National Institute of Neurological Disorders and Stroke Biorepository:

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

Biospecimen Collection, Processing, and Shipment Manual for **C**linical **T**rial **R**eadiness for MSA (CTR-MSA)

Table of Contents

1.0 PURPOSE

The purpose of this manual is to provide collection site staff (PIs, study coordinators, and the sample collection and processing teams) at various study sites with instructions for collection and submission of biological samples. It includes instructions for biospecimen submission to the BioSpecimen Exchange for Neurological Disorders (BioSEND) located at Indiana University.

This manual includes instructions for the collection, processing, aliquoting and shipping of the following samples:

- Serum
- Plasma
- Buffy Coat (for DNA extraction)
- Whole Blood
- CSF
- Skin Biopsy

These procedures are relevant to all study personnel responsible for processing blood specimens to be submitted to BioSEND.

2.0 ABBREVIATIONS

BioSEND	BioSpecimen Exchange for Neurological Disorders
EDTA	Ethylene Diamine Tetra-acetic Acid
IATA	International Air Transport Association
PDBP	Parkinson's Disease Biomarkers Program
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute

3.0 BioSEND Information

3.1 BioSEND Contacts

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Phone: 317-274-2218

Claire Wegel, Project Manager
Phone: 317-278-6158
Email: cwegel@iu.edu

General BioSEND Contact Information
Email: biosend@iu.edu
Website: www.BioSEND.org

Sample Shipment Mailing Address
BioSEND
Indiana University School of Medicine
351 W. 10th Street. TK-217
Indianapolis, IN 46202-5188

3.2 Hours of Operation

Indiana University business hours are from 8 AM to 5 PM Eastern Time, Monday through Friday.

Frozen samples must be shipped Monday- Wednesday only.

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.

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 19	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 to inbound shipments around the Thanksgiving and Christmas holidays. In addition to sending advance notification of these closures to sites, dates will be posted on the BioSEND website. Frozen specimens collected during this period should be held at your site to ship after the first business day in January. If you are ever unsure if it is safe to ship samples, please email biosend@iu.edu to confirm.

4.0 BioSEND Sample Requirements

NINDS approves each study for a specific biospecimen collection protocol. Studies and study sites should make every effort to meet their approved biospecimen collection requirements. The expected number of samples from each site that should be returned to BioSEND are listed in sections 4.1-4.2.

If a sample is not obtained at a particular visit, this should be recorded in the notes section of the Specimen Collection and Processing Form **(see Appendix I).** This form is submitted with your frozen sample shipment to BioSEND.

4.1 Protocol Schedule for Biospecimen Submission to BioSEND – CTR-MSA

Visit (month)	BL	6M	12M
Serum aliquots, 1.5ml	6	6	6
Plasma aliquots, 1.5ml	6	6	6
Buffy Coat	2	2	2
Whole Blood, 3ml	2	2	2
CSF, 1.5ml	10	10	10
Skin Biopsy	2	2	2

5.0 SPECIMEN COLLECTION KITS, SHIPPING KITS AND SUPPLIES

Research specimen collection kits (except dry ice and equipment listed in Section 5.7) will be provided by BioSEND. BioSEND will provide a sufficient number of labels only for those specimens that are to be shipped back to the BioSEND repository. Any specimens 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

Kits and individual kit components (ie, "Extra Supplies") can be ordered as required through the kit request module. Sites are advised to proactively confirm kits are on hand ahead of study visits.

The link to the kit request module is shown below:

- CTR-MSA: https://redcap.link/CTR-MSA

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

5.2 Specimen Collection Kit Contents

Collection kits contain the following (for each subject) as designated per your protocol and/or NINDS resource development agreement. 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*

CTR-MSA Blood Collection Kit	
Supply	**Quantity**
Cryovial (Sarstedt®) with purple cap, 2ml	6
Cryovial (Sarstedt®) with clear cap, 2ml	2
Cryovial (Sarstedt®) with red cap, 2ml	6
EDTA (glass) tube, 10ml	2
Serum (glass) tube, 10ml	2
EDTA (plastic) tube, 3ml	2
Bubble-tube sleeve	6
Disposable pipet, 3ml	3
Cryobox, 25 cell	1
Label set (kit & specimen labels)	1
Plastic Biohazard bag with absorbent sheet	2
UPS Airbill Sleeve	1
Shipping box/Styrofoam container	1
UN3373 Category B Label	1
Fragile label	1
Dry ice label	1

CTR-MSA CSF Processing Kit	
Supply	**Amount**
Conical tube (individually wrapped), 15ml	2
Conical tube (individually wrapped), 50ml	2
Cryovial (Sarstedt®) with clear cap, 2ml	10
Filter Straw	1
LP Tray (22G or 24G)	1

Quantity	Lumbar Puncture Tray Components
1	*Sprotte® needle, 24G x 90mm OR Sprotte® needle, 22G x 90mm*
1	*Introducer needle, 1 mm x 30 mm*
1	*Hypodermic needle, 22G x 1.5”*
1	*Plastic syringe, (3 ml, luer lock) with 25G x 5/8” needle attached*
4	*Polypropylene syringe (6 ml, luer lock)*
1	*Needle stick pad*
1	*Adhesive bandage*
1	*Drape, fenestrated, 2 tabs, paper, 18” x 26”*
2	*Towel, 13.5” x 18”*
6	*Gauze pad, 2” x 2”*
3	*Sponge stick applicator*
1	*Lidocaine 1%, 5 ml*
1	*Povidone-Iodine Topical Solution, 0.75 oz*

