Parkinson's Precision Medicine Initiative (PPMI)

Parkinson's Precision Medicine Initiative

Biospecimen Collection, Processing, and Shipment
Manual of Procedures

Version: August 2026

Log of Changes

Title	PPMI Biologics Manual			
Description	Biospecimen Collection, Processing, and Shipment Manual of Procedures (Expanded Collection)			
Created By	Indiana University PPMI Biorepository			
Date Created	June 10, 2020			
Maintained By	Indiana University PPMI Biorepository			
Version Number	Modified By	Modifications Made	Date Modified	Status
Feb 2026	Indiana University	• Updated Shipping Instructions • Updated Biorepository Contacts • Reorganization/Updated Table of Contents • Added label types • Change from 1 x 10mL EDTA tube to 3 x 10mL EDTA tubes for plasma & buffy coat. • Change from 2 x PAXgene™ RNA tubes to 1 x PAXgene™ RNA tube • Change from 2 x 3mL EDTA tubes to 1 x 3mL EDTA tube • Removal of formalin fixed skin biopsy • Addition of all sites to collect 2 x frozen skin biopsies • Removal of ambient shipping information • Canadian sites to use FedEx for sample shipment • Addition of Electronic Sample Form	2/1/2026	ACTIVE
August 2026	Indiana University	• Update PPMI study name to: Parkinson's Precision Medicine Initiative. • Updated links throughout • Updated shipping instructions • For sites shipping to the IU biorepository – updated shipment window for SC samples from Monday-Friday to Monday-Wednesday only. No changes for sites shipping to the BioRep biorepository • Added repository address to Sample Form – one version per recipient repository	8/4/2026	

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1. ABBREVIATIONS

BL	Baseline
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
EDC	Electronic Data Capture
EDTA	Ethylene Diamine Tetra-acetic Acid
IATA	International Air Transport Association
IU	Indiana University
LONI	Lab of Neuro Imaging
LP	Lumbar Puncture
PD	Parkinson's Disease
PPMI	Parkinson's Precision Medicine Initiative
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RNA	Ribonucleic Acid
RPM	Revolutions Per Minute
SMC	Site Management Core
US DOT	United States Department of Transfer
WBLD	Whole Blood

2. BIOREPOSITORY INFORMATION

2.1. Biorepository Contacts

2.1.1. Indiana University Study Support:

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

Sample Shipment Mailing Address:
PPMI Biorepository
Indiana University School of Medicine
351 W. 10th St, TK-217
Indianapolis, IN 46202

Indiana University Contacts:

PPMI Team Contact Information:
Phone: 317-274-5722
International Phone: (00+1) 317-274-5744
Email: ppmibio@iu.edu

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Study Coordinator
Phone: (317)278-3131
Email: saealexa@iu.edu

2.1.2. Milan BioRep Study Support:

Milan BioRep business hours are from 8 AM to 7 PM Europe Central Time, Monday through Friday.

Milan BioRep can accommodate 8AM to 12PM when necessary.

Sample Shipment Mailing Address:

BioRep Srl
c/o DIBIT2 Palazzina San Michele Via Olgettina 60
20132 Milano – Italy

Milan BioRep Contacts:

Paola Casalin
Repository and Biobank Director Phone: +39 02 58029768
After Hours: +39 348 0716024
Fax: +39 02 58018471
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Molecular Biology Laboratory Manager Phone: +39 02 58029725
After Hours: +39 348 0716025
Fax: +39 02 58018471
Email: ppmi@biorep.it

2.1.3. Tel Aviv Study Support

Sample Shipment Mailing Address

6 Weizmann St. (The Genetic Institute – R&D)
Tel Aviv 64239, Israel

Tel Aviv Contacts

Mali Gana-Weisz
Phone: 972-3-6947271, 972-3-6973628
Fax: 972-3-6973628
Email: maligw@tlvmc.gov.il

2.2. Holiday Schedules

Please note that courier service providers may observe a different set of holidays. Verify shipping dates with your courier prior to any holiday. Weekend/holiday delivery must be arranged in advance with biorepository staff. Individual collection site questions should be directed toward the respective repository.

2.2.1. Holiday Observations – United States

Holiday
New Year's Day
Martin Luther King, Jr Day
Memorial Day
Juneteenth
Independence Day (observed)
Labor Day
Thanksgiving

Friday after Thanksgiving
Christmas Day

Between December 24th and January 2nd Indiana University will be open for essential operations ONLY. Normal operations will resume on January 2nd. **Biological specimens for submission to Indiana University should NOT be shipped between December 24th and January 2nd.** If samples are collected during this period, please store at -80ºC and ship on dry ice to Indiana University until after the holidays.

2.2.2. Holiday Observations – Canada

Holiday
New Year's Day
15th February
Good Friday
Victoria Day
Quebec National Day
Canada Day
Civic Holiday
Remembrance Day
Christmas Day
Boxing Day

2.2.3. Holiday Observations – Europe

Holiday
New Year's Day
6th January
Easter
Easter Monday
25th April
1st May
2nd June
15th August
1st November
7th and 8th December
25th and 26th December

2.2.4. Holiday Observation – Tel Aviv

Holiday
Purim
Passover
Memorial Day
Independence Day
Shavuot
Rosh Hashanah
Yom Kippur
Sukkot
Chanukah

3. SPECIMEN COLLECTION KITS, SHIPPING KITS, AND SUPPLIES

3.1. Biospecimen Kits and Supplies Provided by IU

Biospecimen Supplies: Research Specimen Collection Kits will be provided by IU. IU provides most materials needed for biospecimen collection. Their contents are detailed in Appendix A. Sites must order Specimen Collection kits through the Kit Request Module at https://redcap.uits.iu.edu/surveys/?s=9TJ9XE89MWFCTPKH . .
Note: Sites can order certain supplies individually, on demand, if they need to be replaced, or additional supplies are needed.

Assessments: IU can provide sites with paper copies of cognitive assessments including the Benton Line Judgment Orientation (BLJO) test, the Hopkins Verbal Learning test (HVLT), the Boston Naming Test (BNT), and the University of Pennsylvania Smell Identification Test (UPSIT). Assessments should be ordered through the same Kit Request Module: https://redcap.uits.iu.edu/surveys/?s=9TJ9XE89MWFCTPKH.

When Ordering Supplies:

- Kits should be ordered **at least two weeks** in advance of when they are needed. This ensures all necessary supplies can be prepared and delivered before the visit.
- Sites should only order supplies that they intend to use within 2 months of receipt. This ensures that supplies do not expire before they can be used.
- Materials and equipment not provided by Indiana University are listed in the tables below.

3.2. Supplies Provided by BioRep

BioRep provides shipping materials for sites shipping to their facility (see contact information in section 2.1.2).

3.3. Biospecimen Kits and Supplies Provided by Labcorp

Clinical lab supplies will be provided to sites by Labcorp Drug Development and will include all materials needed for collecting and shipping clinical blood samples (this does not include dry ice for Screening and Baseline labs). These samples will be shipped to Labcorp Drug Development after collection.

Automatic Resupply: Labcorp Drug Development will anticipate the number of kits needed at each site and resupply based on the number of complete kits that have been shipped back to Labcorp Drug Development. Please note that this service can result in extra kits being supplied to the sites to ensure appropriate kits quantities are on hand.

Sites are responsible for independently monitoring inventory and supply status (expiration date, damage, etc.). Should additional supplies be needed, a minimum of **10 working days** is required for kit resupply. Delivery times may vary in extended delivery areas. Please refer to the Labcorp Drug Development Lab Manual or contact Labcorp Drug Development with questions.

3.4. Supplies and Equipment Sites to Obtain Independently

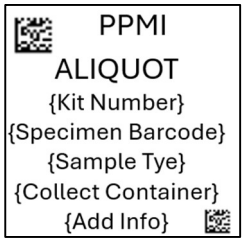
Materials
Pelleted Dry Ice
Alcohol Prep Pads
Bandages and Steri-Strips
Tourniquets
Gloves
Pipettes and Pipette Tips
Lidocaine for Skin Biopsy
Crushed Ice
Gauze Pads
Butterfly Needles
Wire Tube Racks (2 mL to 10 mL)
Sharps Bin and Lid
Lidocaine for LP
Equipment
4°C Refrigerated Centrifuge
-80°C Freezer
Room Temperature Centrifuge
4°C Refrigerator

4. SAMPLE COLLECTION AND PROCESSING

4.1. Sample Labels

Labels must be affixed on all collection and aliquot tubes to ensure unique specimen identity. The PPMI Biorepository Team at IU provides labels for all research samples being collected under the PPMI Clinical protocol. 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.

4.1.1. Label Types

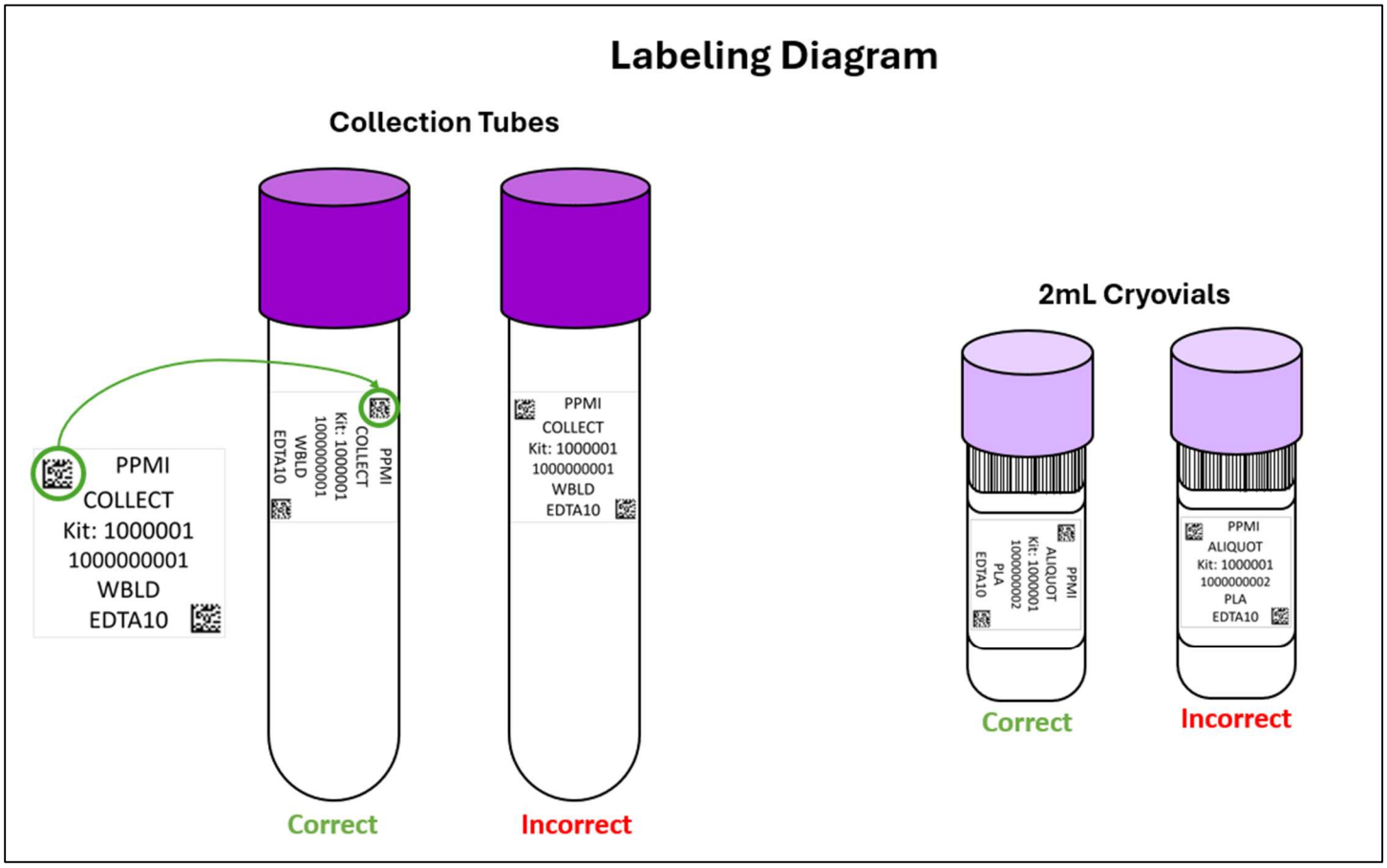
Label Template	Example Label	Label Type
		**Collection Specimen Label**: placed on all collection tubes & devices as described in applicable appendices.
		**Aliquot Specimen Label**: placed on aliquot tubes as described in applicable appendices.
		**Kit Number Label**: link all specimens collected from a single participant at one visit. These labels are used on the sample form, cryobox lid, and sample bag.

4.1.2. Labeling Procedures

- Kit Numbers
  - Each participant visit is identified by a seven-digit kit number. This kit number can be found on every label. All samples for a single participant visit should have labels with the same exact kit number.
  - **A given kit number should NOT be used for more than one participant visit.**
  - It is critical to record the kit number for a participant on the Sample Form (Appendix J). This kit number connects samples to a specific participant visit. **If the Kit Number is not recorded properly in the EDC and on the**

Sample Forms, there is an increased risk that the samples will not be connected back to the participant.

- **Any labels that are left over from a specific participant visit should be discarded. <u>Left over or extra labels should not be repurposed for other participant visits.</u>**
- Label Placement
    - Labels should be placed on the collection and aliquot tubes before sample collection, processing, or freezing.
    - Place Collect and Aliquot Specimen Labels horizontally on the tube, so that the left-hand barcode is near the cap. The clear tail should wrap around the tube.
    - On 2 mL cryovials, the label should be placed horizontally and just below the ridges of the aliquot tubes (see labeling diagram below). There is enough space on the aliquot tube for the label to be placed without overlapping the ridges.
    - Take a moment to ensure the specimen label is completely adhered to each tube. It may be helpful to roll the tube between your fingers after applying the label.



4.2. Collection Schedule

	Visit	SC	BL	V02	V04	V05	V06	V08	V10	V12	V13	V14	V15	V16	V17	V18	V19	Annual
	Months	-2	0	6	12	18	24	36	48	60	72	84	96	108	120	132	144	156+
HC	Blood & Urine	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	CSF	X			X		X	X	X	X		X		X		X		X^L
	Skin biopsy	X^R	X^j				X		X									
Prodromal	Blood & Urine	X			X		X	X	X	X	X	X	X	X	X	X	X	X
	CSF	X			X		X	X	X	X		X		X		X		X^L
	Skin biopsy	X^R	X^j				X		X			X						
PD	Blood & Urine	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	CSF	X			X		X	X	X	X		X		X		X		X^L
	Skin biopsy	X^R	X^j				X		X									

X^R Completed at Screening or Baseline based on site preference.
X^j Only collect at Baseline Visit if not collected at Screening Visit.
X^L Cadence should continue to follow every 2 years.

4.3. Blood Collection – Order of Draw

Tubes should be filled in the following order:

1. 1 x 2.5 ml PAXgene™ Tube
2. 3 x10 ml EDTA Tubes for Plasma and Buffy Coat
3. 1 x 3 ml EDTA Purple Top for Whole Blood
4. 1 x 10 ml Serum Determination Red Top
5. General clinical lab tubes (REFER TO LABCORP DRUG DEVELOPMENT LAB MANUAL)

4.3.1. General Clinical Labs

General clinical lab samples (using Labcorp Drug Development kits), if collected, should be drawn after all research samples have been collected. Please refer to the Labcorp Drug Development Manual for detailed instructions on collection and shipment of samples to Labcorp Drug Development.

4.3.2. Skin Biopsy

4.3.2.1. Double Punch (2 x Frozen Tissue Biopsies) – Appendix I

US/Canadian sites will collect double punch skin biopsies from all participants. Collections will occur at SC/BL, V06, and V10.

4.4. Collection and Processing Procedures

- APPENDIX B: Filling Aliquot Tubes
- APPENDIX C: Whole Blood with 1 x 2.5mL PAXgene™ Tube for RNA
- APPENDIX D: Whole Blood with 3 x 10mL EDTA Tube for Plasma and Buffy Coat
- APPENDIX E: Whole Blood with 1 x 3 ml EDTA Tubes
- APPENDIX F: Whole Blood with 1 x 10 ml Serum Red Top Tube
- APPENDIX G: Urine Collection and Processing
- APPENDIX H: CSF Collection and Processing
- APPENDIX I: Skin Biopsy – Double Frozen Punch

4.5. Sample Form: Appendix J

4.5.1. When completing the sample form, please ensure that the correct visit is listed. Under AM4, research biospecimens (blood, urine), CSF, and skin punches should be collected at the SC visit. Skin punches may be collected at BL if not collected at SC.

5. PACKAGING AND SHIPPING INSTRUCTIONS

5.1. Frozen Shipping to the IU Biobank – US Sites

Please refer to Appendix L for detailed instructions regarding frozen sample shipment to IU.

5.2. Frozen Shipping to the IU Biobank – Canadian Sites

Please refer to Appendix M for detailed instructions regarding frozen sample shipment to IU.

5.3. Frozen Shipping to the Milan BioRep Biobank – European and African Sites

Please refer to Appendix N for detailed instructions regarding frozen sample shipment to BioRep.

6. SAMPLE QUALITY CHECKS AND FEEDBACK TO SITES

For each sample collected, the recipient biorepository monitors sample shipment, count, and condition. Sites must strive to collect the requested amount of each fluid as they are able. Samples must be packed well with enough dry ice to avoid a thawing event while in transit. If any issues or nonconformances are identified, the recipient biorepository will complete a Nonconformance Report (Appendix Q) to provide feedback to the site and the SMC.

Issues of concern that may impact sample collect, processing, or future analyses may also be escalated to the PPMI Steering Committee for review.

7. DATA QUERIES AND RECONCILIATION

A predetermined dataset pertaining to the collection of each sample must be entered into the study EDC on the day of sample collection to accurately capture sample processing details. The data captured will be used to complete sample data reconciliation and provide information essential to future analyses.

IU will collaborate with LONI to reconcile information captured in the EDC database with data from samples accessioned at IU. Any discrepant information will be queried.

Data queries may include:

- Apparent missing samples at the recipient biorepository and/or corresponding data.
- Incorrect samples collected and shipped to the recipient biorepository.
- Damaged or incorrectly prepared samples.
- Unlabeled samples, samples labeled with incomplete information, or mislabeled samples.
- Discrepant information between the IU Sample Form and the information available in the EDC.

