Concussion Assessment, Research, and Education for Kids (CARE4Kids) Study Manual of Procedures

Update: Version 04.2025

April 2025

Section	Change
Document Footer	Updated to "Version (April 2025)"
Throughout Document	Minor changes made to phrasing and wording. No alterations to procedures were made.

National Institute of Neurological Disorders and Stroke Biorepository:

BioSpecimen **E**xchange for **N**eurological **D**isorders, **BioSEND**

Concussion Assessment, Research and Education for Kids (CARE4KIDS) Protocol

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:

- Plasma collected from EDTA tube, P100 tube, and Tasso HemoLink™ device
- Whole Blood (for RNA extraction)
- Buffy Coat (for DNA extraction)

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
CARE	Concussion Assessment, Research and Education
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

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 46202-4118

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)
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. Should it be necessary to ship blood samples for DNA extraction to Indiana University during this period, please contact the Indiana University staff before December 24th by e-mailing biosend@iu.edu, so that arrangements can be made to have staff available to process incoming samples. 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 section 4.1.

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).** This form is submitted with your sample shipment to BioSEND.

4.1 Protocol Schedule for Biospecimen Submission

Sample type	Visit	Tube Provided	Blood Volume Collected	Sample Volume	Samples shipped to BioSEND	Cryovial Color
Plasma	T1, T3	EDTA tube (purple-top)	10ml	500ul per cryovial	Up to 10 cryovials	Purple Cap
Buffy Coat	T1, T3			750ul	1 cryovial	Clear Cap
Plasma	T1, T3	P100	2ml	200ul per cryovial	Up to 5 cryovials	Lavender Sticker
Plasma	T1, T3	Tasso™ HemoLink w/ EDTA microtainer	500ul	200ul per cryovial	1 cryovial	White Sticker
RNA	T1, T3	PAXgene™ tube	2.5ml	N/A	1 tube	n/a

5.0 SPECIMEN COLLECTION KITS, SHIPPING KITS AND SUPPLIES

Research specimen collection kits as well as clinical lab supplies (except dry ice and equipment listed in Section 5.7) will be provided by BioSEND. These materials include blood tubes, boxes for plasma and buffy coat aliquots, as well as partially completed shipping labels to send materials to 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 Section 4.1); 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

Each individual site will be responsible for ordering collection kits from BioSEND. We advise sites to proactively confirm kits are on hand ahead of study visits.

Within the kit request module, there is a drop down menu to request kits based on the Principal Investigator at that site. Kits and individual items can be ordered as required through the kit request module.

The link to the kit request module is shown below:

- CARE4Kids: http://kits.iu.edu/biosend/care4kids

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 15-25° C until use.* Note that "supplemental" kits will be provided should you require additional supplies from those contained in the visit specific kits.

BioSEND Supplies

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

General Items
25 cell cryobox
Cryovial tubes (0.5 ml) with lavender and white stickered-caps
Cryovial tubes (2 ml) with purple and clear caps
Airway bill envelope
Shipping container for dry ice shipment (shipping and Styrofoam® box)
Plastic biohazard bag
Warning label packet
Blood Collection Items
Purple-top EDTA blood collection tube (10 ml) plastic
Tasso™ HemoLink Device (0.5ml)
P100 EDTA blood collection tube (2ml)
PAXgene™ RNA tube (2.5 ml)

We realize there may be instances where additional supplies are needed; therefore, one supplemental kit will be provided with the initial kit shipment for new studies. Replacement supplemental kits can be requested on the kit request website. In addition, individual supplies can be requested as well.

