An Intermediate Expanded Access Protocol with CNM-Au8 for Amyotrophic Lateral Sclerosis for Protocol CNMAU8.EAP04 (NIH Grant RFA-NS-23-012)

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March 2025

Section	Change
Document Footer	Updated to "Version (March 2025)"
Section 4.0	Adding 36 week visit
Appendix I	Adding 36 week visit

National Institute of Neurological Disorders and Stroke Biorepository:

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

Biospecimen Collection, Processing, and Shipment Manual for

An Intermediate Expanded Access Protocol with CNM-Au8 for Amyotrophic Lateral Sclerosis for Protocol CNMAU8.EAP04 (NIH Grant RFA-NS-23-012)

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:

- LiHep tube for Creatinine Testing
- Plasma
- Buffy Coat
- Whole Blood Aliquots

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
EAP	Expanded Access Program
EDTA	Ethylene Diamine Tetra-acetic Acid
IATA	International Air Transport Association
LiHep	Lithium Heparin
PST	Plasma Separator Tube
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute
WBC	White Blood Cells

3.0 BioSEND Information

3.1 BioSEND Contacts

Tatiana Foroud, PhD, Principal Investigator

Claire Wegel, MPH, Project Manager
Email: cwegel@iu.edu

Ashley Garwood, CCRP, Clinical Research Coordinator
Email: hoviousa@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 reports 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
June 19th	Juneteenth (observed)
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 BioSEND has extended closures surrounding the week of Thanksgiving and the last two weeks of the year. BioSEND will post notice of these closures on biosend.org and send email reminders in advance. Please contact biosend@iu.edu if you are unsure whether the day you plan to ship is permissible.

For up-to-date holiday closure information and instructions, please visit https://biosend.org/holiday-closures.

4.0 Protocol Schedule for Biospecimen Submission to BioSEND – CNMAu8

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.

If a sample is not obtained at a particular visit, this should be recorded in the notes section of the Sample Collection and Processing Form**.** This form should be submitted to BioSEND on or prior to the date of shipment. A physical copy should also be included in the sample shipment to BioSEND.

Note: All samples in this protocol will ship frozen on dry ice to BioSEND

Samples collected at visit weeks 1 (Screening/Baseline), 12, 24, 36, 48, 72, 96, 120, and 144 or ET					
Draw Order	**Collection Tube**	**Specimen Type**	**Aliquot Volume**	**Total Number of Aliquots**	**Cryovial Cap Color**
1	1 PST LiHep (Green-top) Tube (4.5ml)	Plasma for creatinine testing	2ml	1	Orange
2	2 EDTA (purple-top) Tubes (10ml)	Plasma for Quanterix Nfl and UCHL1 analysis	1.5ml	6	Purple
		Buffy Coat for DNA extraction	~750ul	2	Clear
3	1 EDTA (purple-top) Tube (3ml)	Whole Blood for NadMED analysis	0.5ml	6	Green

*For females of child-bearing potential only: A urine pregnancy test should take place at the Screening/Baseline visit and as clinically indicated thereafter (See Appendix H for details).

5.0 Specimen Collection Kits and Supplies

BioSEND will provide labels and supplies only for those specimens that are to be shipped back to the BioSEND repository. Any tubes that will remain at the collection site or shipped to other repositories should be labeled accordingly. Links to each study's kit request module, sample submission forms, and other information can be found at https://biosend.org/coordinate-studies/active-studies

5.1 Ordering Kits and Supplies

Study sites are responsible for ordering study kits. We advise sites to proactively confirm kits are on hand ahead of study visits.

Kits and individual items can be ordered as required through the BioSEND kit request module:

- **CNMAu8.EAP04**: http://kits.iu.edu/biosend/CNMAu8EAP04

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

5.2 Kit Contents

Collection kits contain the following (for each participant) and provide the necessary supplies to collect samples from a given participant visit. 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.*

In-Clinic Collection Kit	
Supply	**Quantity**
Cryovial (Sarstedt®) with green cap, 2ml	6
Cryovial (Sarstedt®) with purple cap, 2ml	6
Cryovial (Sarstedt®) with clear cap, 2ml	2
Cryovial (Corning®) with orange cap, 2ml	1
LiHep (plastic) tube, 4.5ml	1
EDTA (plastic) tube, 10ml	2
EDTA (plastic) tube, 3ml	1
Disposable pipet, 3ml	4
Cryobox, 25 cell	1
hCG pregnancy dipstick test	1
Urine cup	1
Label set (kit & specimen labels)	1

Each In-Clinic Collection Kit contains the supplies to collect samples for one study visit

In-Clinic Shipping Kit – Standard Size	
Supply	**Quantity**
Plastic Biohazard bag with absorbent sheet	4
Bubble-wrap tube sleeve	2
UPS Airbill Sleeve	1
Shipping box/Styrofoam container, standard size	1
UN3373 Category B Label	1
Fragile label	1
Dry ice label	1

The standard size In-Clinic Shipping Kit contains the supplies to ship up to two participant visits' worth of samples (that is, up to 4 biohazard bags may be shipped in a single shipper

In-Clinic Shipping Kit – Bulk Size	
Supply	**Quantity**
Plastic Biohazard bag with absorbent sheet	8
Bubble-wrap tube sleeve	4
UPS Airbill Sleeve	1
Shipping box/Styrofoam container, bulk size	1
UN3373 Category B Label	1
Fragile label	1
Dry ice label	1

The bulk size In-Clinic Shipping Kit contains the supplies to ship up to four participant visits' worth of samples (that is, up to 8 biohazard bags may be shipped in a single shipper

Remote Collection and Shipping Kit	
Supply	**Quantity**
Cryovial (Sarstedt®) with green cap, 2ml	6
Cryovial (Sarstedt®) with purple cap, 2ml	6
Cryovial (Sarstedt®) with clear cap, 2ml	2
LiHep (plastic) tube, 4.5ml	1
EDTA (plastic) tube, 10ml	2
EDTA (plastic) tube, 3ml	1
Disposable pipet, 3ml	3
Bubble-wrap tube sleeve	1
Cryobox, 25 cell	1
hCG pregnancy dipstick test	1
Urine cup	1
Label set (kit & specimen labels)	1
Plastic Biohazard bag with absorbent sheet	2
UPS Airbill	1
Shipping box/Styrofoam container	1
UN3373 Category B Label	1
Fragile label	1
Dry ice label	1

Remote Collection and Shipping Kits contain the supplies to collect and ship samples for one study visit

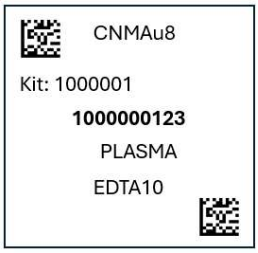
6.0 Specimen Labels

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. If labels are provided but the sample is not collected, please discard the unused labels.