APPENDIX A: SPECIMEN COLLECTION KITS AND SUPPLIES PROVIDED BY IU

Blood + Urine Collection Kit – RNA, Plasma, Buffy Coat, Whole Blood, Serum, Urine

Quantity	Item
1	6-tube bubble pouch
1	Cryobox
16	Cryogenic vials (2 mL) – 3 red cap, 12 purple cap, 1 clear cap
3	Screw-top centrifuge tubes (15 mL)
1	Screw-top centrifuge tubes (50 mL)
1	Specimen cup
1	PAXgene™ tube (2.5 mL)
3	Purple-top EDTA tube (10 mL)
1	Purple-top EDTA tube (3 mL)
1	Red-top serum tube (10 mL)
1	Transfer pipette (3mL)
1	Plastic biohazard bag with absorbent sheet

CSF Collection Kit

Quantity	Item
15	Cryogenic vials (2 mL) – 1 purple cap, 14 clear cap
2	Screw-top centrifuge tubes (15 mL)
1	Lumbar Puncture Tray (24G) UPON REQUEST ONLY
1	Medication Filter Straw
1	Screw-top centrifuge tube (50mL)
1	Transfer pipette (3mL)

Skin Biopsy Collection Kit (Double Frozen Punch)

Quantity	Kit Component
1	Sterile drape
1	Tweezers
2	Gauze pads
2	Alcohol prep pads
1	Scissors
2	Skin biopsy punch tools with plungers
1	Gelfoam sterile compressed sponge
1	Vaseline ointment packet
1	Coverlet adhesive dressing
1	Transparent film dressing
2	Low binding cryovial tube (2mL)
1	Plastic biohazard bag with absorbent sheets
1	Cryobox

Supplemental Kit

Quantity	Item
10	6-tube bubble pouches
5	Cryoboxes
50	Cryogenic vials 2 mL with red caps
50	Cryogenic vials 2 mL with purple caps
50	Cryogenic vials 2 mL with clear caps
5	Needles – Sprotte spinal with introducer
5	Medication transfer filter straws (for lidocaine)
30	Screw-top centrifuge tubes (15 mL)
5	Screw-top centrifuge tubes (50 mL)
5	Specimen cups
10	PAXgene™ tube (2.5 mL)
5	Purple-top EDTA tubes (10 mL)
10	Purple-top EDTA tubes (3 mL)
5	Red-top serum tubes (10 mL)
5	Transfer pipettes
5	Plastic biohazard bags with absorbent sheets
5	Warning label packets
5	UPS® or FedEx® shipping pouch

Frozen Shipping Kit

Quantity	Item
1	Large Dry Ice Shipper
1	Warning Label Packet
1	UPS® or FedEx® shipping pouch

International Shipping Kit

Quantity	Item
1	Warning Label Packet
1	UPS® or FedEx® shipping pouch

Visit Label Roll

Quantity	Item
50	Sample Labels • 1 x PAXgene Collect Label • 3 x WBLD 10ml EDTA Collect Label • 13 x Plasma Aliquot Label • 1 x Buffy Coat Aliquot Label • 1 x WBLD 3ml EDTA Collect Label • 1 x WBLD 10ml Serum Collect Label • 4 x Serum Aliquot Label • 1 x CSF Collect Label • 20 x CSF Aliquot Label • 2 x Frozen Tissue Label • 1 x Urine Collect Label • 2 x Urine Aliquot Label
12	Kit Number Labels

APPENDIX B: FILLING ALIQUOT TUBES

Filling Aliquot Tubes with Biofluid (Plasma, Buffy Coat, Serum, and CSF)

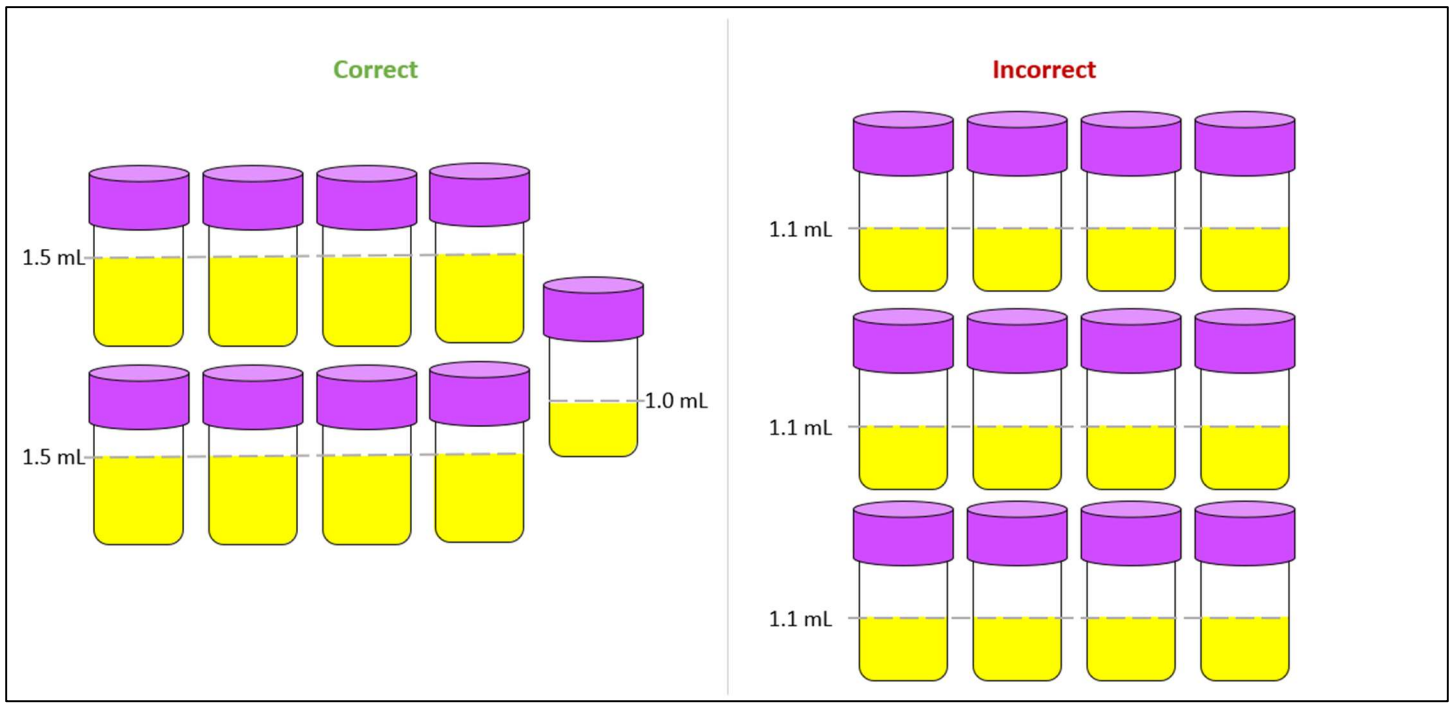
To assist in the preparation and aliquoting of specimens, colored caps are used for the aliquot tubes. The chart below summarizes the correspondence between cap color and type of aliquot, if used.

Cap Color	Specimen Type
Purple	Plasma
Purple	CSF for local lab
Red	Serum
Clear	CSF
Clear	Buffy coat

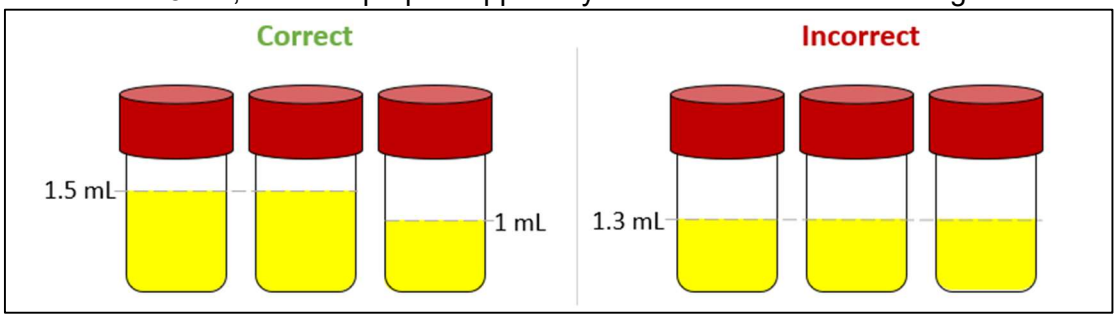
To ensure that the biorepository receives enough of the specimen for processing and storage, and to avoid cracking of the tubes, each aliquot tube should be filled to 1.5 milliliters after processing is completed (refer to detailed processing instructions per specimen type in the applicable subsequent appendices). A 1.5 mL aliquot will reach the bottom of the ridged section of the cryovial as shown below. Over-filled tubes may burst once frozen, resulting in loss of specimen.

For a given specimen type, fill aliquot tubes as close to 1.5 mL as possible. If there is less than 1.5 mL of biologic material remaining, that remaining residual amount should be sent in a partially filled aliquot tube. This residual tube should be noted on the sample form (Appendix J).

Example Scenario A: if 13 mL of plasma is obtained, the site should fill 8 purple capped cryovial tubes each with 1.5 mL, and one additional purple capped cryovial tube with the remaining 1.0 mL.



Example Scenario B: if 4 mL of serum is obtained, the site should fill 2 purple capped cryovial tubes each with 1.5 mL, and one purple capped cryovial tube with the remaining 1 mL.



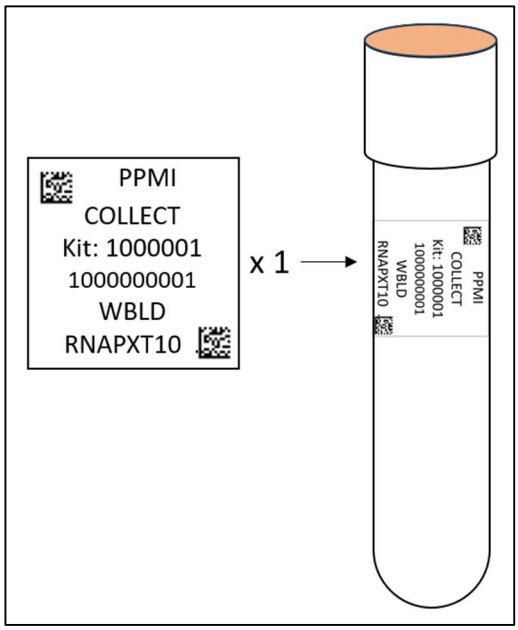
APPENDIX C: WHOLE BLOOD COLLECTION WITH 1 x PAXGENE™ RNA TUBE

See collection schematic on following pages.

1. **CRITICAL STEP:** Store PAXgene™ RNA Tube at room temperature, 64°F - 77°F (18°C to 25°C) before use.

2. **CRITICAL STEP:** The PAXgene™ RNA Tube should be the drawn first during the blood collection procedure (before CBC, plasma, etc.).

3. Place the "RNAPXT10" specimen label on the PAXgene™ Tube prior to blood draw.



4. Using a blood collection set and a holder, collect blood into the PAXgene™ RNA Tube using site recommended procedure for standard venipuncture technique.

The following techniques can 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.

5. Allow at least 10 seconds for a complete blood draw to take place. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The vacuum within the PAXgene™ RNA Tube is designed to draw 2.5 mL of blood into the tube.

6. **CRITICAL STEP:** Immediately after blood collection, gently invert/mix (180 degree turns)

the PAXgene™ RNA Tube 8-10 times.

7. **CRITICAL STEP:** Incubate the PAXgene™ RNA Tube upright at room temperature, 64°F - 77°F (18°C to 25°C) for 24 hours.

8. Record Kit Number, time of draw, and date of draw in the study database.

9. After 24 hours at room temperature, place the PAXgene™ RNA Tube upright in a wire or plastic test tube rack and transfer the rack into a -80°C freezer. **DO NOT** store/freeze samples in a solid Styrofoam test tube holder.

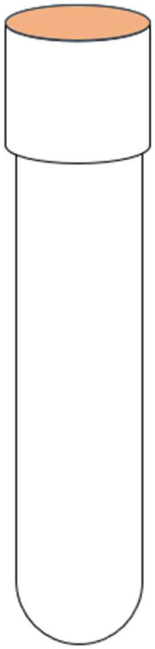
   Note: If blood is drawn on a Friday and the sample will not be accessible on Saturday, transfer the tube into the -80°C freezer as late as possible on Friday. Sample must sit at room temperature for a minimum of 2 hours.

10. Keep the PAXgene™ RNA Tube at -80°C until packed for shipment on dry ice.

11. Samples should be shipped following the instructions in Appendix L (US sites shipping to IU), Appendix M (Canadian sites shipping to IU), or Appendix N (sites shipping to BioRep).

12. Enter remaining sample collection data into the study database.

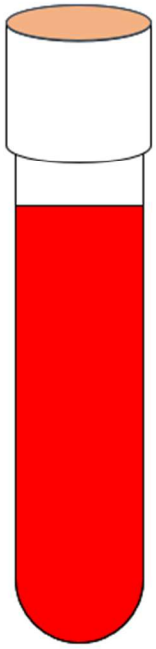
Whole Blood for RNA Collection & Processing Schematic



Store PAXgene™ tube at room temp.

Ensure tube is not expired.

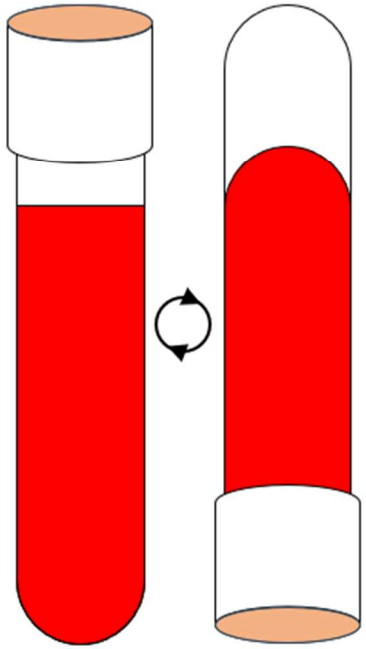
Label tube prior to sample collection.



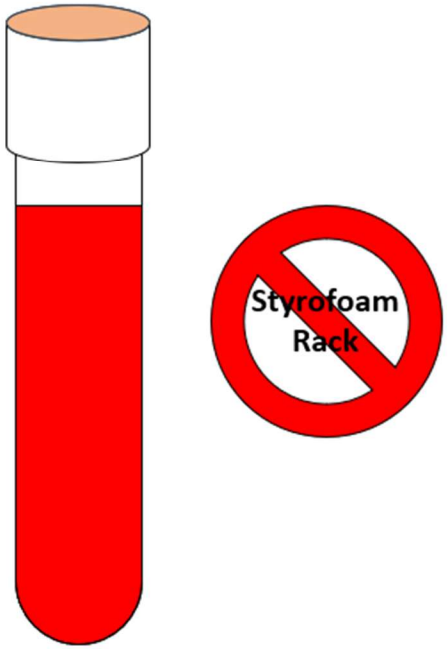
Collect Blood into 1 x PAXgene™ tube.

Allow blood to flow for at least 10 seconds.

Ensure blood flow has stopped before ending collection.



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



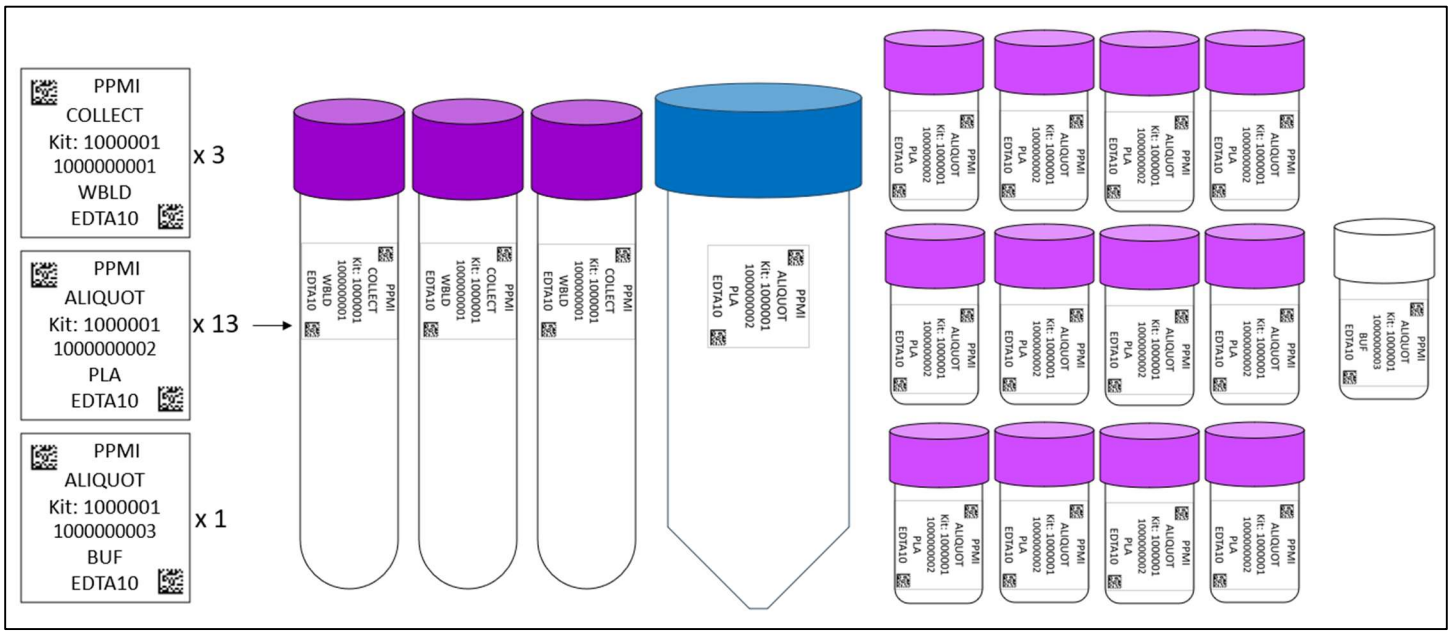
After 24 hours incubation at room temperature, store sample upright at -80°C in a wire rack until shipment.

APPENDIX D: WHOLE BLOOD COLLECTION WITH 3 X 10mL EDTA TUBES FOR PLASMA & BUFFY COAT

See collection schematic on following pages.

1. **CRITICAL STEP:** Store 10 mL EDTA Tubes at room temperature, 64°F - 77°F (18°C to 25°C) before use.

2. Complete labeling prior to blood draw.
   a. Place a “WBLD, EDTA10” Label on each of the 3 x 10mL EDTA tubes.
   b. Place “PLA, EDTA10” specimen labels on the 50 mL Centrifuge Tube and the purple capped 2 mL aliquot tubes (up to 12).
   c. Place “BUF, EDTA10” specimen label on the clear capped 2 mL aliquot tube.



3. Using a blood collection set and a holder, collect blood into the 3 x 10 mL EDTA Tubes using site recommended procedure for standard venipuncture technique.

   The following techniques can be used to prevent possible backflow:
   a. Place donor’s arm in a downward position.
   b. Hold tube in a vertical position, below the donor’s arm during blood collection.
   c. Release tourniquet as soon as blood starts to flow into tube.

d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

4. Allow at least 10 seconds for a complete blood draw to take place. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The vacuum within the 10 mL EDTA Tube is designed to draw 10 mL of blood into the tube.

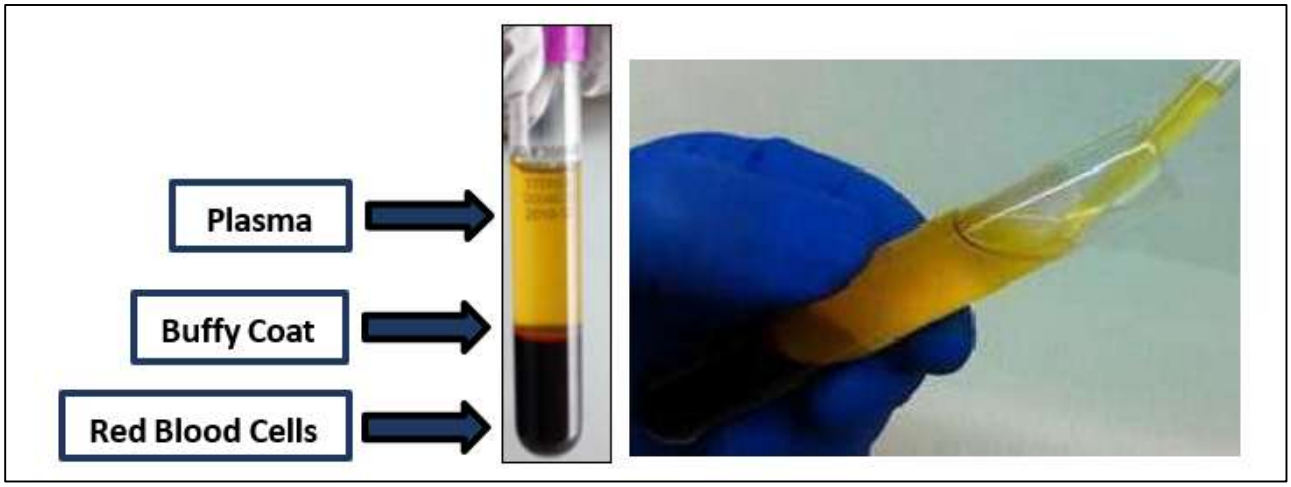
5. **CRITICAL STEP:** Immediately after blood collection, gently invert/mix (180 degree turns) the 10 mL EDTA Tubes 8-10 times.

6. Record Kit Number, time of draw, and date of draw in the study database.

7. Within 30 minutes of collection, centrifuge balanced 10 mL EDTA Tubes at **4°C for 15 minutes at 1500 x g.** It is critical that the tubes are centrifuged at the appropriate speed to ensure proper separation. For assistance, see Appendix M.