5.3 Specimen Collection Kit Contents – CARE4Kids

Quantity	Component
3	Set protective bubble pouches for tubes
1	Purple-top EDTA tube (10 ml)
1	P100 EDTA blood collection tube (2ml)
1	PAXgene™ RNA tube (2.5 ml)
10	Cryogenic vials (2 ml) with purple caps
1	Cryogenic vials (2 ml) with clear caps
5	Cryogenic vials (0.5 ml) with lavender sticker cap
1	Disposable transfer pipette
1	Cryobox, 25 slot
2	95 kPa biohazard bag with absorbent sheet
1	Shipping container for dry ice shipments
1	Shipping label packet
1	UPS clear sleeve
1	Kit & specimen label set

*As the HemoLink device will not be used with all participants, each site will be sent a shipment of HemoLink supplies separate from the standard collection kit

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
- Wet ice

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) with refrigeration to 4°C
- -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 prevent sample mix-ups and ensure chain-of-custody tracking. 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. **If labels are provided but the sample is not collected, please discard the unused labels.**

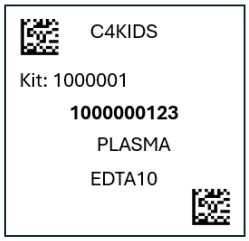
6.1 Types of Labels

Label Type Summary

1. Kit Label
2. Collection and Aliquot Tube Label for Blood

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

The **Kit Labels** link all specimens collected from a single participant at one visit. There will be a different Kit Number for each visit a subject has. These labels are used on packaging materials; see Appendix K for further instructions.

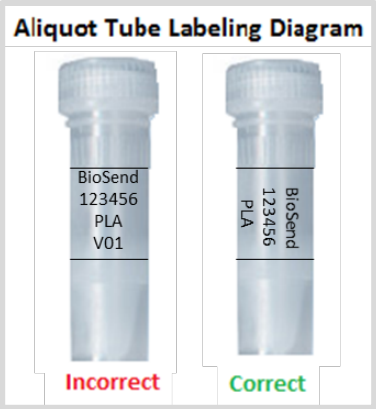


The **Specimen Labels** are placed on all blood collection and aliquot tubes. Each Specimen Label has a unique barcode that is tied to the Kit. The labels indicate the specimen type, as well as the collection tube for that specimen. For example, the label to the right would be placed on a plasma aliquot generated from a 10ml EDTA tube.

6.2 Affixing Labels

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

- Place blood collection and aliquot labels on **ALL** collection and aliquot tubes **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 and aliquot 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) and **just below the ridges** of the aliquot tubes (see attached labeling diagram).



- Take a moment to ensure the label is **completely affixed** to each tube. It may be helpful to roll the tube between your fingers after applying the label.

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 Specimen Collection

Blood collection should be performed in the following order:

1. PAXgene™ tube for RNA
2. EDTA (purple top) blood collection for plasma and buffy coat
3. P100 EDTA blood collection tube (2ml)
4. Tasso™ HemoLink Device (0.5ml)

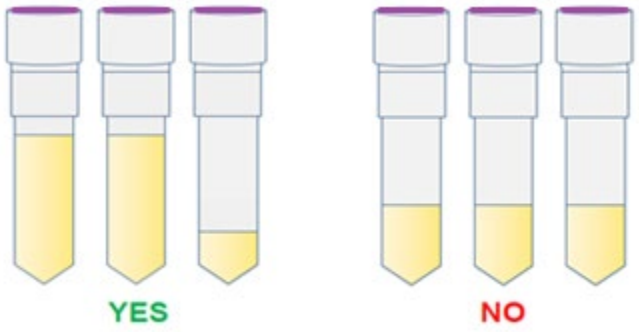
7.2 Blood Collection Protocols

1. PAXgene™ tube for RNA **(Appendix A)**
2. EDTA (purple top) blood collection for plasma and buffy coat (EDTA) **(Appendix B)**
3. Whole Blood Collection for Isolation of Plasma (P100) **(Appendix C)**
4. Whole Blood Collection for the Isolation of Plasma (Tasso HemoLink™) **(Appendix D)**

7.4 Filling Aliquot Tubes

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.

Cap Color	Specimen Type
Purple	Plasma (EDTA)
Clear	Buffy coat
Lavender	Plasma (P100)
White	Plasma (Tasso HemoLink™)



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 shipment notification Form(s). 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 (all visits). Reference Appendix K for frozen shipping instructions.