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.

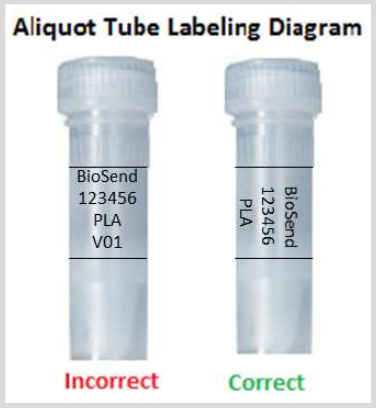


The **Specimen Labels** are placed on specimen tubes shipped to BioSEND. Each Specimen Label has a unique barcode that is tied to the Kit. The specimen type and collection tube will be noted on the tube. For example, the label to the left would be placed on a plasma aliquot generated from a 10ml EDTA tube.

6.2 Affixing Labels

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

- Label all tubes prior to 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.
- BioSEND provides extra labels for all specimen types in each kit. These can be used if, for example, a difficult draw occurs, and a new tube (and label) is needed. If you do not need the extra labels, please dispose of them at your site after the visit.
- The specimen 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.**

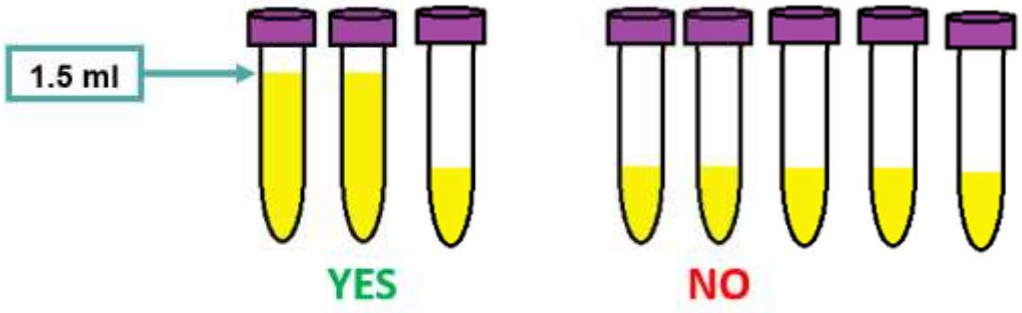
7.1 Blood Collection Protocols

1. Whole Blood Collection and Processing for Plasma Creatinine (Appendix A)
2. Whole Blood Collection for Plasma and Buffy Coat (Appendix B)
3. Whole Blood Collection and Processing (Appendix D)

7.2 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.

Please generate as many standard-sized (1.5ml) plasma and (0.5ml) whole blood aliquots as possible. Fewer standard sized aliquots (with potentially one residual aliquot) is preferable to more aliquots with non-standard volume. Buffy Coats will generally be between 0.5ml-1ml in volume.



To assist in the preparation and aliquoting of samples, colored caps are used for the aliquot tubes. The chart below summarizes the association between cap color and type of aliquot.

Cap Color	Specimen Type
Green	Whole Blood
Orange	LiHep Plasma
Purple	Plasma (EDTA)
Clear	Buffy Coat

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/).

We encourage all studies to use our custom UPS shipping interface to create shipping waybills and schedule package pickups. More information can be found at https://biosend.org/shipping-resources.

8.1 Sample Collection and Processing Form

The Specimen Collection and Processing Form should be completed for all samples submitted to BioSEND. For in-clinic visits, please see Appendix I for further instructions. For remote visits, please see Appendix J.

8.2 Shipping Instructions

All samples are shipped frozen. Reference Appendix K for frozen shipping instructions and Appendix Q for generating airway bills and scheduling pick-ups. Note that for remote visits, a pre-printed UPS airway bill will be included in each kit.

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

9.0 Reconciliation and Non-Conformance

Sample forms 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.

BioSEND will contact the site as soon as possible when a discrepancy or issue is found with either the samples or paperwork.

Common non-conformance issues that will result in BioSEND staff contacting your site include:

- Missing samples (samples documented on the sample form that are not physically present in the shipment)
- Incorrect samples collected and shipped
- Damaged or incorrectly prepared samples
- Unlabeled or mislabeled samples
- Samples frozen and stored longer than three months at the site

10.0 Appendices

Appendix A: Whole Blood Collection and Processing for Plasma Creatinine

Appendix B: Whole Blood Collection and Processing for Plasma and Buffy Coat

Appendix D: Whole Blood Collection and Processing

Appendix E: Accessing Creatinine Results

Appendix F: Creatinine Requisition Form

Appendix H: hCG Pregnancy Test

Appendix I: In-Clinic Visit Sample Collection and Processing Form

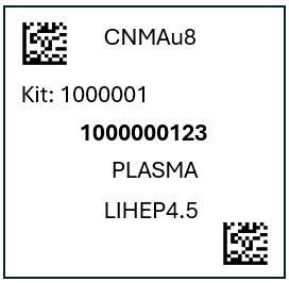
Appendix J: Remote Visit Sample Collection and Processing Form

Appendix K: Frozen Shipping Instructions

Appendix Q: UPS ShipExec™ Thin Client Instructions

Appendix A – Whole Blood Collection and Processing for Plasma Creatinine Testing

One 4.5ml Green-Top PST LiHep Tube is provided by BioSEND for Collection and Processing of Plasma for Creatinine Testing



1. Store empty tubes and collection supplies at room temperature, 64°F - 77°F (18°C to 25°C) before use.

2. Place “PLASMA LIHEP” specimen label on the 4.5ml green-top LiHep tube prior to blood draw.

3. Store empty tubes and collection supplies at room temperature, 64°F - 77°F (18°C to 25°C) before use.

4. Place “PLASMA LIHEP” specimen label on the 4.5ml green-top LiHep tube prior to blood draw.

5. Using a blood collection set and a holder, collect whole blood into the 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. Ensure that the blood has stopped flowing into the tube before removing the tube from the holder. Immediately after blood collection, gently invert/mix (180 degree turns) the tube 8-10 times. Do not shake the tube!