8. While centrifuging, record the time of centrifuge start in the study database.

9. Transfer the separated plasma into the 50 mL centrifuge tube. This should be completed at room temperature.
   a. While aliquoting, tilt the collection tube, placing the pipette tip along the lower side of the tube wall to ensure the buffy coat and packed red blood cells at the bottom of the collection tube are not agitated. See picture below.
   b. **Note:** When pipetting plasma from the plasma tube into the 50 mL centrifuge tube, be very careful to pipette the plasma top layer only, leaving the buffy coat and the red blood cell layers

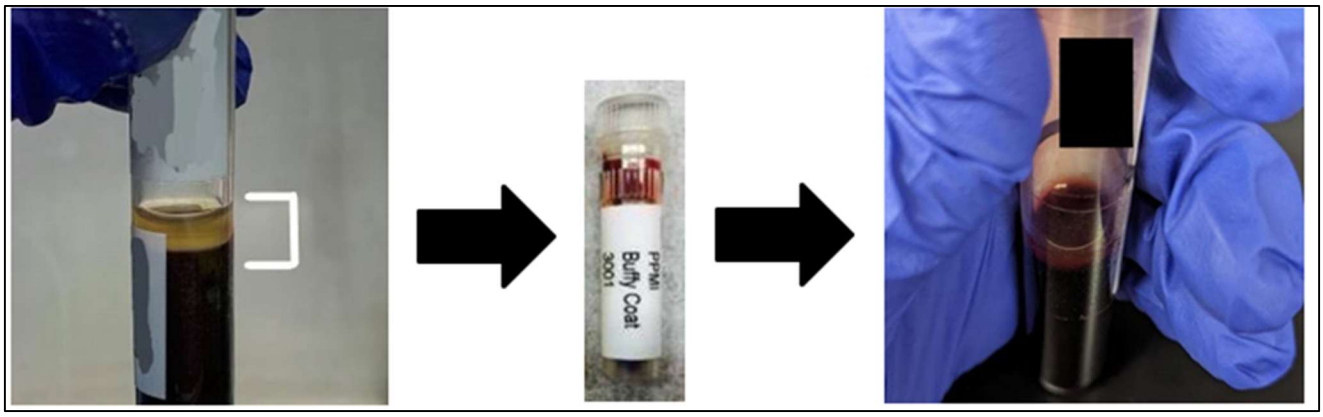


10. Mix the plasma gently by inverting the 50 mL centrifuge tube 3-4 times.

11. Pipette 1.5 mL of plasma from the 50 mL centrifuge tube into each "PLASMA" labeled 2 mL aliquot tube. This collection should yield, on average, 12 - 18 mL of blood plasma, for a total of 8 - 12 aliquot tubes

per participant. Seal each aliquot tube with a purple cap.

12. Using a clean transfer pipette (micropipette preferred), transfer the buffy coat layer from one (1) of the EDTA Tubes into the “BUFFY COAT” labeled aliquot tube. Seal the aliquot tube with a clear cap. A small amount of residual plasma above the buffy coat and some of the RBCs underneath the buffy coat will be collected in this process. View the diagram below for a brief visual representation of what this isolation will look like.
    a. The buffy coat from the other two EDTA tubes can be discarded with the RBCs.



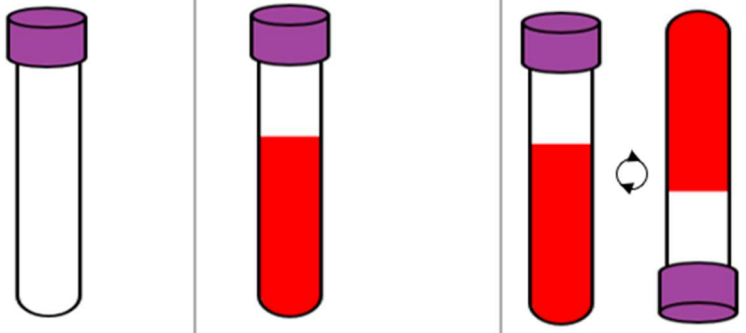
13. Within 60 minutes of sample collection, freeze and store samples at -80°C. Samples should be frozen and stored upright. A cryobox is provided for this purpose.

14. Discard the used 10 mL EDTA Tubes and 50 mL centrifuge tubes according to site guidelines for disposing of biomedical waste.

15. Enter remaining sample collection data into the study database.

16. Samples should be shipped following the instructions in Appendix L (US sites shipping to IU), Appendix M (Canadian sites shipping to IU), or Appendix N (sites shipping to BioRep).

Whole Blood for Plasma & Buffy Coat Collection & Processing Schematic



Store tubes at room temp

Ensure tubes are not expired

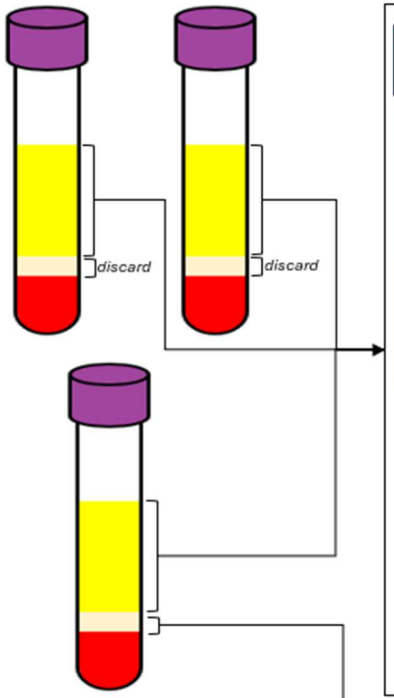
Label tubes prior to sample collection

Collect Blood into 3 x 10 mL EDTA tubes

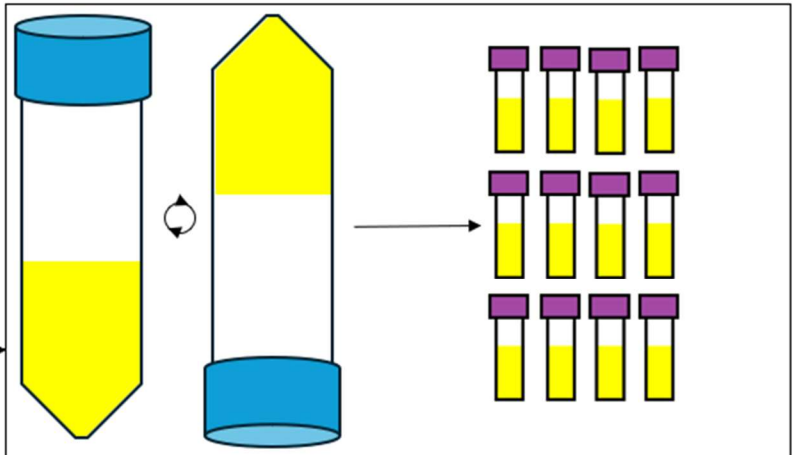
Allow blood to flow for at least 10 seconds

Ensure blood flow has stopped before ending collection

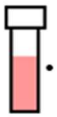
Immediately after blood draw, invert tube 8-10 times to mix sample



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



- Using a clean transfer pipette, transfer all plasma into a 50 mL conical tube
- Mix gently by inverting 3-4 times
- Aliquot 1.5 mL plasma into each "ALIQUOT, PLA" pre-labeled cryovial with purple cap (up to 12)
- Store aliquots upright at -80°C in provided cryobox until shipment



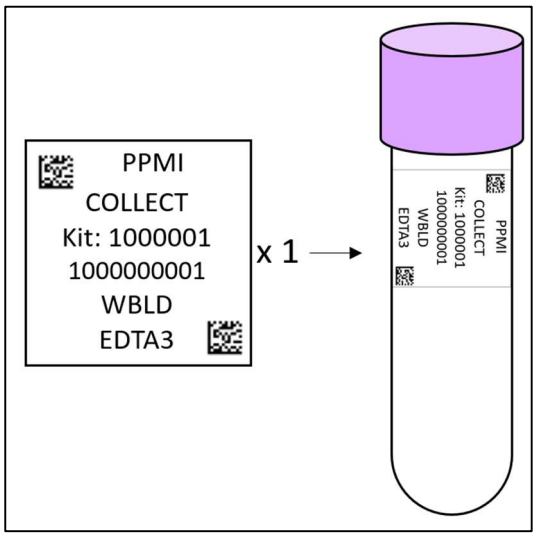
- Using a clean transfer pipette, collect the buffy coat from one (1) of the EDTA tubes (will include residual plasma & some RBCs).
- Transfer the buffy coat to the "ALIQUOT, BUF" pre-labeled cryovial with clear cap.
- Store buffy coat aliquot upright at -80°C in provided cryobox until shipment

APPENDIX E: WHOLE BLOOD COLLECTION USING 1 X 3 mL EDTA TUBE

See collection schematic on following pages

1. **CRITICAL STEP:** Store 3 mL EDTA Tube at room temperature, 64°F - 77°F (18°C to 25°C) before use.

2. Complete labeling prior to blood draw.
   a. Place a “WBLD EDTA3” COLLECT label on the 3 mL EDTA Tube.



3. Using a blood collection set and a holder, collect blood into the 3 mL EDTA Tube using site recommended procedure for standard venipuncture technique.

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

4. Allow at least 10 seconds for a complete blood draw to take place. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The vacuum within the 3 mL EDTA Tube is designed to draw 3 mL of blood into the tube.

5. **CRITICAL STEP:** Immediately after blood collection, gently invert/mix (180 degree turns) the 3 mL EDTA tube 3 - 4 times.

6. Record Kit Number, time of draw, and date of draw in the study database.

7. Immediately transfer tube to -80°C Freezer. The sample should be frozen and stored upright in a wire or plastic test tube rack.

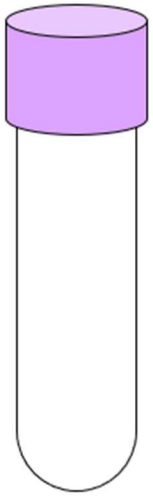
8. Transfer the rack into a -80°C freezer. DO NOT store/freeze samples in a solid Styrofoam test tube holder.

9. Enter remaining sample collection data into the study database.

10. Keep the 3 mL EDTA Tube at -80°C until packed for shipment on dry ice.

11. Samples should be shipped following the instructions in Appendix L (US sites shipping to IU), Appendix M (Canadian sites shipping to IU), or Appendix N (sites shipping to BioRep).

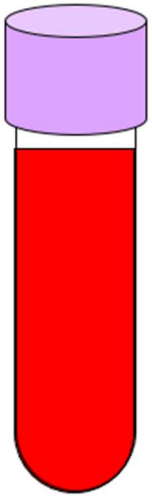
Whole Blood Collection & Processing Schematic
1 x 3 mL EDTA Tube



Store tube at room temp.

Ensure tube is not expired.

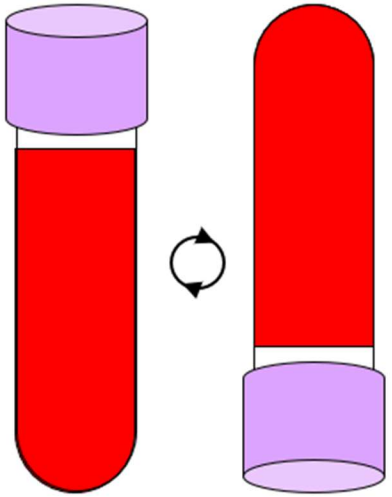
Label tube prior to sample collection.



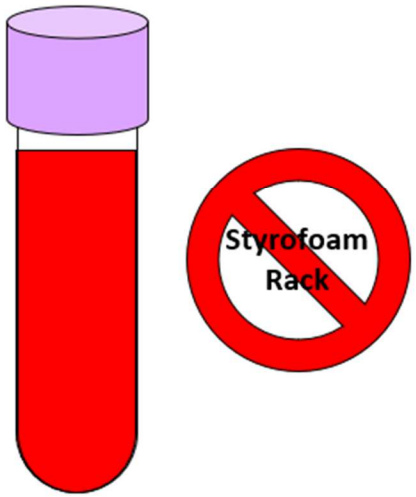
Collect Blood into 1 x 3 mL EDTA tube.

Allow blood to flow for at least 10 seconds.

Ensure blood flow has stopped before ending collection.



Immediately after blood draw, invert tube 3-4 times to mix sample.



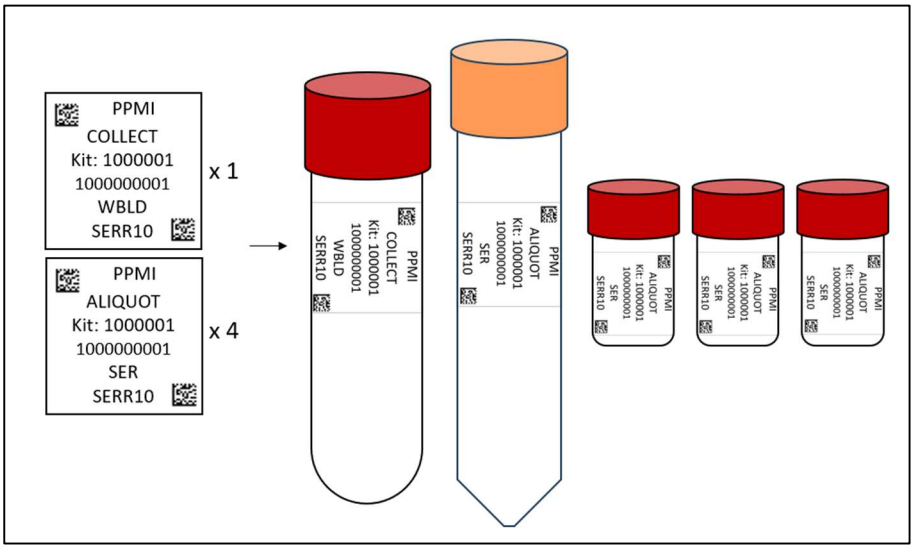
Immediately following inversion, store sample upright at -80°C in a wire or plastic rack until shipment.

APPENDIX F: WHOLE BLOOD COLLECTION WITH 1 X 10 ML SERUM TUBE FOR SERUM

See collection schematic on following pages.

1. **CRITICAL STEP:** Store Serum Determination Tube at room temperature, 64°F - 77°F (18°C to 25°C) before use**.**

2. **Complete labeling prior to blood draw.**
   a. Place the “WBLD, SER10” COLLECT label on the 10 mL Serum Tube.
   b. Place “SER, SER10” ALIQUOT labels on the 15 mL Centrifuge tube and the 3 x red capped 2 mL cryovials**.**



3. Using a blood collection set and a holder, collect blood into the Serum Determination Tube using site recommended procedure for standard venipuncture technique.

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

4. Allow at least 10 seconds for a complete blood draw to take place. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The vacuum within the Serum Determination Tube is designed to draw 10 mL of blood into the tube.

5. **CRITICAL STEP:** Immediately after blood collection, gently invert/mix (180 degree turns)

the Serum Determination Tube 8-10 times.

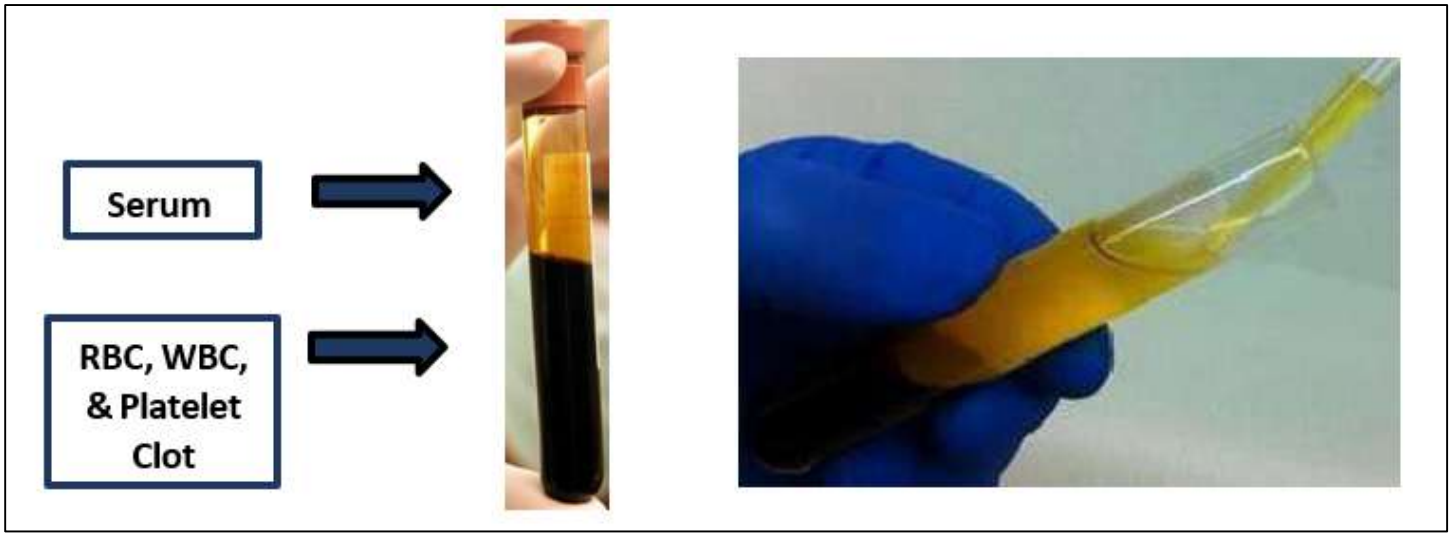
6. **CRITICAL STEP:** Allow blood to clot at room temperature for at least 15 minutes.

7. Record Kit Number, time of draw, and date of draw in the study database.

8. Within 60 minutes of collection (after 15 minutes of clotting at room temperature) centrifuge balanced Serum Determination Tube at 4°C for 15 minutes at 1500 x g. It is critical that the tubes are centrifuged at the appropriate speed to ensure proper separation. For assistance, see Appendix A.

9. Record the time of centrifuge start in the study database.

10. Transfer the separated serum into the 15mL centrifuge tube. This should be completed at room temperature.
    e. While aliquoting, tilt the tube and place the pipette tip along the lower side of the tube wall to ensure the clot at the bottom of the collection tube is not agitated. See picture below.



11. Mix the serum gently by inverting the 15 mL centrifuge tube 3-4 times.

12. Pipette 1.5 mL of serum from the 15 mL centrifuge tube into each “SERUM” labeled 2 mL aliquot tube. This collection should yield, on average, 4.5 mL of serum, for a total of 2-3 aliquot tubes per participant. Seal each aliquot tube with a red cap.

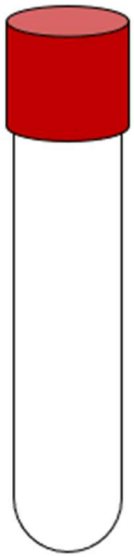
13. Within 60 minutes of sample collection, freeze and store samples at -80°C. Samples should be frozen and stored upright. A cryobox is provided for this purpose.

14. Discard the used Serum Determination Tube and 15 mL centrifuge tube according to site guidelines for disposing of biomedical waste.

15. Enter remaining sample collection data into the study database.

16. Samples should be shipped following the instructions in Appendix L (US sites shipping to IU), Appendix M (Canadian sites shipping to IU), or Appendix N (sites shipping to BioRep).

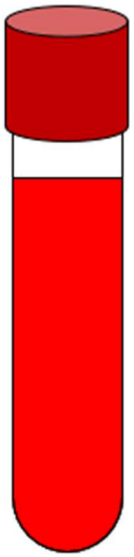
Whole Blood for Serum Collection & Processing Schematic



Store 10ml red-top tube at room temp.

Ensure tube is not expired.

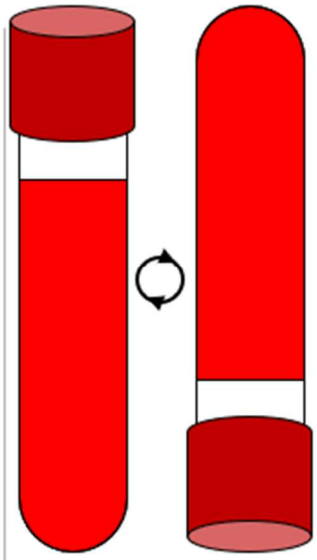
Label tube prior to sample collection.