Double Punch Biopsy Kit Components	
Supply	**Quantity**
Sterile drape	1
Tweezers	1
Gauze pads	2
Alcohol prep pads	2
Scissors	1
Skin biopsy punch tool with plunger	2
Gelfoam sterile compressed sponge	1
Vaseline ointment packet	1
Coverlet adhesive dressing	1
Transparent film dressing	1
Cryovial (Sarstedt®) with clear cap, 2ml	2
Cryobox, 25-slot	1
Biohazard Bag with absorabent sheet	1

5.4 Site Required Equipment

The following materials and equipment are necessary for the processing of specimens at the collection site and are to be **supplied by the local site**:

- Personal Protective Equipment: lab coat, nitrile/latex gloves, safety glasses
- Tourniquets
- Alcohol Prep Pads
- Gauze Pads
- Bandages
- Butterfly needles and hubs
- Microcentrifuge tube rack
- Test tube rack
- Sharps bin and lid

In order to process samples consistently across all projects and ensure the highest quality samples possible, project sites must have access to the following equipment:

- Centrifuge capable of ≥ 1500 rcf (1500 x g) with refrigeration to 4°C
- -80°C Freezer

In order to ship specimens, you must provide:

- Dry ice (minimum 10 pounds per shipment)

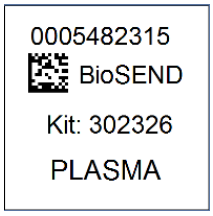
6.0 SPECIMEN LABELS

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

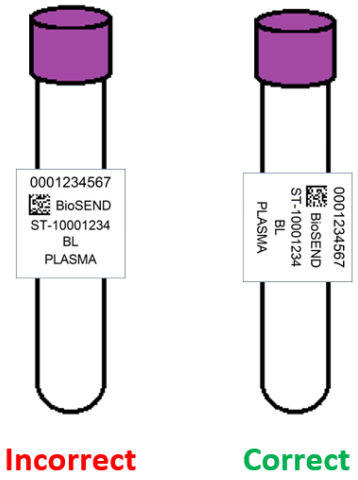


The **Specimen Labels** are placed on all sample collection and aliquot tubes. See processing appendices for further instructions.

6.2 Affixing Labels

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

- Place specimen labels on **ALL** collection tubes and cryovials **BEFORE** sample collection, sample processing, or freezing. This will help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.
- The blood collection tube labels contain a 2D barcode on the left hand side of the label. When turned horizontally, the barcode should be closer to the top (cap end) of the tube.
- Place label **horizontally** on the tube (wrapped around sideways if the tube is upright); see below.



- 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

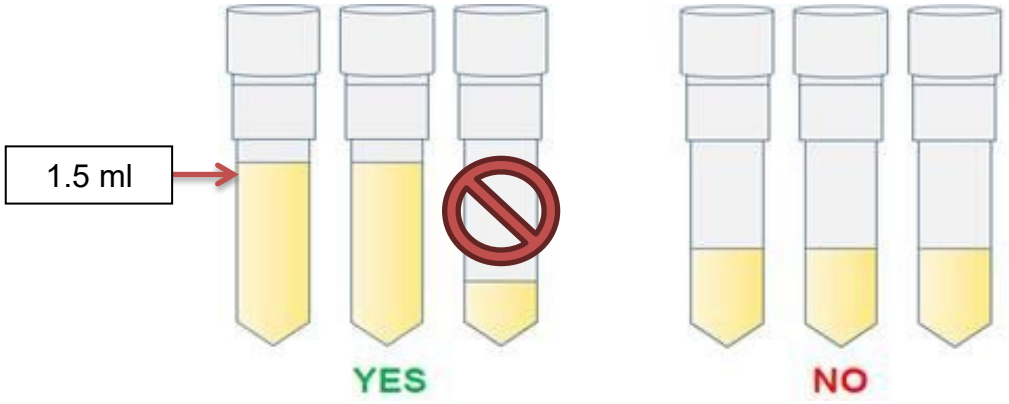
Consistency in sample collection and processing is essential for biomarker studies. All samples are drawn in the same order and then processed in a uniform fashion. **Please read the instructions before collecting any specimens. Have all your supplies and equipment out and prepared prior to drawing blood.**

7.1 Blood Collection Protocols

- Appendix B: Whole Blood Collection for Isolation of Plasma and Buffy Coat
- Appendix D: Whole Blood Collection (No Processing)
- Appendix F: Whole Blood Collection for the Isolation of Serum

7.4 Filling Aliquot Tubes (Plasma, Serum, and CSF)

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.



8.0 Packaging and Shipping Instructions

ALL study personnel responsible for shipping should be certified in biospecimen shipping. If not available at your institution, 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 Specimen Collection and Processing Form

The Specimen Collection and Processing Form should be completed for all samples submitted to BioSEND. Please see Appendix I for further instructions.

8.2 Shipping Instructions

Please reference Appendix K for frozen shipping instructions and Appendix Q for generating airway bills and scheduling pick-ups.

8.3 Shipping Address

All samples are shipped to the BioSEND laboratory:

BioSEND
Indiana University School of Medicine
351 W. 10th Street. TK-217
Indianapolis, IN 46202-5188

9.0 Reconciliation and Non-Conformance

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.

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 B: Whole Blood Collection for Isolation of Plasma and Buffy Coat

Appendix D: Whole Blood Collection (No Processing)

Appendix F: Whole Blood Collection for the Isolation of Serum

Appendix G: Cerebrospinal Fluid Processing

Appendix H: Skin Biopsy

Appendix I: Specimen Collection and Processing Form

Appendix K: Frozen Shipping Instructions

Appendix Q: UPS ShipExec™ Thin Client Instructions

Appendix B – Whole Blood Collection for Plasma and Buffy Coat

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

1. Store empty EDTA (glass) 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.

3. Pre-chill the labeled cryovials on wet ice for at least 5 minutes.

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

5. 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.

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.

