Study in Parkinson Disease of Exercise: SPARX3

Update: Version 03. 2025

March 2025

Section	Change
Document Footer	Updated to "Version (March 2025)"
Section 3.1	Added contact information for Carolyn Dunifon, updated general BioSEND phone number
Section 3.4	Updated link to BioSEND website
Section 6.1	Updated kit and specimen label format
Appendix B	Updated kit and specimen label format

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

Study in Parkinson Disease of Exercise: SPARX3

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)
- Whole Blood (for banking)

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

Carolyn Dunifon, Clinical Coordinator
Phone: 317-274-5751
Email: cdunifon@iu.edu

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

Sample Shipment Mailing 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 the shipping courier website to make sure impending weather events (blizzards, hurricanes, etc.) will not impact the shipping or delivery of the samples. Couriers often report anticipated weather delays on their website.

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)
June 19	Juneteenth (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. 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 4.1-4.2.

If a sample is not obtained at a particular visit, this should be recorded in the notes section of the **Sample Record and Shipment Notification Form (see Appendix I).** These forms are submitted with your sample shipment to BioSEND.

4.1 Protocol Schedule for Biospecimen Submission to BioSEND-SPARX3

Visit	BL	6M	12M	18M	24M
Serum aliquots, 1.5ml	6	6	6	6	6
Plasma aliquots, 1.5ml	6	6	6	6	6
Buffy Coat	2	2	2	2	2
Whole blood, 3ml	2	2	2	2	2

5.0 SPECIMEN COLLECTION KITS, SHIPPING KITS AND SUPPLIES

Research specimen collection kits (except dry ice and equipment listed in Section 5.7) will be provided by 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 4.1-4.2); 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

Only the baseline kit will need to be ordered by the site. From that point forward, all follow-up kits will be sent automatically on a schedule calculated from the baseline collection date. Because follow-up visits will likely not occur exactly 6M from the BL collection date, for example, we will send the kits about a month in advance of the calculated visit date. Please see below example of shipping timeline:

Baseline Collection Date	Anticipated 6M Visit Date	Anticipated 6M Visit Date -30 Days	6M Visit Kit Shipped
06/07/22	12/07/2022	11/07/2022	11/01/2022

Each month, BioSEND will contact your site prior to shipping to confirm the list of kits to ship. Please reply to this email to confirm that this list is correct or let us know if there are any issues (eg, a subject has discontinued the study and no longer needs kits). You will receive another email with tracking information when the kits ship.

The link to the kit request module is shown below:

- SPARX3: http://kits.iu.edu/biosend/sparx3

Please allow **TWO weeks** for kit orders to be processed and delivered.

5.2 Specimen Collection Kit General Contents

Collection kits contain the following (for each subject) as designated per your protocol and/or NINDS resource development agreement. Kits provide the necessary supplies to collect samples from a given subject. Do not replace or supplement any of the tubes or kit components provided with your own supplies unless you have received approval from the NINDS/BioSEND Study team to do so. *Please store all kits at room temperature until use.* Note that "supplemental" kits can be provided should you require additional supplies from those contained in the visit specific kits. Replacement supplemental kits can be requested on the kit request website. In addition, individual supplies can be requested as well.

BioSEND Supplies

Available upon request from the online kit request module (Section 5.1)

General Items
48 Slot Micronic Tube Rack
Cryogenic Vials
Airway bill envelope
Shipping container for dry ice shipment (shipping and Styrofoam® box)
Sterile pipettes (3ml)
Plastic biohazard bag with absorbent sheet
Shipping label packet (dry ice, fragile, and UN3373 label)
Blood Collection Items
Red-top serum blood collection tubes (glass, 10ml)
Purple-top EDTA blood collection tubes (glass, 10 ml)
Purple-top EDTA blood collection tubes (plastic, 3 ml)

5.3 Specimen Collection Kit Contents – SPARX3

SPARX3 BL/Annual Collection Kit	
Supply	**Amount**
Purple cryogenic vial, 2ml	6
Grey cryogenic vial, 2ml	2
Red cryogenic vial, 2ml	6
Serum (glass) tube, 10ml	2
EDTA (glass) tube, 10ml	2
EDTA (plastic) tube, 3ml	2
Bubble-tube sleeve	6
Disposable pipet, 3ml	2
Cryobox, 25 cell	1
Biohazard bag w/ absorbent sheet	2
Fragile label	1
UN3373 label	1
Dry ice label	1
Airway bill envelope	1
Frozen shipper	1
Label set (case & specimen labels)	1