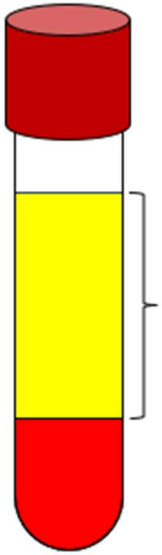
Collect Blood into 1 x 10 mL Serum tube.

Allow blood to flow for at least 10 seconds.

Ensure blood flow has stopped before ending collection.

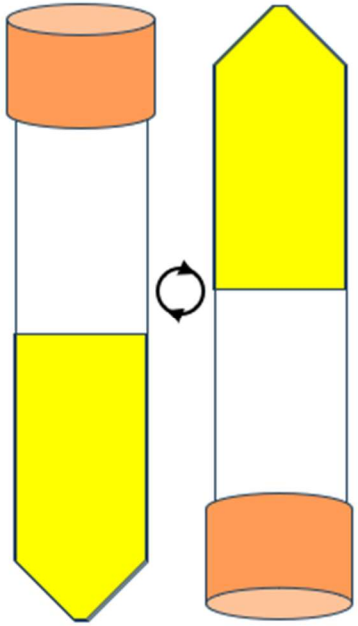


Immediately after blood draw, invert tube 8-10 times to mix sample.



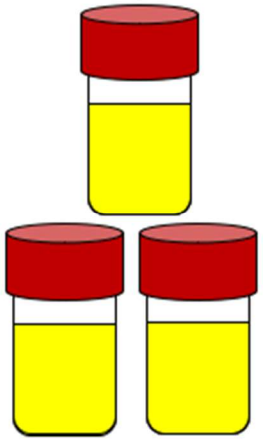
Allow blood to clot at room temperature for at least 15 minutes.

Within 60 minutes of blood draw, centrifuge sample at 4°C for 15 minutes at 1500 x g.



Using a clean transfer pipette, transfer all serum into 1 x 15 mL conical tube.

Mix gently by inverting 3-4 times.

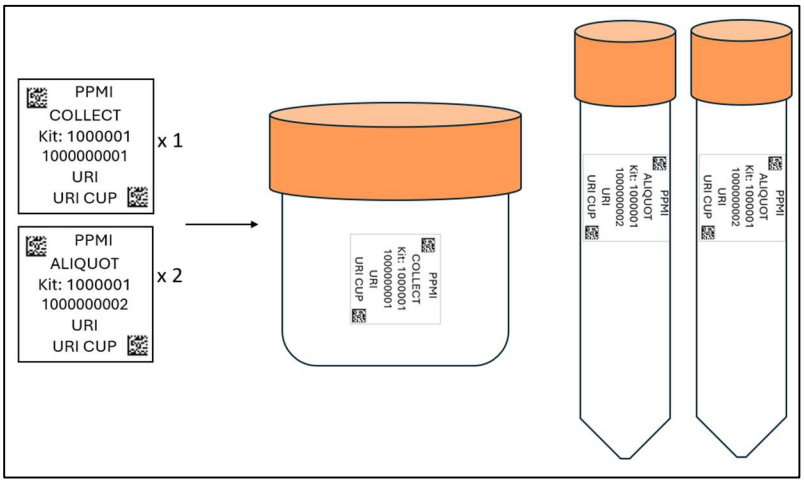


Aliquot 1.5 mL of serum into each "SERUM" labeled cryovial.

Store aliquots upright at -80°C in provided cryobox until shipment.

APPENDIX G: URINE COLLECTION AND PROCESSING

1. Complete labeling prior to blood draw.
    a. Place the "URI" COLLECT label on the urine collection cup.
    b. Place a "URI" ALIQUOT label on each of the 2 x 15 mL centrifuge tubes.



2. Ask study participant to collect a urine specimen in the collection cup. Urine should be collected midstream to remain as sterile as possible.

3. Pour urine specimen into one labeled 15 mL centrifuge tube, ensure the tube is <u>at least</u> half full (7.5 mL). Cap the centrifuge tube with the included screw-cap.

4. Within 30 minutes of collection, centrifuge the tube **at 4°C, for 15 minutes, at 2500 x g** to remove sediment and cells.

5. While centrifuging, record the time of collection and centrifuge start in the study database.

6. Record Kit Number, time of draw, and date of draw in the study database.

7. Using a clean transfer pipette, carefully transfer supernatant from the 15 mL processing centrifuge tube into the second, labeled, 15 mL conical tube.

8. Firmly cap with the included screw cap.

9. Discard the original processing tube per your institution's guidelines.

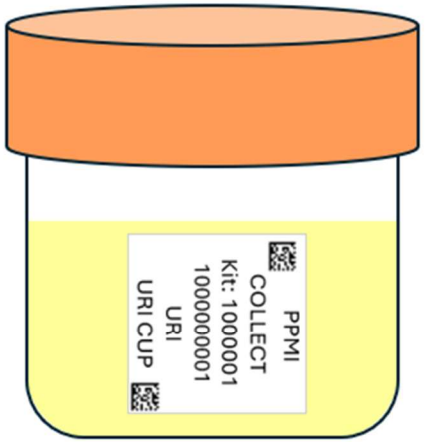
10. Place the labeled tube upright in dry ice or at -80°C and allow the sample to freeze completely.

11. Within 60 minutes of sample collection, freeze and store samples at -80°C. Samples should be frozen and stored <u>upright</u>.

12. Enter remaining sample data into the study database.

13. Samples should be shipped following the instructions in Appendix L (US sites shipping to IU), Appendix M (Canadian sites shipping to IU), or Appendix N (sites shipping to BioRep).

Urine Collection & Processing Schematic



Label 15mL urine collection cup and 15ml conical tubes.

Subject provides sample in urine collection cup

Record date & time of sample collection

Pour approximately 10mL of urine specimen into a 15mL centrifuge tube with pre-printed "URI" ALIQUOT label

Within 30 minutes of collection, centrifuge sample at 4°C for 15 minutes at 2500 x g

Record temperature and rate of centrifuge as well as time of centrifugation

Using a clean transfer pipette, transfer the supernatant from the centrifuged tube into new 15mL conical tube that has been pre-labeled with a "URI" ALIQUOT label. Secure sample with orange screw cap.

Freeze in dry ice
&
Store in -80°C Freezer

Place urine upright in dry ice and allow the sample to completely freeze.

Within 60 minutes of collection, freeze and store sample upright at -80°C

Record time sample was placed in freezer

APPENDIX H: DETAILED CSF SAMPLE PREP, COLLECTION AND PROCESSING PROCEDURE

PARTICIPANT PREP – LOW-FAT DIET MENU SUGGESTIONS

Due to the interference of lipid content in blood and CSF specimens collected for biomarker evaluation in the PPMI study, **it is strongly advised that CSF and biofluid samples be collected after an 8 hour fast (no food or drink except fluids such as water, tea, black coffee).** If fasting is not achievable, a participant should be on a low-fat diet for at least 8 hours prior to blood or CSF collection.

Below is a list of suggested sample menus that could be consumed prior to blood collection or lumbar puncture. These lists are not all inclusive and sites should use their best judgment in this process.

Sample Breakfast Items:	Sample Lunch Items
• Dry whole wheat toast • Poached egg white or egg substitute • Plain oatmeal or other whole grains topped with fresh or dried fruit • Dry cereal • Fruit salad • Clear tea or coffee • Fruit or vegetable juice	• Turkey breast sandwich on whole wheat bread with lettuce, tomato, and mustard • Plain pasta with plain marinara sauce • Assorted vegetables (steamed or raw) and green salads • Steamed chicken breast – lean, without skin • Clear broth with vegetables and pasta • Fruit salad, flavored gelatin • Clear beverages
For all suggestions above, avoid: • Oils • Butter • Dressings • Cheese • Avocado • Red Meats • Nuts • Granola • Milk • Cream	

Additional foods to avoid prior to blood and CSF collection:

Avoid: All fats and nuts such as:

- Butter
- Cream
- Bacon fat
- Lard
- All oils
- All margarine
- All nuts
- Peanut butter
- Coconut
- Whole seeds such as pumpkin and sunflower

Avoid: All milk and dairy products such as:

- All cheese
- All whole milk products
- All products containing cheese
- Cheese spreads such as cream cheese
    - Sour cream
    - All ice cream
    - Milk chocolate
    - Yogurts

Avoid: High fat prepared foods and foods naturally high in fat:

    - All red meats or meats containing fat such as pork
    - Fatty meats such as:
        - Luncheon meats
        - Organ meats
        - Bacon
    - Fatty fish such as:
        - Salmon
        - Mackerel
- Salad dressing and mayonnaise
    - Buttered, au gratin, creamed, or fried vegetables
    - Fried Foods
    - Fried snacks such as:
        - Chips
        - Crackers
    - French Fries
- Gravies and sauces
- Baked goods and Frosting

CSF COLLECTION PREPARATION

1. Setting Up a sterile field for an LP:
   1.1. 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 participant.
   1.2. 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.
   1.3. 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 or 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.

2. Maintaining a sterile field:
   2.1. Keep in mind that there is usually a lot of 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 away 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.

3. Tips for Clinicians Performing Lumbar Puncture:
   *Optimizing participant comfort and minimizing the risk of adverse events.
   3.1. Talk to the participant through the procedure so that there are no surprises.
   3.2. Use of a Sprotte 24g atraumatic spinal needle and careful technique are optimal for reducing post-LP headache risk. A pencil point spinal needle such as Spinocan 22g or 24g may also be used.
   3.3. Use of medication transfer filter straw is required to decrease any chance of microscopic glass particles from breaking of the glass ampule. Guidance is provided in the document: Filter Straw Directions for Use. File may be found in Florence under Biologics and Labcorp Manuals, Documents
   3.4. 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 participant 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).
   3.5. Increasing fluid intake immediately after LP is helpful.
      3.5.1. Be sure to give post-LP care instructions verbally to the participant (see below). A follow-up call will be placed by the study coordinator 2-3 days after the procedure to assess for adverse events.

4. Post-LP care instructions:
   4.1. Advise the participant to refrain from exertion (e.g., exercise, housework, gardening, lifting, sexual activity, or any other strenuous activities) for 24 hours after the LP.

4.2. Advise the participant to continue with increased fluid intake.

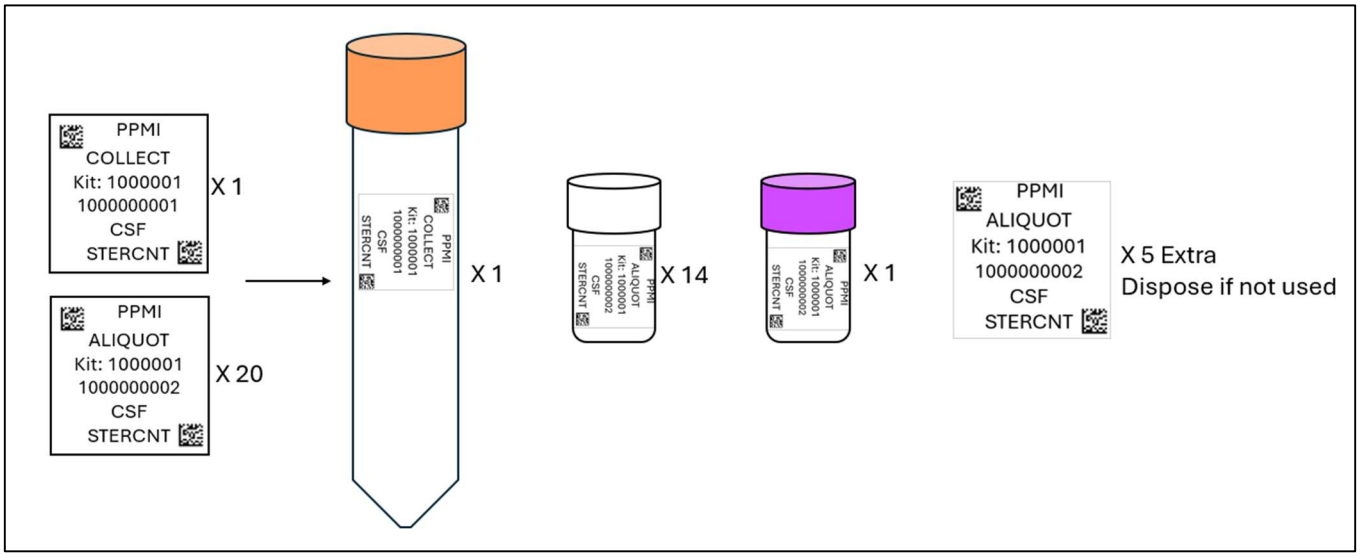
5. Mild to moderate headache after a lumbar puncture:
   5.1. Mild to Moderate headache following lumbar puncture usually resolves within 3-4 days.
   5.2. Treatment of Mild to Moderate headache:
      5.2.1. Limit physical activity as much as possible.
      5.2.2. Oral fluids and caffeine are helpful. Drinking a can of Mountain Dew soft drink (for example) is preferable to coffee, which has some diuretic activity.
      5.2.3. Acetaminophen (e.g., Tylenol) should be used for symptomatic relief. If a participant cannot tolerate acetaminophen, ibuprofen should be used. Avoid aspirin. If these do not relieve the headache, Tylenol with codeine or an equivalent could be considered.

6. Severe headache after a lumbar puncture:
   6.1. If the headache becomes severe, posturally sensitive (relieved by supine posture), or is accompanied by nausea, vomiting, tinnitus, and/or visual disturbances, the participant should contact the site study staff for further instruction per standard clinical care.

DETAILED CSF PROCESSING PROCEDURE

IMPORTANT:
CSF is processed at room temperature (64°F – 77°F or 18°C – 25°C).
A portion of the CSF must be sent to your clinical lab and analyzed within 4 hours of collection.

1. Complete labeling prior to blood draw.
   1.1. Place the "CSF" COLLECT label on the 15 mL centrifuge tube.
   1.2. Place a "CSF" ALIQUOT label on the 2 mL cryovials with clear caps and the single 2 mL cryovial with purple cap.
   Note: the kit will come with 20 CSF ALIQUOT labels. Dispose of the extra 5 labels if not needed for the visit.



2. Pre-cool labeled aliquot tubes by placing on wet ice prior to the procedure.

3. Perform lumbar puncture using the atraumatic technique.

4. Collect CSF into syringes (if a noticeably blood tap, discard the first 1-2 mL).
   4.1. Note: Bedside LP should be performed at the L3-S1 interspace, as distal as possible. Fluoroscopy-guided LP is preferably performed at L3-L4 but may occur at L2-L3 if considered safe, at the discretion of the radiologist.

5. After the LP has begun and fluid is being collected, take the first 1-2 mL of CSF from the first syringe and place in the CSF labs tube (PURPLE TOP).
   5.1. Send this sample to the local lab for routine diagnostic tests. Do not freeze this sample. Send at room temperature to local clinical lab for basic CSF analysis.
   NOTE: Sample must be analyzed within 4 hours of collection
   - Cell Count (erythrocytes first)
   - Total Protein
   - Glucose

6. Collect an additional 15-18 mL of CSF and transfer to the labeled 15 mL conical polypropylene tubes at room temperature. Mix gently by inverting 3-4 times.

7. Record the Kit Number, time of draw, and date of draw in the study database.

8. Within 15 minutes of collection, spin the remaining CSF sample down at **2000 x g** for **10 minutes at room temperature**, 64°F – 77°F (18°C to 25°C).

9. While centrifuging, record the time of centrifuge start in the study database.

10. Using a clean transfer pipette, transfer CSF from both 15 mL conical tubes into a 50 mL conical tube, leaving debris undisturbed at the bottom of the 15 ml tubes.

11. Gently invert the 50 mL conical tube 3-4 times to mix the sample.

12. Using a pipette (micropipette preferred), transfer 1.5 mL of supernatant directly into the labeled, pre-cooled, 2 mL CSF aliquot tubes. This will yield, on average, 10-14 aliquot tubes per participant. Use more aliquot tubes if needed. **Do not discard any CSF**. Seal each aliquot tube with a clear cap.

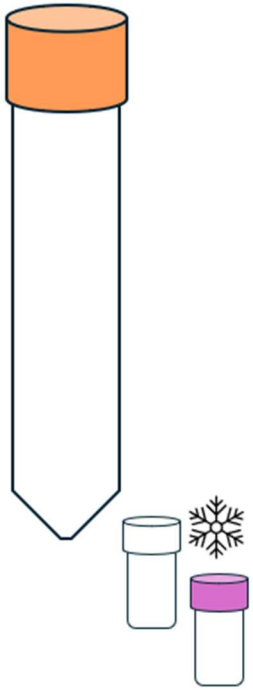
13. Within 60 minutes of CSF collection, freeze aliquoted samples on dry ice and stored at -80°C until they are prepared for shipment on dry ice.
    13.1. Samples should be <u>frozen at -80°C</u> and <u>stored upright</u>. A cryobox is provided for proper storage purposes.

14. Complete the remainder of the sample data entry in the study database.
    14.1. **NOTE**: If both gravity and suction collection techniques are used, defer to entering suction in the EDC.

15. Samples should be shipped following the instructions in Appendix L (US sites shipping to IU), Appendix M (Canadian sites shipping to IU), or Appendix N (sites shipping to BioRep).

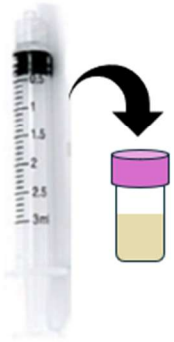
See Collection Schematic Below for additional detail.

CSF Collection & Processing Schematic



Label all tubes prior to sample collection.

Pre-chill all labeled cryovials on wet ice.



Collect CSF into the 3mL luer lock syringe.

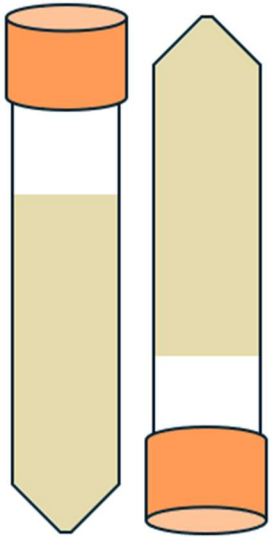
Dispense 1-2mL into the purple-capped cryovial.

Send to local lab for testing.

Collect CSF into the 6mL luer lock syringe.

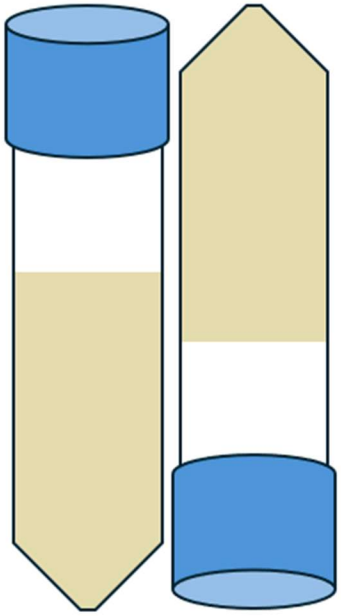
Collect 15-20mL total, including the amount sent for local lab testing.

Transfer the CSF to 15mL tubes.



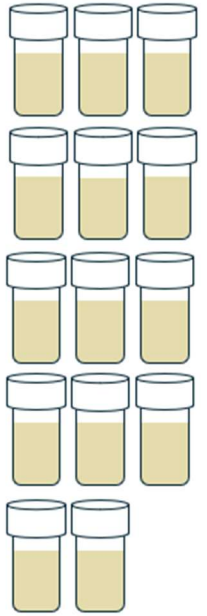
Immediately after collection, gently invert the 15mL tubes 3-4 times to mix the sample.

Within 15 minutes of collection, centrifuge sample at room temp for 10 minutes at 2000 x g.



Using a clean transfer pipette, transfer all CSF into one 50mL tube, leaving debris in the bottom of the 15mL tubes.

Gently invert the 50mL tube 3-4 times to mix the sample.



Aliquot 1.5mL into each pre-labeled, pre-chilled cryovial with clear cap.

Store CSF aliquots upright at -80°C in provided cryobox until shipment.