- Frozen 0.5 ml aliquots of plasma (EDTA)
- Frozen 0.2 ml aliquots of plasma (P100)
- Frozen 0.2 ml aliquot of plasma (Tasso HemoLink™)
- Frozen aliquot of buffy coat
- Frozen PAXgene™ Tube

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 46202-4118

*****Important Note*****

In order to have room for a sufficient amount of dry ice to keep samples frozen up to 24, Include samples for no more than 2-3 subjects per shipping container

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 data collection 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 study 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 documented on the BioSEND Sample Form compared to information entered into the study database.
- Samples frozen and stored longer than three months at the site

10.0 APPENDICES

Appendix A: Whole Blood Collection for Isolation of RNA

Appendix B: Whole Blood Collection for Isolation of Plasma (EDTA) and Buffy Coat

Appendix C: Whole Blood Collection for Isolation of Plasma (P100)

Appendix D: Whole Blood Collection for the Isolation of Plasma (Tasso HemoLink™)

Appendix I: Sample Record and Shipment Notification Form

Appendix K: Frozen Shipping Instructions

Appendix Q: UPS ShipExec™ Thin Client Instructions

Appendix A – Whole Blood Collection for Isolation of RNA

Whole Blood Collection for Isolation of RNA: 2.5 ml PAXgene® tubes are provided by BioSEND for the collection of blood for RNA isolation.

See training video for blood collection for RNA: (http://www.preanalytix.com/videos/rna-tube-collection-video/)

1. Store PAXgene® tube(s) at room temperature 64^0F - 77^0F (18°C to 25°C) before use.

2. Place pre-printed "**RNA**" label on the PAXgene® tube prior to blood draw.

3. Using a blood collection set and a holder, collect blood into the PAXgene® 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. 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 PAXgene® tube with its vacuum is designed to draw 2.5 ml of blood into the tube.

5. Immediately after blood collection, gently invert/mix (180 degree turns) the PAXgene® tube 8 – 10 times. Do not shake the tube!

6. Place the PAXgene® tube upright in a wire or plastic rack. Allow the filled PAXgene® tube to incubate upright at room temperature for 24 hours.

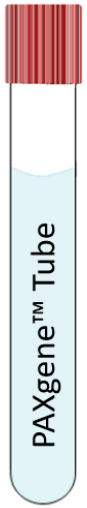
7. Complete the Sample Record and Shipment Notification form (Appendix I).

8. After samples have incubated at room temperature for 24 hours, transfer the **wire or plastic** rack with the PAXgene® tube to -80°C freezer. Do **NOT** use a Styrofoam rack. This will cause the PAXgene® tube to crack when frozen. Store all samples at -80°C until shipped to BioSEND on dry ice.

9. Ship frozen samples to BioSEND according to Appendix K – Frozen Shipping Instructions.

Whole Blood Collection and Preparation – 2.5 ml PAXgene™ RNA Tube

Step One

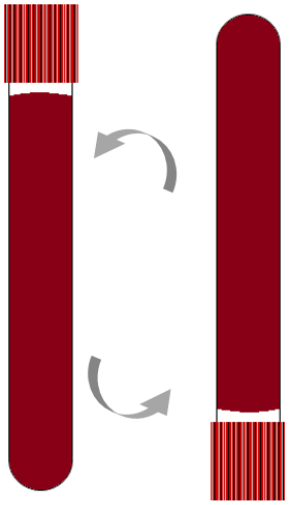


- Store tube at room temperature.
- Label tubes with preprinted RNA label prior to blood draw.

Step Two

- Collect blood in PAXgene™ tube, 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

- Incubate the tubes at room temperature for **2 to 24 hours**.
- Freeze tubes upright at -80^{o}C until shipment.

Appendix B – Whole Blood Collection for Isolation of Plasma (EDTA) and Buffy Coat

Whole Blood Collection for Isolation of Plasma: 10 ml EDTA tube(s) and cryotubes are provided by BioSEND for the collection of plasma.

1. Store empty EDTA tubes at room temperature 64°F – 77°F (18°C to 25°C) prior to use.

2. Place pre-printed **PLASMA EDTA** label on 10 ml EDTA tube and on 10 x 2 ml (purple cap) cryotube tubes. Label one 2ml (clear cap) cryotube with a **BUFFY COAT** label.

3. Pre-chill the labeled cryotubes 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 to 4°C will depend on your centrifuge model.