SPARX3 Supplemental Kit	
Supply	**Amount**
Purple cryogenic vial, 2ml	10
Grey cryogenic vial, 2ml	10
Red cryogenic vial, 2ml	10
Serum (glass) tube, 10ml	5
EDTA (glass) tube, 10ml	5
EDTA (plastic) tube, 3ml	5
Bubble-tube sleeve	10
Disposable pipet, 3ml	10
Cryobox, 25 cell	2
Biohazard bag w/ absorbent sheet	5
Fragile label	5
UN3373 label	5
Dry ice label	5

5.4 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

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)
- Wet ice
- -80°C Freezer

In order to ship specimens, you must provide:

- Dry ice (approximately 10 pounds per shipment)

6.0 SPECIMEN LABELS

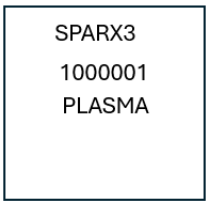
Labels must be affixed on all collection and aliquot tubes to ensure unique specimen identity. BioSEND provides labels for all samples being collected and returned to BioSEND. The site is responsible for providing labels for biospecimens that will be retained at the site.

6.1 Types of Labels

Each kit contains all labels required for the return of biospecimens to BioSEND.

The **Kit Labels** do not indicate a specimen type, but are affixed on BioSEND forms and on specific packing materials. See Appendix K for further instructions.

The **Collection Tube Labels for Blood** are placed on all blood collection tubes. Note that the tube type and volume will be printed on the label. For example, the label on the right would be placed on whole blood sample collected into a 3ml EDTA tube.

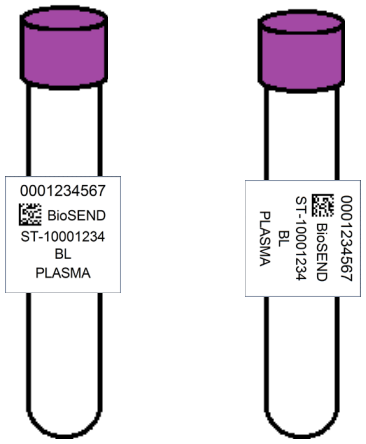


Plasma, Serum, and Buffy Coat Aliquot labels are placed on cryovials. The tube itself with have a unique barcode printed in both 2D format (on bottom of tube) and human readable formats (alongside of tube). The barcoded cryovials in a kit are linked to the ID on the labels provided in that kit in the BioSEND LIMS.

6.2 Affixing Labels

In order to ensure the label adheres properly and remains on the tube, follow these instructions:

- Place specimen labels on **ALL** collection tubes and cryovials **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.
- The blood collection tube labels contain a 2D barcode on the left hand side of the label. When turned horizontally, the barcode should be closer to the top (cap end) of the tube.
- Place label **horizontally** on the tube (wrapped around sideways if the tube is upright); see below.



- For plasma and buffy coat labels, ensure the specimen label is placed on the correct cryovial. The purple cryovials should be labeled for plasma, grey cryovials should be labeled for buffy coat, and red cryovials should be labeled for serum.
- The barcoded cryovials in a kit are linked to the ID on the labels provided in that kit in the BioSEND LIMS. **Please do not mix the labels and cryovials from different kits.**

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 Draw for BioSEND Collection Tubes