APPENDIX I: SKIN BIOPSY COLLECTION – DOUBLE FROZEN PUNCH

Note: The Double Frozen Punch Protocol is intended for all PPMI sites. If a participant refuses a second biopsy, a single frozen punch biopsy should be obtained.

Note: Frozen skin biopsy samples may be shipped on dry ice and packaged with other frozen samples (plasma, serum, etc.). Screening Visit samples should be shipped as soon as possible. Samples from all other visits 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 expiration dates 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.

Double Frozen 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	Low binding 2mL cryovial
1	25-cell Cryobox
1	Biohazard Bag with absorbent sheet

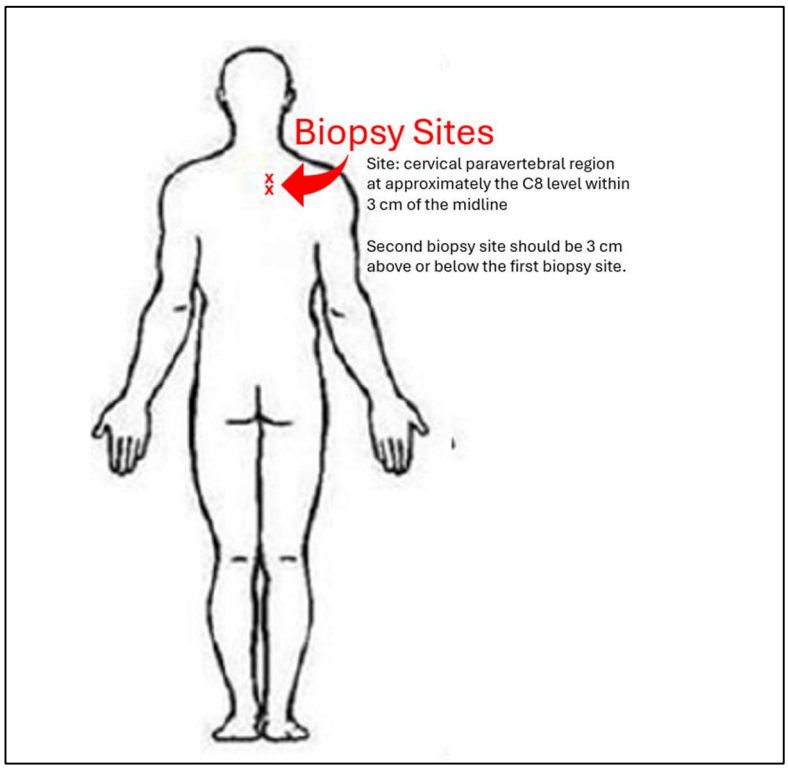
2. Setting up the Kit

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 participant.

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.

3. Skin Sample Collection

3.1. Two skin biopsies will be obtained from the cervical paravertebral region at approximately the C8 level within 3 cm of the midline (see figure below). Both biopsies will be frozen, without additive, in 2mL cryovials.



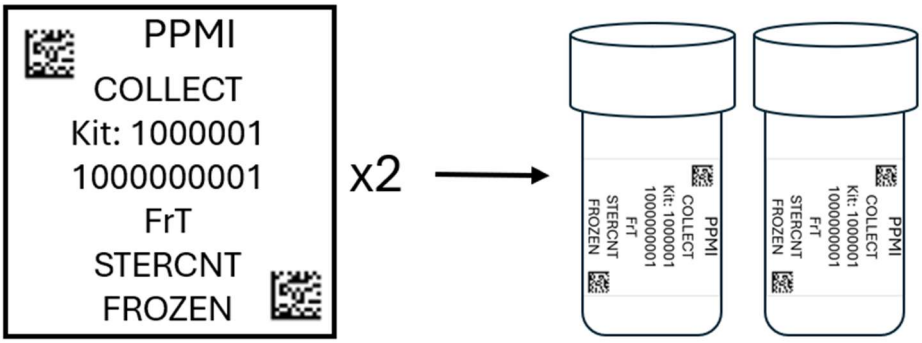
3.2. Pre-Collection Preparation Step: Preparation of Participant

3.2.1. Prepare participant for procedure per institution guidelines.

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

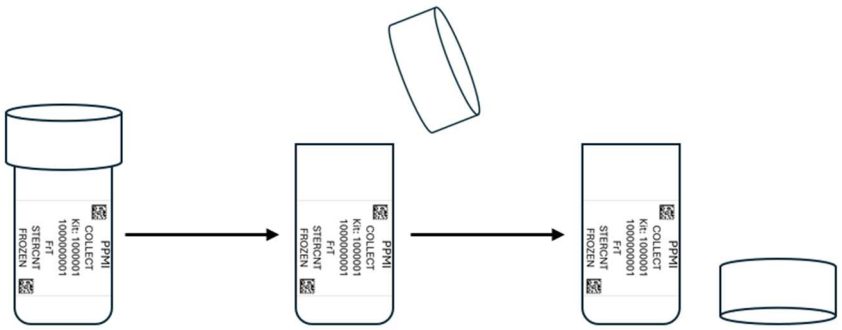
3.3. Pre-Collection Preparation Step: Cryovial for Frozen Tissue Collection

3.3.1. Place a printed “FrT, STERCNT, FROZEN” label on each of the 2 mL cryovial with clear caps.



3.3.2. Record the Kit number on the Sample Form (Appendix J).

3.3.3. Open the cryovial and place cryovial lid to the side in a sterile location. Repeat this step for the second cryovial.



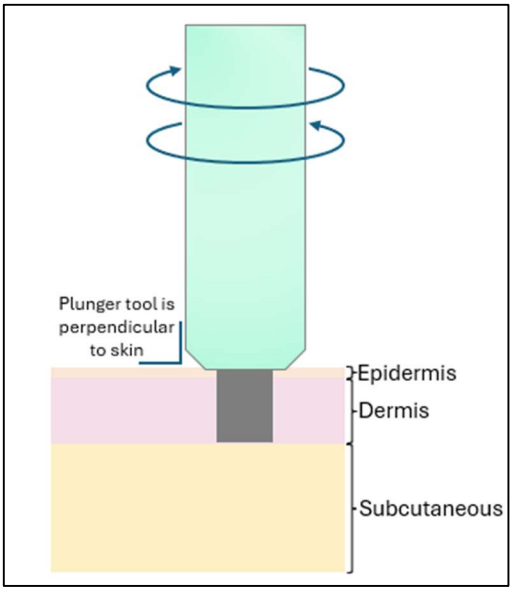
3.4. Biopsy Collection Procedure

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.

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.

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 participant 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.

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.

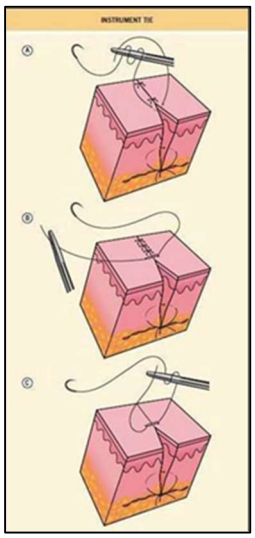


3.4.5. Depress the plunger to remove the specimen. Forceps may be needed to remove the specimen. If the specimen remains connected at the level of the subcutaneous fat, it may be necessary to cut at the base of the specimen to remove it. Do not try to tear a specimen that remains connected, as it may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

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

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.

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).



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

CRITICAL STEP: Place the specimen directly into the second prepared “FrT” cryovial and close the cryovial cap securely.

3.4.10. 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.

3.4.11. Be sure to give post care instructions verbally to the participant as found in the Operations Manual. A follow-up call will be placed by the study coordinator 2-3 days after the procedure to assess for adverse events.

3.5. Double Frozen Punch Biopsy Processing

3.5.1. CRITICAL STEP: Place the cryovials in the provided cryobox and freeze at -80ºC as soon as possible.

3.5.2. Record Time Frozen in the EDC. Store at -80ºC until shipped to repository on dry ice.

APPENDIX J: SAMPLE FORM

PPMI Sample Record Summary and Shipment Notification Form
For Indiana University

Instructions: ***Ship frozen samples Monday – Wednesday ONLY****. This form must be completed electronically for all research sample shipments. One form should be completed per participant visit. Use the link below to complete an electronic copy, which will notify the recipient repository automatically. Place a copy of the completed form in the shipment box and retain a copy for site record. The site will be contacted if any sample/form issues are noted upon receipt.*

Site:_____

Site Investigator:_____ Coordinator:_____

Site Telephone:_____ Site Email:_____

PPMI ID:	Participant Gender:	Participant Year of Birth:
Kit Number:	Visit:	

Specimen Type	# of Tubes	Sample Volume	Draw Date	Date Frozen	Notation of problems (for biorepository to complete)
Total # Tubes:					

Shipment Type:☐Frozen Date Shipped:_____ Tracking Number:_____

IMPORTANT!
AN ELECTRONIC VERSION OF THIS FORM MUST BE COMPLETED AND SUBMITTED BEFORE SHIPPING:
https://redcap.link/PPMISampleForm

Sample Shipment Mailing Address:
PPMI Biorepository
Indiana University School of Medicine
351 W. 10th St, TK-217
Indianapolis, IN 46202
Email: ppmibio@iu.edu

PPMI Sample Record Summary and Shipment Notification Form
For BioRep in Milan, Italy

Instructions: ***Ship frozen samples Monday – Wednesday ONLY****. This form must be completed electronically for all research sample shipments. One form should be completed per participant visit. Use the link below to complete an electronic copy. Place a copy of the completed form in the shipment box and retain a copy for site record. The site will be contacted if any sample/form issues are noted upon receipt.*

Site:_____

Site Investigator:_____ Coordinator:_____

Site Telephone:_____ Site Email:_____

PPMI ID:	Participant Gender:	Participant Year of Birth:
Kit Number:	Visit:	

Specimen Type	# of Tubes	Sample Volume	Draw Date	Date Frozen	Notation of problems (for biorepository to complete)
Total # Tubes:					

Shipment Type:☐Frozen Date Shipped:_____ Tracking Number:_____

IMPORTANT!
AN ELECTRONIC VERSION OF THIS FORM MUST BE COMPLETED AND SUBMITTED BEFORE SHIPPING:
https://redcap.link/PPMISampleForm

Sample Shipment Mailing Address:
BioRep Srl
c/o DIBIT2 Palazzina San Michele Via Olgettina 60
20132 Milano – Italy
Email: ppmi@biorep.it

PPMI Sample Record Summary and Shipment Notification Form
For Tel Aviv, Israel

Instructions: ***Ship frozen samples Monday – Wednesday ONLY****. This form must be completed electronically for all research sample shipments. One form should be completed per participant visit. Use the link below to complete an electronic copy. Retain a copy for site record.*

Site:_____

Site Investigator:_____ Coordinator:_____

Site Telephone:_____ Site Email:_____

PPMI ID:	Participant Gender:	Participant Year of Birth:
Kit Number:	Visit:	

Specimen Type	# of Tubes	Sample Volume	Draw Date	Date Frozen	Notation of problems (for biorepository to complete)
Total # Tubes:					

Shipment Type:☐Frozen Date Shipped:_____ Tracking Number:_____

IMPORTANT!
AN ELECTRONIC VERSION OF THIS FORM MUST BE COMPLETED AND SUBMITTED BEFORE SHIPPING:
https://redcap.link/PPMISampleForm

APPENDIX K: RATE OF CENTRIFUGE WORKSHEET

Please complete and email to the PPMI Biorepository at ppmibio@iu.edu.The PPMI Biorepository team will calculate and return a correct RPM. This must be noted in the Site PPMI Biologics Manual.

Submitter Information

Name: **Site:**

Email:

Centrifuge Information

Please answer the following questions about your centrifuge:

Centrifuge Type

Fixed Angle Rotor: ☐ Swing Bucket Rotor: ☐

Radius of Rotation (mm):

Determine the centrifuge's radius of rotation (in mm) by measuring distance from the center of the centrifuge spindle to the bottom of the device when inserted into the rotor (if measuring a swing bucket rotor, measure to the middle of the bucket).

$$RPM = \sqrt{\frac{RCF}{r \times 1.118}} \times 1,000$$

Calculating RPM from G-Force:

RCF = Relative Centrifugal Force (G-Force)
RPM = Rotational Speed (revolutions per minute)
R= Centrifugal radius in mm = distance from the center of the turning axis to the bottom of centrifuge

Comments:

Please send this form to the PPMI Biorepository team at ppmibio@iu.edu

APPENDIX L: PPMI FROZEN SHIPPING INSTRUCTIONS – US SITES

Preparing Frozen Sample Packaging/Shipment to Indiana University

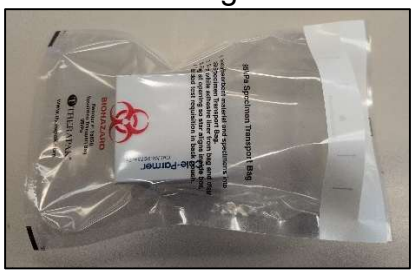
Samples Shipped on Dry Ice:

- Frozen whole blood in 1 x 3 mL plastic EDTA tube
- Frozen whole blood in 1 x PAXgene™ RNA tube
- Frozen plasma in 2 mL polypropylene tubes (up to 12)
- Frozen serum in 2 mL polypropylene tubes (up to 3)
- Frozen buffy coat in 1 x 2 mL polypropylene tube
- Frozen urine in 1 x 15 mL conical tube
- Frozen CSF in 2 mL polypropylene tubes (up to 14)
- Frozen tissue in a 2 x 2mL polypropylene tube

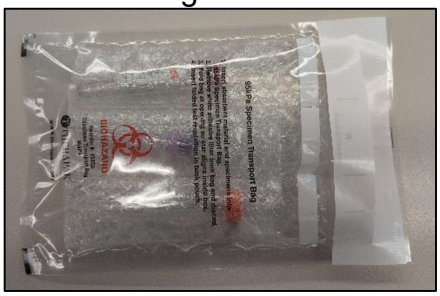
IMPORTANT
SHIP FROZEN SAMPLES MONDAY-WEDNESDAY ONLY. **Only ONE set of samples may be shipped in a single package.** **AM4 Screening Visit Frozen samples should be shipped as soon as possible (M-W only).** **Samples from all other visits should be shipped within two weeks of collection.**

1. Contact UPS® to confirm service is available and schedule package pickup.
2. Notify Indiana University of shipment by completing the electronic version of the Sample Form at this link: https://redcap.link/PPMISampleForm.
    a. Please note that completion of this survey will automatically notify Indiana University of an incoming shipment.
    b. Save a PDF copy of the completed form for your site records.
    c. Print a hard copy of the Sample Form to include in the shipment.
3. Place all frozen 2 mL aliquot vials in the provided cardboard cryobox. Label the outside of the cryobox with the participant ID and visit number.
4. If using an 81-cell cryobox, you must use a large biohazard bag. If using a 25-cell cryobox, you can use a large biohazard bag or a small biohazard bag.
5. *If using large biohazard bag:*
    a. Place the cryobox in the large clear plastic biohazard bag (do NOT remove the absorbent material found in the bag).
    b. Place frozen 3ml EDTA tube, PAXgene™ tube, and urine tube in provided bubble wrap tube sleeves. To avoid broken or cracked tubes, it is advised to package the bubble wrapped tubes with additional padding.
    c. Put the packaged tubes into the same biohazard bag as the 81-cell cryobox.
    d. Seal biohazard bag according to the instructions on the bag.

6. *<u>If using small biohazard bag:</u>*
    a. Place the cryobox into a clear plastic biohazard bag with the absorbent sheet and seal according to the instructions on the bag.



    b. Insert frozen EDTA, PAXgene™, and urine tubes into the provided bubble wrap pouch. To avoid broken or cracked tubes, it is advised to package the bubble wrapped tubes with additional padding.
    c. Place the bubble-wrapped tubes into the second clear plastic biohazard bag with the absorbent sheet and seal according to the instructions on the bag.



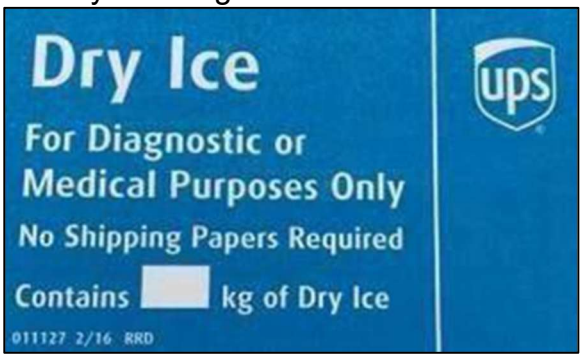
7. Place approximately 2-3 inches of dry ice in the bottom of the Styrofoam-lined shipping carton.
8. Place the biohazard bag(s) containing the cryobox and tubes into the Styrofoam-lined shipping carton, on top of the dry ice. Please ensure that the cryobox is placed so that the cryovials are upright in the shipping container.

9. Fill the remaining space in the shipping carton with dry ice, ensuring ice surrounds the bag and reaches the top of the carton, as shown below:

10. Replace the lid on the Styrofoam carton, place the completed Sample Form (printed completed form from RedCap) on top of the carton, and close and seal the outer cardboard shipping carton with packing tape.

IMPORTANT!
Failure to complete the required fields on the UPS® Dry Ice label may result in UPS® rejecting or returning your package.

11. To Ship:
    a. Log in to the Indiana University UPS® portal at (https://kits.iu.edu/ups). Click on the Shipping drop down menu and choose Shipping and Rating.
    b. Choose your study from the Study Group drop down menu. Click on the magnifying glass icon to search for your site in the address book and click the select button to populate your site's shipping address into the label.
    c. Enter the weight of the package in the Package Weight field (enter the weight of dry ice in Dry Ice Weight field). It is important that the weight of dry ice entered in this field matches the weight on the dry ice label.
    d. Click on the blue Pickup Request button, fill out the pickup information needed, and click Save.
    e. Click the ship button and print the air waybill.
    f. Place the printed waybill in the clear sleeve, peel the back off and stick to the carton, and place the package at the UPS® pickup location as indicated on the Pickup Request fields or at an already established UPS® pickup location at your site.
    g. Complete the UPS Dry Ice Label (blue sticker) with the following information:
        i. Net Weight of dry ice in kg



    h. Optional: Add "KEEP FROZEN" penguin sticker to the outside of the box. Stickers can be ordered via the kit request module.

    i. Do not cover any part of this label with other stickers, including pre-printed address labels.
    j. Apply all provided warning labels to the outside of the package, taking care not to overlap labels.
    k. Hold packaged samples in a -80°C freezer until the time of UPS® pickup.

APPENDIX M: PPMI FROZEN SHIPPING INSTRUCTIONS – CANADIAN SITES

Preparing Frozen Sample Packaging/Shipment to Indiana University

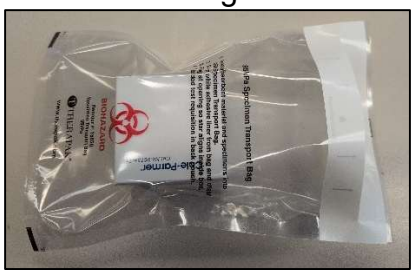
Samples Shipped on Dry Ice:

- Frozen whole blood in 1 x 3 mL plastic EDTA tube
- Frozen whole blood in 1 x PAXgene™ RNA tubes
- Frozen plasma in 2 mL polypropylene tubes (up to 12)
- Frozen serum in 2 mL polypropylene tubes (up to 3)
- Frozen buffy coat in 1 x 2 mL polypropylene tube
- Frozen urine in 15 mL conical tube
- Frozen CSF in 2 mL polypropylene tubes (up to 14)
- Frozen Tissue in 2mL polypropylene tube

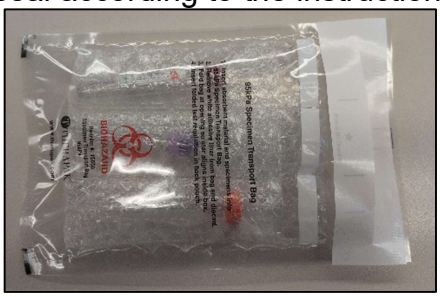
IMPORTANT
SHIP FROZEN SAMPLES MONDAY-WEDNESDAY ONLY. **Only ONE set of samples may be shipped in a single package.** **AM4 Screening Visit Frozen samples should be shipped as soon as possible (M–W only).** **Samples from all other visits should be shipped within two weeks of collection.**

1. Contact FedEx® to confirm service is available and schedule package pickup.
2. Notify Indiana University of shipment by completing the electronic version of the Sample Form at this link: https://redcap.link/PPMISampleForm.
    a. Please note that completion of this survey will automatically notify Indiana University of an incoming shipment.
    b. Save a PDF copy of the completed form for your site records.
    c. Print a hard copy of the Sample Form to include in the shipment.
3. Place all frozen 2 mL aliquot vials in the provided cardboard cryobox. Label the outside of the cryobox with the Kit Number label.
4. If using an 81-cell cryobox, you must use a large biohazard bag. If using a 25-cell cryobox, you can use a large biohazard bag or a small biohazard bag.
5. *If using large biohazard bag:*
    a. Place the cryobox in the large clear plastic biohazard bag (do NOT remove the absorbent material found in the bag).
    b. Place frozen 3ml EDTA tube, PAXgene™ tube, and urine tube in provided bubble wrap tube sleeves. To avoid broken or cracked tubes, it is advised to package the bubble wrapped tubes with additional padding.
    c. Put the packaged tubes into the same biohazard bag as the 81-cell cryobox.
    d. Seal biohazard bag according to the instructions on the bag.

