Parkinson's Progression Markers Initiative (PPMI)

Parkinson's Progression Markers Initiative

Biospecimen Collection, Processing, and Shipment Manual of Procedures

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**
1.0	IU	• Initial Version	June 10, 2020	Retired
2.0	IU	• Section 2.3 – addition of items related to skin biopsyto list of site-provided supplies • Section 3.0 – addition of note accompanying the SOA to indicate when skin biopsy should be performed • Section 5.1 – addition of listing skin biopsy shipping appendix • Section 5.2 – addition of note to ship skin biopsy samples M-Th only • Appendix B – addition to include shipment information for skin biopsysamples • Appendix E – addition to include supply listing for skin biopsy kits and skin biopsy supplemental kits • Appendix G – addition to include skin biopsy as a sample type • Addition of new appendices(R, S, and T) for skin biopsy collection, processing, and shipping	August 12, 2020	Retired
3.0	IU	• Addition of Section 2.7 regarding assessments • Section 3.0 – clarification of note accompanying the SOA • Appendix L – correction regarding number of tubesto be collected	August 18, 2020	Retired
4.0	IU	• Section 2.3 – addition of saline for skin biopsy and clarification regarding lidocaine • Section 2.5 – clarification regarding ordering BL kits	April 7, 2021	Retired

		• Section 3.0 – clarificationon skin note regarding ancillary punch protocol • Appendix A – addition of site 404 to drop-down menu • Appendix B – removal of skin biopsy information (separate appendix created) • Appendix D – updated couriers used • Appendix E – addition of ancillary skin biopsy kit, updates to skin and LP supplies, correction of errorin kit list titles • Appendix F – addition of assessments • Appendix G – addition of ancillary skin protocol • Appendix J – addition of site 404 to drop-down menu • Appendix N – had been cutoff in previous version • Appendix O – clarified fasting instructions • Appendix R – update of kit setup step 2, removal of note that containers will be pre-labeled with a formalin label, correction in processing step 7 regardingshipping appendix • Appendix T – update for option of ancillary skin protocol • Addition of new appendices(U, V, and W) for skin biopsy collection and processing (ancillary protocol) and skin biopsy shipping form		
5.0	IU	• Section 2.3 – further details regarding saline for skin biopsy, removed formalin and fixative disposal bin from list of site-supplied items • Section 3.0 – further clarification on skin note regarding ancillary punch protocol • Appendix E – updates to skin supplies to reflect that pre-filled formalin	May 3, 2021	Retired

		containers will be used going forward • Appendix G – clarificationon fasting prior to sample collections • Appendix Q – clarificationon fasting prior to sample collections • Appendix R – correction to reflect that post-biopsy call should occur 2-3 days after collection, updates to reflect that pre-filled formalin containers will be used going forward, note about formalin shipping • Appendix S - updates to reflect that pre-filled formalin containers will beused going forward • Appendix T - note about formalin shipping • Appendix U - correction to reflect that post-biopsy call should occur 2-3 days after collection, updates to reflect that pre-filled formalin containers will be used going forward, note about formalin shipping • Appendix V - updates to reflect that pre-filled formalin containers will be used going forward		
6.0	IU	• Addition of appendices X-Z for sample shipments from Canadian sites • Section 1.1 – IU staff update • Section 1.2 – addition of Canadian holidays • Appendix A – correction of formatting display issue • Appendix C – modifications to UPS shipping portal instructions • Appendix E – addition of FedEx® and UPS® supplies to kit componentand supply lists • Appendix H – included in this version; had been omitted from previous version	August 18, 2021	Retired

7.0	IU	• Removals: o Removal of the word "ancillary" and replaced with double punch o Removal of PPMI 2.0 o Removal of Covance and replacement with LabCorp DrugDevelopment • Appendix I – Removal of "optional" for CSF for local lab • Additions: o Table of contents –Expanded o Section 1.0 – Updated to Abbreviations Table • Rearrangements: o Section 2.0 – Biorepository Information o Section 3.0 – Specimen Collection Kits, Shipping Kits, and Supplies o Section 4.0 – Sample Collection Schedule o Section 5.0 – Sample collection o Section 6.0 – Sample Processing o Section 7.0 - Packaging and Shipping Instructions o Section 8.0 - Sample Quality Checks and Feedback to Sites o Section 9.0 – Data Queries and Reconciliation • Corrections/Changes: o Appendix C – Correction of https://kits.iu.edu.UPS to https://kits.iu.edu/ups o Appendix T – Correction of https://kits.iu.edu.UPS to https://kits.iu.edu/ups o Appendix Z – Update from freeze cold packs to refrigerate cold packs at 4°C o Throughout document – Addition of hyperlinks (references to appendices and sections within the document) o Section 2.1.1, Appendix B, Appendix W – Updated IU fax number and phone number	October 20, 2021	Active

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1.0 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 Progression Markers Initiative
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RNA	Ribonucleic Acid
RPM	Revolutions Per Minute
US DOT	United States Department of Transfer

2.0 BIOREPOSITORY INFORMATION

2.1 Biorepository Contacts

2.1.1 Indiana University Study Support

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

Sample Shipment Mailing Address

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

Contacts

General PPMI Contact Information
Phone: 1-317-278-1148
International Phone: (00+1) 317-274-5744
Fax: 317-321-2003
Email: ppmibio@iu.edu

Tatiana Foroud, PhD
Core Leader
Phone: 317-274-2218

Jan Hamer, BS, PMP, CCRP
Project Manager
Email: jehamer@iu.edu

Karla Gonzalez, BS, CCRP
Study Coordinator
Phone: 317-278-1148
Email: gonzalka@iu.edu

Caitlin Schulz, BA
Study Specialist
Phone: 317-278-1166
Email: caschu@iu.edu

2.1.2 BioRep Study Support

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

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

Sample Shipment Mailing Address

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

Contacts

Paola Casalin
Project Manager
Phone: +39 02 58029768
After Hours: +39 348 0716024
Fax: +39 02 58018471
Email: ppmi@biorep.it

Giulia Malferrari
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

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 repositories.

Frozen samples must be shipped Monday – Wednesday only.

2.2.1 Holiday Observations – United States

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

Please note that between December 24th and January 2nd Indiana Universitywill be open for essential operations ONLY. Normal operations will resume onJanuary 2nd. **If possible, biological specimens for submission to IndianaUniversity should NOT be shipped between December 24th and January2nd.** If samples are collected during this period and cannot be shipped, please store at -80ºC and ship on dry ice to Indiana University AFTER January 1.

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 Observations – Tel Aviv

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

3.0 Specimen Collection Kits, Shipping Kits, and Supplies

3.1 LabCorp Drug Development Clinical Lab Collection Kits

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.

3.2 LabCorp Drug Development Resupply

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.

3.3 Research Biospecimen Collection Kits and Supplies

Research Specimen Collection Kits will be provided by Indiana University (IU). IU provides most materials needed for biospecimen collection. Materials and equipment not provided by Indiana University are listed in the tables below.

Research Kits will include collection tubes, specimen storage containers, and tube labels with pre-printed study information. IU also provides shipping supplies necessary for sending samples back to the PPMI biorepository.

Table 3.3.1 Materials Provided by Site

Dry Ice	Crushed Ice
Alcohol Prep Pads	Gauze Pads
Bandages and Steri-Strips	Butterfly Needles
Tourniquets	Tube Racks (2 mL to 10 mL)
Gloves	Sharps Bin and Lid
Pipettes and Pipette Tips	Lidocaine for LP (Non-US Sites Only)
Lidocaine for skin biopsy (if performing)	Normal, non-sterile saline (if performing skin biopsy)

BioRep also provides shipping materials for sites shipping to their facility (see contact informationin Section 2.1.2).

Table 3.3.2 Equipment Provided by Site

4°C Refrigerated Centrifuge	Room Temperature Centrifuge
-80°C Freezer	4°C Refrigerator

Important Note:

To ensure consistent, high quality sample, sites must have access to this equipment.

3.4 Research Biospecimen Collection Kit Contents

Each Research Specimen Collection Kit provides the necessary supplies to collect samples from one subject at one study visit**. Do not replace or supplement any kit components provided by Indiana University unless MJFF/Indiana University has approved the substitution**.

See Appendix E for visit-specific kit contents.

Note: IU Supplemental Kits provide extra collection materials to sites. Supplemental Kit contents may be used in the event a kit component needs replaced.

3.5 Automatic Kit and Label Distribution

After an enrolled subject has completed a BL visit, subsequent longitudinal collection kits are automatically sent to sites. The subject's baseline visit date is collected from the EDC and used to calculate their projected visit schedule. This determines when automatic kits are distributed to the site. Kits will arrive at least 15 days prior to the

start of the 90-day study visit window. Sites may still order kits and labels on demand Section 2.6 through the kit ordering module Appendix F in the event of an unscheduled visit, lost labels, etc.

3.6 Kit and Label Ordering on Demand

Sites must order BL visit kits through the kit ordering module. Kits should be ordered with as much advance notice as possible to ensure all necessary supplies can be prepared and delivered before the BL visit.

Sites may request kits and labels at any time. Refer to the sample collection schedule (Section 4.0) to verify which kits are needed for a particular visit.

See Appendix F for details.

3.7 Assessments

Indiana University will provide sites with paper copies of cognitive assessments including the Benton Line Judgment Orientation test, the Hopkins Verbal Learning test, the Boston Naming Test, and the University of Pennsylvania Smell Identification Test (UPSIT).

Site staff may request additional assessments through the kit ordering module.

4.0 SAMPLE COLLECTION SCHEDULE

4.1 Collection Schedule Table

Visit	BL	V02	V04	V05	V06	V08	V10	V12	V13	V14	V15	V16	V17	V18	V19	V20
Month	**0**	**6**	**12**	**18**	**24**	**36**	**48**	**60**	**72**	**84**	**96**	**108**	**120**	**132**	**144**	**156**
Study Arm																
HC, PD																
Prodromal																
Study Visit Kit 1 (Blood for: RNA, plasma, buffy coat, serum, and whole blood. CSF, Urine)																
Study Visit Kit 2 (Blood for: RNA, plasma, buffy coat, serum, and whole blood. Urine)																

4.2 Skin Biopsy

4.2.1 Single Punch

For approved sites, a single punch skin biopsy should be collected from subjects transitioning from the previous PPMI schedule of activities to the current PPMI schedule of activities at the first feasible post-transition visit

The single punch skin biopsy will be collected for newly enrolled PPMI subjects at BL, V06, and V10.

4.2.2 Double Punch

Sites with Amendment 2 approval will collect a double punch skin biopsy from newly enrolled, HC, idiopathic PD and prodromal subjects. Collections will occur at BL, V06, and V10. Sites must have Amendment 2 approval before executing this collection.

5.0 SAMPLE COLLECTION

- See Appendix G for an overview of the visit-specific sample collection.
- See Appendix K for Whole Blood Collection with PAXgene™ RNA Tube Collection Instructions.
- See Appendix L for Whole Blood Collection for Plasma and Buffy Coat Isolation Collection Instructions.
- See Appendix M for 6ml EDTA Tube Whole Blood Collection Instructions.
- See Appendix N for Whole Blood Collection for Serum Isolation Collection Instructions.
- See Appendix O for Urine Sample Collection Instructions.
- See Appendix P for CSF Sample Collection Instructions.
- See Appendix R for Single Punch Skin Biopsy Sample Collection Instructions.
- See Appendix U for Double Punch Skin Biopsy Sample Collection Instructions.