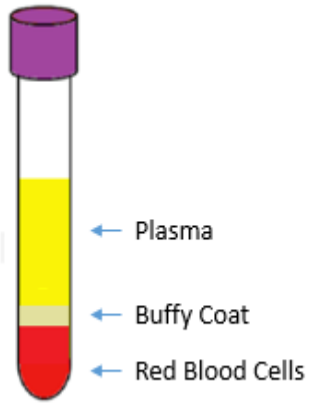
7. Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tubes 8 – 10 times. **Do not shake the tubes!**

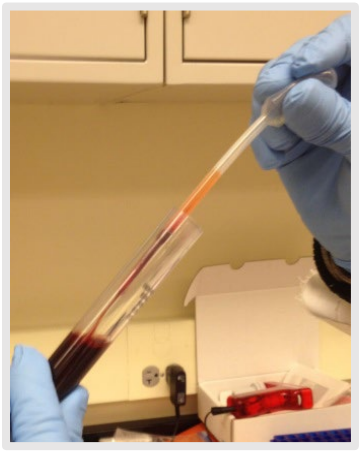
8. Within 30 minutes of blood collection, centrifuge balanced tubes for 15 minutes at 1500 RCF (x g) at 4°C. It is critical that the tubes be centrifuged at the appropriate speed and temperature to ensure proper plasma separation.

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 purple-capped cryovials. Aliquot 1.5 ml per cryovial. If you cannot obtain 6 plasma aliquots, please note "low volume draw" on the Sample Record

and Shipment Notification form (Appendix I) under “Notification of Problems”. Each 10 ml EDTA tube should yield approximately 4-5 ml of plasma.

10. After plasma has been removed from the EDTA tubes, aliquot buffy coat layer (see figure below) into clear-capped cryovial using a disposable graduated micropipette. All of the buffy coat from a single 10 ml EDTA tube will be placed into one cryovial, resulting in two buffy coat spceimens. The buffy coat aliquot is expected to have a reddish color from the red blood cells.





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

12. Complete the Sample Collection and Processing Form (Appendix I).

13. Place the labeled cryovials in the 25 slot cryobox. Place the cryobox UPRIGHT on dry ice. Transfer to **-80°C freezer as soon as possible, within 2 hours of blood draw**. Store all samples at -80°C until shipped to BioSEND on dry ice.

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

Plasma and Buffy Coat Collection and Preparation – 10ml K3 EDTA (glass) Tube

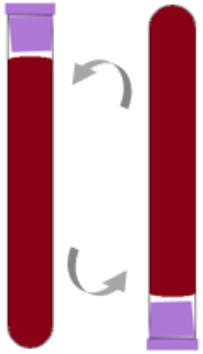
Step One

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

Step Two

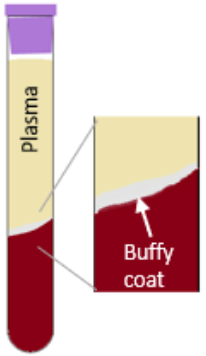
- Collect blood in EDTA tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



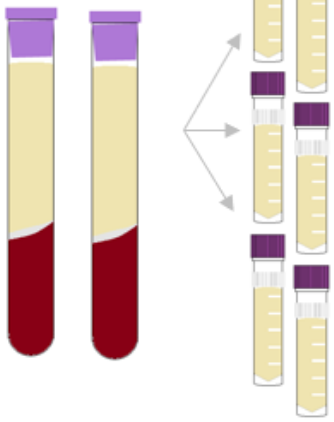
- Immediately after blood draw, gently invert tubes 8-10 times to mix samples.

Step Four



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

Step Five



- Aliquot 1.5 ml plasma into each purple-capped cryovial
- Store plasma aliquots upright at -80°C until shipment.

Step Six

- Using a clean transfer pipet, collect the buffy coats (will include residual plasma and some red blood cells).
- Transfer the buffy coat from one EDTA tube into a clear-capped cryovial. Repeat for second EDTA tube.
- Store plasma and buffy coat aliquots upright at -80°C until shipment.

Appendix D – Whole Blood Collection (No Processing)

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

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

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

3. Using a blood collection set and a holder, collect whole blood into the 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 tubes 8-10 times. Do not shake the tube!**

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

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

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

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

Step One

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

Step Two

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

Step Three

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

Step Four

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

Appendix F – Whole Blood Collection for Isolation of Serum

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

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

2. Place pre-printed specimen labels noted as "SERUM" on the serum determination red-top tubes and on six of the 2 ml cryovials prior to blood draw. Six cryovials will be shipped to BioSEND; the remaining cryovials will be retained by the site and labeled accordingly.

3. Pre-chill labeled cryovials on wet ice for at least 5 minutes or longer.

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

5. Using a blood collection set and a holder, collect blood into the 10 ml red-top serum (glass) tubes using your institution's recommended procedure for standard venipuncture technique.

   The following techniques shall be used to prevent possible backflow:
   a. Place donor's arm in a downward position
   b. Hold tube in a vertical position, below the donor's arm during blood collection
   c. Release tourniquet as soon as blood starts to flow into tube.
   d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

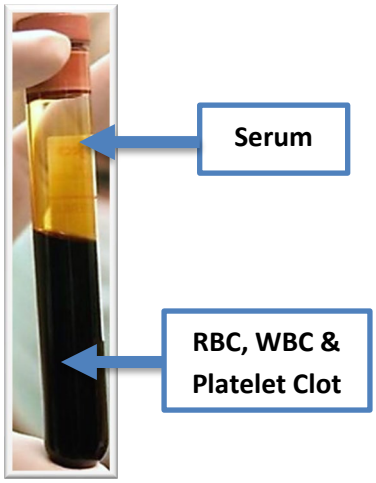
6. Allow at least 10 seconds for a complete blood draw to take place in each tube. Ensure that the blood has stopped flowing into the tube before removing the tube from the holder. The tube with its vacuum is designed to draw 10 ml of blood into the tube.