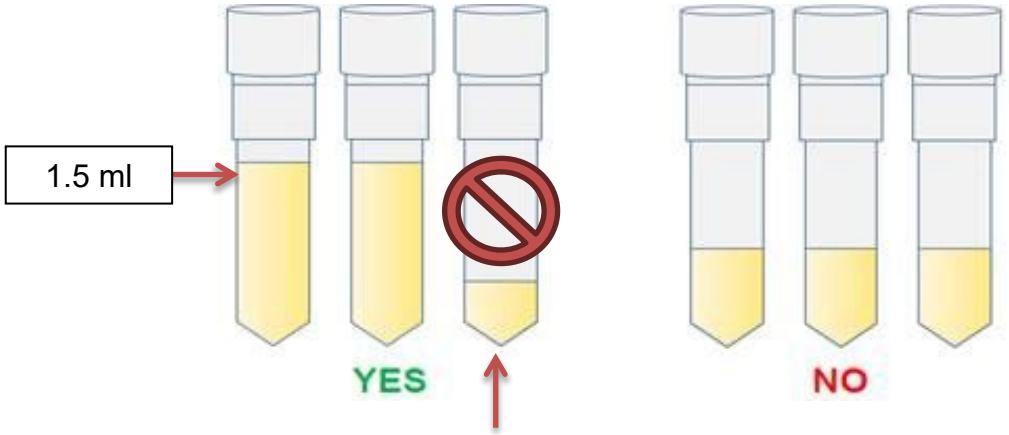
1. Serum (red top, 10ml) x 2 for serum
2. EDTA (purple top, 10ml) x 2 for plasma and buffy coat
3. EDTA (purple top, 3ml) x 2 for whole blood

7.2 Blood Collection Protocols

1. Serum (red top) blood collection for serum **(Appendix F)**
2. EDTA (purple top) blood collection for plasma **(Appendix B)**
3. EDTA (purple top) blood collection for Buffy Coat **(Appendix C)**
4. EDTA (purple top) blood collection for whole blood **(Appendix D)**

7.4 Filling Aliquot Tubes (Plasma , Serum and Buffy Coat)

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). Specimens collected in addition to those described in Section 4 are collected at the site's discretion and are not returned to BioSEND.



Please note: It is critical for the integrity of future studies using these samples that study staff **not submit** residual aliquot tubes (anything under 1.5 ml) to BioSEND.

Each aliquot cryovial will be have a color-coded cap as follows:

Cap Color	Specimen Type
Purple	Plasma
Grey	Buffy Coat
Red	Serum

Please be sure to use the appropriate colored top for the appropriate sample type.

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 Sample Record and Shipment Notification Form

All sample shipments to BioSEND must include the BioSEND Sample Record and Shipment Notification Form. The completed forms are:

- Emailed to BioSEND@iu.edu at the time the samples are being shipped
- And the original document should be Included in the shipment with the samples

8.2 Shipping Instructions

Frozen Shipment (baseline and follow-up). Reference Appendix K for frozen shipping instructions.

- Frozen 1.5 ml aliquots of plasma and serum
- Frozen Buffy Coat
- Frozen 3 ml EDTAs for whole blood

*****Important Note*****
Please do not include more than 2 visits' worth of samples in a single shipper (ie, no more than 4 biohazard bags of samples in one shipper).

Please ship samples to BioSEND within two weeks of collection.

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

Appendix I must be completed the day that samples are collected to capture information related to sample collection and processing. This form includes information that will be used to reconcile sample collection and receipt, as well as information essential to future analyses.

The SPARX3 Study 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 clinical 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 reported in the clinical database compared to information on Appendix I
- Samples frozen and stored longer than three months at the site

10.0 APPENDICES

Appendix B: Whole Blood Collection for Isolation of Plasma

Appendix C: Whole Blood Collection for Isolation of Buffy Coat

Appendix D: Whole Blood Collection for Banking

Appendix F: Whole Blood Collection for the Isolation of Serum

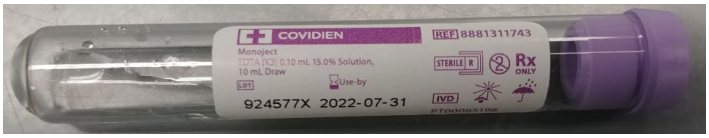
Appendix I: Sample Record and Shipment Notification Form

Appendix K: Frozen Shipping Instructions

Appendix Q: UPS ShipExec™ Thin Client Instructions

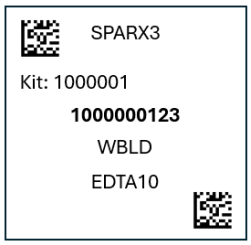
Appendix B – Whole Blood Collection for Isolation of Plasma

Whole Blood Collection for Isolation of Plasma: 10 ml Purple-Top EDTA tubes and cryovials are provided by BioSEND for the collection of plasma.