6. *<u>If using small biohazard bag:</u>*
    a. Place the cryobox into a clear plastic biohazard bag with the absorbent sheet and seal according to the instructions on the bag.



    b. Insert frozen EDTA, PAXgene™, and urine tubes into the provided bubble wrap pouch. To avoid broken or cracked tubes, it is advised to package the bubble wrapped tubes with additional padding.
    c. Place the bubble-wrapped tubes into the second clear plastic biohazard bag with the absorbent sheet and seal according to the instructions on the bag.

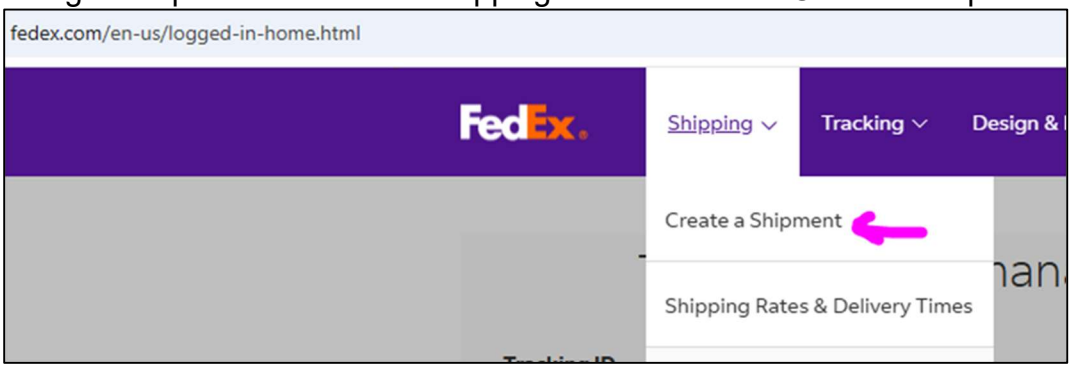


7. Place approximately 2-3 inches of dry ice in the bottom of the Styrofoam-lined shipping carton.
8. Place the biohazard bags containing the cryobox and tubes into the Styrofoam-lined shipping carton, on top of the dry ice. Please ensure that the cryobox is placed so that the cryovials are upright in the shipping container.

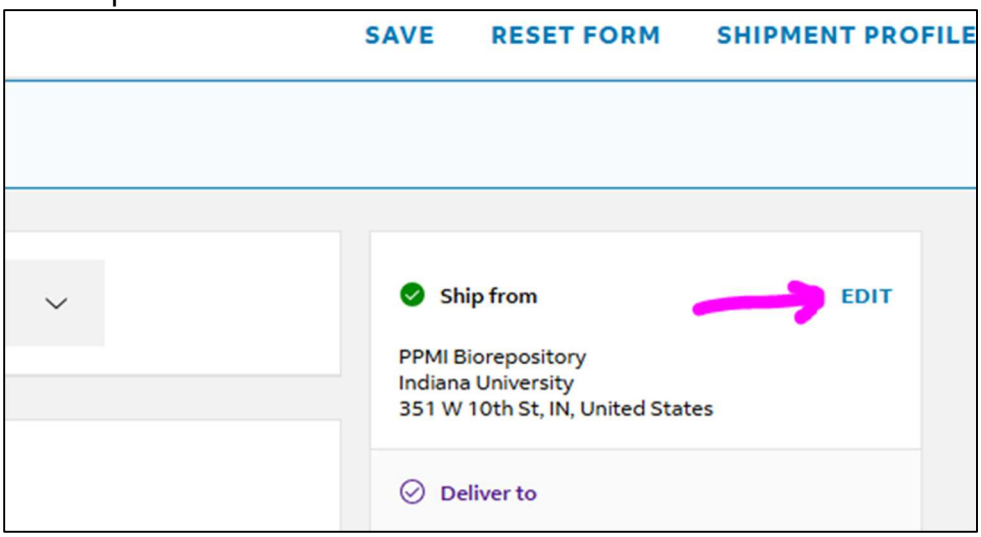
9. Fill the remaining space in the shipping carton with dry ice, ensuring ice surrounds the bag and reaches the top of the carton, as shown below:

10. Replace the lid on the Styrofoam carton, place the completed Sample Sample Form (printed completed form from RedCap) on top of the carton, and close and seal the outer cardboard shipping carton with packing tape.

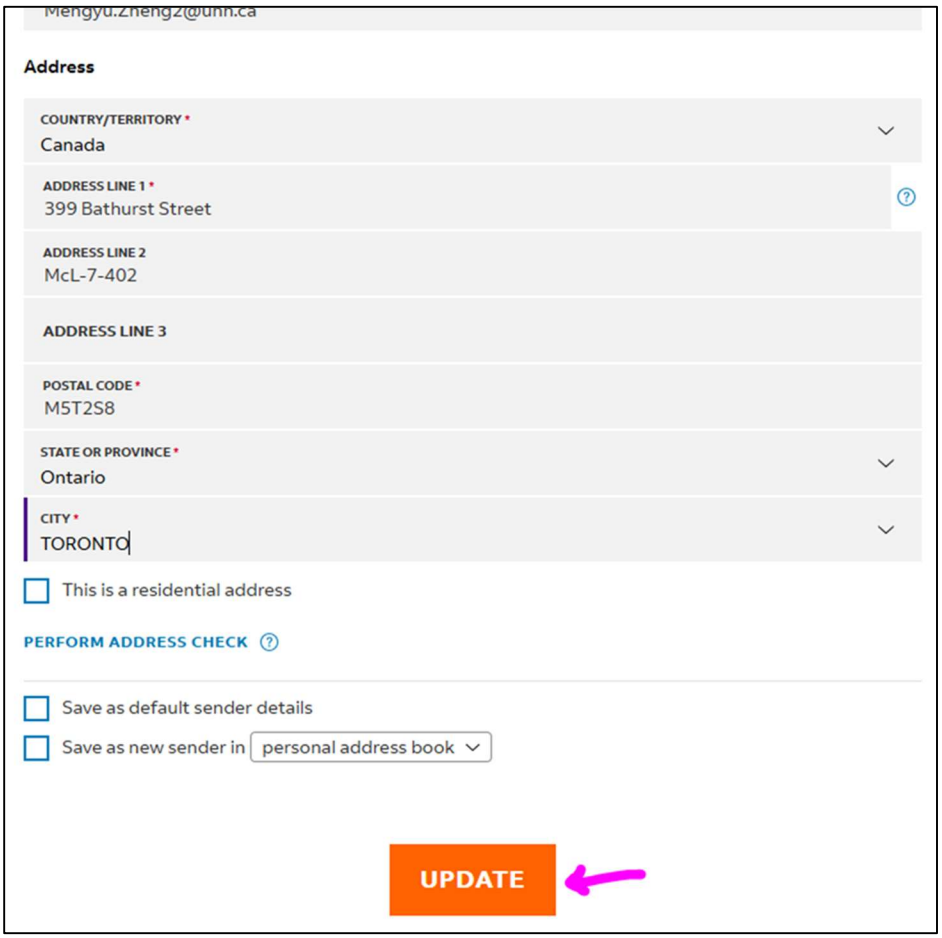
11. To ship, head to the FedEx® website(https://www.fedex.com/en-us/home.html).
    a. Along the top tool bar find the Shipping Tab and select “Create a Shipment”.



    b. This will open the “Deliver To” page, here you can go to the right-side corner and locate the “Ship From” section.

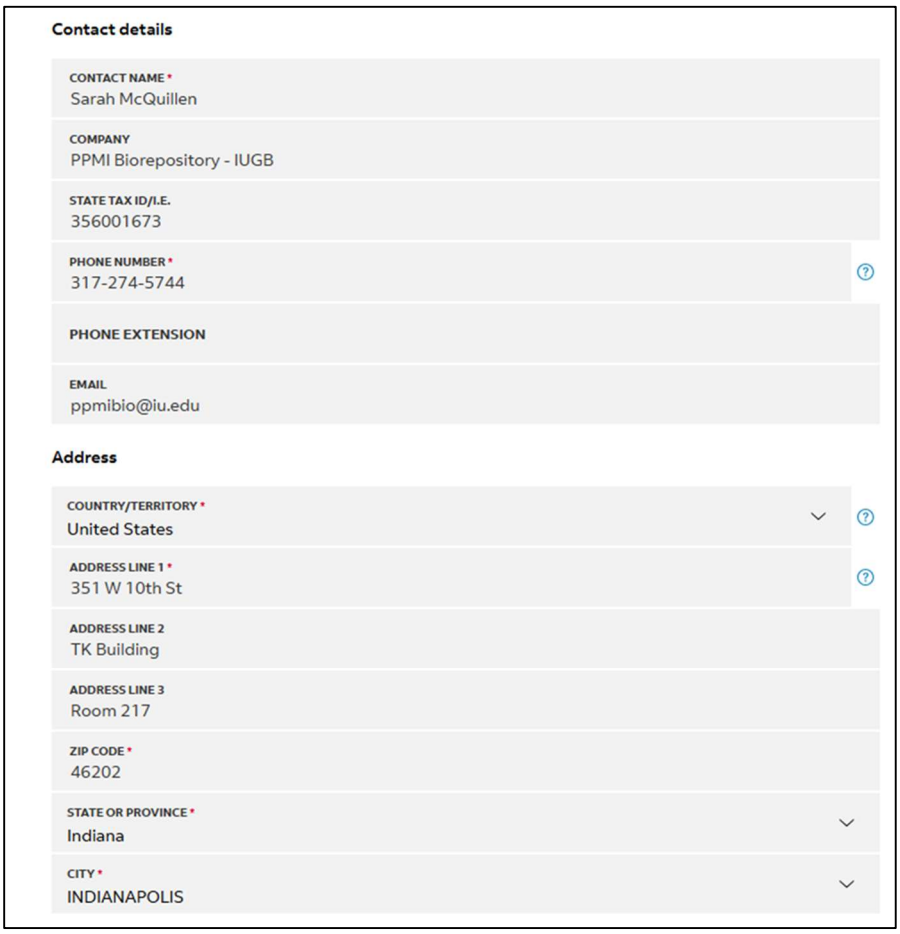


    c. Click “EDIT”. Fill in your information for pickup (the example on the following images uses Toronto’s site information).

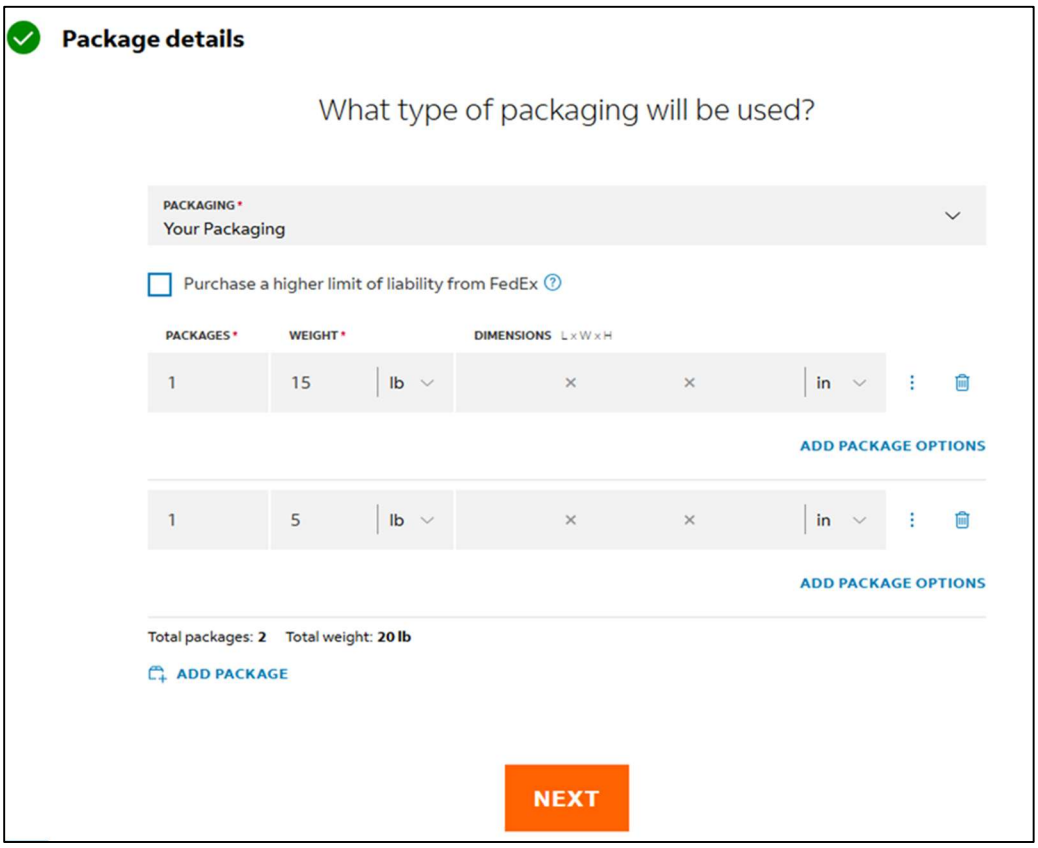


d. Click UPDATE when finished.
e. This will take you back to the “Deliver To” section, where you will fill out information for the Biorepository here in Indianapolis.

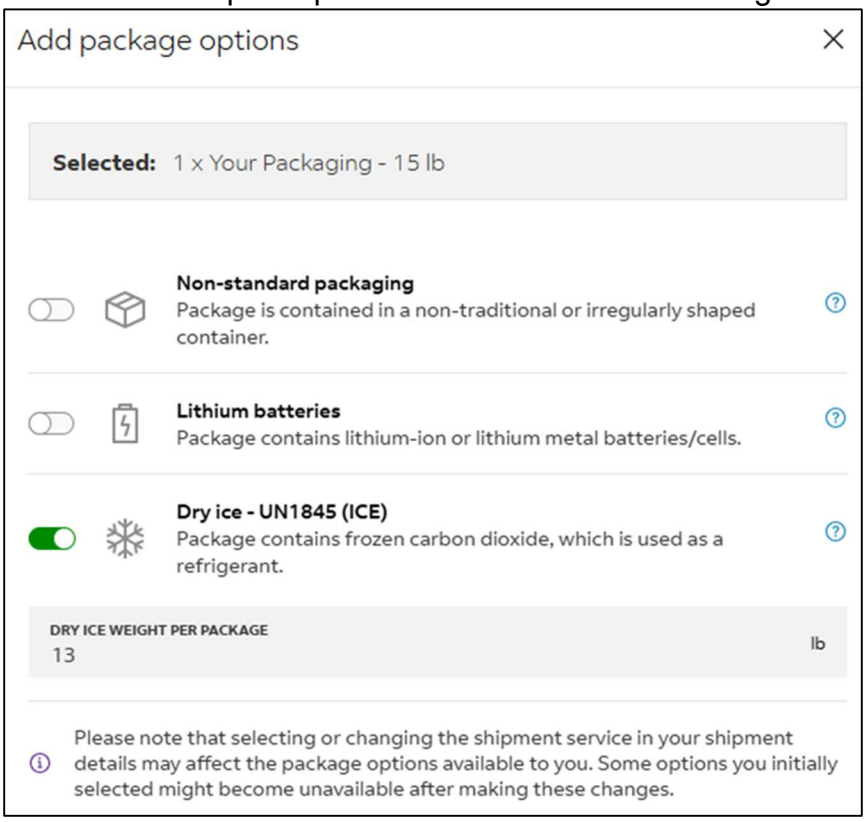
Contact Name: Sarah McQuillen
Company: PPMI Biorepository – IUGB
Phone Number: 317-274-5744
Email: ppmibio@iu.edu
Country/Territory: United States
Address Line 1: 351 W 10th St
Address Line 2: TK Building
Address Line 3: Room 217
Zip Code: 46202
State or Provice: Indiana
City: Indianapolis



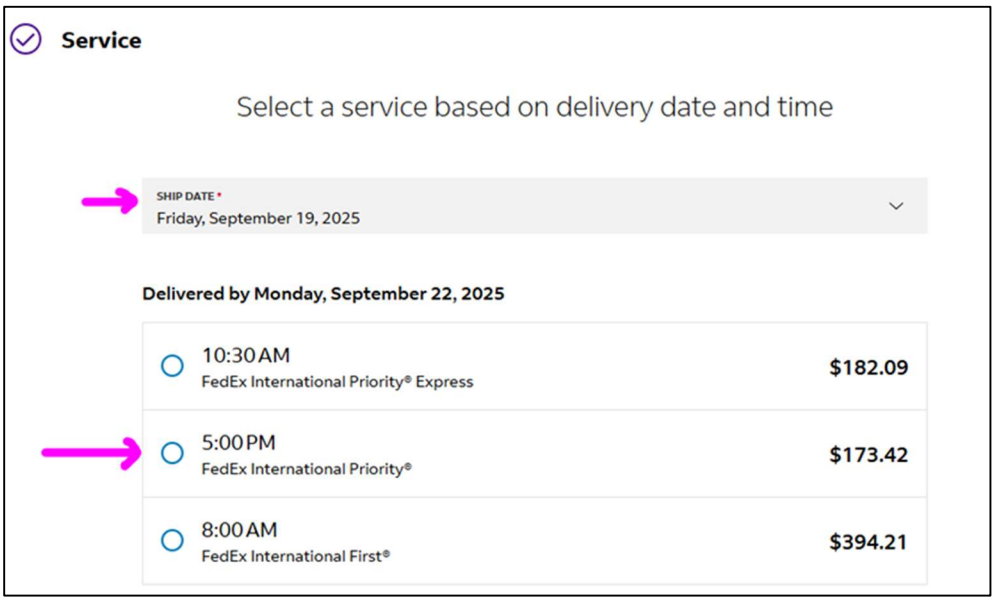
f. Click “Next” at the bottom of the page when finished.
g. The next section is for Package details. Select “Your Packaging” and then input how many shipment boxes you have and their weights.
    i. For Ambient Skin Shipments put 5lbs for weight.
    ii. For Frozen Shipments put 15lbs for weight.
h. You can also add a separate package here too if you are shipping more than one type of sample shipment.
    i. Ex. 1 Frozen Shipment and 1 Ambient Shipment.