5. Using a blood collection set and a holder, collect blood into the **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.

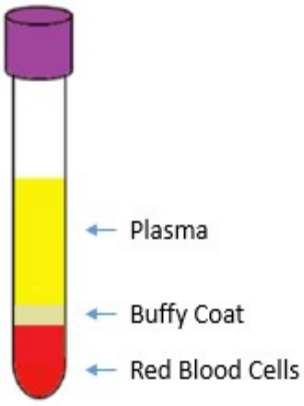
6. Allow at least 10 seconds for a complete blood draw to take place in each tube. Ensure that the blood has stopped flowing into the tube before removing the tube from the holder. The tube vacuum is designed to draw 10 ml of blood into the tube.

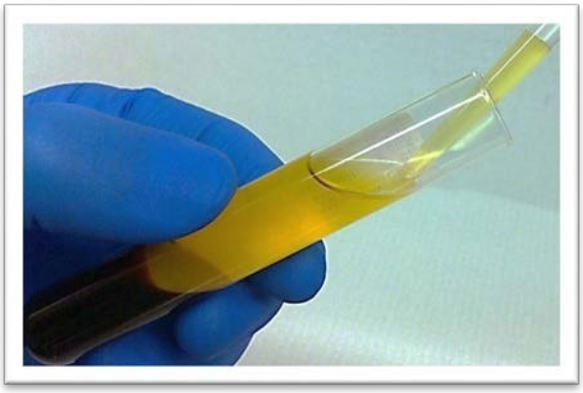
7. Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tube(s) 8 – 10 times. **Do not shake the tubes!**

8. Within 30 minutes of blood collection, centrifuge balanced tubes for 15 minutes at 1500 RCF (x g) at 4°C. It is critical that the tubes be centrifuged at the appropriate speed and temperature 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**

tube so that the plasma is not contaminated (see below). Using a disposable tipped micropipette, transfer plasma into the pre-labeled cryotubes. Aliquot 500 ul per cryotube. This will generate up to 10 aliquots.





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

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

12. Place the labeled cryotubes in the 25 slot cryobox. **Freeze cryotubes upright at -80°C within 90 minutes of draw.** Store all samples at -80°C until shipped to BioSEND on dry ice.

13. Ship frozen samples to BioSEND according to **Appendix K – Frozen Shipping Instructions.**

Plasma and Buffy Coat Collection and Preparation – 10ml EDTA Tube

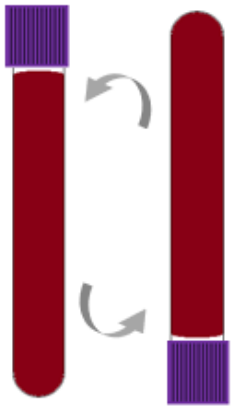
Step One

- Store tubes at room temperature.
- Label collection tube and cryotubes prior to blood draw.
- Pre-chill cryotubes on wet ice for at least 5 minutes.

Step Two

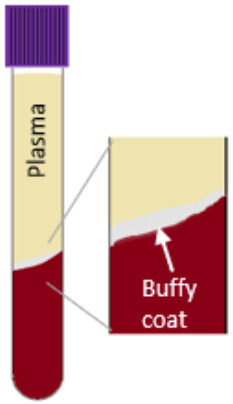
- Collect blood in EDTA tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



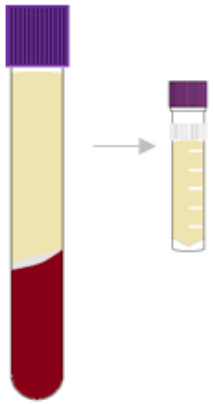
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Four



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

Step Five



- Using a clean transfer pipet, aliquot 500ul of plasma into each purple cryotube.
- Generate up to 10 x 500ul plasma aliquots.

Step Six

- Label clear-capped cryotubes with preprinted buffy coat labels.
- Using a clean transfer pipet, collect the buffy coat (will include residual plasma and some red blood cells).
- Transfer each buffy coat to separate cryotubes.
- Within 90 minutes of draw, freeze plasma and buffy coat aliquots upright at -80°C. Store at -80°C until shipment.