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

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

   - ❖ Within 30 to 60 minutes from blood collection, centrifuge balanced tubes for 15 minutes at 1500 RCF (x g) at 4°C. It is critical that the tubes be centrifuged at the appropriate speed and temperature to ensure proper serum separation.

9. Remove the serum by tilting the tube and placing the pipette tip along the lower side of the tube wall. Use caution to pipet only the serum layer and not the red blood cell layer. Using a disposable tipped micropipette, transfer serum into the pre-labeled cryovials. Aliquot 1.0 ml per cryovial. Send 6 x 1.5 ml aliquots to BioSEND.



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

11. Place the labeled cryovials in the 25 slot cryovial box. Transfer to -80°C Freezer as soon as possible, **ensuring cryovials are frozen upright**. Store all samples upright at -80°C until shipped to BioSEND on dry ice.

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

Serum Preparation –10 ml Serum (Red Top) Tube

Step One

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

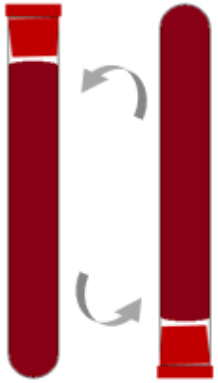
Step Two

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

Step Three

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

Step Four

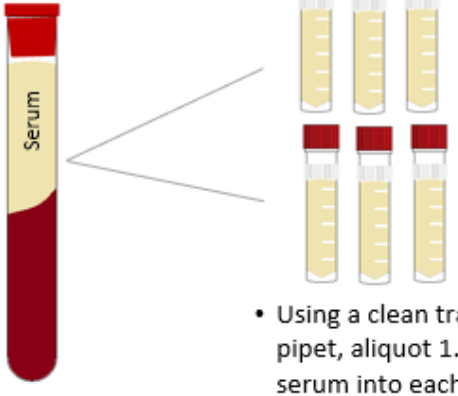


- Immediately after blood draw, gently invert tube 8-10 times to mix samples.

Step Five

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

Step Six



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

Appendix G — Cerebrospinal Fluid Collection

Important Note

CSF should be collected in the morning between 8am – 10am, preferably fasted.

1. Lumbar Puncture Supplies

The lumbar puncture tray contains the following items, which will be used to perform the lumbar puncture. Check the dates of expiration: these reflect the expiration date of the lidocaine and sterile seal. Supplies for shipment of CSF are sent with the blood collection kit.

a. Lumbar Puncture Tray Components

Quantity	Lumbar Puncture Tray Kit Components
1	Sprotte® needle, 24G x 90mm*
1	Introducer needle, 1 mm x 30 mm
1	Hypodermic needle, 22G x 1.5”
1	Plastic syringe, (3 ml, luer lock) with 25G x 5/8” needle attached
4	Polypropylene syringe (6 ml, luer lock)
1	Needle stick pad
1	Adhesive bandage
1	Drape, fenestrated, 2 tabs, paper, 18” x 26”
2	Towel, 13.5” x 18”
6	Gauze pad, 2” x 2”
3	Sponge stick applicator
1	Lidocaine 1%, 5 ml
1	Povidone-Iodine Topical Solution, 0.75 oz

*Trays with 22G x 90mm Sprotte® needle and introducer available upon request.

Sterile, individually packaged 50 ml conical tubes are provided for sites who are completing the Lumbar Puncture through the use of the gravitational method. Please ensure that all supplies necessary for a participant draw are available at your site at least two weeks prior to the appointment.

2. Setting Up the LP

a. On an overbed table, remove the contents of the LP kit from the outer plastic packaging, leaving the contents wrapped in their sterile drape. Leave everything wrapped until the person performing the LP is seated and begins examining the subject.

b. Feel the outside of the LP kit (still wrapped) to determine which end contains the spongy swabs. Turn this end toward the person performing the LP and begin unwrapping the kit.

c. Touch only the outside of the paper wrapper. When you grab an edge to unfold it, touch only the folded under portions of the outside of the wrapper. Also, don't let the outside of the wrapper touch any part of the inside. If you touch any part of the paper wrapper, or if any non-sterile object outside of the wrapper touches any part of the inside of the wrapper, discard the kit and start over. If you are in doubt as to whether something touched the inside of the paper wrapper, throw the kit away and start over.

3. Maintaining the sterile field

a. Keep in mind that there are usually many staff in the room during an LP, and a big part of assisting with the LP is keeping the field sterile—keeping people away from it, and reminding them to be careful around it. If anyone touches the inside of the paper wrapper or any part of the contents of the kit, throw the kit away and start over. If you are in doubt as to whether someone touched the kit, throw it away and start over. Also, you are the monitor for whether the person performing the LP has broken sterility usually by touching something not sterile with a sterile gloved hand. Feel free to speak up and inform people if need be. Be assertive.

4. Tips for Clinicians Performing Lumbar Puncture: Optimizing patient comfort and minimizing the risk of adverse events.

a. Talk the patient through the procedure so that there are no surprises.

b. Use of a Sprotte® 24g or 22g atraumatic spinal needle and careful technique are optimal for reducing post-LP headache risk. This Sprotte® 24g or 22g atraumatic spinal needle is included in the BioSEND LP Tray; additional needles may be ordered upon request. A pencil point spinal needle such as Whitacre® 24g, Spinocan® 22g, or other 24g may also be used.

c. Use adequate local anesthesia. Use the 25g 1/2" needle and inject lidocaine to raise a skin wheal. Then, inject lidocaine using the pattern of a square— first the center, and then to all 4 corners. If the subject is thin, do not insert the deep infiltration needle

OR the spinal introducer all the way. Use only about 2/3 of their length (to prevent entering the subarachnoid space with anything other than the 24g pencil point spinal needle).

d. Encourage fluid intake immediately after LP is helpful.

e. Be sure to give post-LP care instructions verbally to the subject (see below).