6.0 SAMPLE PROCESSING

- See Appendix K for Whole Blood Collection with PAXgene™ RNA Tube Processing Instructions.
- See Appendix L for Whole Blood Collection for Plasma and Buffy Coat Isolation Processing Instructions.
- See Appendix M for 6ml EDTA Tube Whole Blood Processing Instructions.
- See Appendix N for Whole Blood Collection for Serum Isolation Processing Instructions.
- See Appendix O for Urine Sample Processing Instructions.
- See Appendix P for CSF Sample Processing Instructions.
- See Appendix R for Single Punch Skin Biopsy Sample Processing Instructions.
- See Appendix U for Double Punch Skin Biopsy Sample Processing Instructions.

7.0 PACKAGING AND SHIPPING INSTRUCTIONS

Important Notes

Include a sample set for only one subject per shipping carton. This allows space for enough dry ice to keep samples frozen during transit.

Ship frozen samples Monday through Wednesday only.

Ship biopsy samples Monday through Thursday only.

7.1 Shipping to Indiana University

US Sites:

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

Please refer to Appendix T for detailed instructions regarding biopsy sample shipment.

Canada Sites:

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

Please refer to Appendix Z for detailed instructions regarding biopsy sample shipment.

7.2 Shipping to BioRep

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

8.0 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 J) to provide feedback to the site.

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

9.0 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 the 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.

10.0 APPENDICES

Please see applicable appendices for information on sample collection, kit components and ordering, nonconformance, and shipping.

APPENDIX A: 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 B: SAMPLE FORM – FROZEN

PPMI Sample Record Summary and Shipment Notification Form – Frozen

Site: **Site Investigator:**

Coordinator: **Telephone:** **Email:**

Instructions: **Ship frozen samples 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 contact 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**
Subject ID #	**Visit**		**Gender**	
Specimen Type	**# of Tubes**	**Date of Draw**	**Tube Volume (if less than standard)**	**Notation of problems**
Total # of tubes:				

Date Shipped:

Tracking #:

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

Indiana University	**BioRep**
ppmibio@iu.edu	**ppmi@biorep.it**
Fax: 317-321-2003	**Fax: +39 02 58018471**
Phone: 317-278-1148	**Phone: +39 02 58029768**

APPENDIX C: PPMI FROZEN SHIPPING INSTRUCTIONS – USA

Preparing Frozen Sample Packaging/Shipment to Indiana University

Samples Shipped on Dry Ice:

- Frozen whole blood in 6 mL plastic EDTA tube
- Frozen whole blood in PAXgene™ RNA tubes
- Frozen plasma in 2 mL polypropylene tubes
- Frozen serum in 2 mL polypropylene tubes
- Frozen buffy coat in 2 mL polypropylene tube
- Frozen urine in 15 mL conical tube
- Frozen CSF in 2 mL polypropylene tubes

IMPORTANT!
FROZEN SAMPLES MAY BE SHIPPED MONDAY-WEDNESDAY ONLY! **Only ONE set of samples may be shipped in a single package.**

1. Contact UPS® to confirm service is available and schedule package pickup.

2. Notify Indiana University of shipment by e-mailing ppmibio@iu.edu (preferred) or faxing (317-321-2003) a copy of the completed Sample Record Summary and Shipment Notification Form (Appendix B)

3. Place all frozen 2 mL aliquot vials in the provided cardboard cryobox. Label the outside of the cryobox with the subject ID and visit number.

4. Place the cryobox into a clear plastic biohazard bag with the absorbent sheet and seal according to the instructions on the bag.

5. 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.

6. Place the bubble-wrapped tubes into the 2nd 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 Record Summary and Shipment Notification Form 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.

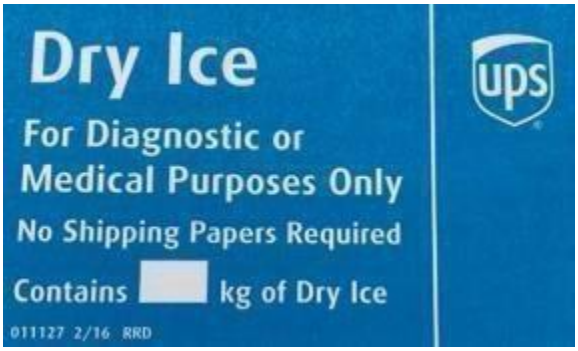
d. Click on the blue Pickup Request button, fill out the pickup information needed, and click Save.

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

i. Apply all provided warning labels to the outside of the package, taking care not to overlap labels.

j. Hold packaged samples in a -80°C freezer until the time of UPS® pickup.

APPENDIX D: PPMI FROZEN SHIPPING INSTRUCTIONS – EUROPE, AFRICA

Preparing Frozen Sample Packaging/Shipment to BioRep

Samples Shipped on Dry Ice:

- Frozen whole blood in 6 mL plastic EDTA tube
- Frozen whole blood in PAXgene™ tubes
- Frozen plasma in 2 mL polypropylene tubes
- Frozen serum in 2 mL polypropylene tubes
- Frozen buffy coat in 2 mL polypropylene tube
- Frozen urine in 15 mL conical tube
- Frozen CSF in 2 mL polypropylene tubes

IMPORTANT!
FROZEN SAMPLES MAY BE SHIPPED MONDAY-WEDNESDAY ONLY! **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. Notify BioRep of shipment by emailing PPMI@biorep.it (preferred) or faxing (+39 02 58018471) a copy of the completed Sample Record Summary and Shipment Notif ication Form (Appendix B).

3. Place the cryobox into a clear plastic biohazard bag with the absorbent sheet.

4. Seal according to the instructions on the bag.

5. 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.

6. Place the bubble-wrapped tubes into the 2nd 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 Record Summary and Shipment Notification Form on top of the carton, and close and seal the outer cardboard shipping carton with packing tape.

11. 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

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

13. Apply all provided warning labels and the completed air waybill to the outside of the package, taking care not to overlap labels.

14. Hold packaged samples in a -80°C freezer until the time of shipment pickup.

APPENDIX E: KIT COMPONENTS

Study Visit Kit 1 – RNA, Plasma, Buffy Coat, Whole Blood, Serum, Urine, CSF

Quantity	Item
1	5-tube bubble pouch
1	Cryobox
22	Cryogenic vials (2 mL) – 3 red cap, 4 purple cap, 15 clear cap
1	Lumbar puncture tray – Lidocaine (for US sites only)
1	Medication transfer filter straw (for Lidocaine)
6	Screw-top centrifuge tubes (15 mL)
1	Screw-top centrifuge tubes (50 mL)
1	Shipping container for dry ice shipments
1	Specimen cup
2	PAXgene™ tube (2.5 mL)
1	Purple-top EDTA tube (10 mL)
1	Purple-top EDTA tube (6 mL)
1	Red-top serum tube (10 mL)
1	Transfer pipette
2	Plastic biohazard bag with absorbent sheets
1	Warning label packet
1	UPS® or FedEx® shipping pouch
1	Shipping instruction sheet

Study Visit Kit 2 – RNA, Plasma, Buffy Coat, Whole Blood, Serum, Urine

Quantity	Item
1	5-tube bubble pouch
1	Cryobox
7	Cryogenic vials (2 mL) – 3 red cap, 3 purple cap, 1 clear cap
4	Screw-top centrifuge tubes (15 mL)
1	Shipping container for dry ice shipments
1	Specimen cup
2	PAXgene™ tube (2.5 mL)
1	Purple-top EDTA tube (10 mL)
1	Purple-top EDTA tube (6 mL)
1	Red-top serum tube (10 mL)
1	Transfer pipette
2	Plastic biohazard bag with absorbent sheets
1	Warning label packet
1	UPS® or FedEx® shipping pouch
1	Shipping instruction sheet

Skin Biopsy Visit Kit (Single Punch Protocol)

Quantity	Kit Component
1	Sterile drape
1	Tweezers
2	Gauze pads
2	Alcohol prep pads
1	Scissors
1	Skin biopsy punch tool with plunger
1	Gelfoam sterile compressed sponge
1	Vaseline ointment packet
1	Coverlet adhesive dressing
1	Transparent film dressing
1	Tissue specimen storage container, pre-filled with 10% buffered formalin
1	Tissue cassette
2	Sponges
2	Cold packs – REFRIGERATE AT 4° UPON RECEIPT
1	Plastic biohazard bag with absorbent sheets
1	Warning label packet
1	UPS® or FedEx® shipping pouch
1	Shipping instruction sheet

Skin Biopsy Visit Kit (Two Punch Protocol)

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
1	Tissue specimen storage container (empty)
1	Tissue specimen storage container, pre-filled with 10% buffered formalin
2	Tissue cassettes
4	Sponges
2	Cold packs – REFRIGERATE AT 4° UPON RECEIPT
2	Plastic biohazard bag with absorbent sheets
1	Warning label packet
1	UPS® or FedEx® shipping pouch
1	Shipping instruction sheet

Supplemental Kit Components

Quantity	Item
10	5-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)
5	Purple-top EDTA tubes (6 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

Supplemental Skin Biopsy Kit Components

Quantity	Item
1	Needle driver
1	Suture pack
4	Cold packs – REFRIGERATE AT 4° UPON RECEIPT
2	Skin biopsy punch tools with plunger
2	Sterile forceps
2	Gauze sponges
1	Sterile scissors
1	Coverlet adhesive dressings
1	Sterile drapes
2	Gelfoam sterile compressed sponges
5	Vaseline ointment packets
5	Transparent film dressings
5	Alcohol prep pads
3	Tissue specimen storage containers, pre-filled with 10% buffered formalin
4	Tissue cassettes
4	Cassette sponges
4	Plastic biohazard bag with absorbent sheets
4	Warning label packet
4	UPS® or FedEx® shipping pouch
4	Shipping instruction sheet