Appendix C – Whole Blood Collection for Isolation of Plasma (P100)

Whole Blood Collection for Isolation of Plasma: 2 ml P100 tube(s) and cryotubes are provided by BioSEND for the collection of plasma.

1. Store empty P100 tubes at room temperature 64°F – 77°F (18°C to 25°C) prior to use.

2. Place pre-printed **PLASMA P100** label on 10 ml P100 tube and on 5 x 0.5 ml (lavender dot on cap) cryotube tubes.

3. Pre-chill the labeled cryotubes 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 to 4°C will depend on your centrifuge model.

5. Using a blood collection set and a holder, collect blood into the **2 ml P100 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 2 ml of blood into the tube.

7. Immediately after blood collection, gently invert/mix (180 degree turns) the P100 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) at 4°C. It is critical that the tubes be centrifuged at the appropriate speed and temperature 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. Using a disposable tipped micropipette, transfer plasma into the pre-labeled cryotubes. Aliquot 200 ul per cryotube. This will generate up to 5 aliquots.

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

11. Place the labeled cryotubes in the 25 slot cryobox. **Freeze cryotubes upright at -80°C within 90 minutes of draw.** Store all samples at -80°C until shipped to BioSEND on dry ice.

12. Ship frozen samples to BioSEND according to **Appendix K – Frozen Shipping Instructions.**

Plasma Collection and Preparation – 2ml P100 Tube

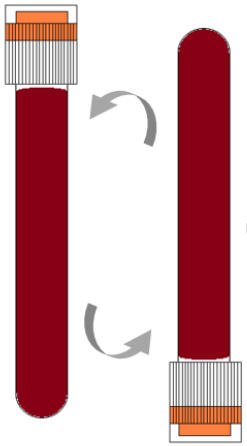
Step One

- Store tubes at room temperature.
- Label collection tube and cryotubes prior to blood draw.
- Pre-chill cryotubes on wet ice for at least 5 minutes.

Step Two

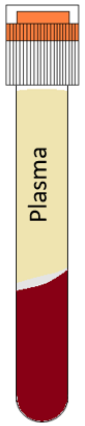
- Collect blood in P100 tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



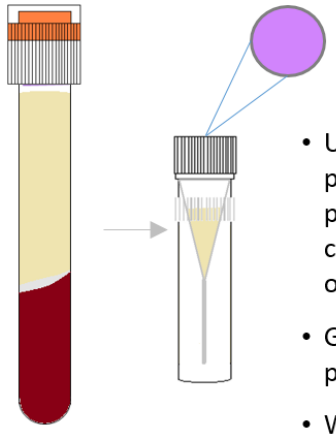
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Four



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

Step Five



- Using a clean transfer pipet, aliquot 200ul of plasma into each 0.5ml cryotube (lavender dot on top).
- Generate up to 5 x 500ul plasma aliquots.
- Within 90 minutes of draw, freeze plasma and buffy coat aliquots upright at -80°C. Store at -80°C until shipment.

Appendix D – Whole Blood Collection for the Isolation of Plasma (Tasso HemoLink™)

Whole Blood Collection for Isolation of Plasma: Tasso HemoLink™ device and cryotubes are provided by BioSEND for the collection of plasma.

1. Store unused Tasso HemoLink™ device(s) and cryotube(s) at room temperature 64°F – 77°F (18°C to 25°C) prior to use.

2. Place pre-printed **PLASMA TASSO** label on collection tube in the Tasso HemoLink™ kit and on 1 x 0.5 ml (white dot on cap) cryotube tubes.

3. Pre-chill the labeled **white dot cryotube** on wet ice for at least 5 minutes. Do not pre-chill the collection tube that is inserted into the Tasso HemoLink™ device.

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. Clean subject's deltoid with an alcohol swab and allow skin to dry.

6. Peel off backing to expose adherent surface and place device on patient's deltoid with the tube pointing to the floor. The adherent will allow the device to stick to the skin.