7. Within 30 minutes of blood collection, centrifuge tube at room temperature for 15 minutes at 1500 RCF (x g). If any deviations occur during processing, please note them on the collection and processing form.

Note: 4.5ml LiHep tube can be centrifuged at the same time as the 10ml EDTA tubes for plasma and buffy coat (Appendix B).

6. After centrifugation, 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 packed red blood cells at the bottom of the tube so that the plasma is not contaminated. Using a disposable tipped micropipette, transfer plasma into the orange-capped cryovial.

   Note: Aliquot all the plasma from the collection tube into the orange capped cryovial. Each tube should yield approximately 2mls of plasma.

7. **For in-clinic visits**: After centrifugation, freeze the tube upright in a -80°C freezer. Tube should remain frozen until shipment to BioSEND.

8. **For remote visits**: After centrifugation, place the tube in one of the provided biohazard bags and seal. Wedge the tube in the dry ice so that the tube is upright.

9. Complete the In-Clinic Collection and Processing Form (http://kits.iu.edu/biosend/CNMAu8EAP04SampleForm) or the Remote Collection and Processing Form (Appendix J), depending on visit type.

10. Ship the tube to BioSEND according to Appendix K.

Whole Blood Collection and Processing for Plasma Creatinine – 4.5ml PST LiHep (green top) Tube

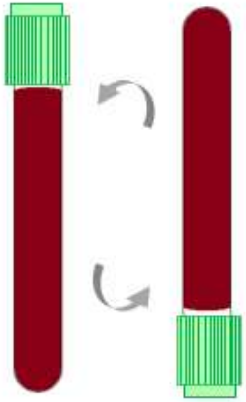
Step One

- Store collection supplies at room temperature prior to use.
- Label collection tube and orange-caped cryovial with PLASMA LIHEP label prior to blood draw.

Step Two

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

Step Three

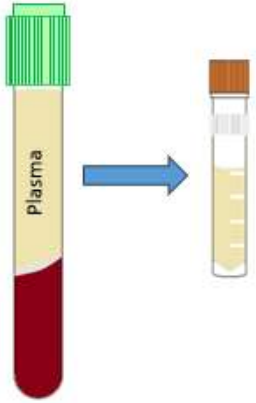


- Immediately after blood draw, **gently** invert tube 8-10 times to mix samples.

Step Four

- Within 30 minutes of blood draw, centrifuge sample at room temperature at 1500 x g for 15 minutes.

Step Five

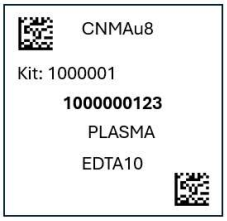


- After centrifugation, remove the plasma by tilting the tube and placing the pipette tip along the lower side of the wall. Use caution not to touch the packed red blood cells at the bottom of the tube so that the plasma is not contaminated.
- Aliquot all plasma into the orange-capped cryovial.
- Within 2 hours of collection, freeze sample in -80 freezer or on dry ice.

Appendix B – Whole Blood Collection and Processing for Plasma and Buffy Coat

Whole Blood Collection for Plasma and Buffy Coat using two 10 ml EDTA (plastic) tubes

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



2. Place "PLASMA" specimen labels on 10 ml EDTA tubes and on the six purple-capped 2 ml cryovial tubes. Place "BUFFY COAT" specimen labels on the two clear-capped 2ml cryovial tubes. Extra labels are provided in the kit, should they be needed. If you do not need the extra labels, please dispose of them at your site.

3. Using a blood collection set and a holder, collect blood into the purple top 10 ml EDTA (plastic) 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 the 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 tube vacuum is designed to draw 10 ml of blood into the tube.

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

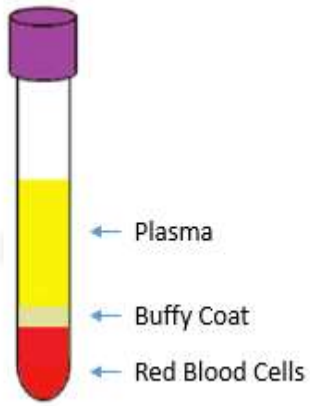
6. Within 30 minutes of blood collection, centrifuge tubes at room temperature for 15 minutes at 1500 RCF (x g). If any deviations occur during processing, please note them on the collection and processing form.

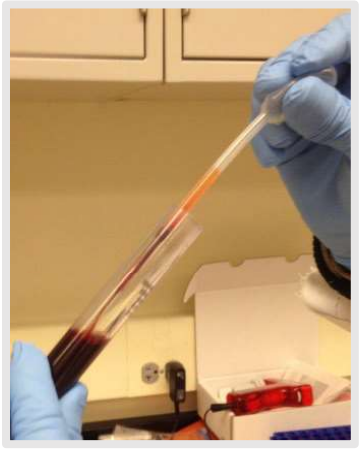
   Note: The 10ml EDTA tubes can be centrifuged at the same time as the 4.5ml LiHep tube (Appendix A).

7. 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 graphic on next page). Using a disposable tipped micropipette, transfer plasma into the purple-capped cryovials. Aliquot 1.5 ml per cryovial. Each EDTA tube should yield approximately 4-5 ml of plasma.

   Note: If a low volume draw occurs, please generate as many 1.5ml aliquots as possible. Fewer standard size aliquots are preferred over six aliquots of non-standard size.

8. After plasma has been removed from the EDTA tubes, aliquot buffy coat layer from one of the EDTA tubes (see figure below) into a clear-capped cryovial using a disposable graduated micropipette. Repeat for the second EDTA tube. Each 10ml EDTA tube will produce 1 buffy coat aliquot, resulting in a total of two buffy coat aliquots. The buffy coat aliquot is expected to have a reddish color from the red blood cells. If the buffy coat looks like the plasma (ie, yellow), it was not properly captured.





9. After plasma and buffy coat has been aliquoted into cryovials, **discard** the 10ml EDTA collection tubes. Do not send these tubes to BioSEND.