APPENDIX F: KIT ORDERING MODULE

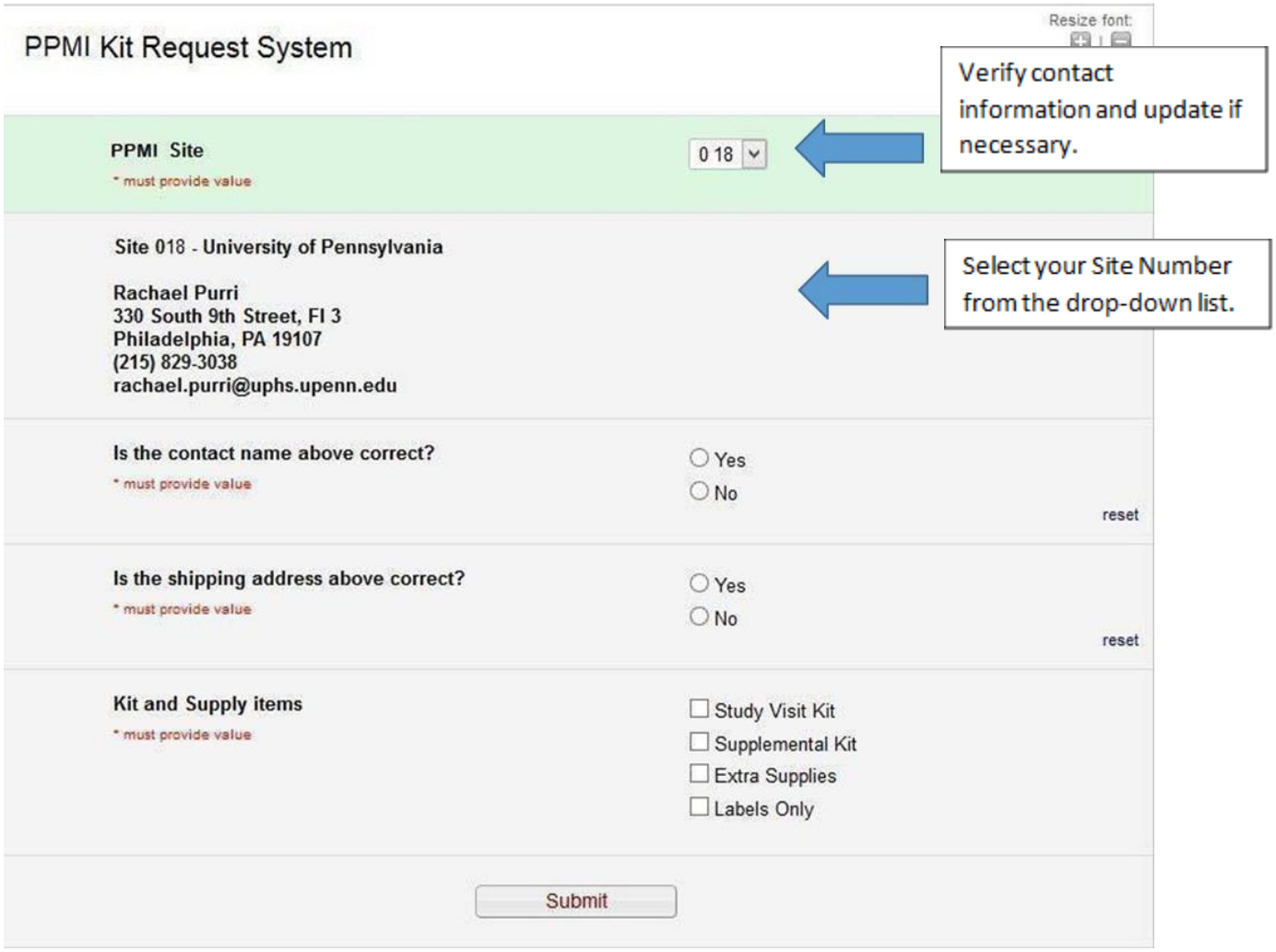
- Upon site startup, each site will receive one Supplemental Kit filled as well as an initial supply of three (3) study visit kits with labels.

- Sites are responsible for monitoring kit supplies. Kits, extra supplies, and paper assessments must be ordered through the "electronic kit ordering" module. Site coordinators should periodically check their stock of kits and supplemental supplies and order replacements as needed. Expiration dates should be monitored as well.

- Orders placed through the kit module will ship within three to five (3-5) business days; please provide as much notice as possible when ordering.

- To order kits or supplies, visit http://kits.iu.edu/ppmi.

To complete a kit order via the PPMI Kit Ordering Module:



APPENDIX G: SAMPLE COLLECTION INFORMATION

1. Sample Collection

The following samples will be collected at PPMI study visits:

- Serum, plasma, and buffy coat suitable for proteomic, metabolomic, and other analyte studies
- Whole blood
- Cerebrospinal fluid
- Urine
- Skin biopsy

If a sample is not obtained at a visit, this should be recorded in the study database and a reason should be provided.

Subjects should be fasting prior to CSF and biofluid collections. If fasting isn't possible, the suggested low-fat diet options may be used. See Appendix Q.

When a visit includes DaTScan imaging and biospecimen collection, all biospecimens MUST be collected prior to DaTscan tracer injection.

Otherwise, biospecimens should be collected after a minimum of 6 hours post DaTscan injection.

2. Sample Collection Volumes

Sample Type	Amount
Whole Blood for RNA	2 x 2.5 mL
Whole Blood for Plasma and Buffy Coat	10 mL
Whole Blood for Serum	10 mL
Whole Blood	6 mL
Urine	10 – 15 mL
CSF	15 – 20 mL
Skin Biopsy	1 punch, ~ 3mm and 3 mm deep. 2 punches for sites with Amendment 2 approval)

3. Blood Collection – Order of Draw

Tubes should be filled in the following order:

1. 2 x 2.5 ml PAXgene™
2. 1 x10 ml Plasma and Buffy Coat EDTA Purple Top
3. 1 x 6 ml Whole Blood EDTA Purple Top
4. 1 x 10 ml Serum Determination Red Top
5. General clinical lab tubes (REFER TO LABCORP DRUG DEVELOPMENT LAB MANUAL)

4. General Clinical Labs

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

5. Video List

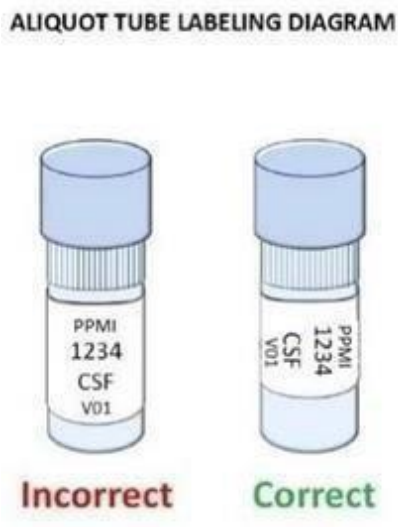
Training videos are available to assist with PPMI specimen processing, aliquoting, and shipping processes. Please contact the repository at ppmibio@iu.edu for more information.

APPENDIX H: SAMPLE LABELING INFORMATION

Labeling Samples

To ensure the label adheres properly and remains on the tube:

- Place labels on ALL collection and aliquot tubes BEFORE sample collection, sample pro cessing, or freezing. This should help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.
- Place label horizontally on the tube (wrapped around sideways if the tube is upright) and just below the ridges of the aliquot tubes (see attached labeling diagram). There is enough space on the aliquot tube for the label to be placed without overlapping the ridges.
- Take a moment to ensure the label is completely adhered to each tube. It may be helpful to roll the tube between your fingers after applying the label.

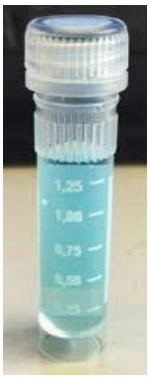


APPENDIX I: FILLING ALIQUOT TUBE

Filling Aliquot Tubes (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

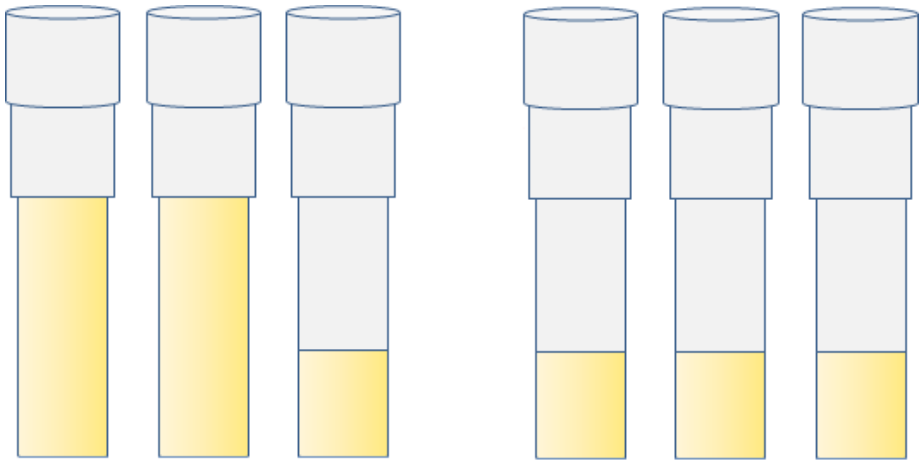


To ensure that the biorepository receives enough of the specimen for processing and storage, and to avoid cracking of the tubes prior to shipment, each aliquot tube should be filled to 1.5 milliliters (see picture, right) after processing is completed (refer to detailed processing instructions per specimen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

If there is biologic material remaining that will not fill a subsequent aliquot tube to 1.5 mL, that remaining amount should be sent in a partially filled aliquot tube.

All collected material should be shipped to the biorepository. After processing, aliquot the recommended volume (1.5 mL) into as many aliquot tubes as available sample will allow.

For example, if 3.7 mL of total specimen is obtained, fill 2 cryovials with 1.5 mL each, and one additional cryovial with the remaining 0.7 mL specimen volume (see example below).



YES NO

APPENDIX J: SAMPLE SUBMISSION NONCONFORMANCE REPORT

Repository Name: IU

Subject ID: Site #:

Received Date: Visit Type:

Shipping Issues Noted:

- ☐ Shipment notification not received.
- ☐ 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:

APPENDIX K: WHOLE BLOOD COLLECTION WITH PAXGENE™ RNA TUBE

See collection schematic on following pages

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

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

3. Place "RNA" collection tube labels on the PAXgene™ Tubes prior to blood draw (per Appendix H).

4. Using a blood collection set and a holder, collect blood into the **first of the two** PAXgene™ RNA Tubes using your institution's recommended procedure for standard venipuncture technique.

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

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. Repeat steps 4 – 6 to collect a second PAXgene™ RNA Tube.