7. Remove plastic cap and firmly press red button until "pop" is felt and the micro-lancets are deployed.

8. Keep patient as still as possible during the blood collection process. Blood should collect within 3-4 minutes. **Be sure to keep the collection tube pointing towards the floor.**

9. When the collection tube is full, slowly peel the device from patient's arm, and place bandage on draw site. While removing device, Keep device upright; do not put it down or lay it flat.

10. Remove collection tube by rotating it and pulling down and cap tube. Discard Tasso HemoLink™ device in sharps container.

11. Invert the collection blood tube several times to ensure anti-coagulant mixes well with the blood.

12. Within 30 minutes of blood collection, centrifuge tube 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 plasma separation.

13. Remove the plasma by tilting the tube and placing the pipette tip along the lower side of the wall. Using a disposable tipped micropipette, transfer plasma into the 0.5ml (white dot) cryotube. Aliquot 200 ul per cryotube.

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

15. Place the labeled cryotube in the 25 slot cryobox. **Freeze cryotube upright at -80°C within 90 minutes of draw.** Store all samples at -80°C until shipped to BioSEND on dry ice.

16. Ship frozen samples to BioSEND according to **Appendix K – Frozen Shipping Instructions.**

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 also be physically included in the shipper. The form can be completed via REDCap by following the bellow link:

- **Link to Sample Collection and Processing Form: https://redcap.link/Care4KIDSSampleForm**

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.

It is preferred that you complete the form online via the REDCap link above. However, a copy of the printed form is available on the following pages, should you need a back-up option. Please note that if you do not complete the form online, you will need to email a copy of the form directly to biosend@iu.edu prior to shipment.

CARE4Kids Specimen Collection And Processing Form

Please complete the Specimen Collection and Processing Form, below.

Study Site	◯ Children's National Hospital ◯ Seattle Children's Hospital ◯ University of California - Los Angeles ◯ University of Rochester ◯ University of Texas - Southwestern ◯ Wake Forest University
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.	_____
CARE4Kids Subject ID:	_____
Subject's biological sex (used for DNA quality control)	◯ Male ◯ Female ◯ Other
Visit	◯ T1 ◯ T3
IU Kit Number	_____

Blood Collection and Processing

Date of venipuncture blood collection

_____

Time of venipuncture blood collection

_____
(Use 24 Hour clock.)

Was EMLA used?

◯ Yes
◯ No

Date participant last ate

_____

Time participant last ate

_____
(Use 24 Hour clock.)

1. RNA (PAXGene™ tube, 2.5 mL)

Was blood collected for RNA?

◯ Yes
◯ No

Number of PAXGene™ tubes collected for RNA

_____

Blood volume collected for RNA

Note: Max collection volume per tube is 2.5 ml.

_____
(mL)

Reason volume was less than standard

◯ Difficult stick/poor veins
◯ Patient dehydrated
◯ Bad tube vacuum
◯ Other

Date RNA was frozen

_____

Time RNA was placed in freezer

_____
(Use 24 Hour clock.)

RNA storage temperature

_____
(degrees Celsius)

RNA notes

_____

2. PLASMA and BUFFY COAT (Purple-top EDTA tube, 10 mL)

Was blood collected and processed for PLASMA EDTA?

◯ Yes
◯ No

Blood volume collected for PLASMA EDTA

_____ (mL)

What volume of EDTA tube was used to collect plasma?

◯ 10mL
◯ 6mL

Reason volume was less than standard

◯ Difficult stick/poor veins
◯ Patient dehydrated
◯ Bad tube vacuum
◯ Other

Time of PLASMA EDTA tube centrifugation

_____ (Use 24 Hour clock.)

Duration of PLASMA EDTA tube centrifugation

_____ (minutes)

Rate of PLASMA EDTA tube centrifugation

_____ (x g)

Temperature of PLASMA EDTA tube centrifugation

_____ (degrees Celsius)

Total volume of PLASMA EDTA collected

_____ (mL)

Number of PLASMA EDTA aliquots created

_____ (Each aliquot should be 0.5 mL)

Was the BUFFY COAT collected?