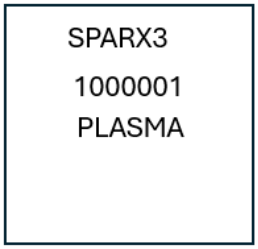


1. **CRITICAL STEP: Store empty purple-top EDTA tubes at room temperature 64°F – 77°F (18°C to 25°C) prior to use.**

2. Place provided "**PLASMA**" labels on 10 ml purple-top EDTA tube(s); place the plasma aliquot labels on the six 2 ml purple cryovial tubes. These six cryovials will be shipped to BioSEND. Any remaining cryovials can be retained by the site and labeled per site standards. Labels for aliquots kept by the site are not provided by BioSEND.



Collection Tube Label



Label on cryovials

3. Pre-chill the cryovials 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 will depend on your centrifuge model.

5. Using a blood collection set and a holder, collect blood into the **purple top 10 ml EDTA tube(s)** using your institution's recommended procedure for standard venipuncture technique.

   The following techniques shall 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.

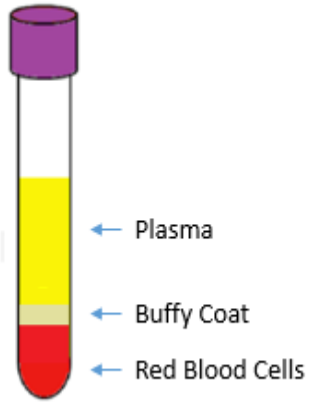
6. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube vacuum is designed to draw 10 ml of blood into the tube.

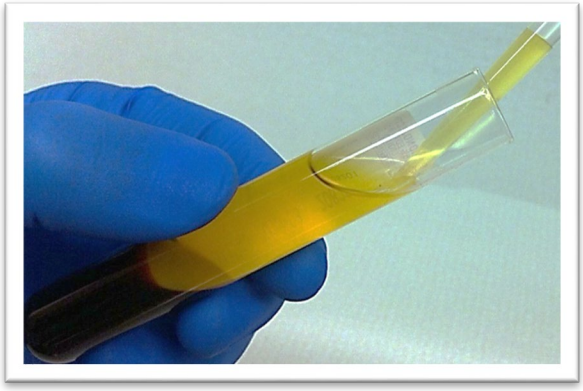
7. **CRITICAL STEP: Immediately after blood collection, gently invert/mix (180 degree turns) the purple-top EDTA tube(s) 8 – 10 times. Do not shake the tubes!**

8. Within 30 minutes of blood collection, centrifuge balanced tubes for 15 minutes at 1500 RCF (x g). **It is critical that the tubes be centrifuged at the appropriate speed to ensure proper plasma separation.**

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 collection tube so that the plasma is not contaminated** (see below).

10. Using a disposable pipette, aliquot 1.5 ml into each cryovial. Send 6 x 1.5 ml aliquots to BioSEND. If you cannot obtain the requested number of aliquots, please note "low volume draw" on the Sample Record and Shipment Notification form (Appendix I) under "Notification of Problems". Each 10 ml EDTA tube should yield, on average, 4-5 ml of plasma.



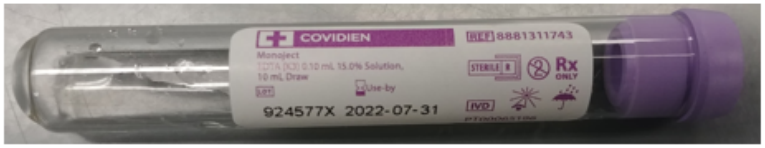


11. Complete the **Sample Record and Shipment Notification form (Appendix I).**

12. Place the labeled cryovials 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.

13. Ship the frozen plasma aliquots to BioSEND according to **Appendix K – Frozen Shipping Instructions.**

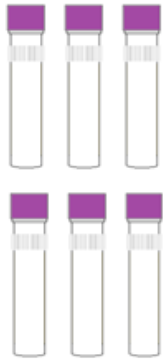
Plasma Preparation –10 ml EDTA (Purple Top) Tube



Step One

- Store tubes at room temperature.
- Label tubes with preprinted plasma labels prior to blood draw.