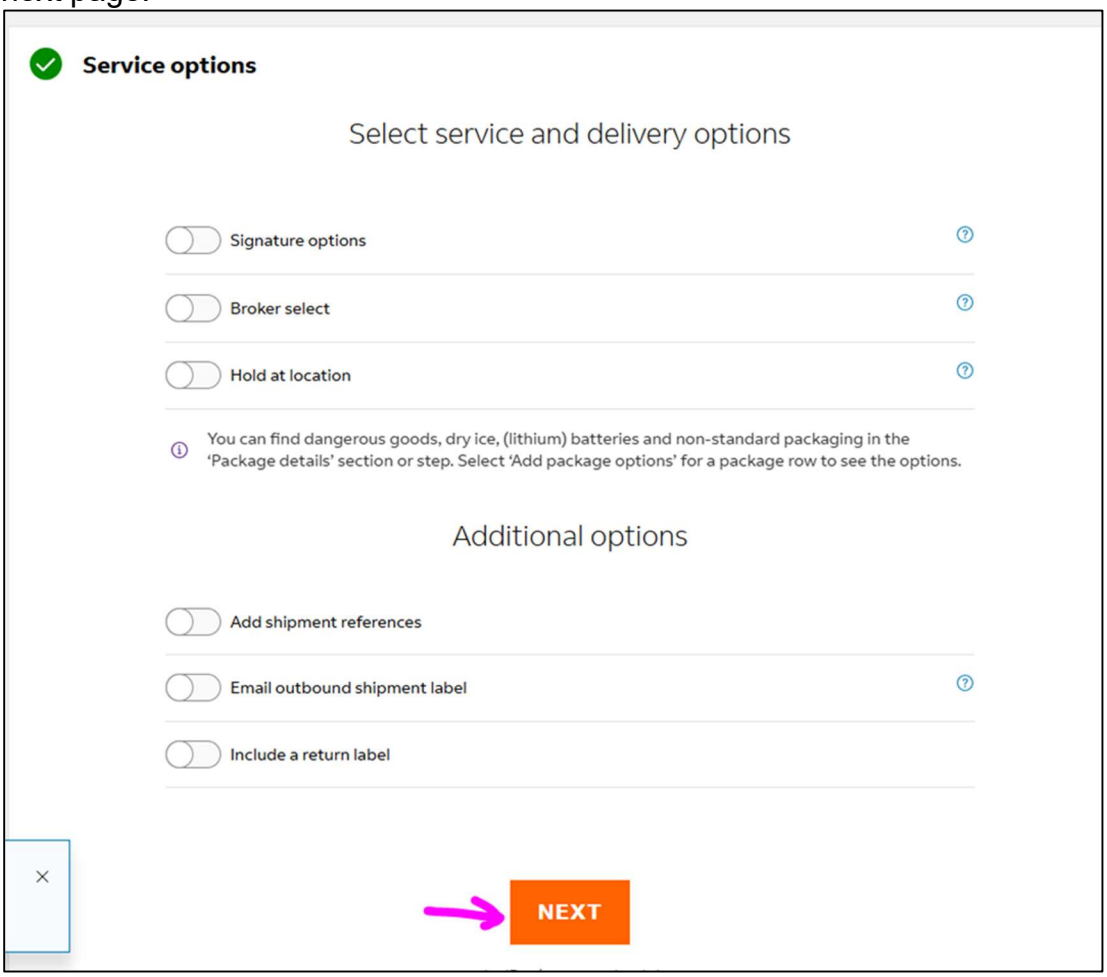
i. To add Dry Ice, click on “Add Package Options” and add dry ice there. The weight should be a couple of pounds lower than the total weight.



j. When finished, the next page will be for selecting service type.
    i. Select “FedEx International Priority” this is the equivalent of next day shipping.

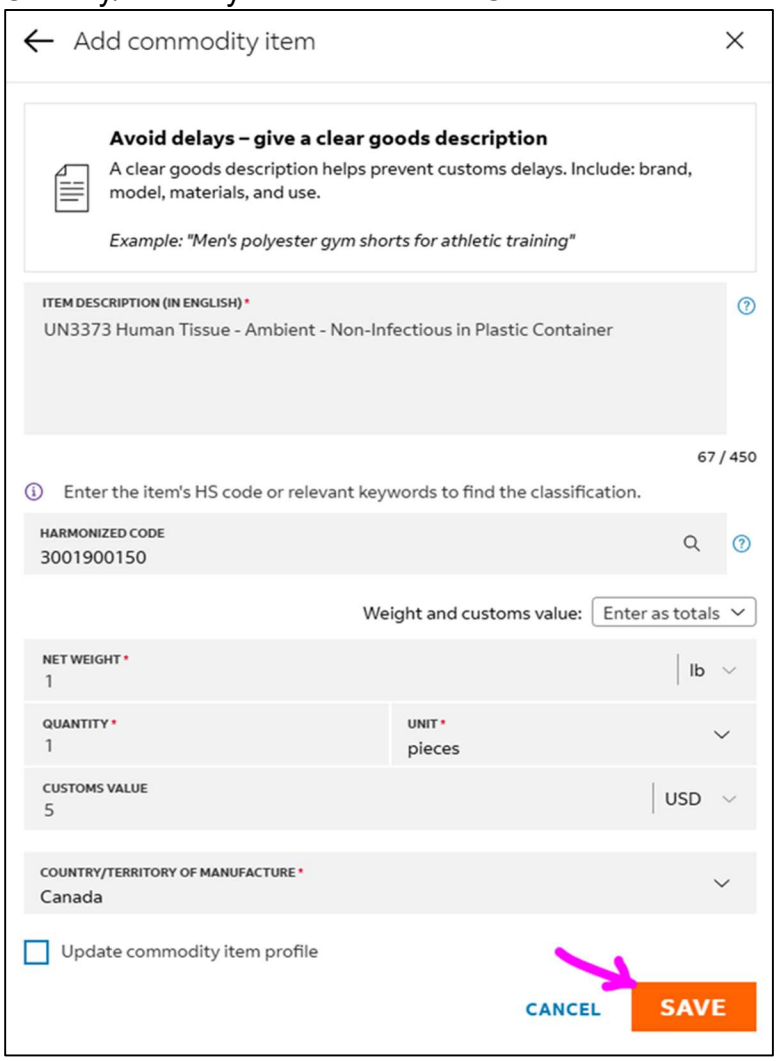


k. The next page will be Service Options. Don't select anything here and continue to the next page.

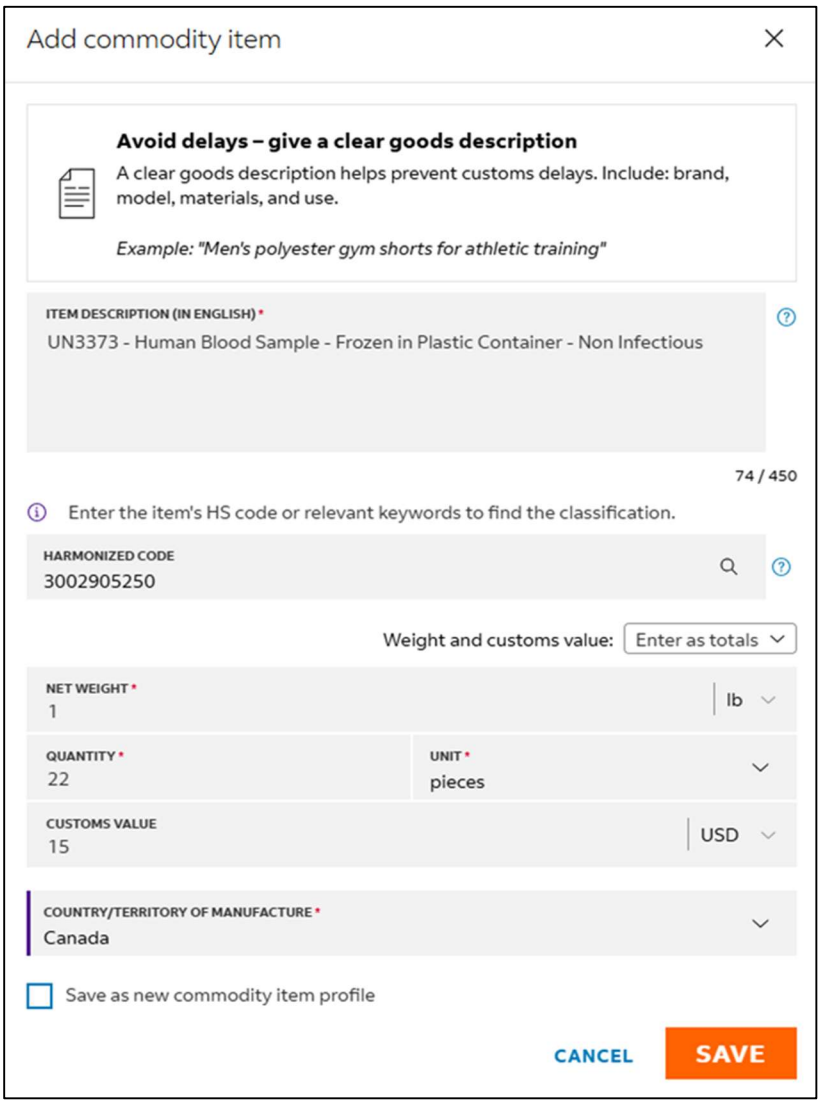


l. This next page will be your Package Contents. Here is where you will input what you are shipping and make the commercial invoice.
    i. First, you'll want to add your new item. This will either be Ambient Tissue or a Shipment of Blood Samples.

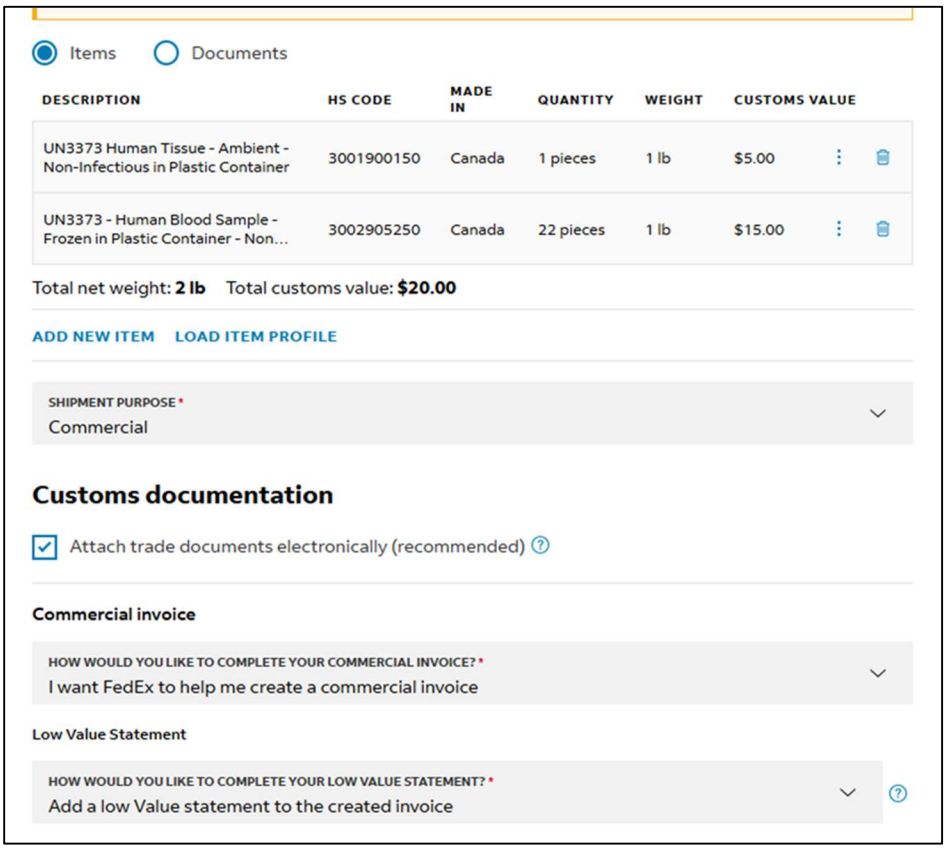
ii. For Ambient Tisue
    1. Item Description: UN3373 Human Tissue – Ambient – Non-Infectious in Plastic Container
    2. Harmonized Code: 3001900150
    3. Net Weight: 1 lb
    4. Quantity: 1
    5. Unit: Pieces
    6. Customs Value: 5 USD
    7. Country/Territory of Manufacture: Canada



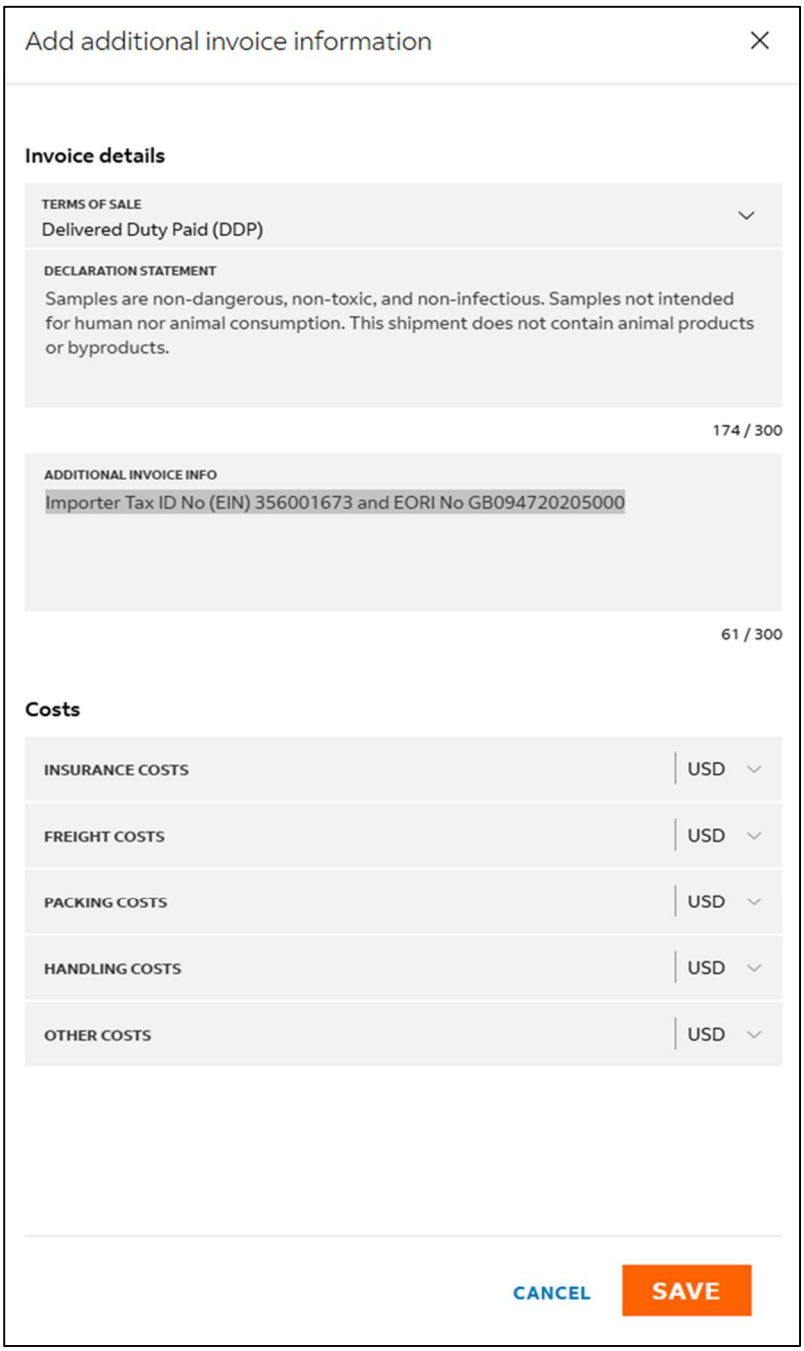
iii. For Frozen Blood Samples
    1. Item Description: UN3373 - Human Blood Sample - Frozen in Plastic Container - Non-Infectious
    2. Harmonized Code: 3002905250
    3. Net Weight: 1
    4. Quantity: # of Tubes
    5. Unit: Pieces
    6. Customs Value: 15 USD
    7. Country/Territory of Manufacture: Canada



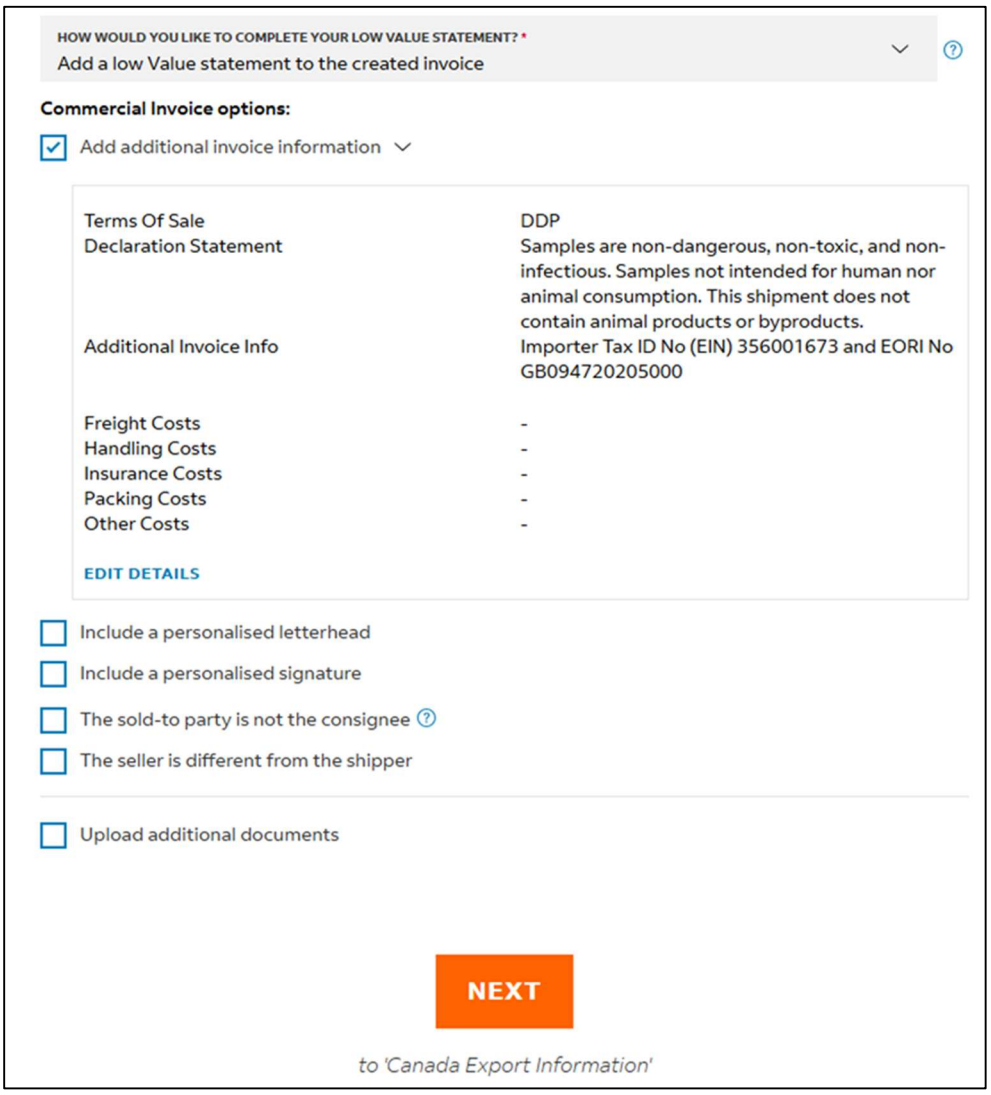
m. Click “SAVE” and make sure that “Attach trade documents electronically” is checked.
n. Select “I want FedEx to help me create a commercial invoice”.
    i. If you have any other documents that you would like to add on your own, you can add them here on this page too.
o. Leave the Low Value Statement selection as “Add a low value statement to the created invoice”.