◯ Yes
◯ No

Time PLASMA EDTA and BUFFY COAT were placed in freezer

_____ (Use 24 Hour clock.)

PLASMA EDTA and BUFFY COAT storage temperature

_____ (degrees Celsius)

PLASMA EDTA notes

_____

3. PLASMA (P100 tube, 2 mL)

Was blood collected and processed for PLASMA P100?

◯ Yes
◯ No

Blood volume collected for PLASMA P100

_____ (mL)

Reason volume was less than standard

◯ Difficult stick/poor veins
◯ Patient dehydrated
◯ Bad tube vacuum
◯ Other

Time of PLASMA P100 tube centrifugation

_____ (Use 24 Hour clock.)

Rate of PLASMA P100 tube centrifugation

_____ (x g)

Duration of PLASMA P100 tube centrifugation

_____ (minutes)

Temperature of PLASMA P100 tube centrifugation

_____ (degrees Celsius)

Total volume of PLASMA P100 collected

_____ (mL)

Number of PLASMA P100 aliquots created

_____ (Each aliquot should be 0.2 mL)

Time PLASMA P100 were placed in freezer

_____ (Use 24 Hour clock.)

PLASMA P100 storage temperature

_____ (degrees Celsius)

PLASMA P100 notes

_____

4. PLASMA (Tasso™ HemoLink Device, 0.4-0.6 mL)

Was blood collected and processed for PLASMA TASSO?

◯ Yes
◯ No

Date of capillary blood collection

_____

Time of capillary blood collection

_____ (Use 24 Hour clock.)

Blood volume collected for PLASMA TASSO	_____ (mL)
Reason volume was less than standard	◯ Difficult stick/poor veins ◯ Patient dehydrated ◯ Bad tube vacuum ◯ Other
Time of PLASMA TASSO tube centrifugation	_____ (Use 24 Hour clock.)
Rate of PLASMA TASSO tube centrifugation	_____ (x g)
Duration of PLASMA TASSO tube centrifugation	_____ (minutes)
Total volume of PLASMA TASSO collected	_____ (mL)
Number of PLASMA TASSO aliquots created	_____ (Each aliquot should be 0.2 mL)
Time PLASMA TASSO were placed in freezer	_____ (Use 24 Hour clock.)
PLASMA TASSO storage temperature	_____ (degrees Celsius)
PLASMA TASSO notes	_____

CARE4Kids 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:

- ◯ Children's National Hospital
- ◯ Seattle Children's Hospital
- ◯ University of California - Los Angeles
- ◯ University of Rochester
- ◯ University of Texas - Southwestern
- ◯ Wake Forest University
- ◯ Washington University

CARE4Kids Subject ID: _____

Visit:

- ◯ T1
- ◯ T3

IU Kit Number: _____

Date of blood collection: _____

PLASMA EDTA

Number of PLASMA EDTA aliquots shipped: _____

Number of BUFFY COAT aliquots shipped: _____

PLASMA P100

Number of PLASMA P100 aliquots shipped: _____

PLASMA TASSO

Number of PLASMA TASSO aliquots shipped: _____

RNA

Number of PAXGene™ 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?

_____

Appendix K – Frozen Shipping Instructions

IMPORTANT!

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

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, place labeled tubes in bubble sleeves and seal.

8. Place the tubes 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.

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.

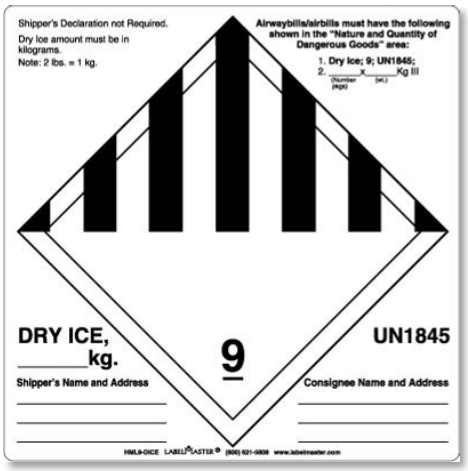
11. If including additional biohazard bags in package, include a layer of dry ice (approximately 2 inches) between each biohazard bag.

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 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.

BioSEND
IU School of Medicine
351 W. 10th Street
TK-217
Indianapolis, IN 46202

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.