Step Two

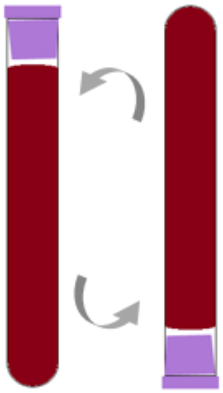


- Pre-chill cryotubes on wet ice for at least 5 minutes.

Step Three

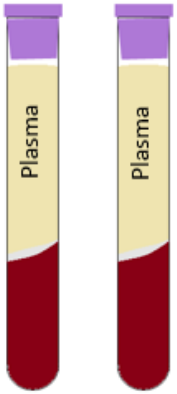
- Collect blood in EDTA tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Four



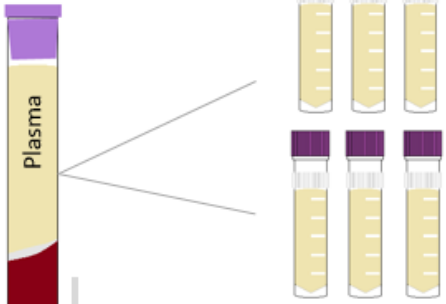
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Five



- Within 30 minutes of blood draw, centrifuge sample at <u>1500 x g at 4°C</u> for <u>15 minutes</u>.

Step Six

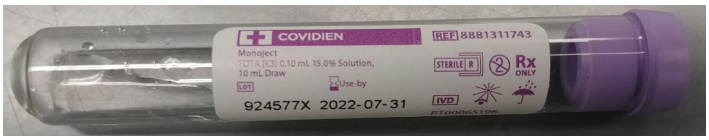


- Using a clean transfer pipet, aliquot 1.5 ml plasma into each purple cryotube.
- Store plasma aliquots <u>upright</u> at -80°C until shipment.

- See Appendix C for instructions on buffy coat collection.

Appendix C –Whole Blood Collection for Isolation of Buffy Coat

Whole Blood Collection for Isolation of Buffy Coat: 10 ml Purple-Top EDTA tubes and cryovials are provided by BioSEND for the collection of the buffy coat.

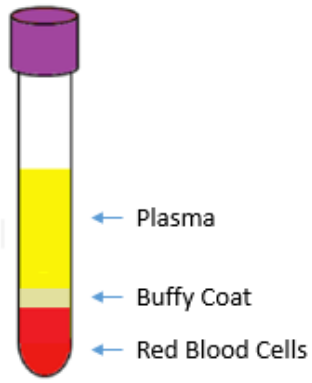


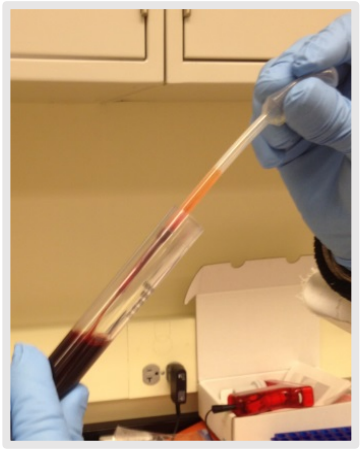
1. **CRITICAL STEP: Store Purple-Top EDTA tubes at room temperature 64^{0}F - 77^{0}F (18°C to 25°C) before use.**

2. Label grey cryovials with "**Buffy Coat**" labels and set to chill on wet ice for at least 5 minutes.

3. Set centrifuge to 4°C to pre-chill before use. Time needed to pre-chill the centrifuge will depend on your centrifuge model.

4. After plasma has been removed from the EDTA purple-top tube (see Appendix B), aliquot buffy coat layer (see figure below) into labeled cryovial with grey cap using a disposable graduated micropipette. All of the buffy coat from a single 10 ml purple-top EDTA tube will be placed into one cryovial. The buffy coat aliquot is expected to have a reddish color from the red blood cells.



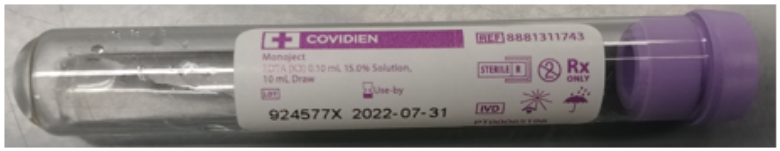


5. Complete the **Sample Record and Shipment Notification form (Appendix I).**

6. Freeze cryovial(s) in upright position on dry ice. Transfer to a **-80°C Freezer when possible**. Store all samples UPRIGHT at **-80°C until shipped** to BioSEND on dry ice.

7. Ship the frozen buffy coat aliquots to BioSEND according to **Appendix K – Frozen Shipping Instructions.**

Buffy Coat Preparation –10 ml EDTA (Purple Top) Tube



Step One

- Store tubes at room temperature.
- Label tubes with preprinted plasma labels prior to blood draw.

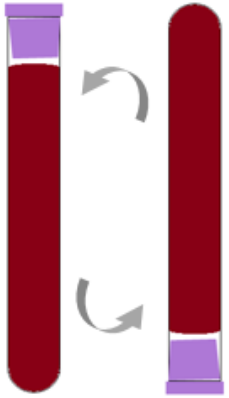
Step Two

- Pre-chill cryotubes on wet ice for at least 5 minutes.

Step Three

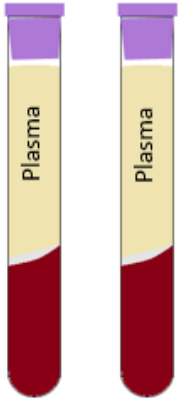
- Collect blood in EDTA tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Four



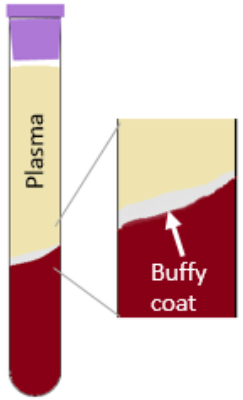
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Five



- Within 30 minutes of blood draw, centrifuge sample at <u>1500 x g at 4°C</u> for <u>15 minutes</u>.

Step Six



- Using a clean transfer pipet, collect the buffy coat (will have residual plasma and some RBCs included).
- Transfer the buffy coat from each EDTA tube into a grey cryotube.
- Store plasma aliquots <u>upright</u> at -80°C until shipment.

Appendix D – Whole Blood Collection (No Processing)

Two 3ml Purple-Top EDTA Tube are provided by BioSEND for Whole Blood collection (to be shipped to BioSEND FROZEN; no processing required).

1. Store empty Whole Blood EDTA tubes at room temperature, 64°F - 77°F (18°C to 25°C) before use.

2. Place pre-printed specimen label (WBLD) on the **two 3ml purple top EDTA tube** prior to blood draw.

3. Using a blood collection set and a holder, collect whole blood into the two 3ml purple-top whole blood tube using your institution's recommended procedure for standard venipuncture technique.
   The following techniques shall 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.

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

5. Complete the Sample Record and Shipment Notification form (Appendix I).

6. Place the Purple-Top EDTAs in a **WIRE** or **PLASTIC** rack. Do **NOT** use a Styrofoam rack. This will cause the tubes to crack when frozen. Place the Purple-Top EDTA tubes immediately to a **-80°C Freezer.**

7. Ship the whole blood tube to BioSEND according to **Appendix K - Frozen Shipping Instructions.**

WBLD Preparation – 2 x 3 ml K2 EDTA (Purple Top) Tube

Step One

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

Step Two

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

Step Three

- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Four

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

Appendix F – Whole Blood Collection for Isolation of Serum

Whole Blood Collection for Isolation of Serum: 10 ml red-top serum (glass) tubes and cryovials are provided by BioSEND for the collection of serum.

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

2. Place pre-printed specimen labels noted as "**SERUM**" on the serum determination red-top tubes and on six of the 2 ml red cryovials prior to blood draw. These six cryovials will be shipped to BioSEND; the remaining cryovials will be retained by the site and labeled accordingly. Labels for aliquots kept by the site are not provided by BioSEND.

3. Pre-chill labeled cryovials 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 **10 ml red-top serum (glass) tubes** using your institution's recommended procedure for standard venipuncture technique.

   The following techniques shall 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.