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

9. Record time and date of draw in the study database.

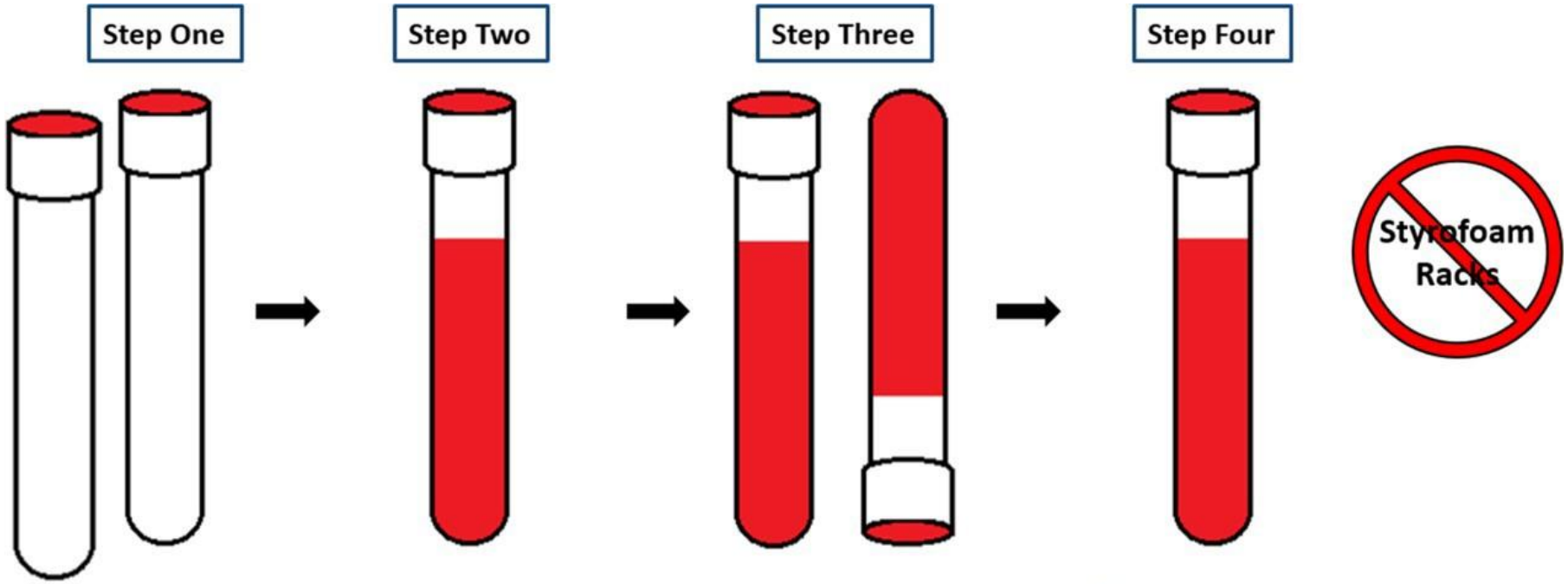
10. After 24 hours at room temperature, place the two PAXgene™ RNA Tubes upright in a wire or plastic test tube rack and transfer the rack into a -80°C freezer. **DO NOT** store/freeze samples in a solid Styrofoam test tube holder.

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

11. Keep the PAXgene™ RNA Tubes at -80°C until packed for shipment on dry ice. Samples should be shipped within two weeks of collection, following the instructions in Appendix C (US sites shipping to IU), Appendix D (sites shipping to BioRep), or Appendix X (Canadian sites shipping to IU).

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

Whole Blood Preparation (2.5 ml PAXgene™ RNA Tube)



Step One

- Store tubes at room temp.
- Label tubes with pre-printed collection tube label prior to blood draw.

Step Two

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

Step Three

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

Step Four

- Store tubes in wire rack at -80°C until shipment
- Do not store tube in Styrofoam racks.

APPENDIX L: WHOLE BLOOD COLLECTION FOR PLASMA/BUFFY COAT ISOLATION

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 (per Appendix H).

    a. Place pre-printed "PLASMA" tube label on the 10 mL EDTA Tube, 15 mL centrifuge Tube, and the purple capped 2 mL aliquot tubes.
    b. Place pre-printed "BUFFY COAT" tube label on the clear capped 2 mL aliquot tube.

3. Using a blood collection set and a holder, collect blood into the 10 mL EDTA Tube using your institution's recommended procedure for standard venipuncture technique.

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

4. Allow at least 10 seconds for a complete blood draw to take place. **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.

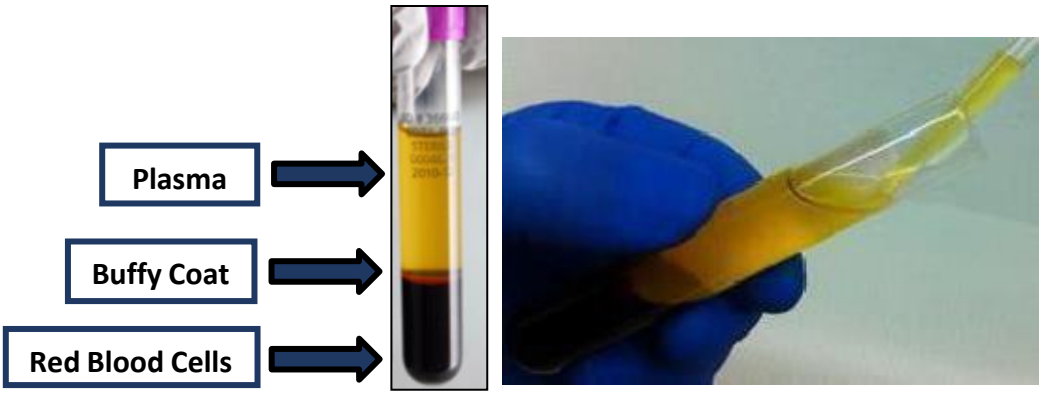
5. **CRITICAL STEP:** Immediately after blood collection, gently invert/mix (180 degree turns) the 10 mL EDTA Tube 8-10 times.

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

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

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

9. Transfer the separated plasma into the 15mL centrifuge tube. This should be completed at room temperature.
    a. While aliquoting, tilt the tube and place 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.



10. Mix the plasma gently by inverting the 15 mL centrifuge tube 3-4 times.

11. Pipette 1.5 mL of plasma from the 15 mL centrifuge tube into each "PLASMA" labeled 2 mL aliquot tube. This collection should yield, on average, 4.5 mL of blood plasma, for a total of 2-3 aliquot tubes per subject. Seal each aliquot tube with a purple cap.

Note: When pipetting plasma from the plasma tube into the 15 mL centrifuge tube, be very careful to pipette the plasma top layer only, leaving the buffy coat and the red blood cell layers

12. Using a clean transfer pipette (micropipette preferred), transfer the buffy coat layer (shown above) into the "BUFFY COAT" labeled aliquot tube. Seal the aliquot tube with a clear cap. Residual plasma and RBCs may also be collected during this isolation process.

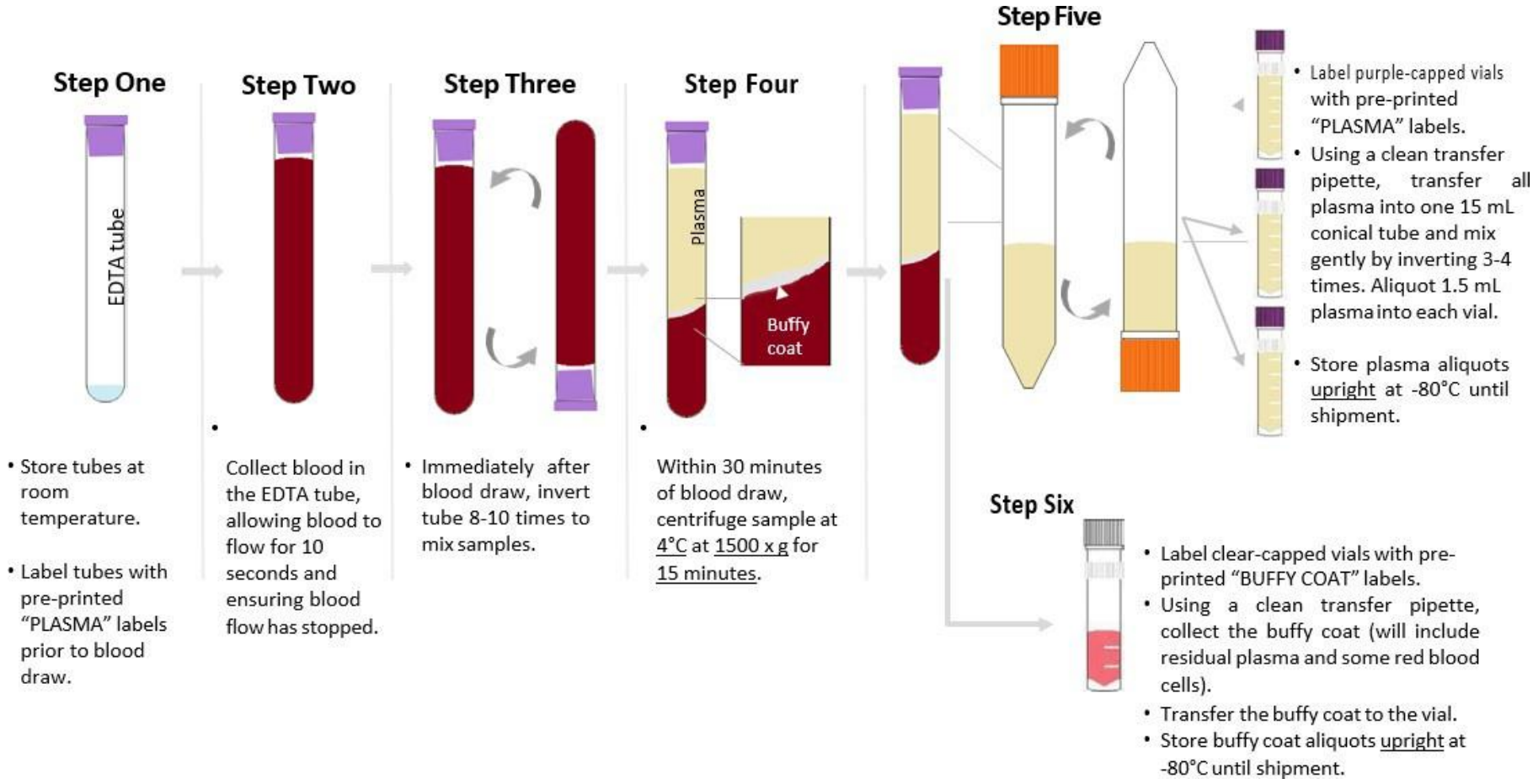
13. Within 60 minutes of sample collection, freeze and store samples at -80°C. Samples should be frozen and stored upright. A cryobox is provided for this purpose.

14. Discard the used 10 mL EDTA Tube and 15 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 within two weeks of collection, following the instructions in Appendix C (US sites shipping to IU), Appendix D (sites shipping to BioRep), or Appendix X (Canadian sites shipping to IU).

COLLECTION SCHEMATIC: PLASMA AND BUFFY COAT PREPARATION (10 mL PURPLE TOP TUBE)



- Store tubes at room temperature.
- Label tubes with pre-printed "PLASMA" labels prior to blood draw.

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

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

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

- Label purple-capped vials with pre-printed "PLASMA" labels.
- Using a clean transfer pipette, transfer all plasma into one 15 mL conical tube and mix gently by inverting 3-4 times. Aliquot 1.5 mL plasma into each vial.
- Store plasma aliquots upright at -80°C until shipment.

- Label clear-capped vials with pre-printed "BUFFY COAT" labels.
- Using a clean transfer pipette, collect the buffy coat (will include residual plasma and some red blood cells).
- Transfer the buffy coat to the vial.
- Store buffy coat aliquots upright at -80°C until shipment.

APPENDIX M: WHOLE BLOOD COLLECTION (6ML EDTA TUBE)

See collection schematic on following pages

1. **CRITICAL STEP:** Store 6 mL EDTA Tubes at room temperature, 64°F - 77°F (18°C to 25°C) before use.

2. Place pre-printed "WBLD" tube label on the 6 mL EDTA Tube. Complete labeling prior to blood draw (per Appendix H).

3. Using a blood collection set and a holder, collect blood into the 6 mL EDTA Tube using your institution's recommended procedure for standard venipuncture technique.

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

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

5. Immediately after blood collection, gently invert/mix (180 degree turns) the 6 mL EDTA Tube 3 to 4 times.

6. Immediately prepare to transfer tube to a -80°C Freezer. The sample should be frozen and stored 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.