5. Post-LP Care Instructions

- Advise the subject to refrain from exertion (e.g., exercise, housework, gardening, lifting, sexual activity, or any other strenuous activities) for 24 hours after the LP.

- Advise the subject to continue with increased fluid intake.

a. Mild to Moderate headache after a lumbar puncture

- Mild to Moderate headache following lumbar puncture usually resolves within 3-4 days.

- Treatment of Mild to Moderate headache:
  - Limit physical activity as much as possible.
  - Oral fluids and caffeine are helpful. Drinking a soft drink (for example) is preferable to coffee, which has some diuretic activity.
  - Acetaminophen should be used for symptomatic relief. If a subject cannot tolerate acetaminophen, ibuprofen should be used. Avoid aspirin. If these do not relieve the headache, acetaminophen with codeine or an equivalent could be considered.

b. Severe headache after a lumbar puncture

If the headache becomes severe, posturally sensitive (relieved by supine posture), or is accompanied by nausea, vomiting, tinnitus, and/or visual disturbances, the subject should contact the site study staff for further instruction per standard clinical care.

6. Detailed Lumbar Puncture Procedure

a. Place the preprinted Collection and Aliquot "**CSF**" labels on the collection and ten 2 ml aliquot tubes. These 10 tubes will be shipped to BioSEND. Prepare the remaining 2 ml aliquot tubes and label per your site's CSF protocols. The remaining tubes will be retained by the site.

b. **CSF cryotubes should remain at room temperature; do not pre-chill these tubes.**

c. Perform lumbar puncture using the atraumatic technique.

d. Collect CSF into syringes or sterile conical tube (if a noticeably bloody tap, discard the first 1-2 mls). After the LP has begun and fluid is being collected, aliquot the first 1-2 mls of CSF from the first syringe into one of the additional cryovials provided by BioSEND, and send it to the local lab for routine diagnostic tests, if applicable to your protocol.

e. Collect additional CSF per your site's protocol and transfer to 50 ml conical polypropylene tube at room temperature. Firmly cap and mix gently by inverting 3-4 times. Record the time of draw (once collection is complete) on the DMR CSF Processing Form. Also ensure that the time of last meal consumed by participant has been documented.

f. Within 15 minutes of collection, transfer the CSF from the 50 ml conical tube to two 15 ml conical tubes ensuring that there is equal volume in each. Spin the CSF samples down at 2000 x g for 10 minutes at **room temperature**, 64°F – 77°F (18°C to 25°C).

g. After centrifugation, pipette the supernatant from both 15 ml conical tubes and transfer to a new 50 ml conical tube. Ensure that debris at the bottom of the 15 ml conical tubes are not disturbed. Firmly cap the 50 ml conical tube and mix gently by inverting 3-4 times.

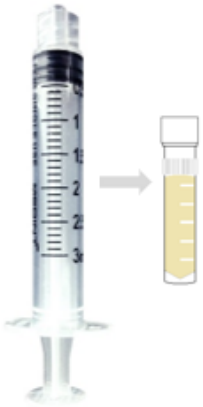
h. Pipette (micropipette preferred) 1.5 ml of CSF directly into each of the pre-labeled aliquot tubes to be sent to BioSEND.

i. Place the labeled cryovials in the 25-slot cryobox and place **upright** on dry ice. Transfer to **-80°C Freezer.** Store all samples at -80°C until shipped to BioSEND on dry ice.

j. Ship the CSF aliquots to BioSEND according to Appendix K – Frozen Shipping Instructions along with Appendix I – Sample Shipment Notification Form

CSF Collection and Preparation

Step One

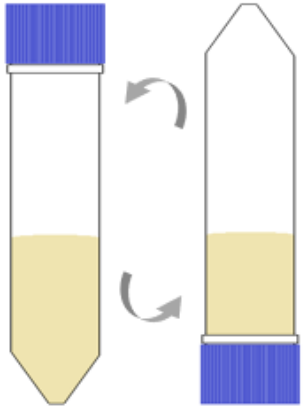


- Collect CSF into the 3ml luer lock syringe or by gravitational pull.
- Dispense 1-2 ml into cryovial.
- Send to local lab for testing, if applicable.

Step Two

- Collect CSF into the 5ml luer lock syringe or by gravitational pull
- Transfer sample into 50 ml conical tube.

Step Three

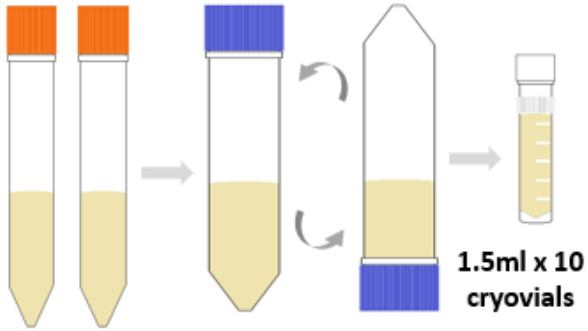


- Immediately after collection, gently invert the 50 ml conical tube 3-4 times to mix the sample.

Step Four

- Transfer CSF into <u>two</u> 15 ml conical tubes.
- Within 15 minutes of collection, centrifuge samples at room temperature at 2000 x g for 10 minutes.

Step Five



- Using a clean transfer pipette, transfer CSF from both 15 ml conical tubes into a 50 ml conical tube, leaving the debris in the bottom.
- Gently invert the 50 ml conical tube 3-4 times to mix the sample.
- Aliquot 1.5 ml into the cryovials.
- Store CSF aliquots <u>upright</u> at -80°C until shipment to BioSEND.