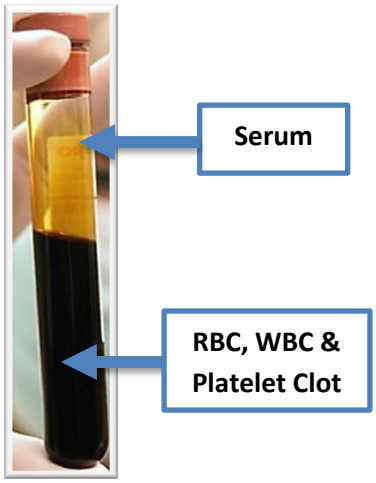
6. Allow at least 10 seconds for a complete blood draw to take place in each tube. Ensure that the blood has stopped flowing into the tube before removing the tube from the holder. The tube with its vacuum is designed to draw 10 ml of blood into the tube.

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

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

9. Within 30 to 60 minutes from blood collection, 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 serum separation.**

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 cryovials. Aliquot 1.5 ml per cryovial. Send 6 x 1.5 ml aliquots to BioSEND. Each 10 ml Serum tube should yield, on average, 4-5 ml of serum.



11. Complete the Sample Record and Shipment Notification form (Appendix I).

12. Place the labeled cryovials in the 25 slot cryovial box. Place the cryobox UPRIGHT on dry ice. Transfer to **-80°C Freezer as soon as possible**. Store all samples UPRIGHT at **-80°C until shipped** to BioSEND on dry ice.

13. Ship the frozen serum aliquots to BioSEND according to **Appendix K – Frozen Shipping Instructions.**

Serum Preparation –10 ml Serum (Red Top) Tube

Step One

- Store tubes at room temperature.
- Label collection tubes and cryovials with serum labels prior to blood draw.

Step Two

- Pre-chill cryotubes on wet ice for at least 5 minutes.

Step Three

- Collect blood in serum tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

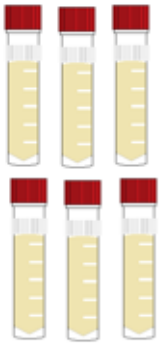
Step Four

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

Step Five

- Allow blood to clot at room temperature for 30 minutes.
- Within 60 minutes of blood draw, centrifuge sample at <u>1500 x g at 4°C</u> for <u>15 minutes</u>.

Step Six



- Using a clean transfer pipet, aliquot 1.5 ml serum into each red cryotube.
- Store serum aliquots <u>upright</u> at -80°C until shipment.

Appendix I – Sample Collection and Processing Form

A Sample Collection and Processing Form must be completed for each subject-visit submitted to BioSEND. This form includes a Frozen Shipping Manifest that should be completed in advance of shipping to BioSEND. A copy of this form should also be included in the shipper. A copy of the form will be emailed to you upon completion. BioSEND will receive an automated notification of your shipment when you click the "Submit" button on the Frozen Shipping Manifest; no additional notification is required. If you are unable to submit the Frozen Shipping Manifest via REDCap, please notify BioSEND at biosend@iu.edu instead.

The form can be completed via REDCap by following the bellow link:

- **Link to Sample Collection and Processing Form:**
  https://redcap.link/SPARX3SampleForm

Please note that there is a Save & Return option at the bottom of the survey. This may be used if, for example, you are ready to complete the Collection and Processing portion of the form, but not yet ready to complete the Frozen Shipping Manifest.

Clicking "Submit" at the end of the Frozen Shipping Manifest portion of the form will send an automatic notification to BioSEND of your shipment.

If unable to access REDCap during collection and processing, the form on the following page may be printed and completed by hand. If your site has a separate processing lab from your coordinating team, you can share this form with them so that they may capture and return this data to you. This form is provided to aid with processing data collection. Data should still be entered into the REDCap (https://redcap.link/SPARX3SampleForm) when site staff is able to do so.

Clinical ID: _____ Visit: ☐ Baseline ☐ 6M ☐ 12M

Kit Number or ST-Number : _____ ☐ 18M ☐ 24M

Sex: ☐M ☐F

Blood Collection:

Date Drawn: _____[DD/MMM/YYYY]	Time of Draw: _____(24 HR)
Date Participant last ate: _____[DD/MMM/YYYY]	Time subject last ate: _____ ☐AM ☐PM

Blood Processing:

10ml Serum (Red-top) Tubes		**10ml EDTA (Purple-top) Tubes**	
Total volume collected for serum	_____ mL	Total volume collected for plasma	_____ mL
Time spin started:	_____ ☐AM ☐PM	Time spin started:	_____ ☐AM ☐PM
Duration of centrifuge:	_____ minutes	Duration of centrifuge:	_____ minutes
Temp of centrifuge:	_____ °C	Temp of centrifuge:	_____ °C
Rate of centrifuge:	_____ x g	Rate of centrifuge:	_____ x g
# of serum aliquots created: (red-capped cryovial)	_____	# of plasma aliquots created: (purple-capped cryovial)	_____
Time serum cryovials frozen:	_____ ☐AM ☐PM	# of buffy coat aliquots created: (grey-capped cryovial)	_____
Storage temperature:	_____ °C	Time plasma and buffy coat cryovials frozen:	_____ ☐AM ☐PM
		Storage temperature:	_____ °C
		3ml EDTA (Purple-top) Tubes	
		Total volume collected for whole blood	_____ mL
		# of whole blood tubes collected:	_____
		Time whole blood tubes frozen:	_____ ☐AM ☐PM
		Storage temperature:	_____ °C

Notes: _____

Appendix K – Frozen Shipping Instructions

IMPORTANT!

Frozen samples must be shipped Monday – Wednesday only, using Next Day Air delivery

Samples should be shipped to BioSEND within two weeks of collection.

Please be aware of holidays and inclement weather and plan your shipments accordingly. Reach out to biosend@iu.edu if you have any questions

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 Appendix Q.

2. Record the tracking number onto the Sample Record and Shipment Notification form (Appendix I).

3. Make a copy of the Sample Record and Shipment Notification form.

4. Place all frozen labeled cryotubes in the cryobox. Only include specimens from one subject in each cryobox.

5. Place the cryobox in a clear plastic biohazard bag (do NOT remove the absorbent material found in the bag), and seal the biohazard bag according to the instructions on the bag. Affix a Case Label to the outside of the biohazard bag.

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

7. If your protocol is collecting frozen whole blood, DNA, or RNA tubes, place labeled tubes in bubble sleeves and seal.

8. Place the tubes in a clear plastic biohazard bag separate from the cryobox (do NOT remove the absorbent material found in the bag), and seal the biohazard bag according to the instructions on the bag. Affix a Case Label to the outside of the biohazard bag.

9. 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).

10. Fully cover the cryobox with approximately 2 inches of dry ice. Do not include more than 2 subjects' worth of samples in a single shipper.

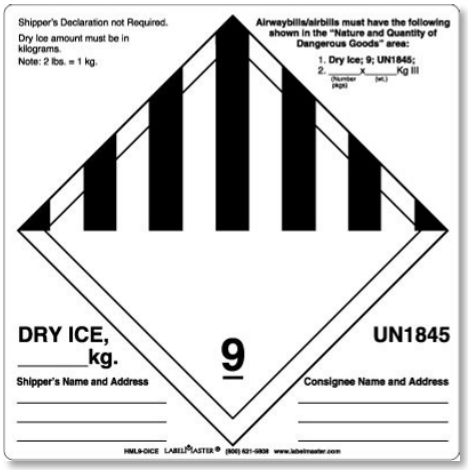
11. If including additional biohazard bags in package, include a layer of dry ice (approximately 2 inches) between each biohazard bag.

12. 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 to ensure the frozen state of the specimens.

13. Replace the lid on the Styrofoam® container. Place the completed Sample Record and Shipment Notification form in the package on top of the Styrofoam® lid for each patient specimen, and close and seal the outer cardboard shipping carton with packing tape.

14. Print a copy of your UPS® airway bill generated through the UPS ShipExec™ Thin Client system (see Appendix Q). Place airway bill into the provided airway bill envelope and affix envelope to package.

15. Complete the Class 9 UN 1845 Dry Ice Label (black and white diamond) 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 airway bill)
    - 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.

IMPORTANT!
Complete the required fields on your airway bill and Class 9 Dry Ice labels, or courier may reject or return your package.

16. Apply all provided warning labels (UN3373, Dry Ice Label and Fragile Label), taking care not to overlap labels with each other or with airway bill.

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

18. 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.

19. **Notify BioSEND by email (biosend@iu.edu) that a shipment has been sent and attach the Sample Record and Shipment Notification form to your email. Do not ship until you've contacted and notified BioSEND staff about the shipment in advance.**

20. Use courier tracking system 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.