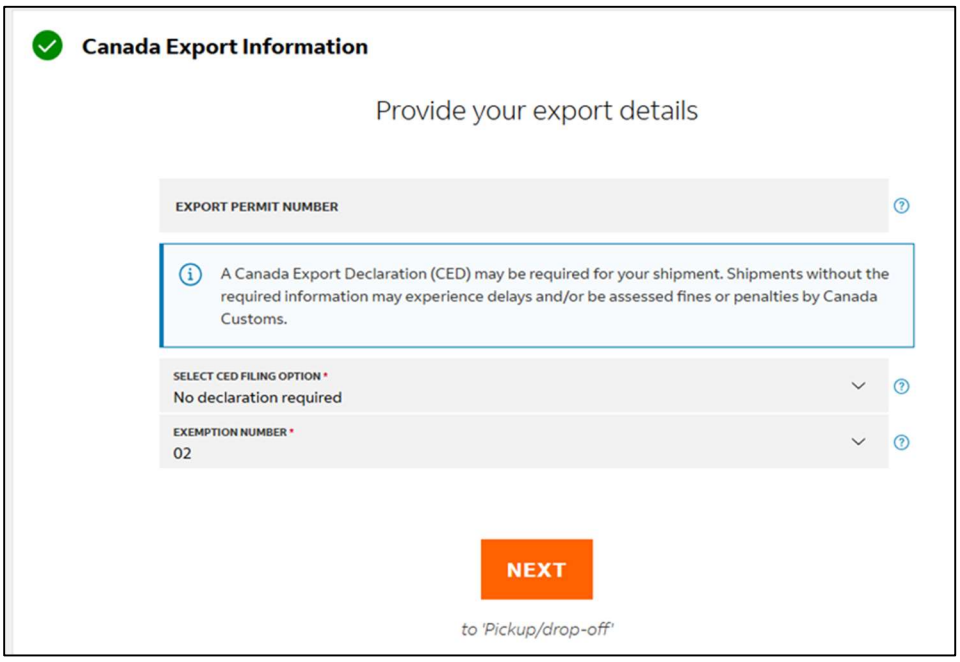
p. Farther below will be the Commercial Invoice Options, select Add Additional Invoice Information.
    i. Terms of Sale: Delivered Duty Paid (DDP)
    ii. Declaration Statement: Samples are non-dangerous, non-toxic, and non-infectious. Samples are not intended for human nor animal consumption. This shipment does not contain animal products or byproducts.
    iii. Additional Invoice Info: Importer Tax ID No (EIN) 356001673 and EORI No GB094720205000



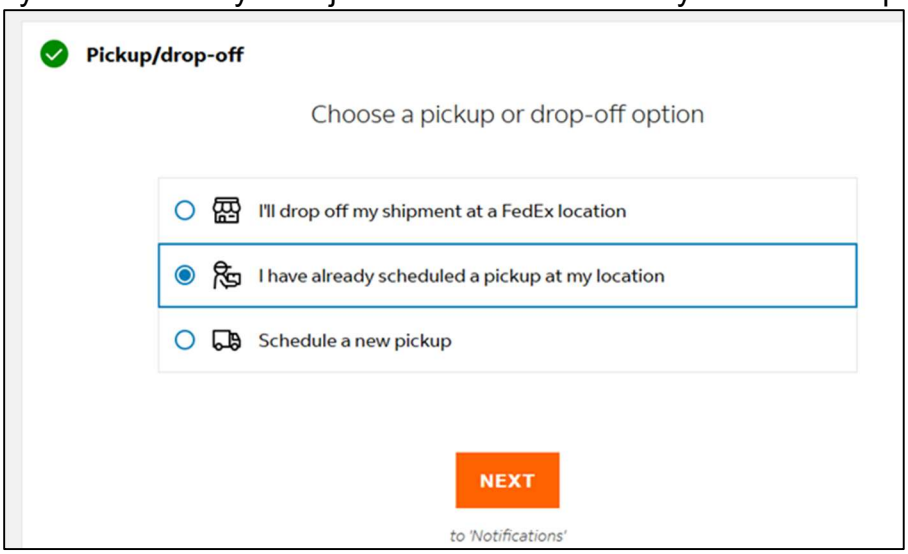
iv. This is also where you can upload any additional documents, as mentioned earlier. Select “Upload Additional Documents” if you have any to add.



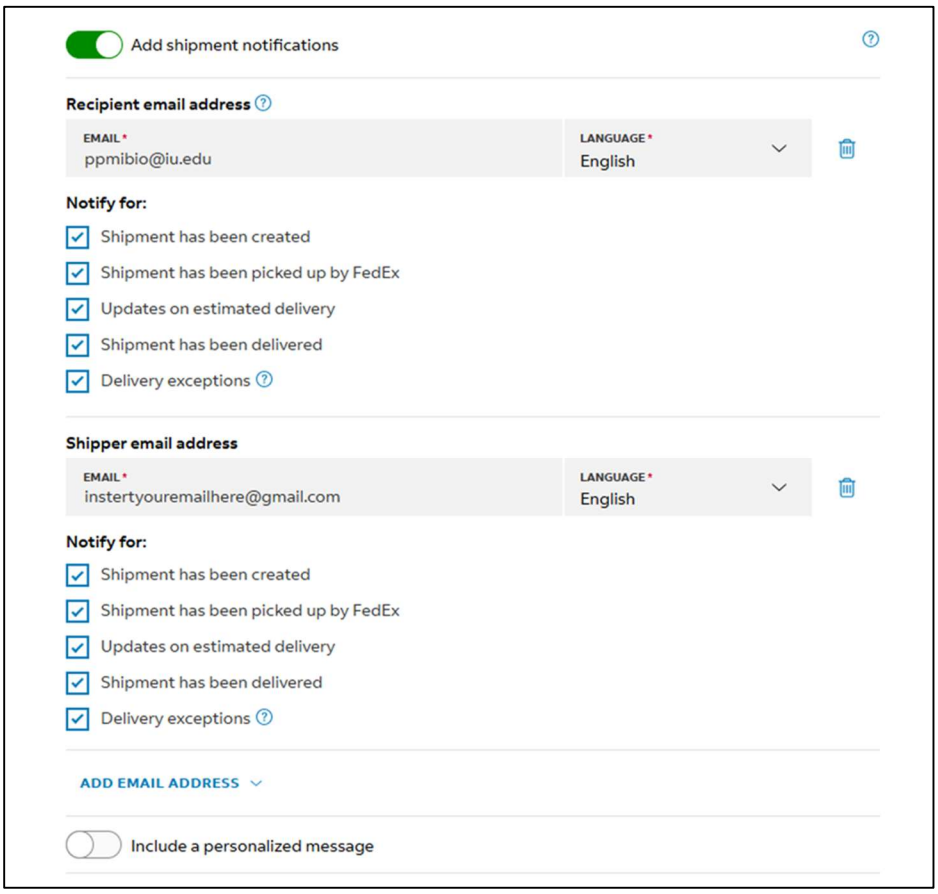
q. Next is the Canada Export Information.
    i. Leave the “Export Permit Number” blank.
    ii. For CED, select “No Declaration Required” and Exemption Number “02”.



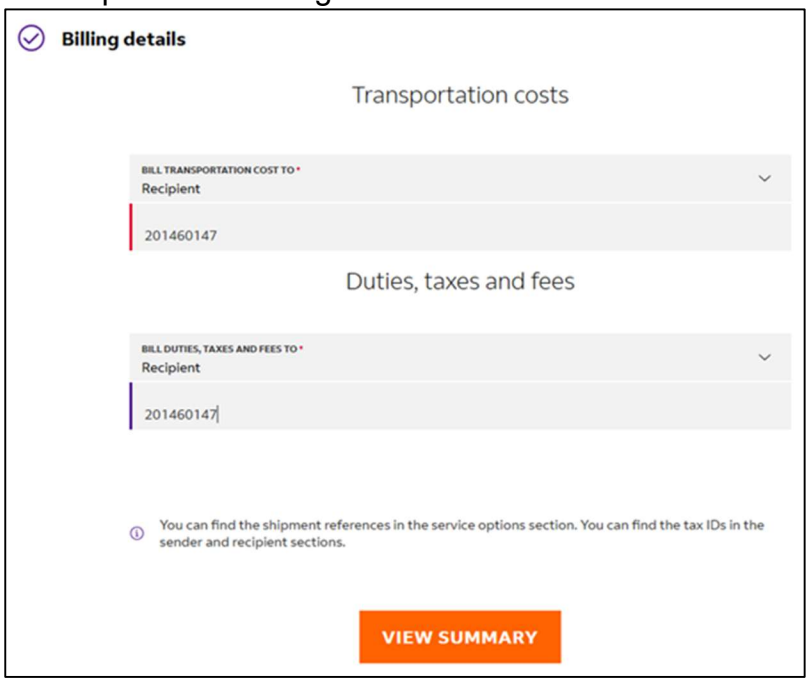
r. Now on the next page you will schedule your Pickup, if you do not have a designated spot in your building with a standing pickup. If you do have a spot that gets picked up by FedEx already then just select “I have already scheduled a pickup at my location”.



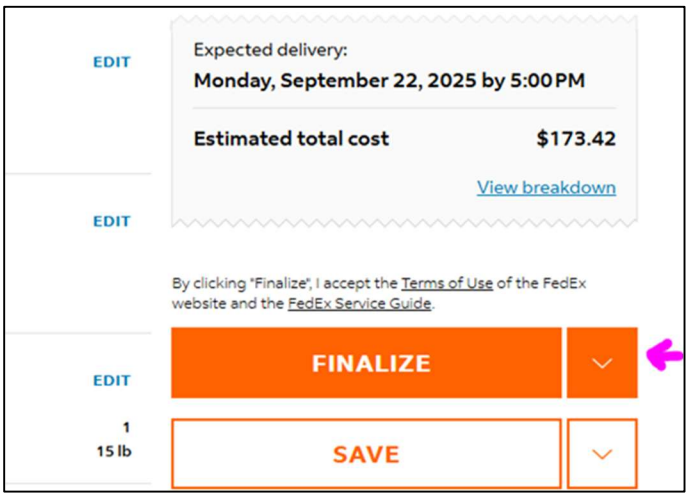
s. On the next page you’ll want to turn on Notifications for your shipment. Add your email address and the ppmibio@iu.edu email to receive updates about the package throughout the trip.



t. For the billing details on the next page, you'll need to update both the billing options to "Recipient" and put the following FedEx Account Number: **201460147**.



u. Next click "Review Summary". This will show you all the details of your shipment, double check to make sure all looks good then hit "Finalize".



v. It will take you to a page where you will see the FedEx tracking number and give you the option to print/download your FedEx Labels and Commercial Invoices. **I highly recommend downloading the labels and commercial invoices, if you close the page too early sometimes you can't get back to it.**

w. Print the box labels and stick them on the boxes. **For the Commercial Invoice, print at least 3 copies per box and put them on top too.** You need at least 3 copies because each customs department will take a copy.

12. Peel the backing from the clear sleeve and attach to the package. Place the printed Labels and Commercial Invoices in the clear sleeve and place the package at your dedicated FedEx® pickup location or drop the package off at a FedEx® store or drop box.
13. Complete the Class 9 UN 1845 Dry Ice label (black and white diamond) with the following information:
    a. Coordinator name and return address
    b. Net weight of dry ice in kg
    c. Consignee name and address:

PPMI Biorepository
IU School of Medicine
351 W 10th Street, TK 217
Indianapolis, IN 46202-4118 USA

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

14. Apply all provided warning labels to the outside of the package, taking care not to overlap labels.
    a. Optional: Add "KEEP FROZEN" penguin sticker to the outside of the box. Stickers can be ordered via the kit request module.

15. Hold packaged samples in a -80°C freezer until the time of FedEx® pickup.
    a. Use FedEx® tracking to ensure the delivery occurs as scheduled and is received by the PPMI biorepository.

APPENDIX N: PPMI FROZEN SHIPPING INSTRUCTIONS – EUROPEAN & AFRICAN SITES

Preparing Frozen Sample Packaging/Shipment to BioRep

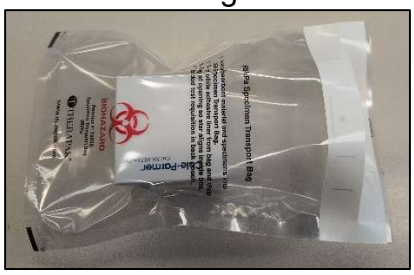
Samples Shipped on Dry Ice:

- Frozen whole blood in 1 x 3 mL plastic EDTA tube
- Frozen whole blood in 1 x PAXgene™ RNA tube
- Frozen plasma in 2 mL polypropylene tubes (up to 12)
- Frozen serum in 2 mL polypropylene tubes (up to 3)
- Frozen buffy coat in 1 x 2 mL polypropylene tube
- Frozen urine in 15 mL conical tube
- Frozen CSF in 2 mL polypropylene tubes (up to 14)
- Frozen tissue in a 1 x 2mL polypropylene tube

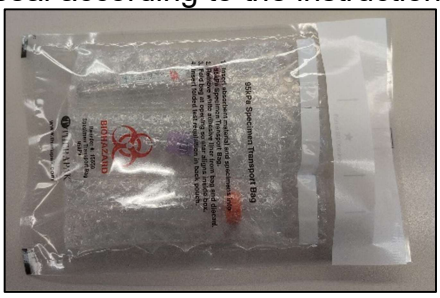
IMPORTANT
ALL SHIPMENTS MUST BE SCHEDULED WITH THE BioRep TEAM **Only ONE set of samples may be shipped in a single package.**

1. Contact BioRep to coordinate shipping via DHL, Marken, or other courier.
2. Complete the electronic version of the Sample Form at this link: https://redcap.link/PPMISampleForm.
    a. Save a PDF copy of the completed form for your site records.
    b. Print a hard copy of the Sample Form to include in the shipment.
3. Email BioRep (ppmi@biorep.it) with the following information prior to sample pick-up:
        - Sample Record Summary (Cumulative) (Appendix P) in Excel format
        - PDF copy of the Sample Form completed in step 2 above
    a. A bulk shipment to BioRep should only contain 6-7 Participant's worth of Visits.
    b. When prepping samples for shipment, separate any AM4 Screening Visit so that they can be packaged together and separately form the other Visits.
4. Place all frozen 2 mL aliquot vials in the provided cardboard cryobox. Label the outside of the cryobox with the Kit Number label.
5. If using an 81-cell cryobox, you must use a large biohazard bag. If using a 25-cell cryobox, you can use a large biohazard bag or a small biohazard bag.
6. *If using large biohazard bag:*
    a. Place the cryobox in the large clear plastic biohazard bag (do NOT remove the absorbent material found in the bag).
    b. Place frozen 3ml EDTA tube, PAXgene™ tube, and urine tube in provided bubble wrap tube sleeves. To avoid broken or cracked tubes, it is advised to package the bubble wrapped tubes with additional padding.
    c. Put the packaged tubes into the same biohazard bag as the 81-cell cryobox.
    d. Seal biohazard bag according to the instructions on the bag.

7. *<u>If using small biohazard bag:</u>*
    a. Place the cryobox into a clear plastic biohazard bag with the absorbent sheet and seal according to the instructions on the bag.



    b. Insert frozen EDTA, PAXgene™, and urine tubes into the provided bubble wrap pouch. To avoid broken or cracked tubes, it is advised to package the bubble wrapped tubes with additional padding.
    c. Place the bubble-wrapped tubes into the second clear plastic biohazard bag with the absorbent sheet and seal according to the instructions on the bag.



8. Place approximately 2-3 inches of dry ice in the bottom of the Styrofoam-lined shipping carton.
9. Place the biohazard bags containing the cryobox and tubes into the Styrofoam-lined shipping carton, on top of the dry ice. Please ensure that the cryobox is placed so that the cryovials are upright in the shipping container.

10. Fill the remaining space in the shipping carton with dry ice, ensuring ice surrounds the bag and reaches the top of the carton, as shown below:

11. Replace the lid on the Styrofoam carton, place the completed Sample Record Summary (Appendix P) and Sample Form (Appendix J- completed in RedCap) on top of the carton, and close and seal the outer cardboard shipping carton with packing tape.
12. Complete the Class 9 UN1845 Dry Ice label (black and white diamond) with the following information:
    a. Shipper name and Return Address
    b. Net weight of dry ice in kg
    c. Consignee name and address: BioRep Srl, c/o DIBIT2 Palazzina San Michele, Via Olgettina 60, 20132, Milano – Italy

13. Do not cover any part of this label with other stickers, including pre-printed address labels.
14. Apply all provided warning labels and the completed air waybill to the outside of the package, taking care not to overlap labels.
15. Hold packaged samples in a -80°C freezer until the time of shipment pickup.

APPENDIX O: INTERNATIONAL COMMERCIAL INVOICE

COMPANY NAME

COMPANY ADDRESS

COMMERCIAL INVOICE

INTERNATIONAL AIR WAYBILL NO.

(NOTE: All shipments must be accompanied by a Federal Express International Air Waybill.)

DATE OF EXPORTATION

EXPORT REFERENCES (1.e., order no., invoice no.)

SHIPPER/EXPORTER (complete name and address)

CONSIGNEE (complete name and address)

PPMI
IU School of Medicine
351W 10th Street, TK217
Indianapolis, IN 46202-4118

COUNTRY OF EXPORT

IMPORTER - IF OTHER THAN CONSIGNEE (complete name and address)

COUNTRY OF MANUFACTURE

COUNTRY OF ULTIMATE DESTINATION

MARKS/NOS.	NO. OF PKGS.	TYPE OF PACKAGING	FULL DESCRIPTION OF GOODS	QTY.	UNIT OF MEA-SURE	WEIGHT	UNIT VALUE	TOTAL VALUE
	TOTAL NO.OF PKGS.					TOTAL WEIGHT		TOTAL INVOICE VALUE

SEE REVERSE SIDE FOR HELP WITH THE ABOVE SECTION

Check one
☐ F.O.B.
☐ C&F
☐ C.I.F.

FOR U.S. EXPORT ONLY: THESE COMMODITIES, TECHNOLOGY, OR SOFTWARE WERE EXPORTED FROM THE UNITED STATES IN ACCORDANCE WITH THE EXPORT ADMINISTRATION REGULATIONS.DIVERSION CONTRARY TO UNITED STATES LAW IS PROHIBITED.

I DECLARE ALL THE INFORMATION CONTAINED IN THIS INVOICE TO BE TRUE AND CORRECT.

SIGNATURE OF SHIPPER/EXPORTER (Type name and title and sign.) DATE

M-1054 REV 2/80 LOGOS116547 WCS

APPENDIX P: SAMPLE RECORD SUMMARY AND SHIPMENT NOTIFICATION CUMULATIVE FILE

SAMPLE RECORD SUMMARY AND SHIPMENT NOTIFICATION_CUMULATIVE

STUDY NAME: PPMI

Site:	Site Investigator:

Coordinator:	Telephone:	Email:

Instructions: **Ship frozen sample Monday-Wednesday ONLY.**
This form must be completed for all biorepository sample
shipments. Notify the recipient repository (e-mail preferred) prior to shipment using the information below.
Place a copy of the completed form in the shipment box and retain a copy for site record. The site will be contacted if any sample/form issues are noted upon receipt.

Completed by Submitter/Site List Subject ID that corresponds to pre-printed labels. List only one Specimen Type per row.					**Completed by Biorepository** **Notation of problems**							
					Specimen Type							
Subject ID #	**Visit**	**Gender**	**Date of Draw**	**Kit Number**	**RNA**	**PLASMA**	**SERUM**	**W. B.**	**BUFFY COAT**	**CSF**	**TISSUE**	**URINE**
Date Shipped:					0	0	0	0	0	0	0	0
Tracking #:												

TOT.

IMPORTANT!
BEFORE SHPPING, E-MAIL (PREFERRED) OR FAX A COPY OF THE COMPLETED FORM TO THE RECIPIENT BIOREPOSITORY:

BioRep
ppmi@biorep.it
Fax: +39 0258018471
Phone: +39 0258029768

APPENDIX Q: SAMPLE SUBMISSION NONCONFORMANCE REPORT

Repository Name:_____

Participant ID:_____ Site #:_____

Received Date:_____ Visit Type:_____

Submission Type: Ambient ☐ Frozen ☐

Shipping Issues Noted:

☐ Shipment notification not received.
☐ Kit Number not included on Sample Form.
☐ Submission form not included in package, incomplete, or inaccurate.
☐ Samples shipped for weekend or holiday delivery.
☐ Samples packaged improperly.
☐ Samples received damaged.
☐ Frozen submission received thawed.
☐ Samples received outside of shipment window.
☐ Other: _____

Sample Collection Issues Noted:

☐ Submitted in non-standard tube(s).
☐ Unlabeled or mislabeled tube(s).
☐ Low volume received.
☐ Sample discolored.
☐ Frozen improperly.
☐ Other: _____

Details/Comments: