

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)			
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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: 1-317-274-5744

International Phone: (00+1) 317-274-5744

Email: ppmibio@iu.edu

Tatiana Foroud, PhD

Core Leader

Jan Hamer, MPH, PMP, CCRP

Project Manager

Email: jehamer@iu.edu

Caitlin Bamrick, CCRP

Study Coordinator

Phone: 317-278-1166

Email: caschu@iu.edu

Sarah McQuillen

Study Coordinator

Phone: 317-278-3131

Email: saealexa@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

Repository and Biobank Director
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 repository.

2.2.1 Holiday Observations – United States

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

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

2.2.2 Holiday Observations – Canada

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

BioRep also provides shipping materials for sites shipping to their facility (see contact information in [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

3.4 Research Biospecimen Collection Kit Contents

Research Specimen Collection Kits provide the supplies necessary to collect samples from one participant 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 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 Kit and Label Ordering

Sites must order Research Specimen Collection kits through the kit ordering module at kits.iu.edu/ppmi. Kits should be ordered with as much advanced notice as possible to ensure all necessary supplies can be prepared and delivered before the 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 on how to order supplies through the kit ordering module.

3.6 Assessments

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

Site staff may request these assessments through the kit ordering module ([Appendix F](#)).

4.0 SAMPLE COLLECTION SCHEDULE

4.1 Collection Schedule Table

Visit	SC	V02	V04	V05	V06	V08	V10	V12	V13	V14	V15	V16	V17	V18	V19	V20
Month	-2	6	12	18	24	36	48	60	72	84	96	108	120	132	144	156
Study Arm																
HC																
PD																
Prodromal																
Blood + Urine and CSF Collection Kits (RNA, Plasma, Buffy Coat, Serum, Whole blood, Urine, CSF)																
Blood + Urine Collection Kits (RNA, Plasma, Buffy Coat, Serum, Whole blood, and Urine)																
Blood + Urine, CSF, and Skin Biopsy Collection Kits (RNA, Plasma, Buffy Coat, Serum, Whole blood, Urine, CSF, and Skin Biopsy)																

4.2 Skin Biopsy

4.2.1 Single Punch (Frozen Collection) – [Appendix R](#)

For sites in Europe, Tel-Aviv, and Africa, a single punch skin biopsy should be collected from:

- 1) Subjects transitioning from the previous PPMI schedule of activities to the current PPMI schedule of activities at the first feasible post-transition visit.
- 2) Newly enrolled PPMI subjects at BL, V06, and V10.

4.2.2 Double Punch (Frozen & Fixed Collections) – [Appendix S](#)

US/Canadian sites will collect double punch skin biopsies from all participants. 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 S](#) 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 S](#) 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.

AM4 Screening Visit collections may be shipped any day of the week.

For all other visits: Ship Frozen samples Monday – Wednesday only.
Ship Ambient samples Monday – 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 V](#) for detailed instructions regarding frozen sample shipment.

Please refer to [Appendix X](#) 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 Blood and Tissue

Site:

Site Investigator:

Coordinator:

Telephone:

Email:

Instructions: **AM4 Screening Visit samples may be shipped any day of the week. Ship frozen samples from all other visits Monday – Wednesday ONLY.**
 This form must be completed for all research sample shipments. Use the contact information below to notify the recipient repository via e-mail prior to shipment. 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					Completed by Biorepository
List Subject ID that corresponds to pre-printed labels. List only one Specimen Type per row.					
Participant ID #		Visit		Gender	
Specimen Type	# of Tubes	Date of Draw	Date Frozen	Tube Volume (if less than standard)	Notation of problems
Total # of tubes:					

Date Shipped:

Kit Number:

Tracking #:

IMPORTANT!	
BEFORE SHIPPING, E-MAIL (PREFERRED) OR FAX A COPY OF THE COMPLETED FORM TO THE RECIPIENT BIOREPOSITORY:	
Indiana University ppmibio@iu.edu Phone: 317-274-5744	BioRep ppmi@biorep.it Fax: +39 02 58018471 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 3 mL plastic EDTA tubes
- 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
- Frozen tissue in a 2mL polypropylene tube

IMPORTANT

AM4 SCREENING SAMPLES MAY BE SHIPPED ANY DAY OF THE WEEK.
SHIP FROZEN SAMPLES FROM ALL OTHER VISITS 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 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.

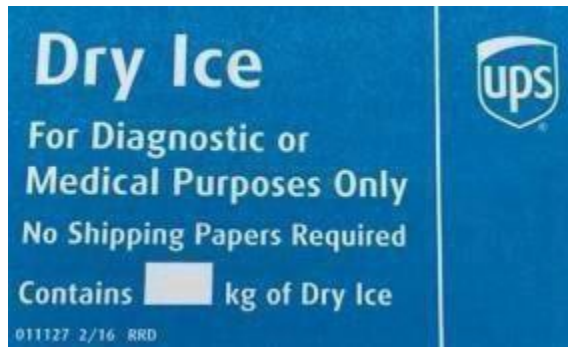
IMPORTANT!

Failure to complete the required fields on the 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 3 mL plastic EDTA tubes
- 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
- Frozen tissue in a 2mL polypropylene tube

IMPORTANT

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

1. Contact BioRep to coordinate shipping via DHL, Marken, or other courier.
2. 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 Notification 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 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.

11. Complete the Class 9 UN1845 Dry Ice label (black and white diamond) with the following information:

- Shipper name and Return Address
- Net weight of dry ice in kg
- 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

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

Quantity	Item
1	6-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	Specimen cup
2	PAXgene™ tube (2.5 mL)
1	Purple-top EDTA tube (10 mL)
2	Purple-top EDTA tube (3 mL)
1	Red-top serum tube (10 mL)
1	Transfer pipette (3mL)
2	Plastic biohazard bag with absorbent sheets

CSF Collection Kit - CSF

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

Skin Biopsy 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	Biohazard Bag
1	Cryobox
1	Low binding cryovial tube (2mL)

Skin Biopsy Visit Kit (Double 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	Low binding cryovial tube (2mL)
1	Tissue specimen storage container, pre-filled with 10% buffered formalin
1	Tissue cassettes
2	Sponges
2	Cold packs – REFRIGERATE AT 4° UPON RECEIPT
2	Plastic biohazard bag with absorbent sheets
1	Cryobox

Skin Biopsy Visit Kit (Double Frozen Punch Protocol)

*Select Sites Only

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

Supplemental Kit Components

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

Frozen Shipping Kit

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

Ambient Shipping Kit

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

International Shipping Kit

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

Visit Label Roll

Quantity	Item
52	Sample Labels
6	Kit Number Labels

APPENDIX F: KIT ORDERING MODULE

Upon site startup, each site will receive one Supplemental Kit as well as an initial supply of three Blood + Urine collection kits, three CSF collection kits, and 3 Skin Biopsy kits.

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 supplies and order replacements as needed. Expiration dates should be monitored as well.

Orders placed through the kit module will ship within two weeks; please provide as much notice as possible when ordering.

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

APPENDIX G: SAMPLE COLLECTION INFORMATION

1. Sample Collection

Per the visit schedule, the following Research Samples can be collected:

- 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 imaging and biospecimen collection, all biospecimens must be collected prior to tracer injection.

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

2. Sample Collection Volumes

Sample Type	Amount
Whole Blood for RNA	2 x 2.5 mL
Whole Blood for Plasma & Buffy Coat	10 mL
Whole Blood for Serum	10 mL
Whole Blood	2 x 3 mL
Urine	10 – 15 mL
CSF	15 – 20 mL
Skin Biopsy	skin punches ~ 3mm and 3 mm deep. 1 or 2 biopsies depending on site-specific protocol.

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

4. **General Clinical Labs**

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

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 or ppmi_smc@indd.org for more information.

APPENDIX H: SAMPLE LABELING INFORMATION

1. Specimen Labels

Labels must be affixed on all collection and aliquot tubes to ensure unique specimen identity. The PPMI Biorepository Team at IU provides labels for all research samples being collected under the PPMI Clinical protocol. The site is responsible for providing labels for biospecimens that will be retained at the site. If labels are provided but the sample is not collected, please discard the unused labels.

1.1 Types of Labels



Kit Labels link all specimens collected from a single participant at one visit. There will be a different Kit Number for each visit participant visit. These labels are used on:

- Kit Bags
- Sample Form (Appendix B)
- Sample Cryobox



Specimen Labels are placed on all sample collection and aliquot tubes, as detailed in sample-specific appendices.

1.2 Label Packaging

Label Rolls will be packaged separately from the sample collection kits. Each roll of labels will contain all labels required for blood, urine, CSF, and skin biopsy collections. The below sticker checklist will be fixed to each bag. Site coordinators should complete the checklist ahead of a visit to ensure all necessary kits are grabbed for any one participant visit. After a single visit collection is complete, any unused labels should be discarded.

PPMI ID: _____

Kit #: _____

Visit #: _____

Blood

CSF

Skin Biopsy

1.3 Recording the Kit Number

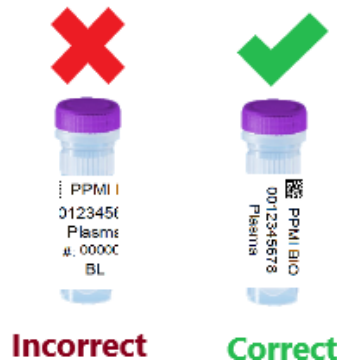
It is critical to record this kit number on the visit Sample Form (Appendix B and/or Appendix U) and in each Research Sample CRF in EDC for that participant visit. This kit number connects samples to a specific patient visit. **If the Kit Number is not recorded properly in the EDC and on the Sample Forms, there is an increased risk that the samples will not be connected back to their donor.**

2. Labeling Samples

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

- Place specimen labels on ALL collection and aliquot tubes BEFORE sample collection, sample processing, or freezing. This will help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.
- Place specimen labels 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 specimen label is completely adhered to each tube. It may be helpful to roll the tube between your fingers after applying the label.

Aliquot Tube Labeling Diagram



- To ensure each label is scanned appropriately upon receipt at the biorepository.
 - Place labels with barcode near the cap as pictured below.
 - Barcodes are scanned via robotic system and must be oriented properly.



APPENDIX I: FILLING ALIQUOT TUBE

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

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

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

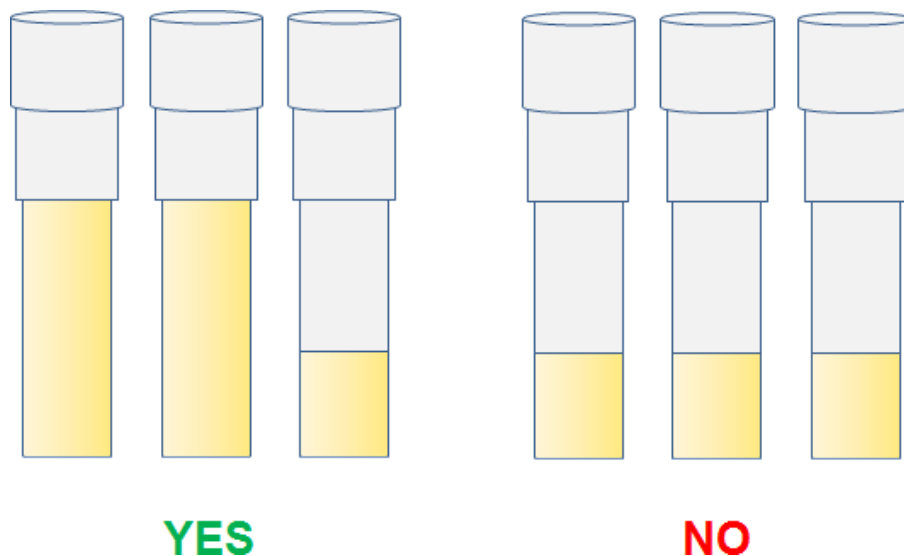
To ensure that the biorepository receives enough of the specimen for processing and storage, and to avoid cracking of the tubes prior to shipment, each aliquot tube should be filled to 1.5 milliliters (see picture, right) after processing is completed (refer to detailed processing instructions per specimen 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).



APPENDIX J: SAMPLE SUBMISSION NONCONFORMANCE REPORT

Repository Name: IU

Subject ID:

Site #:

Received Date:

Visit Type:

Submission Type: Ambient Frozen **Shipping Issues Noted:**

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

Sample Collection Issues Noted:

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

Details/Comments:

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 “RNAPAX10” specimen label on the PAXgene™ Tubes prior to blood draw ([Appendix H](#)).
4. Using a blood collection set and a holder, collect blood into the **first of the two** PAXgene™ RNA Tubes using site recommended procedure for standard venipuncture technique.



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

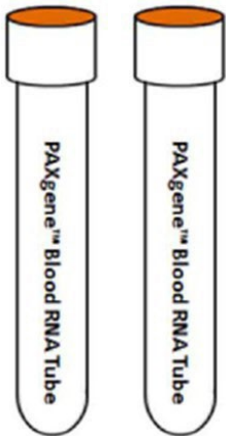
11. Keep the PAXgene™ RNA Tubes at -80°C until packed for shipment on dry ice. AM4 Screening Visit samples should be shipped as soon as possible. Samples from all other visits should be shipped within two weeks of collection, following the instructions in [Appendix C](#) (US sites shipping to IU), [Appendix D](#) (sites shipping to BioRep), or [Appendix V](#) (Canadian sites shipping to IU).

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

Collection Schematic: Whole Blood Preparation (2.5 ml PAXgene™ Tube)

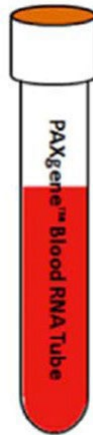


Step One



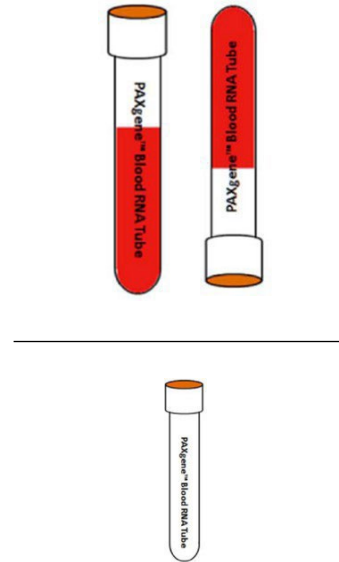
- Store tubes at room temperature.
- Label tubes with pre-printed specimen labels prior to blood draw.

Step Two



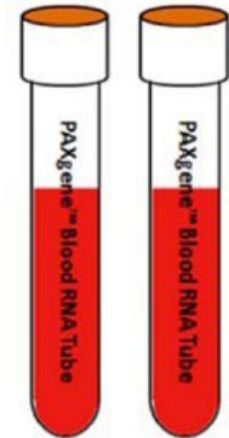
- Collect blood into one PAXgene™ Tube.
- Allow blood to flow for at least 10 seconds.
- Ensure blood flow into tube has stopped before ending collection.

Step Three



- Immediately after blood draw, invert tube 8-10 times to mix sample.
- Repeat Steps 2-3 for each collection tube (2 total).

Step Four



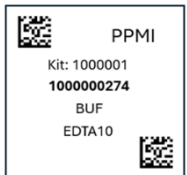
- After 24 hours incubation at room temperature, store upright at -80°C in a wire rack until shipment

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

See collection schematic on following pages.



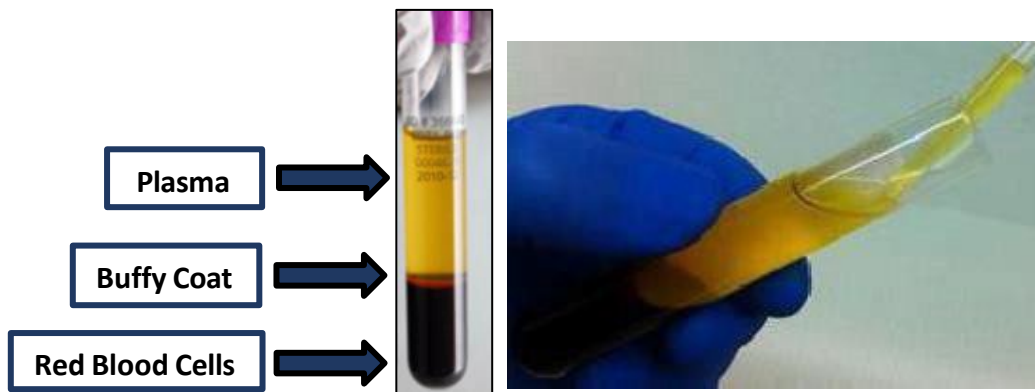
1. **CRITICAL STEP:** Store 10 mL EDTA Tubes at room temperature, 64°F - 77°F (18°C to 25°C) before use.
2. Complete labeling prior to blood draw (per [Appendix H](#)).
 - a. Place a “WBLD, EDTA10” Label on the 10mL EDTA tube
 - b. Place “PLA, EDTA10” specimen labels on the 15 mL Centrifuge Tube and the purple capped 2 mL aliquot tubes.
 - c. Place “BUF, EDTA10” specimen label on the clear capped 2 mL aliquot tube.
3. Using a blood collection set and a holder, collect blood into the 10 mL EDTA Tube using site recommended procedure for standard venipuncture technique.



The following techniques can be used to prevent possible backflow:

- a. Place donor's arm in a downward position.
 - b. Hold tube in a vertical position, below the donor's arm during blood collection.
 - c. Release tourniquet as soon as blood starts to flow into tube.
 - d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.
4. Allow at least 10 seconds for a complete blood draw to take place. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The vacuum within the 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 Kit Number, time of draw, 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 are 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 collection tube, placing the pipette tip along the lower side of the tube wall to ensure the buffy coat and packed red blood cells at the bottom of the collection tube are not agitated. See picture below.



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. A small amount of residual plasma above the buffy coat and some of the RBCs underneath of the buffy coat will be collected in this process. View the diagram below for a brief visual representation of what this isolation will look like.



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. AM4 Screening Visit samples should be shipped as soon as possible. Samples from all other visits 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 V](#) (Canadian sites shipping to IU)

Collection Schematic: Plasma and Buffy Coat Preparation (10 mL Purple Top Tube)

Step One



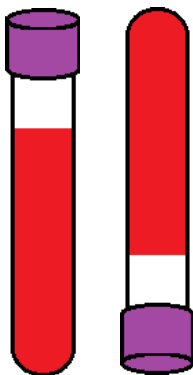
- Store tubes at room temperature until use.
- Label collection tube and cryovials appropriately prior to blood draw.

Step Two



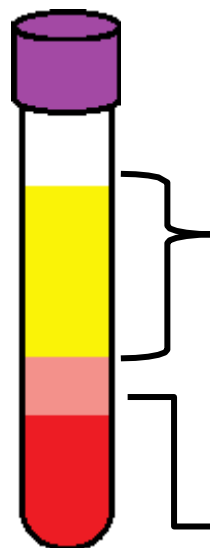
- Collect blood in 10mL EDTA tube.
- Allow blood to flow for at least 10 seconds.
- Ensure blood flow into tube has stopped before ending collection.

Step Three



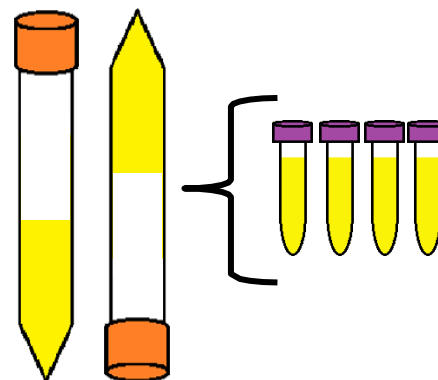
- Immediately after blood draw, invert tubes 8-10 times to mix sample.

Step Four



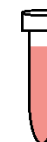
- Within 30 minutes of blood draw, centrifuge sample at 4°C, for 15 minutes, at 1500 x g.

Step Five



- Using a clean transfer pipette, transfer all plasma into one 15mL conical tube
- Mix gently by inverting 3-4 times.
- Aliquot 1.5mL plasma into each "PLASMA" labeled cryovial.
- Store aliquots upright, at -80°C in provided cryobox until shipment.

Step Six



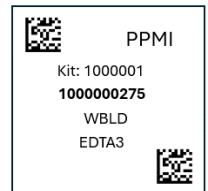
- Using a clean transfer pipette, collect buffy coat (will include residual plasma and some red blood cells).
- Transfer the buffy coat to the "BUFFY COAT" labeled cryovial.
- Store aliquots upright at -80°C in provided cryobox until shipment

APPENDIX M: WHOLE BLOOD COLLECTION (3ML EDTA TUBE)

See collection schematic on following pages



1. **CRITICAL STEP:** Store 3 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 “WBLD EDTA3” specimen label on the 3 mL EDTA Tubes.
3. Using a blood collection set and a holder, collect blood into the 3 mL EDTA Tubes using site recommended procedure for standard venipuncture technique.



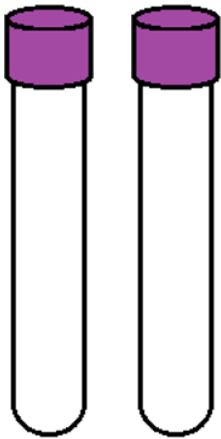
These techniques can be used to prevent possible backflow:

- a. Place donor's arm in a downward position.
 - b. Hold tube in a vertical position, below the donor's arm during blood collection.
 - c. Release tourniquet as soon as blood starts to flow into tube.
 - d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.
4. Allow at least 10 seconds for a complete blood draw to take place. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The vacuum within the 3 mL EDTA Tube is designed to draw 3 mL of blood into the tube.
 5. **CRITICAL STEP:** Immediately after blood collection, gently invert/mix (180 degree turns) the 3 mL EDTA tube 3 - 4 times.
 6. Repeat steps 3-5 for the second 3 mL EDTA tube.
 7. Record Kit Number, time of draw, and date of draw in the study database.
 8. Immediately transfer tubes to a -80°C Freezer. The samples should be frozen and stored upright in a wire or plastic test tube rack.
 9. Transfer the rack into a -80°C freezer. **DO NOT** store/freeze samples in a solid Styrofoam test tube holder.

10. Enter remaining sample collection data into the study database.
11. Keep the 3 mL EDTA Tubes at -80°C until packed for shipment on dry ice.
12. AM4 Screening Visit samples should be shipped as soon as possible. Samples from all other visits 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 V](#) (Canadian sites shipping to IU).

Collection Schematic: Whole Blood Collection and Preparation (2x3mL EDTA Purple Top Tube)

Step One



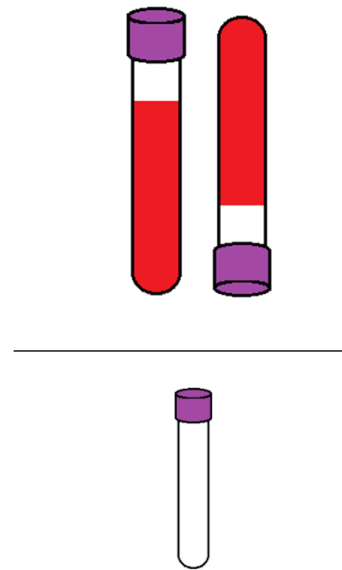
- Store tubes at room temperature.
- Label tubes with "WBLD" specimen labels prior to blood draw.

Step Two



- Collect blood into one 3mL EDTA tube.
- Allow blood to flow for at least 10 seconds.
- Ensure blood flow into tube has stopped before ending collection.

Step Three



- Immediately after blood draw, invert tube 3-4 times to mix sample.
- Repeat steps 2-3 for each collection tube (2 total).

Step Four



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

APPENDIX N: WHOLE BLOOD COLLECTION - SERUM DETERMINATION PROCESSING

See collection schematic on following pages.



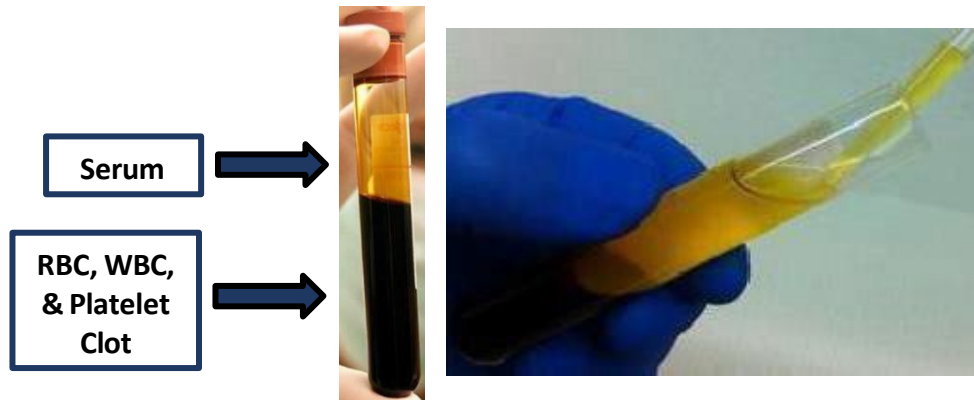
1. **CRITICAL STEP:** Store Serum Determination Tubes at room temperature, 64°F - 77°F (18°C to 25°C) before use.
2. Place “WBLD, SER10” specimen labels on the 10mL Serum Determination Tube
3. Place “SER, SER10” specimen Labels on the 15 mL Centrifuge Tube and the red capped 2 mL aliquot tubes. Complete labeling prior to blood draw (per [Appendix H](#)).
4. Using a blood collection set and a holder, collect blood into the Serum Determination Tube using site recommended procedure for standard venipuncture technique.




The following techniques can be used to prevent possible backflow:

- a. Place donor’s arm in a downward position.
 - b. Hold tube in a vertical position, below the donor’s arm during blood collection.
 - c. Release tourniquet as soon as blood starts to flow into tube.
 - d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.
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 Serum Determination Tube is designed to draw 10 mL of blood into the tube.
 6. **CRITICAL STEP:** Immediately after blood collection, gently invert/mix (180 degree turns) the Serum Determination Tube 8-10 times.
 7. **CRITICAL STEP:** Allow blood to clot at room temperature for at least 15 minutes.
 8. Record Kit Number, time of draw, and date of draw in the study database.
 9. 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](#).

10. Record the time of centrifuge start in the study database.
11. 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.



12. Mix the serum gently by inverting the 15 mL centrifuge tube 3-4 times.
13. 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.
 
14. 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.
15. Discard the used Serum Determination Tube and 15 mL centrifuge tube according to site guidelines for disposing of biomedical waste.
16. Enter remaining sample collection data into the study database.
17. AM4 Screening Visit samples should be shipped as soon as possible. Samples from all other visits 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 V](#) (Canadian sites shipping to IU).



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

Step One



- Store tubes at room temperature until use.
- Label collection tube and cryovials appropriately prior to blood draw.

Step Two



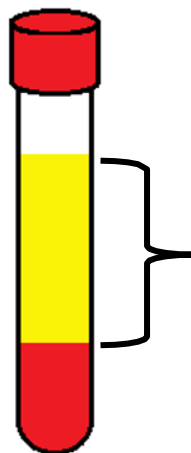
- Collect blood in 10mL Serum tube.
- Allow blood to flow for at least 10 seconds.
- Ensure blood flow into tube has stopped before ending collection.

Step Three



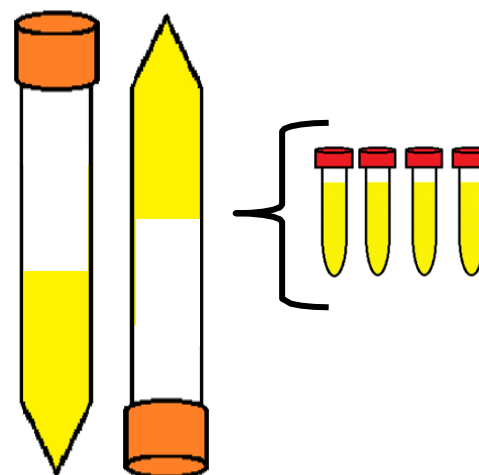
- Immediately after blood draw, invert tubes 8-10 times to mix sample.

Step Four



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

Step Five



- Using a clean transfer pipette, transfer all serum into one 15mL conical tube.
- Mix gently by inverting 3-4 times.
- Aliquot 1.5mL serum into each "SERUM" labeled cryovial.
- Store aliquots upright, at -80°C in provided cryobox 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 "URI-CUP" specimen label.
2. Ask study subject to collect a urine specimen in the collection cup. Urine should be collected midstream to remain as sterile as possible.
3. Pour urine specimen into one labeled 15 mL centrifuge tube, ensure the tube is 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.
5. While centrifuging, record the time of collection and centrifuge start in the study database. Or Record Kit Number, time of draw, and date of draw in the study database?
6. Using a clean transfer pipette, carefully transfer supernatant from the 15 mL processing centrifuge tube into the second, labeled, 15 mL conical tube.
7. Firmly cap with the included screw cap.
8. Discard the original processing tube per your institution's guidelines.
9. Place the labeled tube upright in dry ice or at -80°C and allow the sample to freeze completely.
10. Within 60 minutes of sample collection, freeze and store samples at -80°C. Samples should be frozen and stored upright.
11. Enter remaining sample data into the study database.
12. AM4 Screening Visit samples should be shipped as soon as possible. Samples from all other visits 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 V](#) (Canadian sites shipping to IU).



COLLECTION SCHEMATIC: Urine Collection and Processing

Step One



- Label 15ml centrifuge tubes and urine collection cup with "URINE" specimen label.
- Subject provides sample.
- Record date and time of sample collection

Step Two



- Pour approximately 10mL of urine specimen into one 15mL centrifuge tube.
- Ensure pre-printed label is attached to 15mL centrifuge tube.

Step Three



- Within 30 minutes of sample collection, centrifuge sample at 4°C, for 15 minutes, at 2500 x g.
- Record temperature of centrifuge and time of centrifugation.

Step Four



- Using a clean transfer pipette, transfer 15ml of supernatant from the centrifuged tube into a new, labeled, 15ml conical tube.
- Secure transferred sample with the orange screw cap.

Step Five

DRY
ICE

- Place urine aliquot upright in dry ice and allow the sample to completely freeze.
- Within 60 minutes of collection, freeze and store samples upright at -80°C.
- Record time sample was placed in freezer.

APPENDIX P: DETAILED CSF SAMPLE PROCESSING PROCEDURE

CSF is processed at Room Temperature [64°F – 77°F (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 “CSF” specimen label on the 15 mL centrifuge tubes and 2 mL cryovial aliquot tubes (per [Appendix H](#)).
 - a. Prepare at least 10 aliquot tubes, 14 Clear tops and 1 purple top, 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).



Note: Bedside LP should be performed at the L3-S1 interspace, as distal as possible. Fluoroscopy-guided LP is preferably performed at L3-L4 but may occur at L2-L3 if considered safe, at the discretion of the radiologist.

5. After the LP has begun and fluid is being collected, take the first 1-2 mL of CSF from the first syringe and place in the CSF labs tube (PURPLE TOP).
 - 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.
 7. Record the Kit Number, time of draw, and date of draw in the study database.
 8. Within 15 minutes of collection, spin the remaining CSF sample down **at 2000 x g for 10 minutes at room temperature**, 64°F – 77°F (18°C to 25°C).
 9. While centrifuging, record the time of centrifuge start in the study database.

10. Using a clean transfer pipette, transfer CSF from both 15 mL conical tubes into a 50 mL conical tube, leaving debris undisturbed at the bottom of the 15 ml tubes.
11. Gently invert the 50 mL conical tube 3-4 times to mix the sample.
12. Using a pipette (micropipette preferred), transfer 1.5 mL of supernatant directly into the labeled, pre-cooled, 2 mL CSF aliquot tubes. This will yield, on average, 10-14 aliquot tubes per subject. Use more aliquot tubes if needed. **Do not discard any CSF.** Seal each aliquot tube with a clear cap.
13. Within 60 minutes of CSF collection, freeze aliquoted samples on dry ice and stored at -80°C until they are prepared for shipment on dry ice.
 - a. Samples should be frozen at -80°C and stored upright. A cryobox is provided for proper storage purposes.
14. Complete the remainder of the sample data entry in the study database.

NOTE:

If both gravity and suction collection techniques are used, defer to entering suction in the EDC.

15. AM4 Screening Visit samples should be shipped as soon as possible. Samples from all other visits 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 V](#) (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 of medication transfer filter straw is required to decrease any chance of microscopic glass particles from breaking of the glass ampule. Guidance is provided in the document: *Filter Straw Directions for Use*. File may be found in Florence under Biologics and LabCorp Manuals, Documents
4. Use adequate local anesthesia. Use the 25g 1/2" needle and inject lidocaine to

raise a skin wheal. Then, inject lidocaine using the pattern of a square—first the center, and then to all 4 corners. If the 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).

5. Increasing fluid intake immediately after LP is helpful.
6. 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

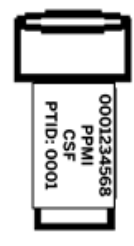
- Mild to Moderate headache following lumbar puncture usually resolves within 3-4 days.
- Treatment of Mild to Moderate headache
- Limit physical activity as much as possible.
- Oral fluids and caffeine are helpful. Drinking a can of Mountain Dew soft drink (for example) is preferable to coffee, which 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.

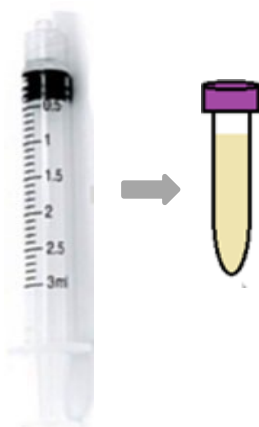
COLLECTION SCHEMATIC: CSF COLLECTION AND PREPARATION

Step One



- Label all cryovials and conical tubes with “CSF” specimen labels prior to collection.
- Pre-chill all labeled cryovials on wet ice.

Step Two



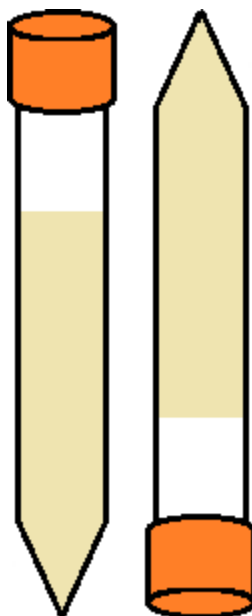
- Collect CSF into the 3mL luer lock syringe.
- Dispense 1-2mL into the purple capped cryovial.
- Send to local lab for testing.

Step Three



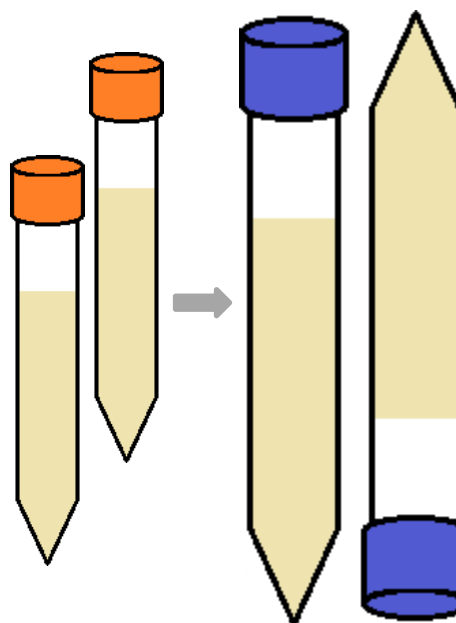
- Collect CSF into the 6mL luer lock syringe.
- Collect 15-20mL total, including the amount sent for local lab testing.
- Transfer the CSF to 15mL conical tubes.

Step Four



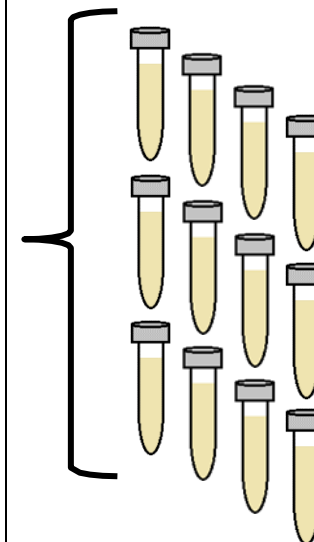
- Immediately after collection, gently invert the 15mL conical tubes 3-4 times to mix the sample.
- Within 15 minutes of collection, centrifuge sample at room temperature, for 10 minutes, at 2000 x g.

Step Five



- Using a clean transfer pipette, transfer all CSF into one 50mL conical tube, leaving debris in the bottom of the 15mL tubes.
- Gently invert the 50mL conical tube 3-4 times to mix the sample.

Step Six



- Aliquot 1.5mL into each clear capped “CSF” cryovial.
- Store CSF aliquots upright, at -80°C in provided cryobox until shipment.

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 collected after an 8 hour fast (no food or drink except fluids such as water, tea, black coffee)**. If fasting is not achievable, a participant should be on a low-fat diet for at least 8 hours prior to blood or CSF collection.

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

<u>Sample Breakfast Items:</u>	<u>Sample Lunch Items:</u>
<ul style="list-style-type: none"> • Dry whole wheat toast • Poached egg white or egg substitute • Plain oatmeal or other whole grains topped with fresh or dried fruit • Dry cereal • Fruit salad • Clear tea or coffee • Fruit or vegetable juice 	<ul style="list-style-type: none"> • Turkey breast sandwich on whole wheat bread with Lettuce, Tomato, and Mustard • Plain pasta with plain marinara sauce • Assorted vegetables (steamed or raw) and green salads • Steamed chicken breast - lean, without skin • Clear broth with vegetables and pasta • Fruit salad, Flavored gelatin • Clear beverages
<p>For all suggestions above, avoid:</p> <ul style="list-style-type: none"> • Oils • Butter • Dressings • Cheese • Avocado • Red Meats • Nuts • Granola • Milk • Cream 	

Low-Fat Diet Menu Suggestions

Additional foods to avoid prior to blood and CSF collection:

Avoid: All fats and nuts such as:

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

Avoid: All milk and dairy products such as:

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

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

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

APPENDIX R: SKIN BIOPSY COLLECTION – SINGLE PUNCH

Note: The Single Punch Protocol is intended for European, Tel Aviv, and African sites. This may also be used by US and Canadian sites should a patient refuse a second biopsy. US and Canadian sites are to otherwise follow the Double Punch protocol.

AM4 Screening Visit samples should be shipped as soon as possible. Samples from all other visits should be shipped within two weeks of collection

1. Skin Biopsy Supplies

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
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	Low protein binding cryovials (2mL)
1	Cryobox
1	Biohazard Bag

1.2. Setting Up the Kit

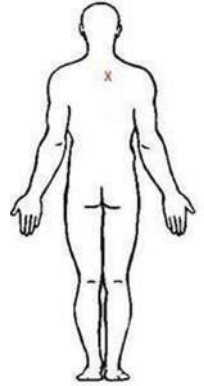
- 1.2.1. On an overbed table, remove the contents of the kit from the outer packaging, leaving all sterile contents wrapped in their packaging. Leave everything wrapped until the person performing the biopsy is seated and begins examining the subject.
- 1.2.2. Open the sterile kit components, touching 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 within 4 cm of the midline (see figure below). This single biopsy will be frozen, without additive, in a 2mL cryovial.

1.3.1. Pre-collection Steps – Preparation of Patient

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

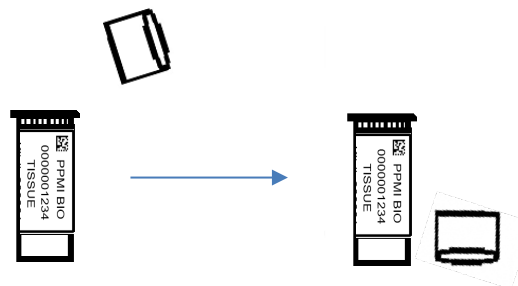


1.3.2. Preparation Steps – Cryovial for Frozen Tissue Collection

- 1.3.2.1. Label the cryovial with a “FrT, STERCNT, FROZEN” specimen label.



- 1.3.2.2. Record the kit number on the Sample Record Summary and Shipment Notification Form ([Appendix B](#)) for frozen tissue and in the EDC.
- 1.3.2.3. Open the cryovial and place cryovial lid to the side in a sterile location.



1.3.3. Biopsy Collection Procedure

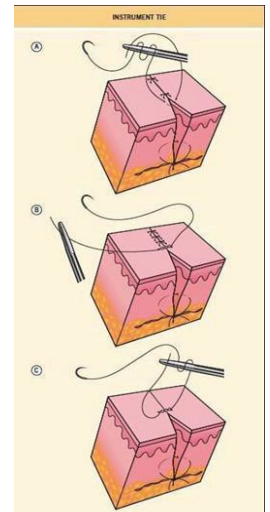
- 1.3.3.1. Ensure that the biopsy site has been properly sterilized with alcohol wipes. A punch biopsy is a clean procedure, not a sterile procedure, and therefore, sterile gloves and gown are not required. Wearing safety glasses is recommended.
- 1.3.3.2. Anesthetize the area by injecting Lidocaine 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. 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:** Place the specimen directly into the prepared “TISSUE” cryovial and close the cryovial cap securely.

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 $\frac{1}{2}$ to $\frac{1}{3}$ 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 exit 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.

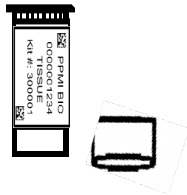


1.4. Single Punch Biopsy Processing

- 1.4.1. **CRITICAL STEP:** Place the cryovial in the provided cryobox and freeze at -80°C as soon as possible.
- 1.4.2. Record Time Frozen in the EDC. Store at -80°C until shipped to repository on dry ice.
- 1.4.3. Place a follow-up call to the subject 2-3 after the procedure to assess for adverse events.

COLLECTION SCHEMATIC: SINGLE PUNCH BIOPSY COLLECTION AND PREPARATION

Step One



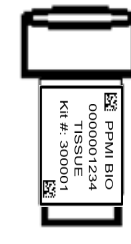
- Label 2ml cryovial with “TISSUE” specimen label.
- Remove lid from 2mL cryovial. Set aside in a sterile location.

Step Two



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

Step Three



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

Step Four



- As soon as possible after collection, store samples upright in the provided cryobox, at -80°C.
- Record time sample was placed in freezer in EDC.
- Ship according to site appropriate Frozen Shipping Appendix

APPENDIX S: SKIN BIOPSY COLLECTION – DOUBLE PUNCH

US AND CANADA SITES ONLY

AM4 Screening Visit Frozen samples should be shipped as soon as possible. Samples from all other visits should be shipped within two weeks of collection

Formalin Fixed skin biopsy samples must be shipped on the day of collection and received at the biorepository the day after collection. **Formalin fixed biopsy collections that are not for an AM4 Screening Visit should not be performed or shipped on Fridays.**

In cases where only one skin biopsy is collected, sites should prioritize processing per the Frozen Tissue Preparation instructions below.

1. Skin Biopsy Supplies

The double punch skin biopsy kit contains the items listed in the table below, which will be used to perform the skin punch biopsy procedure. Check the dates of expiration of all kit components before use. Place cold packs in the refrigerator at 4°C upon arrival the cold pack must be chilled for at least 24 hours ahead of the planned shipment. Note that sutures and needle drivers will be provided in each site's supplemental supplies and should be on hand and ready in case they are necessary for this procedure.

1.1. Double Punch Biopsy Kit Components

Quantity	Kit Component
1	Sterile drape
1	Tweezers
2	Gauze pads
2	Alcohol prep pads
1	Scissors
2	Skin biopsy punch tool with plunger
1	Gelfoam sterile compressed sponge
1	Vaseline ointment packet
1	Coverlet adhesive dressing
1	Transparent film dressing
1	Low binding 2mL cryovial
1	Cryobox
2	Biohazard Bags
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

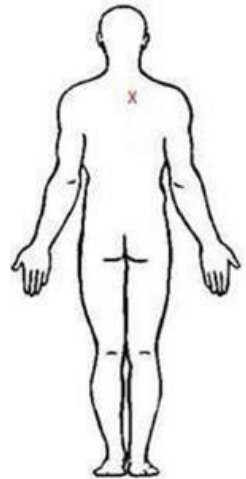
- 1.2.1. On an overbed table, remove the contents of the kit from the outer packaging, leaving all sterile contents wrapped in their packaging. Leave everything wrapped until the person performing the biopsy is seated and begins examining the subject.
- 1.2.2. Open the sterile kit components, touching only the outside of the wrapper. Don't let the outside of the wrapper touch any part of the inside of the kit.

1.3. Skin Sample Collection

Two skin biopsies will be obtained from the cervical paravertebral region at approximately the C8 level within 3 cm of the midline (see figure below). One biopsy will be frozen, without additive, in a 2mL cryovial. The second biopsy will be placed in formalin. Where two biopsies are attempted but only one is collected, sites should process the single biopsy as a [Frozen Tissue Collection](#).

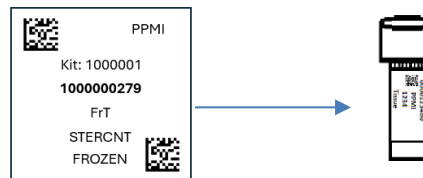
1.3.1. Pre-collection Steps – Preparation of Patient

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



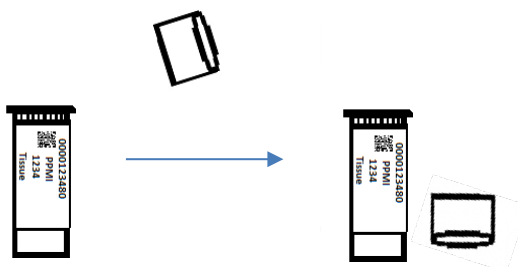
1.3.2. Preparation Steps – Cryovial for Frozen Tissue Collection

- 1.3.2.1. Label the cryovial with a “FrT, STERCNT, FROZEN” specimen label.



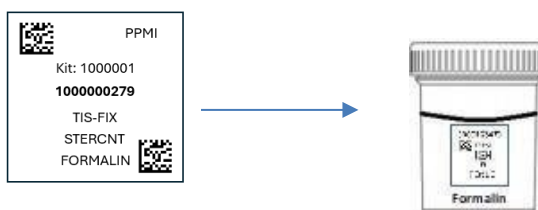
- 1.3.2.2. Record the Kit number on the Sample Record Summary and Shipment Notification Form ([Appendix B](#)).

- 1.3.2.3. Open the cryovial and place cryovial lid to the side in a sterile location.

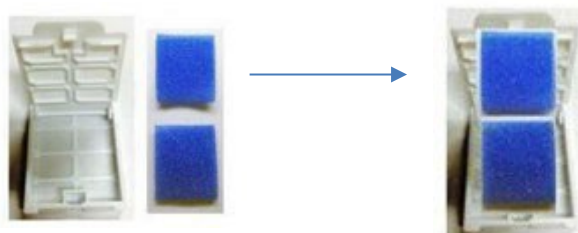


1.3.3. Preparation Steps – Container and Cassette for Fixed/Formalin Tissue Collection

- 1.3.3.1. Label the formalin tissue container with a “TIS-FIX, STERCNT, FORMALIN” specimen label



- 1.3.3.2. Refrigerate cold packs at 4°C for 24 hours before use
- 1.3.3.3. Record the 4-digit barcode label number from the cassette on the Sample Record Summary and Shipment Notification Form ([Appendix U](#)).



- 1.3.3.4. Place the sponges on the top and bottom sections of the cassette.

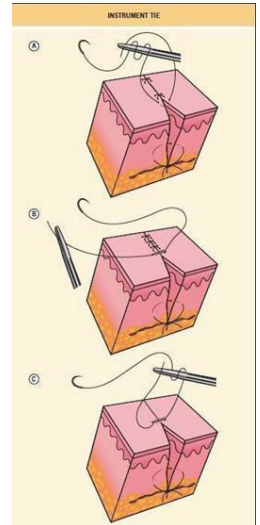
1.3.4. Biopsy Collection Procedure

- 1.3.4.1. Ensure that the biopsy site has been properly sterilized with alcohol wipes. A punch biopsy is a clean procedure, not a sterile procedure, and therefore, sterile gloves and gown are not required. Wearing safety glasses is recommended.
- 1.3.4.2. Anesthetize the area by injecting Lidocaine with epinephrine solution (Lidocaine HCL 1% with epinephrine 1:100,000) just under the epidermis (subepidermally) using a 3-cc syringe just prior to the biopsy. The injection should continue until a “bleb” or small bubble forms under the skin (approximately 3 mm in diameter). The injection will burn slightly (much like a bee sting) due to a pH difference between the skin and the solution. Injecting slowly decreases the burning sensation. The burning will subside quickly, and the site will become numb. It is

acceptable to massage the area. Both biopsy sites can be anesthetized at the same time.

- 1.3.4.3. After the Lidocaine injection, the area anesthetized may be marked using a pen if helpful to the individual completing the biopsy. The area to be biopsied should be checked to ensure the skin is properly anesthetized. This can be tested by gently pressing the needle to the area. If the patient experiences neither pain nor sharp sensation, the area is ready to be biopsied. Experiencing a pressure sensation is normal, but there should be no pain. If the area requires more anesthesia, another injection of Lidocaine solution is made with a new syringe.
- 1.3.4.4. Using a sterile 3 mm skin punch, place the punch perpendicular to the skin, in the paravertebral C8 region, within 3 cm of the midline. Apply constant downward pressure while twirling the punch tool between the thumb and index finger, rotating clockwise and counterclockwise until the blade has pierced the epidermis of the skin and the metal part of the punch tool is buried (there will be a “give” once the punch reaches the subcutaneous fat). Once the tool has reached the lowest point, lift the tool straight up.
- 1.3.4.5. Depress the plunger to remove the specimen. Forceps may be needed to remove the specimen. If the specimen remains connected at the level of the subcutaneous fat, it may be necessary to cut at the base of the specimen to remove it. Do not try to tear a specimen that remains connected, as it may damage the specimen. Using a punch with a plunger should help to ensure that the epidermis is not crushed or damaged during the process.
- 1.3.4.6. **CRITICAL STEP:** Place the specimen directly into the prepared “TISSUE” cryovial and close the cryovial cap securely.
- 1.3.4.7. To restore hemostasis, hold pressure with gauze for approximately 30 seconds. Wipe any excess blood with a sterile 2x2 gauze to expose the site. Pack biopsy site with GelFoam. Apply the Vaseline ointment to the bandage and cover biopsy site. This can be reinforced with gauze and tape if necessary. If the biopsy site is oozing, apply a pressure bandage by applying Vaseline to small gauze and then apply Tegaderm. Other closure options include using a steri-strip and transparent film dressing closure system. In most cases, suturing a wound will not be necessary. Placing a suture can be considered if the wound base is still oozing after packing with GelFoam. To place a suture, grip the needle using the forceps approximately $\frac{1}{2}$ to $\frac{1}{3}$ 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 exit the skin on the opposite side of the wound, again, 2 mm from the wound edge. To tie the suture, hold the needle holder parallel to the axis of the wound and at the center of the wound.

- 1.3.4.8. Wrap the free end of the suture twice around the holder, then grasp the free end and pull through, tightening the knot. Repeat with just looping around the needle holder once for repeat knots. Tie 3 knots (see figure below)
- 1.3.4.9. Collect a second biopsy 3 cm above or below the original collection site on the same side of the midline and following the same procedure.
- 1.3.4.10. Place the second biopsy directly into the sponge-lined cassette.
- 1.3.4.11. Close the cassette securely by bringing the lid down onto the bottom and snapping it.
- 1.3.4.12. The study coordinator or appointed site personnel will be responsible for completing the processing of the tissue once collected using the procedures described in detail below.
- 1.3.4.13. 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. Double Punch Biopsy Processing

- 1.4.1. **CRITICAL STEP:** Place the cryovial in the provided cryobox and freeze at -80°C as soon as possible. Record Time Frozen on the [Appendix B](#). Store at -80°C until shipped to repository on dry ice.
- 1.4.2. **CRITICAL STEP:** Submerge the cassette into the formalin-filled container as soon as possible. Record Time placed in Formalin on the [Appendix U](#).
- 1.4.3. Place Formalin Container in a refrigerator until shipment (shipment should occur day of collection).
- 1.4.4. Ship the formalin-fixed biopsy samples to the repository at 4°C according to [Appendix T](#) (US sites shipping to IU) or [Appendix X](#) (Canadian sites shipping to IU) **on the day of collection**.
- 1.4.5. 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. Please follow all guidelines dictated by your institution for packaging and shipping formalin-fixed tissue samples.

COLLECTION SCHEMATIC: DOUBLE PUNCH SKIN BIOPSY COLLECTION AND PREPARATION

Step One



- Label 2mL cryovial and Formalin Container with "TISSUE" specimen label.
- Remove lid from 2mL cryovial. Set aside in a sterile location.

Step Two



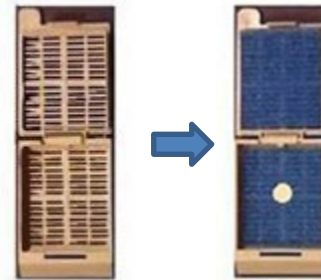
- Using standard punch biopsy procedures, collect two biopsies from the paravertebral C8 region approximately 3mm in diameter and 3mm deep.
- Record time of biopsy collection.

Step Three



- Place one biopsy into the 2mL cryovial.
- As soon as possible after collection, store samples upright in the provided cryobox, at -80°C.
- Record time placed in freezer in the EDC.
- Ship according to site appropriate Frozen Shipping Appendix.

Step Four



- Open the pre-labeled cassette and place the blue sponges into the top and bottom of the cassette.
- Record the cassette label numbers on Appendix U.
- Place a biopsy into the cassette.
- If rinsing the punch is required to release the biopsy, use a saline solution.

Step Five



- As soon as possible after collection, close cassette securely and submerge in the provided formalin cup.
- Record time placed in formalin in EDC.
- Close Formalin cup securely and store at 4°C until shipment.
- Ship on day of collection according to Appendix T procedures.

APPENDIX S.1: SKIN BIOPSY COLLECTION – DOUBLE FROZEN PUNCH

SELECT SITES ONLY

Frozen skin biopsy samples may be shipped on dry ice and packaged with other frozen samples (plasma, serum, etc.) AM4 Screening Visit samples should be shipped as soon as possible. Samples from all other visits should not be shipped on Thursday or Friday.

1. Skin Biopsy Supplies

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

1.1. Double Frozen Punch Biopsy Kit Components

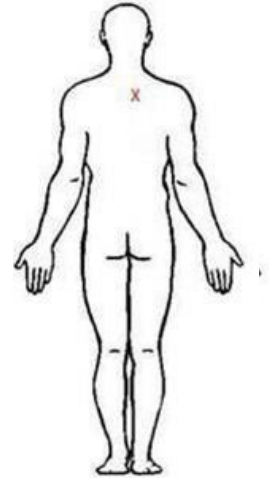
Quantity	Kit Component
1	Sterile drape
1	Tweezers
2	Gauze pads
2	Alcohol prep pads
1	Scissors
2	Skin biopsy punch tool with plunger
1	Gelfoam sterile compressed sponge
1	Vaseline ointment packet
1	Coverlet adhesive dressing
1	Transparent film dressing
2	Low binding 2mL cryovial
1	Cryobox
2	Biohazard Bags

1.2. Setting Up the Kit

- 1.2.1. On an overbed table, remove the contents of the kit from the outer packaging, leaving all sterile contents wrapped in their packaging. Leave everything wrapped until the person performing the biopsy is seated and begins examining the subject.
- 1.2.2. Open the sterile kit components, touching only the outside of the wrapper. Don't let the outside of the wrapper touch any part of the inside of the kit.

1.3. Skin Sample Collection

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

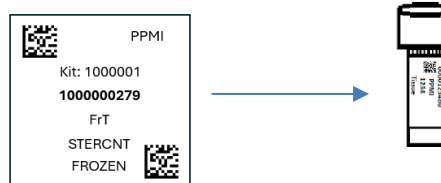


1.3.1. Pre-collection Steps – Preparation of Patient

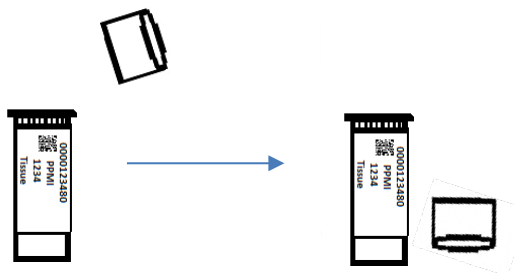
- 1.3.1.1. Prepare patient for procedure per institution guidelines.
- 1.3.1.2. Before the biopsies are collected, the volunteer will be screened and complete the informed consent for the skin biopsy procedure. The doctor will explain the study, and the volunteer will have an opportunity to ask questions.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

1.3.2. Preparation Steps – Cryovials for Frozen Tissue Collections

- 1.3.2.1. Label the cryovials with “FrT, STERCNT, FROZEN” specimen labels.



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



1.3.4. Biopsy Collection Procedure

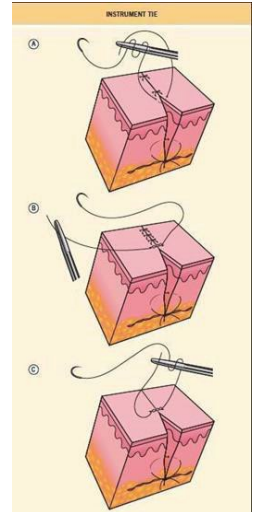
- 1.3.4.1. Ensure that the biopsy site has been properly sterilized with alcohol wipes. A punch biopsy is a clean procedure, not a sterile procedure, and therefore, sterile gloves and gown are not required. Wearing safety glasses is recommended.
- 1.3.4.2. Anesthetize the area by injecting Lidocaine with epinephrine solution

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

- 1.3.4.3. After the Lidocaine injection, the area anesthetized may be marked using a pen if helpful to the individual completing the biopsy. The area to be biopsied should be checked to ensure the skin is properly anesthetized. This can be tested by gently pressing the needle to the area. If the patient experiences neither pain nor sharp sensation, the area is ready to be biopsied. Experiencing a pressure sensation is normal, but there should be no pain. If the area requires more anesthesia, another injection of Lidocaine solution is made with a new syringe.
- 1.3.4.4. Using a sterile 3 mm skin punch, place the punch perpendicular to the skin, in the paravertebral C8 region, within 3 cm of the midline. Apply constant downward pressure while twirling the punch tool between the thumb and index finger, rotating clockwise and counterclockwise until the blade has pierced the epidermis of the skin and the metal part of the punch tool is buried (there will be a “give” once the punch reaches the subcutaneous fat). Once the tool has reached the lowest point, lift the tool straight up.
- 1.3.4.5. Depress the plunger to remove the specimen. Forceps may be needed to remove the specimen. If the specimen remains connected at the level of the subcutaneous fat, it may be necessary to cut at the base of the specimen to remove it. Do not try to tear a specimen that remains connected, as it may damage the specimen. Using a punch with a plunger should help to ensure that the epidermis is not crushed or damaged during the process.
- 1.3.4.6. Place the specimen directly into the prepared “TISSUE” cryovial and close the cryovial cap securely.
- 1.3.4.7. To restore hemostasis, hold pressure with gauze for approximately 30 seconds. Wipe any excess blood with a sterile 2x2 gauze to expose the site. Pack biopsy site with GelFoam. Apply the Vaseline ointment to the bandage and cover biopsy site. This can be reinforced with gauze and tape if necessary. If the biopsy site is oozing, apply a pressure bandage by applying Vaseline to small gauze and then apply Tegaderm. Other closure options include using a steri-strip and transparent film dressing closure system. In most cases, suturing a wound will not be necessary. Placing a suture can be considered if the wound base is still oozing after packing with GelFoam. To place a suture, grip the needle using the

forceps approximately $\frac{1}{2}$ to $\frac{1}{3}$ 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 exit the skin on the opposite side of the wound, again, 2 mm from the wound edge. To tie the suture, hold the needle holder parallel to the axis of the wound and at the center of the wound.

- 1.3.4.8. Wrap the free end of the suture twice around the holder, then grasp the free end and pull through, tightening the knot. Repeat with just looping around the needle holder once for repeat knots. Tie 3 knots (see figure below).
- 1.3.4.9. Collect a second biopsy 3 cm above or below the original collection site on the same side of the midline and following the same procedure.
- 1.3.4.10. Place the second biopsy directly into the sponge-lined cassette.
- 1.3.4.11. Close the cassette securely by bringing the lid down onto the bottom and snapping it.
- 1.3.4.12. The study coordinator or appointed site personnel will be responsible for completing the processing of the tissue once collected using the procedures described in detail below.
- 1.3.4.13. 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.

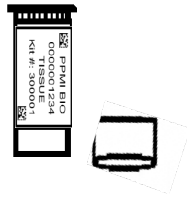


1.4. Double Frozen Punch Biopsy Processing

- 1.4.1. **CRITICAL STEP:** Place the cryovials in the provided cryobox and freeze at -80°C as soon as possible.
- 1.4.2. Record Time Frozen in the EDC. Store at -80°C until shipped to repository on dry ice.
- 1.4.3. Place a follow-up call to the subject 2-3 after the procedure to assess for adverse events.

COLLECTION SCHEMATIC: DOUBLE FROZEN PUNCH SKIN BIOPSY COLLECTION AND PREPARATION

Step One



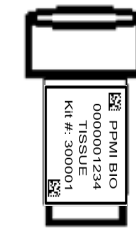
- Label 2mL cryovials with “TISSUE” specimen labels.
- Remove lid from 2mL cryovials. Set aside in a sterile location.

Step Two



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

Step Three



- Place the biopsy into the 2mL cryovial.
- Close the 2mL cryovial securely.
- Repeat steps One through Three for second biopsy.

Step Four



- As soon as possible after collection, store samples upright in the provided cryobox, at -80°C.
- Record time samples were placed in freezer in EDC.
- Ship according to site appropriate Frozen Shipping Appendix

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 in Formalin filled container

IMPORTANT
REFRIGERATE COLD PACKS AT 4°C 24 HOURS BEFORE USE.
AM4 Screening Visit Frozen samples should be shipped as soon as possible. Samples from all other visits should be shipped within two weeks of collection.

1. Contact UPS® to confirm service is available and schedule package pickup.
2. Notify Indiana University of shipment by 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 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. An automatic notice will be sent to UPS® to pick up your package. If you receive an email from UPS® stating it is too late in the day to schedule a pick-up, you will need to make other arrangements to get the package to UPS®.
- 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: SAMPLE FORM – Formalin Fixed Skin Biopsy (US and Canada)

PPMI Sample Record Summary and Shipment Notification Form – Skin Biopsy

Site: _____ **Site Investigator:** _____

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

Instructions: **AM4 Screening Visit samples may be shipped any day of the week. Ship frozen samples from all other visits Monday – Wednesday ONLY.** This form must be completed for all research sample shipments. Use the contact information below to notify the recipient repository via e-mail prior to shipment. 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.

<u>Completed by Submitter/Site</u>			<u>Completed by Biorepository</u>
List Subject ID that corresponds to pre-printed labels.			
Participant ID #	Visit	Gender	Notation of problems

Cassette Label #: _____

Date Shipped: _____

Tracking #: _____

Kit Number: _____

<p>IMPORTANT! BEFORE SHIPPING, E-MAIL (PREFERRED) OR FAX A COPY OF THE COMPLETED FORM TO THE BIOREPOSITORY:</p>
<p>Indiana University ppmibio@iu.edu Phone: 317-274-5744</p>

APPENDIX V: 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
- Frozen Tissue in 2mL polypropylene tubes

IMPORTANT

AM4 Screening Visit Frozen samples should be shipped as soon as possible. Samples from all other visits should be shipped within two weeks of collection.

1. Contact FedEx® to confirm service is available and schedule package pickup.
2. Notify Indiana University of shipment by 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 Kit Number label.
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 W](#))
 - 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 QUANTITE	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 W: INTERNATIONAL COMMERCIAL INVOICE

COMPANY NAME _____

COMPANY ADDRESS _____

COMMERCIAL INVOICE

INTERNATIONAL AIR WAYBILL NO.		<small>(NOTE: All shipments must be accompanied by a Federal Express International Air Waybill.)</small>
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 WEIGHT	TOTAL INVOICE VALUE	

SEE REVERSE SIDE FOR HELP WITH THE ABOVE SECTION

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 _____

- Check one
 F.O.B.
 C & F
 C.I.F.

APPENDIX X: PPMI BIOPSY SHIPPING INSTRUCTIONS – CANADA

Preparing Cold Pack Biopsy Sample Packaging/Shipment to Indiana University

Samples Shipped on Cold Pack:

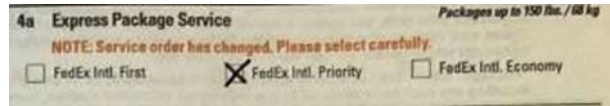
- Skin biopsy sample – 1 tissue cassette

IMPORTANT
REFRIGERATE COLD PACKS AT 4°C 24 HOURS BEFORE USE.
AM4 Screening Visit Frozen samples should be shipped as soon as possible. Samples from all other visits should be shipped within two weeks of collection.

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 U](#)).
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 W](#))
 - 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).

<small>COUNTRY OF ORIGIN & PROVINCE, IF CANADA PAYS D'ORIGINE ET PROVINCE, SI CANADA</small>	<small>DESCRIPTION OF GOODS DESCRIPTION DES MARCHANDISES</small>	<small>QUANTITY QUANTITE</small>	<small>UNIT PRICE PRIX UNITAIRE</small>	<small>TOTAL PRICE PRIX TOTAL</small>
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.