of Medicine - Mt. Sinai
- Contact: Kenny Persaud
- Address 1: 1425 Madison (Icahn Building)
- Address 2: Icahn L4 39
- Address 3: (Empty)
- City: New York
- State/Province: NY
- Postal Code: 10029
- Country/Territory: United States

Shipment Information Section:

- Study Group: SSBC (dropdown menu)
- Weight: 20 (input field) LB (dropdown menu)
- Dry Ice Weight: 10 (input field) LB (dropdown menu)
- Description of Return: Biologic Specimens
- Pickup Request: A blue button.

Annotations:

- "Search for address": A dashed box around the search bar with a blue arrow pointing to it.
- "Choose Study": A dashed box around the "Study Group" dropdown with a blue arrow pointing to it.
- "Enter weight": A dashed box around the "Weight" and "Dry Ice Weight" input fields with a blue arrow pointing to them.
- "Schedule Pickup": A dashed box around the "Pickup Request" button with a blue arrow pointing to it.
- "Click 'Ship'": A dashed box around the "Ship" button at the bottom right with a blue arrow pointing to it.

Buttons:

- Clear (blue button)
- Pickup Request (blue button)
- Reset (blue button)
- Ship (blue button)

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



BILLING: P/P
DESC: Biological Specimens
RETURN SERVICE

Reference No. 1: 4087277

XOL 20.03.09 NV45 83.0A 12/2019



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 serum within 2 hours of collection allows for breakdown of certain proteins and small molecules
- Over/under centrifuging changes specimen 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***

Contacts

Indiana University

General Questions/Shipment Notifications:

biosend@iu.edu

317-278-6158

Request kits:

<https://redcap.link/rapakits>