APPENDIX H: SKIN BIOPSY COLLECTION – DOUBLE PUNCH

Frozen skin biopsy samples may be shipped on dry ice and packaged with other frozen samples (plasma, serum, etc.) Frozen samples should not be shipped on Thursday or Friday.

1. Skin Biopsy Supplies

The double punch skin biopsy kit contains the items listed in the table below, which will be used to perform the skin punch biopsy procedure. Check the dates of expiration of all kit components before use. Note that sutures and needle drivers will be provided in each site's supplemental supplies and should be on hand and ready in case they are necessary for this procedure.

1.1. Double Punch Biopsy Kit Components

Quantity	Kit Component
1	Sterile drape
1	Tweezers
2	Gauze pads
2	Alcohol prep pads
1	Scissors
2	Skin biopsy punch tool with plunger
1	Gelfoam sterile compressed sponge
1	Vaseline ointment packet
1	Coverlet adhesive dressing
1	Transparent film dressing
2	Cryovial (Sarstedt®) with clear cap, 2ml
1	Cryobox, 25-slot
1	Biohazard Bag with absorbent sheet

1.2. Setting Up the Kit

1.2.1. On an overbed table, remove the contents of the kit from the outer packaging, leaving all sterile contents wrapped in their packaging. Leave everything wrapped until the person performing the biopsy is seated and begins examining the subject.

1.2.2. Open the sterile kit components, touching only the outside of the wrapper. Don't let the outside of the wrapper touch any part of the inside of the kit.

1.3. Skin Sample Collection

Two skin biopsies will be obtained from the cervical paravertebral region at approximately the C8 level (see figure below). Both biopsy will be frozen, dry, in a 2mL cryovial.

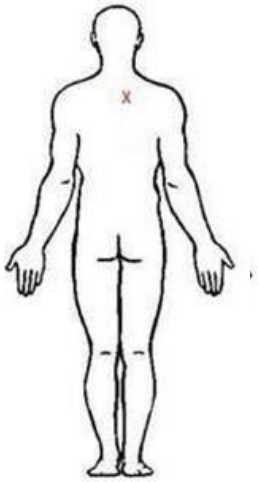
1.3.1. Pre-collection Steps – Preparation of Patient

1.3.1.1. Prepare patient for procedure per institution guidelines.

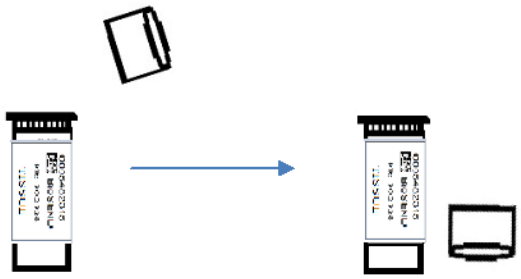
1.3.1.2. Before the biopsies are collected, the volunteer will be screened and complete the informed consent for the skin biopsy procedure. The doctor will explain the study, and the volunteer will have an opportunity to ask questions. Once this discussion is complete, the volunteer is ready for the biopsy procedure.

1.3.2. Preparation Steps – Cryovial for Frozen Tissue Collection

1.3.2.1. Prior to the procedure, label the cryovials with a pre-printed “TISSUE” label.



1.3.2.2. Open the cryovials and place cryovial lids to the side in a sterile location.



1.3.3. Biopsy Collection Procedure

1.3.4.1. Ensure that the biopsy site has been properly sterilized with alcohol wipes. A punch biopsy is a clean procedure, not a sterile procedure, and therefore, sterile gloves and gown are not required. Wearing safety glasses is recommended.

1.3.4.2. Anesthetize the area by injecting Lidocaine with epinephrine solution (Lidocaine HCL 1% with epinephrine 1:100,000) just under the epidermis (subepidermally) using a 3-cc syringe just prior to the biopsy. The injection should continue until a “bleb” or small bubble forms under the skin (approximately 3 mm in diameter). The injection will burn slightly (much like a bee sting) due to a pH difference between the skin and the solution. Injecting slowly decreases the burning sensation. The burning will subside quickly, and the site will become numb. It is acceptable to massage the area. Both biopsy sites can be anesthetized at the same time.

1.3.4.3. After the Lidocaine injection, the area anesthetized may be marked using a pen if helpful to the individual completing the biopsy. The area to be biopsied should be checked to ensure the skin is properly anesthetized. This can be tested by gently pressing the needle to the area. If the patient experiences neither pain nor sharp sensation, the area is ready to be biopsied. Experiencing a pressure sensation is normal, but there should be no pain. If the area requires more anesthesia, another injection of Lidocaine solution is made with a new syringe.

1.3.4.4. Using a sterile 3 mm skin punch, place the punch perpendicular to the skin, in the paravertebral C8 region, within 3 cm of the midline. Apply constant downward pressure while twirling the punch tool between the thumb and index finger, rotating clockwise and counterclockwise until the blade has pierced the epidermis of the skin and the metal part of the punch tool is buried (there will be a “give” once the punch reaches the subcutaneous fat). Once the tool has reached the lowest point, lift the tool straight up.

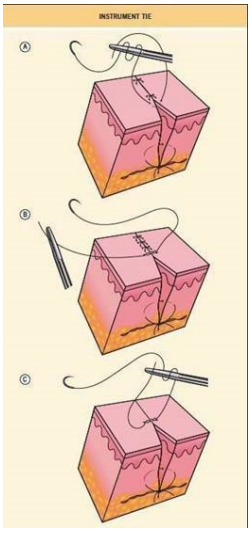
1.3.4.5. Depress the plunger to remove the specimen. Forceps may be needed to remove the specimen. If the specimen remains connected at the level of the subcutaneous fat, it may be necessary to cut at the base of the specimen to remove it. Do not try to tear a specimen that remains connected, as it may damage the specimen. Using a punch with a plunger should help to ensure that the epidermis is not crushed or damaged during the process.

1.3.4.6. **CRITICAL STEP:** Place the specimen directly into the prepared “TISSUE” cryovial and close the cryovial cap securely.

1.3.4.7. To restore hemostasis, hold pressure with gauze for approximately 30 seconds. Wipe any excess blood with a sterile 2x2 gauze to expose the site. Pack biopsy site with GelFoam. Apply the Vaseline ointment to the bandage and cover biopsy site. This can be reinforced with gauze and tape if necessary. If the biopsy site is oozing, apply a pressure bandage by applying Vaseline to small gauze and then apply Tegaderm. Other

closure options include using a steri-strip and transparent film dressing closure system. In most cases, suturing a wound will not be necessary. Placing a suture can be considered if the wound base is still oozing after packing with GelFoam. To place a suture, grip the needle using the forceps approximately ½ to 1/3 of the distance between the suture attachment and the tip of the needle. Place the needle point perpendicular to the skin surface 2 mm away from the wound edge, then turn the wrist to exist the skin on the opposite side of the wound, again, 2 mm from the wound edge. To tie the suture, hold the needle holder parallel to the axis of the wound and at the center of the wound.

1.3.4.8. Wrap the free end of the suture twice around the holder, then grasp the free end and pull through, tightening the knot. Repeat with just looping around the needle holder once for repeat knots. Tie 3 knots (see figure below)



1.3.4.9. Collect a second biopsy 3 cm above or below the original collection site on the same side of the midline and following the same procedure.

1.3.4.10. Place the second biopsy directly into the second prepared “TISSUE” cryovial and close the cryovial cap securely.

1.3.4.11. The study coordinator or appointed site personnel will be responsible for completing the processing of the tissue once collected using the procedures described in detail below.

1.3.4.12. Be sure to give post care instructions verbally to the subject as found in below in section 1.5. A follow-up call will be placed by the study coordinator 2- 3 days after the procedure to assess for adverse events.

1.4. Double Punch Biopsy Processing

1.4.1. Two punch skin biopsies will be collected from either the right or left side

of the paravertebral C8 region within 3 cm of the midline. After collection, each biopsy is placed in a “TISSUE” labeled 2mL cryovial. The cryovial lid is closed securely.

1.4.2. **CRITICAL STEP:** Place the cryovials in the provided cryobox and freeze at -80ºC as soon as possible. Record Time Frozen on the Appendix I. Store at -80ºC until shipped to repository on dry ice.

1.4.3. Place a follow-up call to the subject 2-3 days after the procedure to assess for adverse events.

1.5 Wound Care after a Punch Skin Biopsy

Please read the following instructions regarding your biopsy site(s) care:

1. Leave the original dressing in place and keep dry for 24 hours. The biopsy site(s) should not be washed for 24 hours.
2. Refrain from doing strenuous activity/exertion for the rest of the day of your biopsy (such as running or heavy lifting).
3. After 24 hours, the biopsy site(s) should be cleaned with soap and water daily, leaving any steri-strips in place (if used).
4. A new band-aid with Vaseline or polysporin ointment should be applied daily.
5. The GelForm (if used) may fall out of the biopsy site(s). This is expected.
6. Sutures, if placed, should be removed in 14 days.
7. Steri-strips, if used, will remain in place for approximately 5-7 days. Once the edges begin to peel upward, you may remove the steri-strip, working from the outer edges to the middle on both sides.

The local anesthetic used for the biopsy will usually last for 1 to 2 hours after the procedure. After it wears off, you may have some mild, localized soreness and tenderness at the biopsy site(s) over the next day or two. Use acetaminophen (Tylenol) if you have discomfort. If you are not able to take Tylenol, ask your primary doctor what medicine to take for the discomfort or pain.

It is very rare for people to have any problems during the healing period. It is normal for the biopsy site(s) to bleed a little bit or drain pink fluid for a day or two after the biopsies. They should not bleed excessively (i.e., through the band-aid) after that time. They should never drain pus.

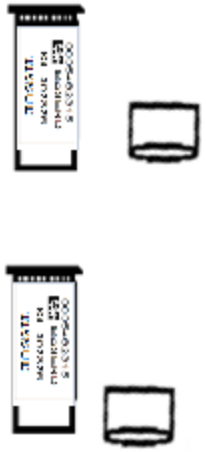
Report any of these symptoms to your study contact:

- Increase in redness
- Swelling
- Infection
- Increasing or severe pain
- Drainage of pus
- Bleeding you cannot stop with firm pressure for 20 minutes
- Fever over 101.5 degrees

Who to Call:
< Insert Site Coordinator Information>

COLLECTION SCHEMATIC: DOUBLE PUNCH BIOPSY COLLECTION AND PREPARATION

Step One



- Label 2ml cryovials with pre-printed "TISSUE" label.
- Remove lids from 2mL cryovials. Set aside in a sterile location.

Step Two

- Using standard punch biopsy procedures, collect two biopsy from the paravertebral C8 region approximately 3mm in diameter and 3mm deep.
- Record time of biopsy collection.

Step Three

- Place the biopsy into the 2mL cryovial.
- Close the 2mL cryovial securely.

Step Four

- As soon as possible after collection, store samples upright in the provided cryobox, at -80ºC.
- Record time sample was placed in freezer on sample form.
- Ship samples to BioSEND per Appendix K

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/CTRMSASampleForm**

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.

CTR-MSA Specimen Collection And Processing Form

Please complete the Specimen Collection and Processing Form, below.

Study	◯ CTR-MSA
Study Site	◯ New York University ◯ Mayo Clinic Rochester ◯ Medizinische Universitat Innsbruck ◯ Vanderbilt University
Email address of staff member completing this form Note: A copy of the completed sample form and the shipping manifest will be sent to this address.	_____
GUID:	_____
Sex (used for DNA quality control)	◯ Male ◯ Female ◯ Other
Visit	◯ BL ◯ 6M ◯ 12M
IU Kit Number	_____

Blood Collection and Processing

Date of venipuncture blood collection

_____

Time of venipuncture blood collection

_____
(Use 24 Hour clock)

Date participant last ate

_____

Time participant last ate

_____

1. SERUM (red-top tubes, 10 mL)

Was blood collected and processed for SERUM?

◯ Yes
◯ No

Time of SERUM tube centrifugation

_____
(Use 24 Hour clock)

Duration of SERUM tube centrifugation

_____
(minutes)

Rate of SERUM tube centrifugation

_____
(x g)

Temperature of SERUM tube centrifugation

_____
(degrees Celsius)

Total volume of SERUM collected

_____
(mL)

Number of SERUM aliquots created for BioSEND

_____
(Each aliquot should be 1.5 mL)

Time SERUM aliquots were placed in freezer

_____
(Use 24 Hour clock.)

SERUM storage temperature

_____
(degrees Celsius)

SERUM notes

_____

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

Was blood collected and processed for PLASMA EDTA?
◯ Yes
◯ No

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)

Number of PLASMA EDTA aliquots created for BioSEND
_____
(Each aliquot should be 1.5 mL)

Number of BUFFY COAT aliquots created for BioSEND
_____

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. WHOLE BLOOD (EDTA tubes, 3 mL)

Was blood collected for WBLD?
◯ Yes
◯ No

Number of WBLD tubes collected
_____
(Two 3ml EDTA tubes expected)

Time WBLD was placed in freezer
_____
(Use 24 Hour clock)

WBLD storage temperature
_____
(degrees Celsius)

WHOLE BLOOD notes

Skin Biopsy

Was skin biopsy collected?
- ◯ Yes
- ◯ No

If no, specify reason
- ◯ Participant declined
- ◯ Procedure attempted unsuccessfully
- ◯ Other

Specify procedure attempted unsuccessfully

_____

Specify other

_____

Date of skin biopsy collection

_____

Time of skin biopsy collection

_____
(Use 24 Hour clock)

Number of biopsies collected

_____
(Two aliquots expected)

Was lidocaine anesthesia administered?
- ◯ No
- ◯ Yes
- ◯ Other anesthetic used

Specify other anesthetic used

_____

On which side of the body was the biopsy performed?
- ◯ Right
- ◯ Left

What type of wound closure was used?
- ◯ Dressing only
- ◯ Steri-strips
- ◯ Suture
- ◯ Other

Specify other wound closure

_____

Skin biopsy storage temperature

_____
(degrees Celsius)

Time skin biopsy frozen

_____
(Use 24 Hour clock)

CSF Processing

Was CSF collected?
◯ Yes
◯ No

Date of CSF collection
_____

Time of CSF collection
_____
(Use 24 Hour clock)

Time of CSF centrifugation
_____
(Use 24 Hour clock)

Duration of CSF centrifugation
_____
(minutes)

Rate of CSF centrifugation
_____
(x g)

Was CSF centrifuged at room temperature?
◯ Yes
◯ No
(degrees Celsius)

Temperature of CSF centrifugation
_____
(degrees Celsius)

Total volume of CSF collected
_____
(mL)

Number of CSF aliquots created
_____

Time CSF aliquots were placed in freezer
_____
(Use 24 Hour clock)

CSF storage temperature
_____
(degrees Celsius)

CSF notes
_____

CTR-MSA 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	◯ CTR-MSA
Study Site:	◯ New York University ◯ Mayo Clinic Rochester ◯ Medizinische Universitat Innsbruck ◯ Vanderbilt University
GUID:	_____
Visit:	◯ BL ◯ 6M ◯ 12M
IU Kit Number:	_____
Date of blood collection:	_____
Date of CSF collection:	_____

SERUM

Number of SERUM aliquots shipped:	_____

PLASMA EDTA

Number of PLASMA EDTA aliquots shipped:	_____
Number of BUFFY COAT aliquots shipped:	_____

WHOLE BLOOD EDTA

Number of WHOLE BLOOD tubes shipped:	_____

CSF

Number of CSF aliquots 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 are unsure whether or not it is safe to ship.

Date of shipment: _____

Did/will you use the IU UPS interface to generate the shipping label?
- ◯ Yes
- ◯ No

Which shipping service did you use?
- ◯ UPS
- ◯ FedEx
- ◯ World Courier
- ◯ Other

What is the shipment tracking number? _____

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.

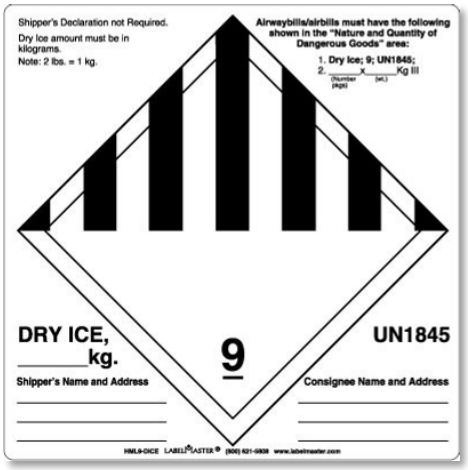
11. If including additional biohazard bags in package, include a layer of dry ice (approximately 2 inches) between each biohazard bag.

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 (black and white diamond) with the following information:
    - Your name and return address
    - Net weight of dry ice in kg (this amount must match the amount recorded on the airway bill)
    - Consignee name and address:



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

- Do not cover any part of this label with other stickers, including pre-printed address labels.

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

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

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

18. Specimens should be sent to the address below. Frozen shipments should be sent Monday through Wednesday only to avoid shipping delays on Thursday or Friday.

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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.