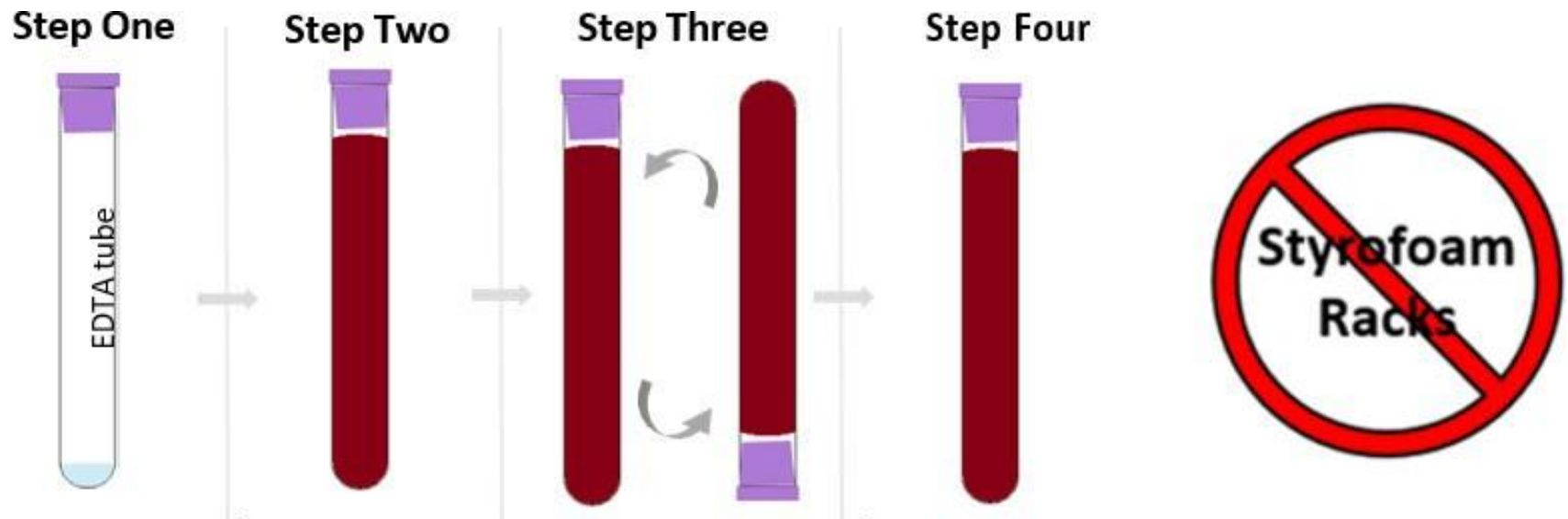
7. Enter remaining sample collection data into the study database.

8. Keep the 6 mL EDTA Tube at -80°C until packed for shipment on dry ice.

9. Samples should be shipped within two weeks of collection, following the instructions in Appendix C (US sites shipping to IU), Appendix D (sites shipping to BioRep), or Appendix X (Canadian sites shipping to IU).

10. Record time and date of draw in the study database.

COLLECTION SCHEMATIC: WHOLE BLOOD COLLECTION AND PREPARATION (6 ML PURPLE TOP TUBE)



- Store tubes at room temperature.
- Label tubes with pre-printed "WBLD" labels prior to blood draw.

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

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

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

APPENDIX N: WHOLE BLOOD COLLECTION - SERUM DETERMINATION PROCESSING

See collection schematic on following pages

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

2. Place pre-printed "SERUM" tube label on the Serum Determination Tube, 15 mL centrifuge Tube, and the red capped 2 mL aliquot tubes. Complete labeling prior to blood draw (per Appendix H).

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

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

4. Allow at least 10 seconds for a complete blood draw to take place. **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.

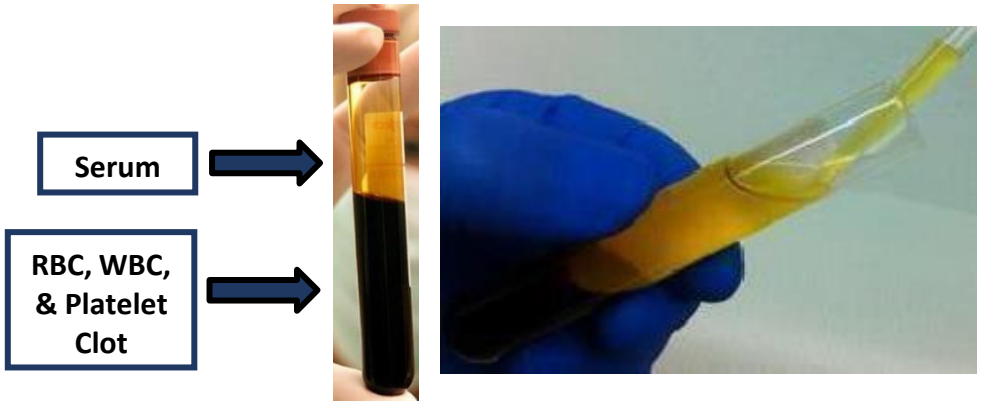
6. **CRITICAL STEP:** Allow blood to clot at room temperature for at least 15 minutes.

7. Record time 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.

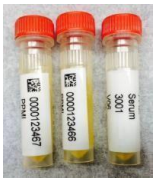
9. While centrifuging, record the time of centrifuge start in the study database.

10. Transfer the separated serum into the 15mL centrifuge tube. This should be completed at room temperature.
    a. 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 subject. 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 within two weeks of collection, following the instructions in Appendix C (US sites shipping to IU), Appendix D (sites shipping to BioRep), or Appendix X (Canadian sites shipping to IU).

COLLECTION SCHEMATIC: SERUM PREPARATION (10 mL RED TOP TUBES)

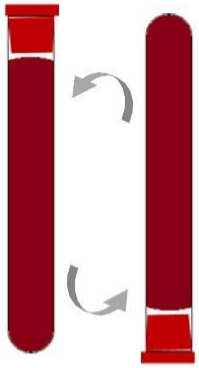
Step One

- Store tubes at room temperature.
- Label tube and red-capped vials with pre-printed "SERUM" label prior to blood draw.

Step Two

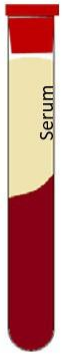
- Collect blood in serum tube, allowing blood to flow for 10 seconds, ensuring blood flow has stopped.

Step Three



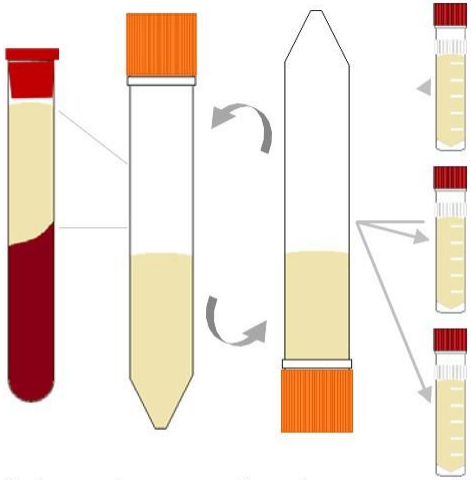
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Four



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

Step Five



- Using a clean transfer pipette, transfer all serum into a 15 mL conical tube.
- Mix gently by inverting 3-4 times.
- Aliquot 1.5 mL into each vial.
- Store serum aliquots upright at-80°C until shipment.

APPENDIX O: Urine Collection and Processing Procedures

1. Label one urine collection cup and two 15 mL centrifuge tubes prior to collection with a pre-printed "URINE" label.

2. Ask study subject to collect a urine specimen in the collection cup. Urine should be collected midstream and should remain as sterile as possible.
3. Pour urine specimen into one labeled 15 mL centrifuge tube, ensure the tube is at least 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. For assistance, see Appendix A.
    - ❖ While centrifuging, record the time of centrifuge start in the study database.
5. Using a clean transfer pipette, carefully transfer supernatant from the 15 mL processing centrifuge tube into the second, labeled, 15 mL conical tube.
6. Firmly cap with the included screw cap.
7. Discard the original processing tube per your institution's guidelines.
8. Place the labeled tube upright in dry ice or at -80°C and allow the sample to freeze completely.
9. Within 60 minutes of sample collection, freeze and store samples at -80°C. Samples should be frozen and stored upright.
10. Enter remaining sample data into the study database.
11. Samples should be shipped within two weeks of collection, following the instructions in Appendix C (US sites shipping to IU), Appendix D (sites shipping to BioRep), or Appendix X (Canadian sites shipping to IU).

APPENDIX P: DETAILED CSF SAMPLE PROCESSING PROCEDURE

CSF is processed at Room Temperature [64^0F – 77^0F (18°C – 25°C)].

A portion of the CSF must be sent to your clinical lab and analyzed within 4 hours of collection.

1. Place a pre-printed "CSF" label on the 15 mL centrifuge tubes and 2 mL cryovial aliquot tubes (per Appendix H).
   a. Prepare at least 10 aliquot tubes based on the collection of 15-20 mL of CSF.

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).

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).
   a. 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.

      1. Cell count (erythrocytes first)
      2. Total protein
      3. Glucose

6. Collect an additional 15-20 mL of CSF and transfer to the labeled 15 mL conical polypropylene tubes at room temperature. Mix gently by inverting 3-4 times. Record the time of draw (once collection is complete) into the study database.

7. 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). For assistance, see Appendix A.

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

9. 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.

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

11. 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 subject. Use more aliquot tubes if needed. **Do not discard any CSF**. Seal each aliquot tube with a clear cap.

12. 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.
    a. Samples should be frozen at -80°C and stored upright. A cryobox is provided for proper storage purposes.

13. Complete the remainder of the sample data entry into the study database.

14. Samples should be shipped within two weeks of collection, following the instructions in Appendix C (US sites shipping to IU), Appendix D (sites shipping to BioRep), or Appendix X (Canadian sites shipping to IU).

See Collection Schematic Below for additional detail

CSF Collection Preparation

Setting Up an LP

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 subject.
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.
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.

Maintaining the sterile field

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.

Tips for Clinicians Performing Lumbar Puncture

**Optimizing patient comfort and minimizing the risk of adverse events.*

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

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. Use adequate local anesthesia. Use the 25g 1/2" needle and inject lidocaine to raise a skin wheal. Then, inject lidocaine using the pattern of a square—first the center, and then to all 4 corners. If the subject is thin, do not insert the deep infiltration needle OR the spinal introducer all the way. Use only about 2/3 of their length (to prevent entering the subarachnoid space with anything other than the 24g pencil point spinal needle).

4. Increasing fluid intake immediately after LP is helpful.

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

Post-LP Care Instructions

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

- Advise the subject to continue with increased fluid intake.

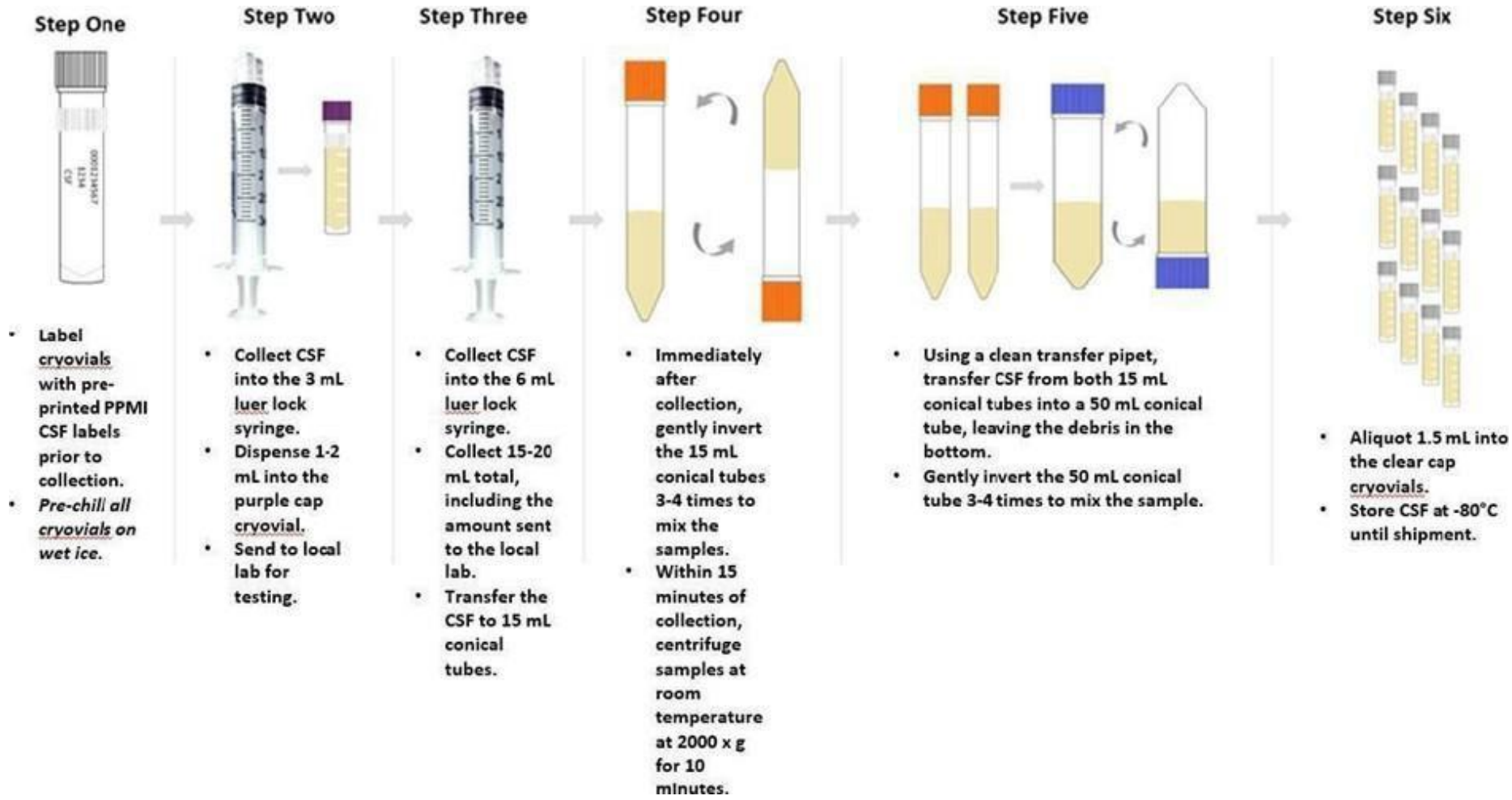
Mild to Moderate headache after a lumbar puncture

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

Severe headache after a lumbar puncture

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

COLLECTION SCHEMATIC: CSF COLLECTION AND PREPARATION



APPENDIX Q: 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 collectedafter an 8 hour fast (no food or drink except fluids such as water, tea, black coffee).** If fastingis not achievable, a subject should be on a low-fat diet for at least 8 hours prior to blood collection.

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

Sample Breakfast Items:	**Sample Lunch Items:**
Dry whole wheat toast Fruit salad • no dressing Clear tea or coffee • no milk or cream Fruit or vegetable juice	Turkey breast sandwich on whole wheat bread Lettuce, Tomato, and Mustard Clear beverage Flavored gelatin
Dry cereal • without nuts/no granola; no milk Clear tea or coffee • no milk or cream Fruit or vegetable juice	Plain pasta with plain marinara sauce • no butter or cheese Side of steamed vegetables or green salad Clear beverage Flavored gelatin
Plain oatmeal or other cooked whole grain cereal • topped with fresh or dried fruit • no butter, milk, or cream Clear tea or coffee • no milk or cream Fruit or vegetable juice	Steamed chicken breast • lean, without skin Side of steamed vegetables or green salad Clear beverage Flavored gelatin
Dry whole wheat toast Poached egg white or egg substitute Clear tea or coffee • no milk or cream Fruit or vegetable juice	Large tossed green salad, assorted vegetables • no dressing or cheese Clear beverage Flavored gelatin
	Cucumber sandwich on whole wheat bread Lettuce, tomatoes, shredded carrots, onions, etc. Clear beverage Flavored gelatin
	Clear broth with vegetables and pasta Fruit salad • no dressing Clear beverage Flavored gelatin

Low-Fat Diet Menu Suggestions

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

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

APPENDIX R: SKIN BIOPSY COLLECTION AND PROCESSING – SINGLE PUNCH

Skin biopsy samples must be shipped on the day of collection and received at the biorepository the day after collection. Biopsy collections should not be performed or shipped on Fridays.

1. Skin Biopsy Using the Punch Biopsy Tool

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

1.1. Punch Biopsy Kit Components

Quantity	Kit Component
1	Sterile drape
1	Tweezers
2	Gauze pads
2	Alcohol prep pads
1	Scissors
1	Skin biopsy punch tool with plunger
1	Gelfoam sterile compressed sponge
1	Vaseline ointment packet
1	Coverlet adhesive dressing
1	Transparent film dressing
1	Tissue specimen storage container, pre-filled with 10% buffered formalin
1	Tissue cassette
2	Sponges
2	Cold packs

1.2. Setting Up the Kit

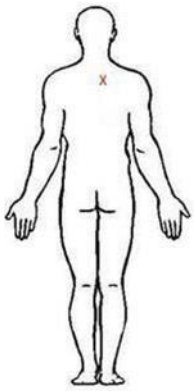
1.2.1. On an overbed table, remove the contents of the kit from the outer packaging, leaving all sterile contents wrapped in their packaging. Leave everything wrapped until the person performing the biopsy is seated and begins examining the subject.

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

1.3. Skin Sample Collection

There will be one skin biopsy obtained from the cervical paravertebral region at

approximately the C8 level (see figure below). The biopsy will be processed in formalin.



1.3.1. Pre-collection Steps for Specialist – Preparation of Patient

1.3.1.1. Prepare patient for procedure per institution guidelines.

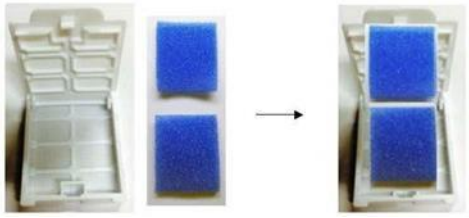
1.3.1.2. Before the biopsy is collected, the volunteer will be screened and complete the informed consent for the skin biopsy procedure. The doctor will explain the study, and the volunteer will have an opportunity to ask questions. Once this discussion is complete, the volunteer is ready for the biopsy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

1.3.2. Preparation Steps for Coordinator – Container and Cassette

1.3.2.1. Prior to the procedure, label the tissue container with the Subject ID and "TISSUE" labels.

1.3.2.2. Record the barcode label from the cassette on the Sample Record Summary and Shipment Notification Form

1.3.2.3. Place the sponges on the top and bottom sections of the cassette.



1.3.3. Biopsy Collection Procedure

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

1.3.3.2. 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.

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

1.3.3.4. Using a sterile 3 mm skin punch, place the punch perpendicular to the skin, in the paravertebral C8 region, within 4 cm of the midline. Apply constant downward pressure while twirling the punch tool between the thumb and index finger, rotating clockwise and counterclockwise until the blade has pierced the epidermis of the skin and the metal part of the punch tool is buried (there will be a “give” once the punch reaches the subcutaneous fat). Once the tool has reached the lowest point, lift the tool straight up.

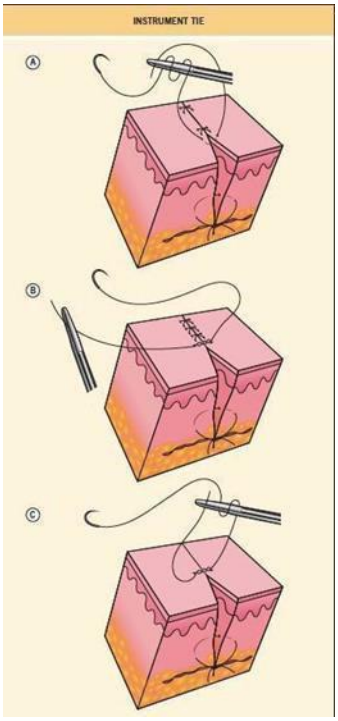
1.3.3.5. Depress the plunger to remove the specimen. Forceps may be needed to remove the specimen. If the specimen remains connected at the level of the subcutaneous fat, it may be necessary to cut at the base of the specimen to remove it. Do not try to tear a specimen that remains connected, as it may damage the specimen. Tissue should be rinsed with a saline solution if it does not release. The specimen should be placed directly into a sponge-lined cassette as instructed. Using a punch with a plunger should help to ensure that the epidermis is not crushed or damaged during the process.

1.3.3.6. **CRITICAL STEP:** Close the cassette securely by bringing the lid down onto the bottom and snapping it.

1.3.3.7. To restore hemostasis, hold pressure with gauze for approximately 30 seconds. Wipe any excess blood with a sterile 2x2 gauze to expose the site. Pack biopsy site with GelFoam. Apply the Vaseline ointment to the bandage and cover biopsy site. This can be reinforced with gauze and tape if necessary. If the biopsy site is oozing, apply a pressure bandage by applying Vaseline to small gauze and then apply Tegaderm. Other closure options include using a steri-strip and transparent film dressing closure system. In most cases, suturing a wound will not be necessary. Placing a suture can be considered if the wound base is still oozing after packing with GelFoam. To place a suture, grip the needle using the forceps approximately ½ to 1/3 of the

distance between the suture attachment and the tip of the needle. Place the needle point perpendicular to the skin surface 2 mm away from the wound edge, then turn the wrist to exist the skin on the opposite side of the wound, again, 2 mm from the wound edge. To tie the suture, hold the needle holder parallel to the axis of the wound and at the center of the wound.

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



1.3.3.9. The study coordinator will be responsible for completing the processing of the tissue once collected using the procedures described in detail below.

1.3.3.10. Be sure to give post care instructions verbally to the subject 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.

1.4. Tissue Processing of Skin Biopsy

1.4.1. One punch skin biopsy will be collected from either the right or left side of the paravertebral C8 region within 4 cm of the midline.