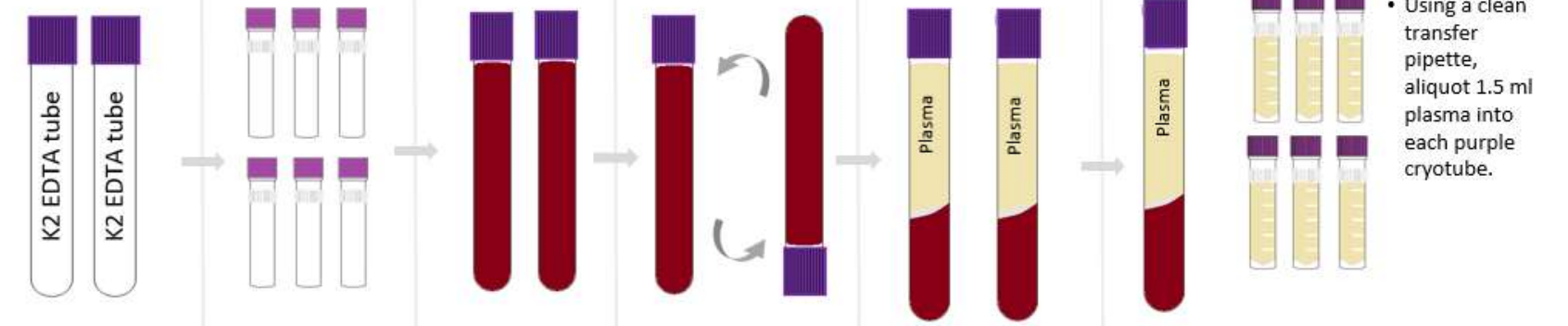
10. Place the labeled cryovials in the 25 slot cryobox.

11. **For in-clinic visits**: Transfer to -80°C Freezer as soon as possible. Store all samples at -80°C until shipped to BioSEND on dry ice. Complete the In-Clinic Collection and Processing Form (Appendix I) at http://kits.iu.edu/biosend/CNMAu8EAP04SampleForm

12. **For remote visits**: Place cryobox in biohazard bag and seal. Note that whole blood aliquots (Appendix D) should also be placed in cryobox before sealing. Transfer the biohazard bag to dry ice as soon as possible, keeping the box upright and level to help the aliquots freeze in an upright position. Complete the Remote Collection and Processing Form (Appendix J).

13. Ship the aliquots to BioSEND according to Appendix K.

Plasma and Buffy Coat Preparation –10 ml EDTA (Purple Top) Tubes



- Store tubes at room temperature.
- Label 6 purple-capped cryotubes for plasma and 2 clear-capped cryotubes for buffy coat.
- Pre-chill cryotubes on wet ice for at least 5 minutes.
- Collect blood in EDTA tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.
- Immediately after blood draw, invert tubes 8-10 times to mix samples.
- Within 30 minutes of blood draw, centrifuge sample at room temperature at 1500 x g for 15 minutes.
- Using a clean transfer pipette, aliquot 1.5 ml plasma into each purple cryotube.

Step Seven

- Using a clean transfer pipette, collect the buffy coat (will have residual plasma and some RBCs included).
- Transfer the buffy coat from each EDTA tube into a clear-capped cryotube.
- Store plasma & buffy coat upright at -80 until shipment.

Appendix D – Whole Blood Collection

One 4ml Purple-Top EDTA Tube is provided by BioSEND for Whole Blood collection for the generation of up to six 0.5ml whole blood aliquots.

1. Store empty tubes and collection supplies at room temperature, 64°F - 77°F (18°C to 25°C) before use.

2. Place a "WBLD" specimen label (WBLD) on the four 2ml cryovials with green caps. Extra labels are provided in the kit, should they be needed. If you do not need the extra labels, please dispose of them at your site.

3. Using a blood collection set and a holder, collect whole blood into the 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.

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

5. Using a disposable pipette, transfer whole blood into the pre-labeled cryovials with green caps. Aliquot 0.5 ml of whole blood per cryovial (total vials = up to 6 with 0.5 ml each). Be sure to only place whole blood in cryovials with green caps and labeled with "WBLD" labels.

Note: If a low volume draw occurs, please generate as many 0.5ml aliquots as possible. Fewer standard size aliquots are preferred over six aliquots of non-standard size.

6. After whole blood has been aliquoted into cryovials, **discard** the 4ml EDTA tube. Do not send the collection tube to BioSEND.

7. Place the labeled cryovials in the 25-slot cryovial box.

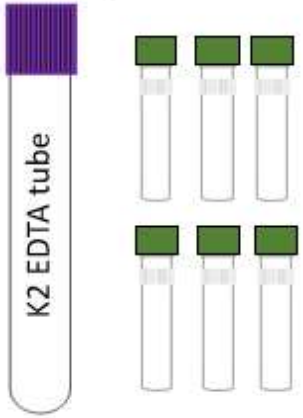
8. **For in-clinic visits**: Transfer to -80°C Freezer within an hour of collection. Store all samples at -80°C until shipped to BioSEND on dry ice. Complete the In-Clinic Collection and Processing Form (Appendix I) at http://kits.iu.edu/biosend/CNMAu8EAP04SampleForm

9. **For remote visits**: Place cryobox in biohazard bag and seal. Note that plasma and buffy coat aliquots (Appendix B) should also be placed in cryobox before sealing. Transfer the biohazard bag to dry ice as soon as possible, keeping the box upright and level to help the aliquots freeze in an upright position. Whole blood aliquots should be frozen within one hour of collection. Complete the Remote Collection and Processing Form (Appendix J).

10. Ship aliquots to BioSEND according to Appendix K.

Whole Blood Preparation – 4 ml K2 EDTA (Purple Top) Tube

Step One

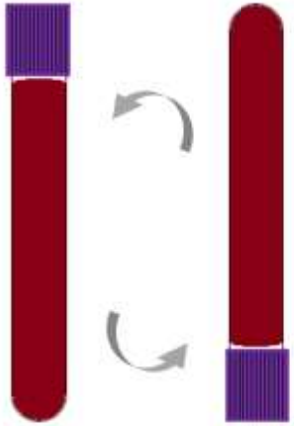


- Store tubes at room temperature.
- Label green-capped cryovials with WBLD labels

Step Two

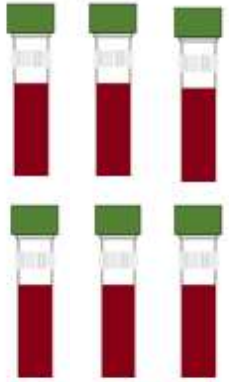
- Collect blood in 4ml K2 EDTA tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



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

Step Four



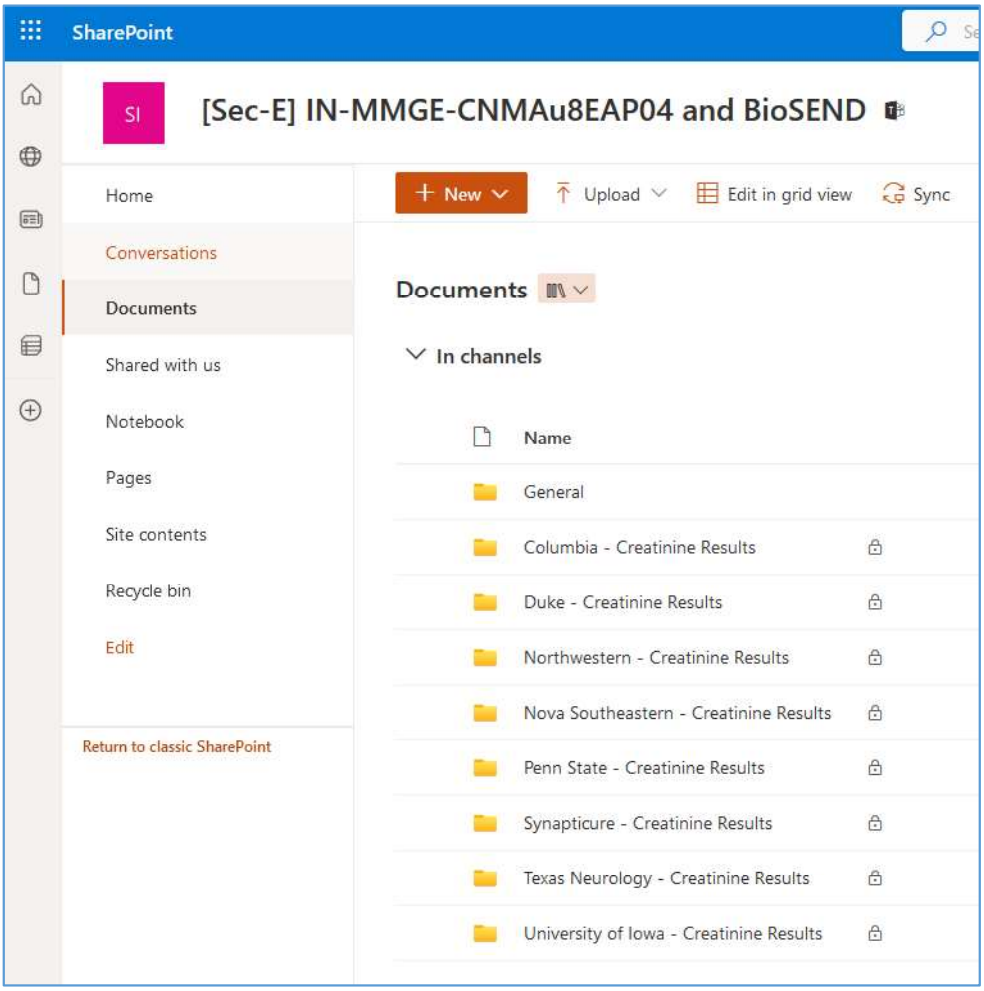
- Aliquot 0.5ml of blood from the tube into each cryovial.
- Transfer aliquots to the cryobox and transfer to a -80 freezer or dry ice, depending on visit type.
- Ship samples to BioSEND according to Appendix K.

Appendix E – Accessing Creatinine Results

1. Once the 4.5ml LiHep tube collected for creatinine testing (Appendix A) has been received at BioSEND, BioSEND will be transfer the tube to the Indiana University Pathology Lab for testing.

2. As results are generated, BioSEND will upload the report into the Microsoft Sharepoint location titled "IN-MMGE-CNMAu8EAP04 and BioSEND".

3. Each site will only have access to its own channel/folder within the "IN-MMGE-CNMAu8EAP04 and BioSEND" location, so that a site may only view results from participants enrolled at their site. These channels/folders are named in the form "[Site Name] – Creatinine Results"



4. **To request access**, please email biosend@iu.edu and hoviousa@iu.edu with the subject line "Creatinine Results Access – Add Users". The email should include the names and email addresses of study staff that should be added to your site's channel.

5. **To remove access**, please email biosend@iu.edu and hoviousa@iu.edu with the subject line "Creatinine Results Access – Remove Users". The email should include the names and email addresses of study staff that should be added to your site's channel.

6. Uploaded results files will be named as "Participant Number_IU Kit Number".

7. Below is an example of how the results will appear. The value in column "Result" is the creatinine value and column "Ref Range" is the reference range.

Collected Date: 5/21/2024 16:02 EDT

Procedure	Result	Units	Ref Range	Received	Verified
Creatinine SerPl QN	0.75	mg/dL	[0.60-1.20]	5/21/2024 16:03 EDT	5/21/2024 16:07 EDT

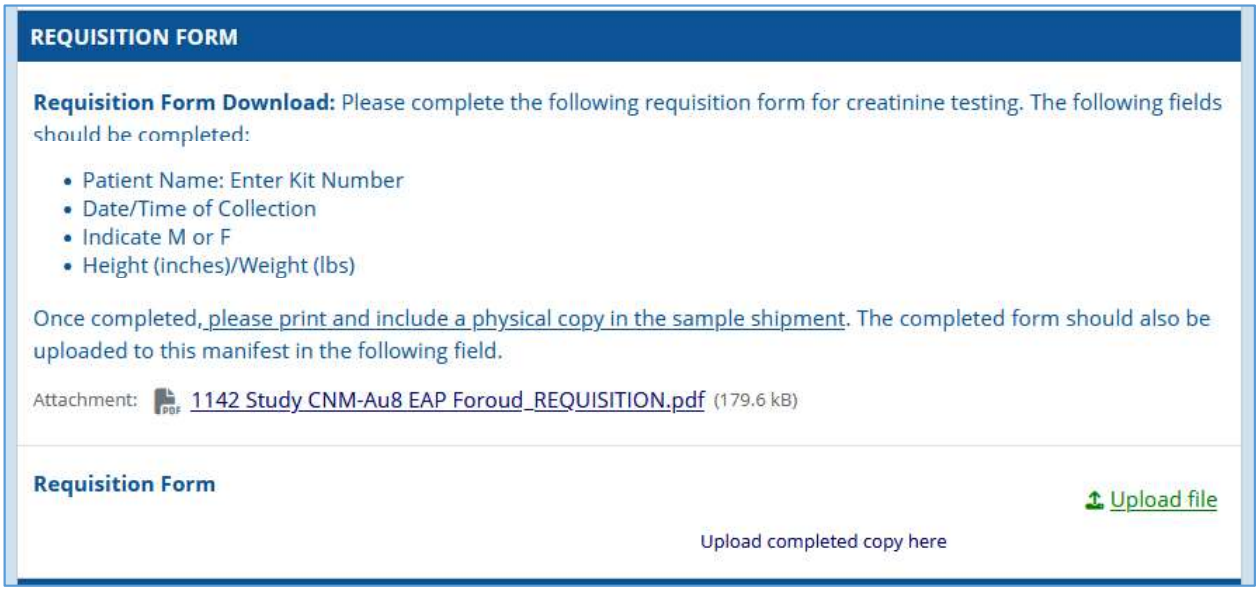
Abnormal results will be note per the following legend: C = Critical, * = Abnormal, H = High, L = Low, T = Textual result, c = Corrected, f = Comment, d = Interp data, r = Performing lab