1.4.2. The biopsy should be placed on the bottom section of a sponge-lined cassette.

1.4.3. Submerge the cassette into the formalin-filled container.

1.4.4. **CRITICAL STEP**: Be sure that the container lid is fully and properly sealed.

1.4.5. Record the time placed in Formalin on the Sample Record Summary and Shipment Notification Form (Appendix W).

1.4.6. Place container in a refrigerator until shipment.

1.4.7. **CRITICAL STEP:** Ship the samples to the repository at 4°C according to Appendix T (US sites shipping to IU) or Appendix Z (Canadian sites shipping to IU) on the same day of collection.

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

NOTE: 10% formalin solutions contain 3-4% formaldehyde and are not regulated for transport by air or highway according to the US DOT and IATA regulations. However, please follow all guidelines dictated by your institution for packaging and shipping formalin-fixed tissue samples.

COLLECTION SCHEMATIC: SKIN BIOPSY COLLECTION AND PREPARATION

NO FRIDAY COLLECTIONS

- Label the outside of the provided container with the subject identifier.

- Using Standard Biopsy procedures, collect a biopsy from the paravertebral C8 region, approximately 3 mm in diameter and 3 mm deep.

- Open the pre-labeled cassette and place the sponges in the top and bottom of the cassette.
- Record the label number on the Sample Record Summary and Shipment Notification Form.

- Place the biopsy into the cassette.
- Record If rinsing the punch tool is required to release the biopsy, use a saline solution.

- Close the cassette securely

- Submerge the cassette in the formalin and note the time on the Sample Record Summary and Shipment Notification form.
- Ensure the container lid is fully and properly sealed. Ship on the day of collection using procedures in Appendix T (US sites shipping to IU) or Appendix Z (Canadian sites shipping to IU).

APPENDIX T: PPMI BIOPSY SHIPPING INSTRUCTIONS - USA

Preparing Cold Pack Biopsy Sample Packaging/Shipment to Indiana University

Samples Shipped on Cold Pack:

- Skin biopsy sample – 1 tissue cassette
  - (2 for sites with Amendment 2 approval)

IMPORTANT!
REFRIGERATE COLD PACKS AT 4°C BEFORE USE **COLLECT AND SHIP BIOPSY SAMPLES MONDAY-THURSDAY ONLY**

1. Contact UPS® to confirm service is available and schedule package pickup.

2. Notify Indiana University of shipment by e-mailing ppmibio@iu.edu (preferred) or faxing (317-321-2003) a copy of the completed Sample Record Summary and Shipment Notification Form (Appendix B)

3. Ensure that tissue containers are completely and properly sealed:
   3.1. Insert a large absorbent sheet into a biohazard bag (both provided)
   3.2. Place a tissue container, with cassette inside, into the bag.
   3.3. Seal the biohazard bag completely.

4. Insert two cold packs into the ambient shipping container provided. Insert the specimen container(s) into the shipping container. If necessary, add paper toweling or other material as padding.

5. Replace the lid on the Styrofoam carton, place the completed Sample Record Summary and Shipment Notification Form on top of the carton, and close and seal the outer cardboard shipping carton with packing tape.

6. To Ship:

   6.1. 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.

   6.2. 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.

   6.3. Enter the weight of the package in the Package Weight field (leave the Dry Ice Weight field blank).

6.4. Click on the blue Pickup Request button, fill out the pickup information needed, and click Save.

6.5. Click the blue ship button and print the air waybill.

6.6. Peel the backing from the clear sleeve and attach to the package. Place the printed waybill in the clear sleeve and place the package at your dedicated UPS® pickup location or drop the package off at a UPS® store or drop box.

7. Apply all provided warning labels to the outside of the package, taking care not to overlap labels.

8. Hold packaged samples in a 4°C refrigerator until the time of UPS® pickup.

9. Ship the samples to Indiana University on the day of collection.

NOTE: 10% formalin solutions contain 3-4% formaldehyde and are not regulated for transport by air or highway according to the US DOT and IATA regulations. However, please follow all guidelines dictated by your institution for packaging and shipping formalin-fixed tissue samples.

APPENDIX U: SKIN BIOPSY COLLECTION AND PROCESSING – DOUBLE PUNCH

Skin biopsy samples must be shipped on the day of collection and received at the biorepository the day after collection. Biopsy collections should not be performed or shipped on Fridays.

NOTE: The two-punch collection should be completed for sites approved under Amendment 2

1. Skin Biopsy Using the Punch Biopsy Tool

The skin biopsy kit contains the items listed in the table below, which will be used to perform the skin punch biopsy procedure. Check the dates of expiration. Place cold packs in the refrigerator upon arrival to chill them. Note that sutures and needle drivers will be provided in each site's supplemental supplies and should be on hand and ready in case they are necessary for this procedure.

1.1. 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
1	Tissue specimen storage container, pre-filled with 10% buffered formalin
2	Tissue cassette
4	Sponges
2	Cold packs

1.2. Setting Up the Kit

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

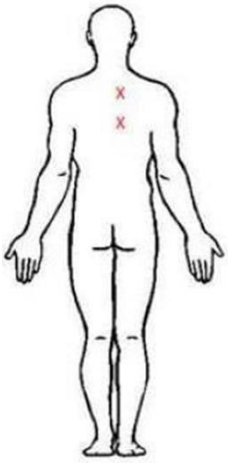
1.2.2. Open the sterile kit components, touching only the outside of the wrapper. Don't let the outside of the wrapper touch any part of the inside.

1.3. Skin Sample Collection

There will be two skin biopsies obtained from the cervical paravertebral region at approximately the C8 level (see figure below). The biopsy will be processed in formalin. One biopsy will be processed in formalin; the other will be placed in saline.

1.3.1. Pre-collection Steps for Specialist – Preparation of Patient

1.3.1.1. Prepare patient for procedure per institution guidelines.



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

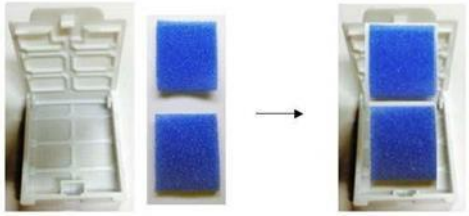
1.3.2. Preparation Steps for Coordinator – Container and Cassette

1.3.2.1. Specimen containers will be pre-labeled with Formalin or Saline labels. Prior to the procedure, label the containers with a “Tissue” label.

1.3.2.2. Fill the saline container 2/3 full of non-sterile normal saline.

1.3.2.3. Record the barcode labels from the cassettes on the Sample Record Summary and Shipment Notification Form (Appendix W).

1.3.2.4. Place the sponges on the top and bottom sections of the cassettes.



1.3.3. Biopsy Collection Procedure

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

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

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

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

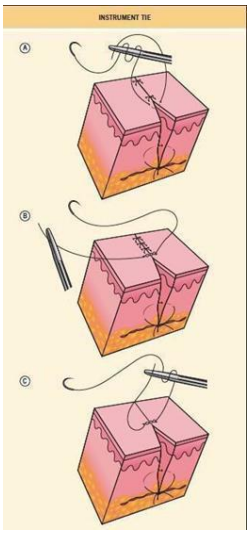
1.3.3.5. Depress the plunger to remove the specimen. Forceps may be needed to remove the specimen. If the specimen remains connected at the level of the subcutaneous fat, it may be necessary to cut at the base of the specimen to remove it. Do not try to tear a specimen that remains connected, as it may damage the specimen. Tissue should be rinsed with a saline solution if it does

not release. The specimen should be placed directly into a sponge-lined cassette as instructed. Using a punch with a plunger should help to ensure that the epidermis is not crushed or damaged during the process.

1.3.3.6. **CRITICAL STEP:** Close the cassette securely by bringing the lid down onto the bottom and snapping it.

1.3.3.7. To restore hemostasis, hold pressure with gauze for approximately 30 seconds. Wipe any excess blood with a sterile 2x2 gauze to expose the site. Pack biopsy site with GelFoam. Apply the Vaseline ointment to the bandage and cover biopsy site. This can be reinforced with gauze and tape if necessary. If the biopsy site is oozing, apply a pressure bandage by applying Vaseline to small gauze and then apply Tegaderm. Other closure options include using a steri-strip and transparent film dressing closure system. In most cases, suturing a wound will not be necessary. Placing a suture can be considered if the wound base is still oozing after packing with GelFoam. To place a suture, grip the needle using the forceps approximately ½ to 1/3 of the distance between the suture attachment and the tip of the needle. Place the needle point perpendicular to the skin surface 2 mm away from the wound edge, then turn the wrist to exist the skin on the opposite side of the wound, again, 2 mm from the wound edge. To tie the suture, hold the needle holder parallel to the axis of the wound and at the center of the wound.

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



1.3.3.9. Collect a second biopsy 3 cm above or below the original collection site on the same side of the midline, and following the same procedure. Place the biopsy into another sponge-lined cassette. Tissue should be rinsed with a saline

solution if it does not release. Follow the same procedure to achieve hemostasis and apply the dressing accordingly.

1.3.3.10. **CRITICAL STEP:** Close the cassette securely by bringing the lid down onto the bottom and snapping it.

1.3.3.11. The study coordinator will be responsible for completing the processing of the tissue once collected using the procedures described in detail below.

1.3.3.12. Be sure to give post care instructions verbally to the subject 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.

1.4. Tissue Processing of Skin Biopsy

1.4.1. Two punch skin biopsies will be collected from either the right or left side of the paravertebral C8 region within 3 cm of the midline.

1.4.2. The biopsies should be placed on the bottom section of a sponge-lined cassettes.

1.4.3. **CRITICAL STEP:** Record the cassette label numbers on the Sample Record Summary and Shipment Notification Form (Appendix W).

1.4.4. Submerge one cassette into the formalin-filled container and the other into the saline-filled container.

1.4.5. **CRITICAL STEP**: Be sure that the container lids are fully and properly sealed.

1.4.6. **CRITICAL STEP:** Record the time placed in Formalin or Saline on the Sample Record Summary and Shipment Notification Form (Appendix W).

1.4.7. Place container in a refrigerator until shipment.

1.4.8. **CRITICAL STEP:** Ship the samples to the repository at 4°C according to Appendix T (US sites shipping to IU) or Appendix Z (Canadian sites shipping to IU) on the same day of collection.

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

NOTE: 10% formalin solutions contain 3-4% formaldehyde and are not regulated for transport by air or highway according to the US DOT and IATA regulations. However, please follow all guidelines dictated by your institution for packaging and shipping formalin-fixed tissue samples.

COLLECTION SCHEMATIC: SKIN BIOPSY COLLECTION AND PREPARATION – Double PUNCH

NO FRIDAY COLLECTIONS

Step One

Label the outside of the provided containers with the subject identifier.

Pour normal non-sterile saline in to the saline-labeled container (about 2/3 full).

Step Two

Using standard punch biopsy procedures, collect two biopsies from the paravertebral C8 region approximately 3 mm in diameter and 3 mm deep.

Step Three

Open the pre-labeled cassettes and place the blue sponges into the top and bottom of each cassette.

Record the label numbers on the Sample Record Summary and Shipment Notification Form (Appendix W).

Step Four

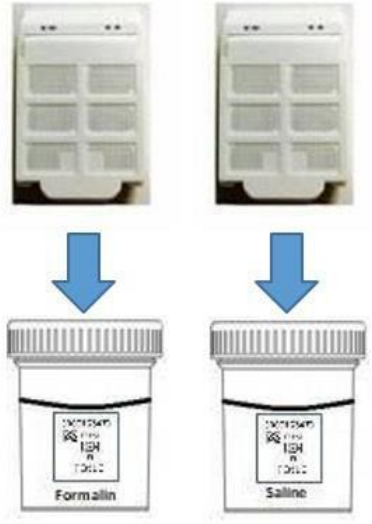
Place a biopsy into each cassette.

If rinsing the punch tool is required to release the biopsy, use a saline solution.

Step Five

Close each cassette securely.

Step Six



Submerge one cassette in formalin and the other in saline and note the times on the Sample Record Summary and Shipment Notification Form (Appendix W).

Ensure that the container lids are fully and properly sealed. Ship on the day of collection using procedures in Appendix T.

APPENDIX W: SAMPLE FORM – SKIN BIOPSY

PPMI Sample Record Summary and Shipment Notification Form – Skin Biopsy

Site: **Site Investigator:**

Coordinator: **Telephone:** **Email:**

Instructions: **Ship skin biopsy samples Monday – Thursday ONLY**. This form must be completed for all biorepository sample shipments. Notify the recipient repository (e-mail preferred) prior to shipment using the contact 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.			**Completed by Biorepository**
Subject ID #	**Visit**	**Gender**	**Notation of problems**

Cassette Label #:	
Time placed in formalin:	
Cassette Label #:	
Time placed in saline*:	

*Sites with Amendment 2 approval only

Date Shipped:

Tracking #:

IMPORTANT!

BEFORE SHIPPING, E-MAIL (PREFERRED) OR FAX A COPY OF THE COMPLETED FORM TO THE BIOREPOSITORY:

Indiana University
ppmibio@iu.edu
Fax: 317-321-2003
Phone: 317-278-1148

APPENDIX X: PPMI FROZEN SHIPPING INSTRUCTIONS – CANADA

Preparing Frozen Sample Packaging/Shipment to Indiana University

Samples Shipped on Dry Ice:

- Frozen whole blood in 6 mL plastic EDTA tube
- Frozen whole blood in PAXgene™ RNA tubes
- Frozen plasma in 2 mL polypropylene tubes
- Frozen serum in 2 mL polypropylene tubes
- Frozen buffy coat in 2 mL polypropylene tube
- Frozen urine in 15 mL conical tube
- Frozen CSF in 2 mL polypropylene tubes

IMPORTANT!
FROZEN SAMPLES MAY BE SHIPPED MONDAY-WEDNESDAY ONLY! **Only ONE set of samples may be shipped in a single package.**

1. Contact FedEx® to confirm service is available and schedule package pickup.

2. Notify Indiana University of shipment by e-mailing ppmibio@iu.edu (preferred) or faxing (317-321-2003) a copy of the completed Sample Record Summary and Shipment Notification Form (Appendix B)

3. Place all frozen 2 mL aliquot vials in the provided cardboard cryobox. Label the outside of the cryobox with the subject ID and visit number.

4. Place the cryobox into a clear plastic biohazard bag with the absorbent sheet and seal according to the instructions on the bag.

5. 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.

6. 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 Record Summary and Shipment Notification Form 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 FedEx® Dry Ice label may result in FedEx® rejecting or returning your package.

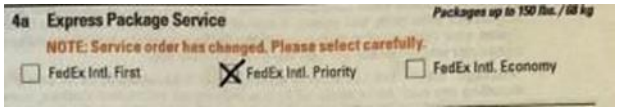
11. To ship, Complete the FedEx® return air waybill with the following information:

    a. **Section 1, From:** Enter the date, coordinator name, phone number, and complete address.

    b. **Section 2, To:** This information will be preprinted with PPMI's return address and phone number.

    c. **Section 3, Shipment Information:** Total Packages, Weight, and Box Dimensions are required. Be consistent between this International FedEx® return airbill and the International Commercial Invoice. Do not declare the value of the shipment to be over $2,500. This would require additional paperwork (a Shipper's Export Declaration form).

    d. **Section 4, Express Package Services:** Please ensure FedEx® Intl. Priority is checked. (Pictured)



    e. **Section 5, Packaging:** Please select "Other".

f. **Section 6a, Special Handling and Delivery Signature Options**: Ensure that under "Does this shipment contain dangerous goods?" that the boxes for "Yes, Shipper's Declaration not required" and "Dry Ice" are checked. Enter the number of packages (1) x the net weight of dry ice in kg.

g. **Section 6b Broker Selection:** leave blank.

h. **Section 7, Payment:** Verify that "Recipient" is checked and that this section is completed with PPMI's FedEx® account number. Duties and Taxes will also be billed to the recipient.

i. **Section 8, Required Signature:** This section must be signed by the sender or department representative.

12. Peel the backing from the clear sleeve and attach to the package. Place the printed waybill 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.

15. Hold packaged samples in a -80°C freezer until the time of FedEx® pickup.

16. International Commercial Invoice (See Appendix Y)
    a. The International Commercial Invoice must be completed and placed with the International return airway bill. Include **ONE** original and **THREE** copies of this completed form with the FedEx® return airway bill.

    b. Complete **Shipped From** with coordinator name, address, and any additional contact information.

    c. Confirm **Shipped To, "Consignee**" with the shipping address information:

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

d. Complete **Number of Packages** and **Shipping Weight** to match the information recorded within the International FedEx® return air waybill.

e. Immediately below the shipping weight is a section asking for the Country of Origin, Description of Goods, Quantity, Unit Price, and Total Price. Please be as detailed as possible within this section (example pictured below).

COUNTRY OF ORIGIN & PROVINCE, IF CANADA *PAYS D'ORIGINE ET PROVINCE, SI CANADA*	DESCRIPTION OF GOODS *DESCRIPTION DES MARCHANDISES*	QUANTITY *QUANTITÉ*	UNIT PRICE *PRIX UNITAIRE*	TOTAL PRICE *PRIX TOTAL*
Canada, Vancouver	Non-Infectious, non-contagious, human Plasma and Buffy Coat sample	1 Box (11 Aliquots)	100.00	100.00

f. Tally the **Total Price** for all goods included in the shipment in the last column. Reminder: the total price/value of the shipment should not exceed $2,500.

g. Complete the final section with a signature.

h. All specimens should be sent to the address above via **FedEx® International Priority**.

i. Use FedEx® tracking to ensure the delivery occurs as scheduled and is received by the PPMI biorepository.

APPENDIX Y: 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 (i.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 MEASURE	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 2/00 LOGOS 116547 WCS

APPENDIX Z: PPMI BIOPSY SHIPPING INSTRUCTIONS – CANADA

Preparing Cold Pack Biopsy Sample Packaging/Shipment to Indiana University

Samples Shipped on Cold Pack:

- Skin biopsy sample – 1 tissue cassette
  - (2 cassettes for sites with Amendment 2 approval)

IMPORTANT!
REFRIGERATE COLD PACKS AT 4°C BEFORE USE **COLLECT AND SHIP BIOPSY SAMPLES MONDAY-THURSDAY ONLY**

Two components are necessary for international shipments:
Section A. International FedEx® Return Air Waybill
Section B. International Commercial Invoice

Section A

1. Contact FedEx® to confirm service is available and schedule package pickup.

2. Notify Indiana University of shipment by e-mailing ppmibio@iu.edu (preferred) or faxing (317-321-2003) a copy of the completed Sample Record Summary and Shipment Notification Form (Appendix W).

3. Ensure that tissue containers are completely and properly sealed. Insert a large absorbent sheet into a biohazard bag (both provided) and place a tissue container, with cassette inside, into the bag and seal it.

4. Insert two cold packs into the ambient shipping container provided. Insert the specimen container(s) into the shipping container. If necessary, add paper toweling or other material as padding.

5. Replace the lid on the Styrofoam carton, place the completed Sample Record Summary and Shipment Notification Form on top of the carton, and close and seal the outer cardboard shipping carton with packing tape.

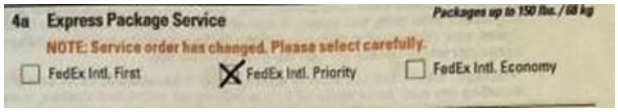
6. To ship, Complete the FedEx® return air waybill with the following information:

   a. **Section 1, From:** Enter the date, coordinator name, phone number, and complete address.

   b. **Section 2, To:** This information will be preprinted with PPMI's return address and phone number.

   c. **Section 3, Shipment Information:** Total Packages, Weight, and Box Dimensions are required. Be consistent between this International FedEx® return airbill and the International Commercial Invoice. Do not declare the value of the shipment to be over $2,500. This would require additional paperwork (a Shipper's Export Declaration form).

   d. **Section 4, Express Package Services:** Please ensure FedEx® Intl. Priority is checked. (Pictured)



e. **Section 5, Packaging:** Please select “Other”.

f. **Section 6, Special Handling and Delivery Signature Options**: Leave Blank

g. **Section 7, Payment:** Verify that “Recipient” is checked and that this section is completed with PPMI’s FedEx® account number. Duties and Taxes will also be billed to the recipient.

h. **Section 8, Required Signature:** This section must be signed by the sender or department representative.

7. Peel the backing from the clear sleeve and attach to the package. Place the printed waybill 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.

Section B

8. International Commercial Invoice (See Appendix Y)

a. The International Commercial Invoice must be completed and placed with the International return airway bill. Include **ONE** original and **THREE** copies of this completed form with the FedEx® return airway bill.

b. Complete **Shipped From** with coordinator name, address, and any additional contact information.

c. Confirm **Shipped To, “Consignee**” with the shipping address information:

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

d. Complete **Number of Packages** and **Shipping Weight** to match the information recorded within the International FedEx® return air waybill.

e. Immediately below the shipping weight is a section asking for the Country of Origin, Description of Goods, Quantity, Unit Price, and Total Price. Please be as detailed as possible within this section (example pictured below).

COUNTRY OF ORIGIN & PROVINCE, IF CANADA *PAYS D'ORIGINE ET PROVINCE, SI CANADA*	DESCRIPTION OF GOODS *DESCRIPTION DES MARCHANDISES*	QUANTITY *QUANTITÉ*	UNIT PRICE *PRIX UNITAIRE*	TOTAL PRICE *PRIX TOTAL*
Canada, Vancouver	Non-Infectious, non-contagious, human Plasma and Buffy Coat sample	1 Box (11 Aliquots)	100.00	100.00

f. Tally the **Total Price** for all goods included in the shipment in the last column. Reminder: the total price/value of the shipment should not exceed $2,500.

g. Complete the final section with a signature.

h. Hold packaged samples in a 4°C refrigerator until the time of FedEx® pickup.

i. Ship the samples to Indiana University on the day of collection.

NOTE: 10% formalin solutions contain 3-4% formaldehyde and are **not** regulated for transport by air or highway according to the US DOT and IATA regulations. However, please follow all guidelines dictated by your institution for packaging and shipping formalin-fixed tissue samples.