8. Please email biosend@iu.edu with any questions.

Appendix F – Creatinine Requisition Form

1. Once the 4.5ml LiHep tube collected for creatinine testing (Appendix A) has been received at BioSEND, BioSEND will transfer the tube to the Indiana University Pathology Lab for testing.

2. To facilitate testing, sites should complete a requisition form.
   a. **In-Clinic Visits**: A fillable PDF of the requisition form is available to download from the Frozen Shipping Manifest portion of the In-Clinic Visit Sample Collection and Processing Form (Appendix I).
      i. Once completed, please print, and include a physical copy in the sample shipment. The completed form should also be uploaded to the REDCap system using the 'Upload File' field.



   b. **Remote Visits**: A paper version of the requisition form will be included in remote kits. If an additional copy is needed, the requisition form can be printed from the following page.

3. The following fields on the requisition form should be completed by sites:
   a. Patient Name: Enter or write-in Kit Number
   b. Date/Time of Collection (24hr clock)
   c. Indicate M or F
   d. Height (inches)/Weight (lbs)
   e. Do NOT enter or check anything in the "STAT" or "Physician" boxes

Patient Name (BioSEND, Kit Number):	DOB:		Date/Time of Collection (24hr clock):
BioSEND, a. _____	01/01/1900	☐ STAT	b.
c. ☐M ☐F	HEIGHT (inches)/WEIGHT (lbs) d.	Physician: Pathnet, Client	

Indiana University Health

Study/Research Lab Orders

IU Health Pathology Laboratory
350 W. 11th Street, Rm 5013
Indianapolis, IN 46202
317.491.6000 or 800.433.0740
Fax: 317.491.6001

SPA & Non-Lab Teams: Do NOT register - Send Directly to Lab

Patient Name (CNM, Kit Number):	DOB:		Date/Time of Collection (24hr clock):
CNM, _____	01/01/1900	☐ STAT	
☐M ☐F	HEIGHT (inches)/WEIGHT (lbs)	Physician: Pathnet, Client	

Client Code: 1142

Study CNM-Au8 EAP Foroud

Study Coordinator: Ashley Garwood

351 W 10th St, TK 321

Phone: (317) 278-6158

Fax: 317-278-1100

Attention Coordinators and/or Nursing staff: Please do not attach Cerner registration labels to study requisitions

Test Code		Test Name
6014	(x)	**Creatinine SerPl QN**

Appendix H – Hcg Pregnancy Test

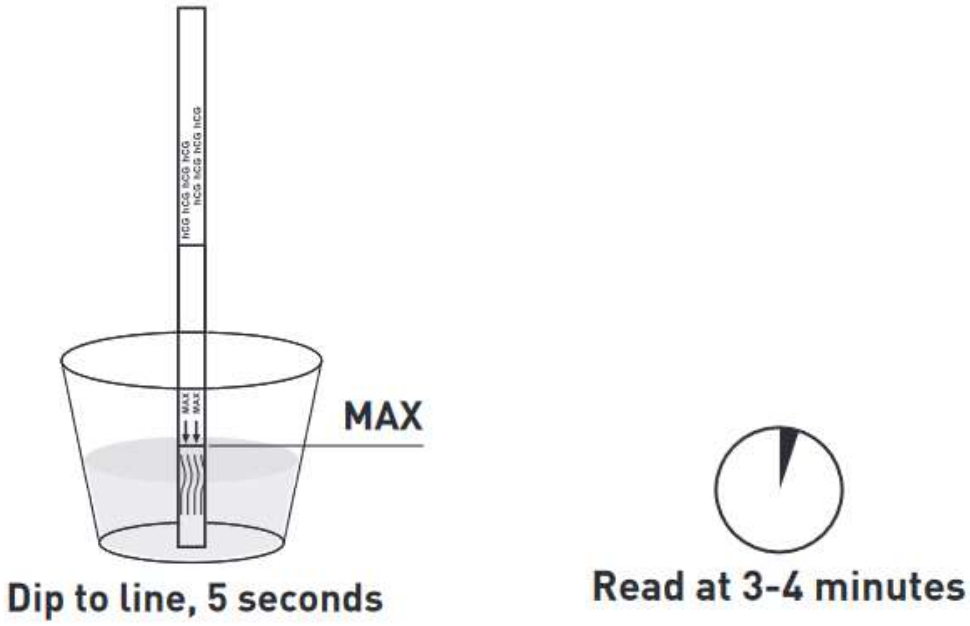
For females of child-bearing potential only. A urine pregnancy test should take place at the Screening/Baseline visit and as clinically indicated thereafter.

1. Store tests and collection supplies at room temperature, 64°F - 77°F (18°C to 25°C) before use.

2. Instruct participant to use the provided collection cup to collect urine. Participant will need to provide enough urine to immerse the test area of the dipstick.

3. Remove the test dipstick from the sealed pouch. Do not open test until ready to use, as test should be used as soon as possible after opening.

4. With arrows pointing toward the urine specimen, immerse the test dipstick vertically in the urine specimen for at least 5 seconds. Do not pass the maximum line (MAX) on the test dipstick when immersing the dipstick (refer to illustration below).



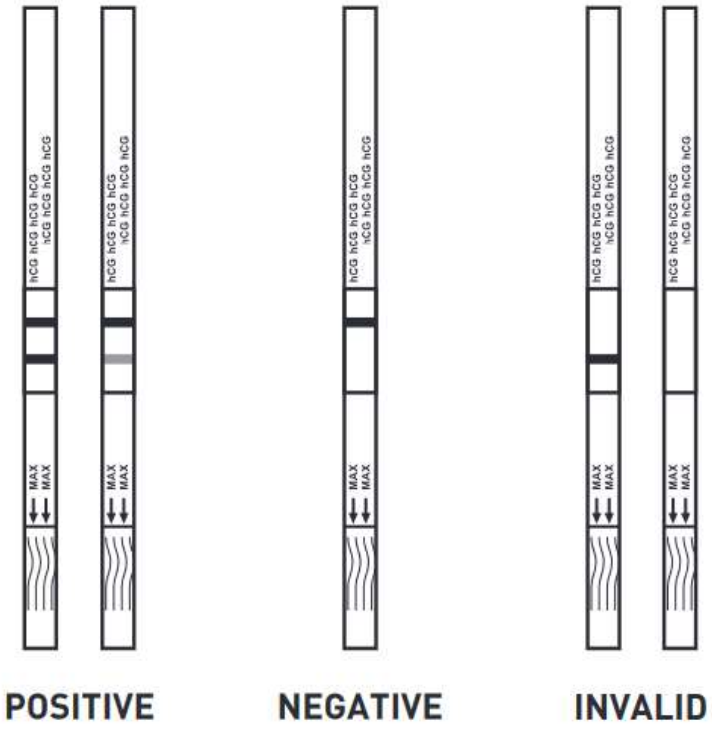
5. Place the test dipstick on a non-absorbent, flat surface, start the timer and wait for the red line(s) to appear. Read the result at 3-4 minutes. Do not interpret results after the appropriate read time. It is important that the background is clear before the result is read.

6. Interpret results:

    a. Positive: Two distinct red lines appear. One line should be in the control region and another line should be in the test region.

    b. Negative: One red line appears in the control region (C). No apparent red or pink line appears in the test region (T).

    c. Invalid: Control line fails to appear. Insufficient specimen volume of incorrect procedural techniques are the most likely reasons for control line failure. In these cases, a new test should be performed.



7. Female participants of childbearing potential must have a negative urine pregnancy test at the Screening/Baseline Visit (Visit 1) prior to enrollment. If a pregnancy test has a positive result, the participant must be withdrawn from the protocol by the site. Please refer to the CNMAu8.EAP04 Protocol document or study leadership for details or questions. BioSEND does not make decisions regarding enrollment.

8. Dispose any remaining urine sample. **Pregnancy tests and urine should not be shipped to BioSEND**. These materials are provided only to determine patient eligibility.

Appendix I – In-Clinic 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 went 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.

For links to your sample processing form, to download the most recent version of of this manual, and access recordings of BioSEND trainings, please visit https://biosend.org/coordinate-studies/active-studies and choose your study from the drop-down list

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

- **Link to Sample Collection and Processing Form: http://kits.iu.edu/biosend/CNMAu8SampleForm**

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.

Appendix J: CNMAu8.EAP04 Remote Collection and Processing Form

Please email this form to biosend@iu.edu on or prior to the date of shipment

Samples collected at visit weeks 1 (Screening/Baseline), 12, 24, 36, 48, 72, 96, 120, and 144 or ET					
Draw Order	**Collection Tube**	**Specimen Type**	**Aliquot Volume**	**Total # of Aliquots**	**Cryovial Cap Color**
1	1 PST LiHep (Green-top) Tube (4.5ml)	Plasma for creatinine testing	2ml	1	Orange
2	2 EDTA (purple-top) Tubes (10ml)	Plasma for Quanterix Nfl and UCHL1 analysis	1.5ml	6	Purple
		Buffy Coat for DNA extraction	~750ul	2	Clear
3	1 EDTA (purple-top) Tube (3ml)	Whole Blood for NadMED analysis	0.5ml	6	Green

*For females of child-bearing potential only: A urine pregnancy test should take place at the Screening/Baseline visit and as clinically indicated thereafter (See Appendix H of BioSEND MOP for instructions). Pregnancy tests should **not** be sent to BioSEND.

To: BioSEND Email: biosend@iu.edu Phone: 317-278-6158

From:_____ **UPS tracking #:**_____

Phone:_____ **Email:**_____

Participant ID: _____ Visit: ☐ BL/Screening ☐ 12 Weeks ☐ 24 Weeks

Kit Number (6-7 digit # on specimen labels):_____ ☐ 36 Weeks ☐ 48 Weeks ☐ 72 Weeks

Height: _____ inches **Weight:**_____pounds ☐ 96 Weeks ☐ 120 Weeks ☐ 144 Weeks ☐ ET

Sex: ☐ M ☐ F If ET, specify week:_____

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 EDTA tubes and 4.5ml PST LiHep Tubes		**Notes**
Total volume collected for plasma and buffy coat	_____ mL	From 10ml EDTA tubes; 20ml expected
Total volume collected for LiHep Plasma	_____ mL	From 4.5ml LiHep tube; 4.5ml expected
Were plasma cryovials pre-chilled on wet ice?	☐ Yes ☐ No	Cryovials should be pre-chilled on wet ice for at least 5 minutes prior to aliquoting
Time spin started:	_____ ☐AM ☐PM	10ml EDTA tubes and 4.5ml LiHep tube should be spun together within 30 minutes of collection
Duration of centrifuge:	_____ minutes	Tubes should be spun for 15 minutes
Temp of centrifuge:	Room Temperature	Tubes should be spun at room temperature. If spun at another temperature, please specify in the "Notes" section below.
Rate of centrifuge:	_____ x g	Tubes should be spun at 1500 x g
# of LiHep aliquots created: **(Orange-capped cryovial)**	_____	Aliquot all plasma from LiHep into the orange-capped cryovial (approx. 2ml). Note that this cryovial will be packaged in its own biohazard bag, rather than the cryobox.
# of plasma aliquots created: **(Purple-capped cryovial)**	_____	From 10ml EDTA tubes. Six 1.5ml plasma cryovials expected. If low volume draw occurs, please generate as many 1.5ml aliquots as possible. Fewer standard size aliquots are preferred over six aliquots of non-standard size.
# of buffy coat aliquots created: **(Clear-capped cryovial)**	_____	From 10ml EDTA tubes. Two buffy coat cryovials expected. Each 10ml EDTA tube will produce 1 buffy coat. Buffy coats are ~750ul.
Time plasma, buffy coat, and LiHep cryovials placed on dry ice:	_____ ☐AM ☐PM	Cryovials should be frozen upright on dry ice within 2 hours of collection.

3ml EDTA tube		**Notes**
Total volume collected for whole blood	_____ mL	From 3ml EDTA tube; 3ml expected
# of whole blood aliquots created: **(Green-capped cryovial)**	_____	Six 0.5ml whole blood cryovials expected. Generate aliquots after collection; this tube is not to be centrifuged. If low volume draw occurs, please generate as many 0.5ml aliquots as possible. Fewer standard size aliquots are preferred over six aliquots of non-standard size.
Time whole blood cryovials placed on dry ice:	_____ ☐AM ☐PM	Cryovials should be frozen upright on dry ice within **one** hour of collection.

Notes:_____

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. For in-clinic visits, samples should be batch shipped on a quarterly basis. For remote visits, samples should be sent as visits occur.

2. **For in-clinic visits**: 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.

3. **For remote visits**: A pre-printed UPS airway bill is included with your kit.

4. Record the tracking number onto the Sample Collection and Processing Form (Appendix I for in-clinic visits, Appendix J for remote visits).

5. Make a copy of the form to include in the shipment.

6. Place frozen buffy coat, whole blood, and plasma (EDTA) cryovials in the cryobox. Only include specimens from one subject-visit in each cryobox.

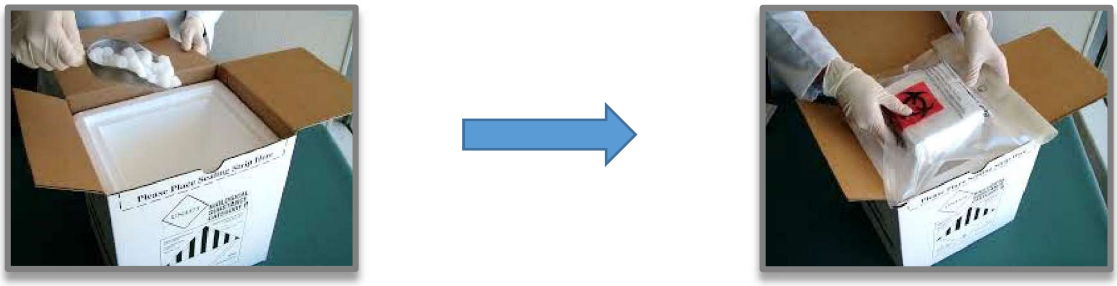
7. Place the cryobox in a clear plastic biohazard bag (do NOT remove the absorbent material found in the bag) and seal the biohazard bag according to the instructions on the bag. Affix a Kit Label to the outside of the biohazard bag.

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

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 on next page).

10. Fully cover the cryobox with approximately 2 inches of dry ice.

11. Place orange-capped LiHep cryovial in a second 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 Kit Label to the outside of the biohazard bag.



12. Place the biohazard bag with the LiHep cryovial into the shipper and fully cover with approximately 2 inches of dry ice.

    - **For remote visits**, please wedge the tube into the ice so that the tube is upright. This will help ensure the cryovial is frozen upright.

13. If including additional biohazard bags in package, include a layer of dry ice (approximately 2 inches) between each biohazard bag.
    - For standard size shippers: Do not package more than two subject visits (or four total biohazard bags) in a single shipper. This size shipper is included in the "Remote Collection and Shipping Kit" and the "In Clinic Shipping Kit – Standard Size".
    - For bulk size shippers: Do not package more than four participant visits (or eight total biohazard bags) in a single shipper. This size shipper is included in the "In Clinic Shipping Kit – Bulk Size".

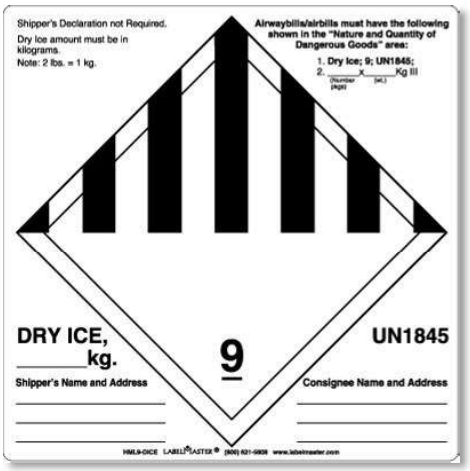
14. The inner Styrofoam® shipping container must contain a minimum of 10 lbs (or 4.5 kg) of dry ice. The dry ice should entirely fill the inner box to ensure the frozen state of the specimens.

15. Replace the lid on the Styrofoam® container. Place the completed Frozen Manifest form in the package on top of the Styrofoam® lid for each visit included in the shipper. Close and seal the outer cardboard shipping carton with packing tape.

16. **For in-clinic visits**: 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.

17. **For remote visits**: Affix the pre-printed UPS® airway bill to the outside of the package.
    Note: If needed, an airway bill can be generated through the UPS ShipExec™ Thin Client system (see Appendix Q).

18. 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

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

19. Apply all provided UN3373 and Fragile labels, taking care not to overlap labels with each other or with airway bill.

20. **For in-clinic visits**, hold packaged samples in -80°C freezer until time of courier pick-up/drop-off.

21. 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

22. **For in-clinic visits**: Notify BioSEND of shipment by completing the In-Clinic Sample Collection and Processing form and Frozen Shipping Manifest in REDCap at http://kits.iu.edu/biosend/CNMAu8EAP04SampleForm (see Appendix I for details). Submission of this form will send an automatic notification of your shipment to the BioSEND team.

23. **For remote visits**: please complete the Remote Sample Collection and Process form (Appendix J). Notify BioSEND of shipment by emailing a copy of this form to biosend@iu.edu on or prior to the day of shipment.

24. 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

1) Airwaybills for in-clinic visits should be generated and printed via the UPS ShipExec™ system. Remote visits kits will contain a pre-printed UPS airwaybill.

2) 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
3) Find the "Shipping" dropdown menu in the top left corner of the screen and click on "Shipping and Rating".
4) 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.
5) After selecting your study, click on the magnifying glass icon on the left side of the screen under "Ship From".
6) 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.
7) 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
8) 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.
9) 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)**
10) After entering the weights, click on the blue "Pickup Request" button.
11) 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.

12) Once you are certain that all the correct information has been entered, click the “Ship” button in the bottom right corner of the screen.
13) 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
14) 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.
15) Place your package in the spot designated in your pickup request, or wherever your daily UPS pickups occur.
16) 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.