Parkinson's Progression Markers Initiative

PPMI

Biospecimen Collection, Processing, and Shipment Manual

Table of Contents

1.0 Biorepository Information

1.1 Biorepository Contacts

1.1.1 Indiana University Study Support

General Study Contact Information
Phone: 317-274-5744
International Phone: (00+1) 317-274-5744
e-mail: ppmibio@iu.edu
Fax: 317-278-1100
International Fax: (00+1) 317-278-1100

Tatiana Foroud, PhD, Core Leader
Phone: 317-274-2218
International Phone: (00+1) 317-274-2218

Danielle Smith, BS, Project Manager
Phone: 317-274-5744
International Phone: (00+1) 317-274-5744

Rose Case, BS, Clinical Research Coordinator
Phone: 317-274-5120
International Phone: (00+1) 317-274-5120

Emma Morone, BS, Clinical Research Specialist
Phone: 317-278-1191
International Phone: (00+1) 317-278-1191

Sample Shipment Mailing Address
PPMI Biorepository
Indiana University School of Medicine
Walther Hall – R3 C102
980 W. Walnut Street
Indianapolis, IN 46202

1.1.2 BioRep Study Support

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

Giulia Malferrari, Molecular Biology Laboratory Manager
e-mail: ppmi@biorep.it
Phone: +39 02 58029725
After Hours: +39 348 0716025
Fax: +39 02 58018471

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

1.1.3 Tel Aviv Study Support

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

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

1.2 Hours of Operation

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

1.2.2 BioRep business hours are from 8 AM to 7 PM Europe Central Time, Monday through Friday (and if necessary on Saturday morning from 8 AM to 12 PM).

1.2.3 Tel Aviv business hours are from 8:30 AM to 5 PM (GMT+2), Sunday through Thursday.

1.3 Holiday Schedules

Please note that courier services may observe a different set of holidays. Please be sure to verify shipping dates with your courier prior to any holiday. **Weekend/holiday delivery must be arranged in advance with the biorepository.** Individual collection site questions should be directed toward the respective repositories.

Frozen samples must be shipped Monday – Wednesday only.

1.3.1 Holiday Observations – United States

Date
New Year's Day
Martin Luther King, Jr. Day
Memorial Day
Independence Day (observed on Friday if the holiday falls on a Saturday, and observed on Monday if it falls on a Sunday)
Labor Day
Thanksgiving Day and following Friday
Christmas Day (observed on Friday if the holiday falls on a Saturday, and observed on Monday if it falls on a Sunday)

Please note that between December 24th and January 2nd, Indiana University will be open Monday through Friday for essential operations **ONLY** and will re-open for normal operations on January 2nd. If at all possible, biological specimens for submission to Indiana University should **NOT** be collected and shipped to Indiana University between December 24th and January 2nd. Should it be necessary to ship blood samples for DNA extraction to Indiana University during this period, please contact the Indiana University staff before December 24th by e-mailing ppmibio@iu.edu so that they can arrange to have staff available to process incoming samples. If samples are collected during this holiday period and cannot be shipped, please store them at -70 or -80 degrees Celsius and ship them on dry ice to Indiana University **AFTER** January 1.

1.3.2 Holiday Observations – Europe

Date
1st January
6th January
Easter and Easter Monday
25th April
1st May
2nd June
15th August
1st November
7th and 8th December
25th and 26th December

1.3.3 Holiday Observations – Tel Aviv

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

2.0 Research and Clinical Laboratory Collection Schedule

The following samples will be collected according to the visit schedule noted below.

- Blood for standard clinical safety analysis (not sent to biorepository)
- Serum, plasma, buffy coat, and whole blood suitable for proteomic, metabolomic, and other analyte studies
- Whole blood for DNA sequencing/genomic analysis
- Whole blood for PBMC collection
- RNA for transcriptomic analysis
- Urine for analyte analysis
- Cerebrospinal fluid for analyte analysis

If a sample is not obtained at a particular visit, this should be recorded on the appropriate data form and a reason should be provided.

2.1 Sample Collection Volumes

Sample Type	Amount
Whole Blood for Clinical Labs	4.5-6 ml
Whole Blood for DNA	6 ml
Whole Blood for RNA	5 ml
Whole Blood for Plasma and Buffy Coat	10 ml
Whole Blood for Serum	10 ml
Whole Blood for Storage	6 ml
Whole Blood for PBMCs	10 ml
Urine	10 ml
Cerebrospinal Fluid	15-20 ml

2.2 Protocol Schedules for Biospecimen Sample Collection

Visit Number	SC	BL	V01	V02	V03	V04	V05	V06	V07	V08	V09	V10	V11	V12	V13	V14	V15	PW	ST	Unsch Visit
Visit Description (±30 days)	**-45 d**	**0**	**3**	**6**	**9**	**12**	**18**	**24**	**30**	**36**	**42**	**48**	**54**	**60**	**72**	**84**	**96**	**---**	**---**	**---**
Parkinson Disease (PD) Subjects (also continuing SWEDD subjects for V07-V12)																				
Blood sample for clinical lab	X					X		X		X		X		X	X	X	X	X	X	X
Blood sample for DNA	X																			
Biomic blood sample (WB, RNA, PL, BC, SER)		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	*X	X	
Blood sample for PBMC collection		X		X			X		X		X		X							
Lumbar puncture (CSF collection)		X		X		X		X		X		X		X	**X	**X	**X	*X	X[k]	
Urine sample		X		X		X		X		X		X		X	X	X	X	*X	X	
Healthy Control (HC) Subjects																				
Blood sample for clinical lab	X					X		X		X		X		X		X		X		X
Blood sample for DNA	X																			
Biomic blood sample (WB, RNA, PL, BC, SER)		X	X	X	X	X	X	X	X	X	X	X	X	X		X		*X		
Blood sample for PBMC collection		X		X			X		X		X		X							
Lumbar puncture (CSF collection)		X		X		X		X		X		X		X		**X		*X		
Urine sample		X		X		X		X		X		X		X		X		*X		
SWEDD Subjects																				
Blood sample for clinical lab	X					X		X										X	X	X
Blood sample for DNA	X																			
Biomic blood sample (WB, RNA, PL, BC, SER)		X	X	X	X	X	X	X										*X	X	
Lumbar puncture (CSF collection)		X		X		X		X										*X	X[k]	
Urine sample		X		X		X		X										*X	X	

[k]Not conducted depending on when ST visit completed – See protocol Sect. 5.3.19.

*If not done in 3 months

**Optional

2.2 Protocol Schedules for Biospecimen Sample Collection (continued)

Visit Number	SC	BL	V01	V02	V03	V04	V05	V06	V07	V08	V09	V10	V11	V12	PW	ST	Unsch Visit
Visit Description (±30 days)	**-45 d**	**0**	**3**	**6**	**9**	**12**	**18**	**24**	**30**	**36**	**42**	**48**	**54**	**60**	**---**	**---**	**---**
PRODROMAL Subjects																	
Blood sample for clinical lab	X					X		X		X		X			X	X	X
Blood sample for DNA		X															
Biomic blood sample (WB, RNA, PL, BC, SER)		X	X	X	X	X	X	X	X	X	X	X			*X	X	
Blood sample for PBMC collection		X		X			X		X		X						
Lumbar puncture (CSF collection)		X		X		X		X		X		X			*X	X[k]	
Urine sample		X		X		X		X		X		X			*X	X	
Genetic Cohort: PD Subjects																	
Blood sample for clinical lab	X					X		X		X		X		X	X	X	X
Blood sample for DNA	X																
Biomic blood sample (WB, RNA, PL, BC, SER)		X		X		X	X	X	X	X	X	X	X	X	*X	X	
Blood sample for PBMC collection		X		X			X		X		X		X				
Lumbar puncture (CSF collection)		X				X		X		X		X		X	*X	X[k]	
Urine sample		X		X		X		X		X		X		X	*X	X	
Genetic Cohort: Unaffected Subjects																	
Blood sample for clinical lab	X					X		X		X		X		X	X	X	X
Blood sample for DNA	X																
Biomic blood sample (WB, RNA, PL, BC, SER)		X		X		X	X	X	X	X	X	X	X	X	*X	X	
Blood sample for PBMC collection		X		X			X		X		X		X				
Lumbar puncture (CSF collection)		X				X		X		X		X		X	*X	X[k]	
Urine sample		X		X		X		X		X		X		X	*X	X	
Genetic Registry Subjects																	
Blood sample for clinical lab		X						X				X				X	X
Blood sample for DNA		X															
Biomic blood sample (WB, RNA, PL, BC, SER)		X						X				X				X	
Urine sample		X						X				X				X	

[k]Not conducted depending on when ST visit completed – See protocol Sect. 5.3.19.

*If not done in 3 months

3.0 Specimen Collection Kits and Supplies

Clinical Lab Collection Kits

Clinical lab supplies will be provided to sites by Covance and will include all materials needed for collecting the clinical blood samples, as well as shipping materials (except for dry ice for screening labs). These samples will be shipped to Covance after collection.

Research Biospecimen Collection Kits and supplies

Research specimen collection kits will be provided to sites by Indiana University. Kits will include most of the materials needed for urine, blood, and CSF collection. Kits will include tube labels, which will be pre-printed with study information and the type of sample being drawn. It is important that you check to be sure that all tubes are properly labeled during processing and at the time of shipment. The kits will also include shipping labels and packaging necessary for sending samples back to the PPMI biorepository.

Each Site Will Need to Provide:

Dry Ice	**Crushed Ice**
Alcohol Prep Pads	**Gauze Pads**
Bandages	**Butterfly Needles**
Tourniquets	**Tube Racks (2 ml to 10 ml)**
Gloves	**Sharps Bin and Lid**
Pipettes and Pipette Tips	**Lidocaine (Non-US Sites Only)**

European sites will also obtain DHL shipping materials through BioRep (see contact information in Section 1.1.2.

3.1 Kits Required at Each Visit Based on Subject's Cohort

	Parkinson's Disease	Healthy Control	SWEDD*	Prodromal	Genetic Cohort	Genetic Registry
SC	~~A~~	~~A~~	~~A~~		A	
BL	~~D~~	~~D~~	~~D~~	~~A+D~~	D+E	A+C
V01	~~B~~	~~B~~	~~B~~	~~B~~		
V02	~~D~~	~~D~~	~~D~~	~~D~~	C+E	
V03	~~B~~	~~B~~	~~B~~	~~B~~		
V04	~~D~~	~~D~~	~~D~~	D	D	
V05	B+E	B+E	B	B+E	B+E	
V06	D	D	D	D	D	C
V07	B+E	B+E	B	B+E	B+E	
V08	D	D	D	D	D	
V09	B+E	B+E	B	B+E	B+E	
V10	D	D	D	D	D	C
V11	B+E	B+E	B		B+E	
V12	D	D	D		D	
V13	D					
V14	D	D				
V15	D					
PW	D	D	D	D	D	C
ST	D		D	D	D	
A	Blood for DNA					
B	Biomic Blood (RNA, Plasma, Serum, Buffy Coat, Whole Blood)					
C	Biomic Blood + Urine					
D	Biomic Blood + Urine + CSF					
A+C	Blood for DNA + Biomic Blood + Urine					
D+E	Blood for PBMC+ Biomic Blood + Urine + CSF					
C+E	Blood for PBMC+ Biomic Blood + Urine					
B+E	Blood for PBMC+ Biomic Blood					

*Only continuing SWEDD subjects complete V07-V12

3.2 Indiana University – Specimen Collection Kit Contents

Research collection kits contain the items listed below under each kit type. Each kit provides the necessary supplies to collect samples from **one** subject. PPMI kit components have been carefully selected to suit the needs of this project. Do not replace or supplement any of the tubes or kit components provided by Indiana University with your own supplies unless you have received approval from MJFF/Indiana University to do so. Note that "supplemental" kits will be provided to sites should you require additional supplies from those contained in the visit specific kits. See Section 7.1 for LP Kit contents.

3.2.1 Kit Type A - Blood for DNA

Quantity	Type A Kit Component
1	Ambient shipping box
1	Airtight shipping canister
1	Absorbent tube sleeve
1	Warning label packet
1	FedEx return Airbill (US Sites and Australia)
1	FedEx/DHL Overpack (US Sites and Australia)
1	Vacutainer – Purple-top EDTA tube (6 ml)
1	Shipping instruction sheet

3.2.2 Kit Type B - Biomic Blood

Quantity	Type B Kit Component
2	100 ml absorbent sheets
1	6-tube bubble pouch
1	Cryobox
7	Cryogenic vials (2 ml) – 3 red cap, 3 purple cap, 1 clear cap
1	FedEx return Airbill (US Sites and Australia)
2	Screw-top centrifuge tubes (15 ml)
1	Shipping container for dry ice shipments
2	STP-714 (Biohazard bag)
2	STP-714 (Tyvek bag)
2	Vacutainer - PAXgene™ tubes (2.5 ml)
1	Vacutainer – Purple-top EDTA tube (10 ml)
1	Vacutainer – Purple-top EDTA tube (6 ml)
1	Vacutainer – Red-top serum tube (10 ml)
1	Transfer pipet
1	Warning label packet
1	Shipping instruction sheet

3.2.3 Kit Type C - Biomic Blood and Urine

Quantity	Type C Kit Component
2	100 ml absorbent sheets
1	6-tube bubble pouch
1	Cryobox
7	Cryogenic vials (2 ml) – 3 red cap, 3 purple cap, 1 clear cap
1	FedEx return Airbill (US Sites and Australia)
4	Screw-top centrifuge tubes (15 ml)
1	Shipping container for dry ice shipments
1	Specimen cup
2	STP-714 (Biohazard bag)
2	STP-714 (Tyvek bag)
2	Vacutainer - PAXgene™ tubes (2.5 ml)
1	Vacutainer – Purple-top EDTA tube (10 ml)
1	Vacutainer – Purple-top EDTA tube (6 ml)
1	Vacutainer – Red-top serum tube (10 ml)
1	Transfer pipet
1	Warning label packet
1	Shipping instruction sheet

3.2.4 Kit Type D with LP– EU - Biomic Blood, Urine, and CSF

Quantity	Type D With LP Kit Component
2	100 ml absorbent sheets
1	6-tube bubble pouch
1	Cryobox
22	Cryogenic vials (2 ml) – 3 red cap, 4 purple cap, 15 clear cap
1	Lumbar puncture tray (no Lidocaine)
6	Screw-top centrifuge tubes (15 ml)
1	Shipping container for dry ice shipments
1	Specimen cup
2	STP-714 (Biohazard bag)
2	STP-714 (Tyvek bag)
2	Vacutainer - PAXgene™ tubes (2.5 ml)
1	Vacutainer – Purple-top EDTA tube (10 ml)
1	Vacutainer – Purple-top EDTA tube (6 ml)
1	Vacutainer – Red-top serum tube (10 ml)
1	Transfer pipet
1	Warning label packet
1	Shipping instruction sheet

3.2.5 Kit Type D – No LP - EU - Biomic Blood, Urine, and CSF

Quantity	Kit Type D – No LP EU Kit Component
2	100 ml absorbent sheets
1	6-tube bubble pouch
1	Cryobox
22	Cryogenic vials (2 ml) – 3 red cap, 4 purple cap, 15 clear cap
6	Screw-top centrifuge tubes (15 ml)
1	Shipping container for dry ice shipments
1	Specimen cup
2	STP-714 (Biohazard bag)
2	STP-714 (Tyvek bag)
2	Vacutainer - PAXgene™ tubes (2.5 ml)
1	Vacutainer – Purple-top EDTA tube (10 ml)
1	Vacutainer – Purple-top EDTA tube (6 ml)
1	Vacutainer – Red-top serum tube (10 ml)
1	Transfer Pipet
1	Warning label packet
1	Shipping instruction sheet

3.2.6 Kit Type D With LP – US - Biomic Blood, Urine, and CSF

Quantity	Kit Type D With LP – US Kit Component
2	100 ml absorbent sheets
1	6-tube bubble pouch
1	Cryobox
22	Cryogenic vials (2 ml) – 3 red cap, 4 purple cap, 15 clear cap
1	FedEx return Airbill
1	Lumbar puncture tray (Lidocaine)
6	Screw-top centrifuge tubes (15 ml)
1	Shipping container for dry ice shipments
1	Specimen cup
2	STP-714 (Biohazard bag)
2	STP-714 (Tyvek bag)
2	Vacutainer - PAXgene™ tubes (2.5 ml)
1	Vacutainer – Purple-top EDTA tube (10 ml)
1	Vacutainer – Purple-top EDTA tube (6 ml)
1	Vacutainer – Red-top serum tube (10 ml)
1	Transfer pipet
1	Warning label packet
1	Shipping instruction sheet

3.2.7 Kit Type A + C – Blood for DNA, Biomic Blood, and Urine

Quantity	Type A + C Kit Component
1	Ambient shipping box
1	Airtight shipping canister
1	Absorbent tube sleeve
1	FedEx Overpack (US Sites and Australia)
2	100 ml absorbent sheets
1	6-tube bubble pouch
1	Cryobox
7	Cryogenic vials (2 ml) – 3 red cap, 3 purple cap, 1 clear cap
2	FedEx return Airbills (US Sites and Australia)
4	Screw-top centrifuge tubes (15 ml)
1	Shipping container for dry ice shipments
1	Specimen cup
2	STP-714 (Biohazard bag)
2	STP-714 (Tyvek bag)
2	Vacutainer - PAXgene™ tubes (2.5 ml)
1	Vacutainer – Purple-top EDTA tubes (10 ml)
2	Vacutainer – Purple-top EDTA tube (6 ml)
1	Vacutainer – Red-top serum tube (10 ml)
1	Transfer pipet
2	Warning label packets
2	Shipping instruction sheets

3.2.8 Kit Type E – Blood for PBMC

Quantity	Type A Kit Component
1	Ambient shipping box
1	Airtight shipping canister
1	Absorbent tube sleeve
1	Warning label packet
1	FedEx return Airbill (US Sites)
1	FedEx Overpack (US Sites)
1	Vacutainer – Green-top Sodium Heparin tube (10 ml)
1	Shipping instruction sheet

3.2.9 Supplemental Kit Components

Quantity	Supplemental Kit Component
5	100 ml absorbent sheets
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	FedEx return Airbills (US Sites and Australia)
10	Needles – introducer
10	Needles – Sprotte spinal
30	Screw-top centrifuge tubes (15 ml)
5	Specimen cups
10	STP-714 (Biohazard bag)
10	STP-714 (Tyvek bag)
10	Vacutainer - PAXgene™ tubes (2.5 ml)
5	Vacutainer – Purple-top EDTA tubes (10 ml)
5	Vacutainer – Purple-top EDTA tubes (6 ml)
5	Vacutainer – Red-top serum tubes (10 ml)
5	Vacutainer – Green-top Sodium Heparin tubes (10 ml)
5	Transfer pipets
5	Warning label packets

3.2.10 Extra Supplies

Quantity	Kit Component
10	100 ml absorbent sheets
20	100 ml absorbent sheets
5	6-tube bubble pouches
10	6-tube bubble pouches
5	Cryoboxes
10	Cryoboxes
50	Cryogenic vials (2 ml) with red caps
100	Cryogenic vials (2 ml) with red caps
150	Cryogenic vials (2 ml) with red caps
50	Cryogenic vials (2 ml) with purple caps
100	Cryogenic vials (2 ml) with purple caps
150	Cryogenic vials (2 ml) with purple caps
50	Cryogenic vials (2 ml) with clear caps
100	Cryogenic vials (2 ml) with clear caps
150	Cryogenic vials (2 ml) with clear caps
5	FedEx return Airbills (US Sites and Australia)
10	FedEx return Airbills (US Sites and Australia)
5	FedEx Overpacks (US Sites and Australia)
5	Lumbar puncture trays (Lidocaine)
10	Lumbar puncture trays (Lidocaine)
5	Lumbar puncture trays (no Lidocaine)
10	Lumbar puncture trays (no Lidocaine)
10	Needles – introducer
10	Needles – Sprotte spinal
10	Screw-top centrifuge tubes (15 ml)
20	Screw-top centrifuge tubes (15 ml)
30	Screw-top centrifuge tubes (15 ml)
1	Ambient shipping box
2	Ambient shipping boxes
3	Ambient shipping boxes
1	Shipping container for dry ice shipments
2	Shipping containers for dry ice shipments
3	Shipping containers for dry ice shipments
5	Specimen cups
10	Specimen cups
15	Specimen cups
5	STP-714 (Biohazard bag)

Quantity	Kit Component
10	STP-714 (Biohazard bag)
5	STP-714 (Tyvek bag)
10	STP-714 (Tyvek bag)
10	Vacutainer - PAXgene™ tubes (2.5 ml)
20	Vacutainer - PAXgene™ tubes (2.5 ml)
30	Vacutainer - PAXgene™ tubes (2.5 ml)
5	Vacutainer – Purple-top EDTA tubes (10 ml)
10	Vacutainer – Purple-top EDTA tubes (10 ml)
15	Vacutainer – Purple-top EDTA tubes (10 ml)
5	Vacutainer – Purple-top EDTA tubes (6 ml)
10	Vacutainer – Purple-top EDTA tubes (6 ml)
15	Vacutainer – Purple-top EDTA tubes (6 ml)
5	Vacutainer – Red-top serum tubes (10 ml)
10	Vacutainer – Red-top serum tubes (10 ml)
15	Vacutainer – Red-top serum tubes (10 ml)
5	Vacutainer – Green-top Sodium Heparin tubes (10 ml)
10	Vacutainer – Green-top Sodium Heparin tubes (10 ml)
15	Vacutainer – Green-top Sodium Heparin tubes (10 ml)
5	Transfer pipets
10	Transfer pipets
5	Warning label packets
10	Warning label packets

3.3 Indiana University – Initial Supply

Each site will be initially supplied with the following items:

- (1) Supplemental Kit

Subsequent kits for SC, BL, ST, PW, and any visits that are scheduled to occur before the target window should be ordered from Indiana University, when needed.

3.4 Indiana University – Automatic Kit and Label Distribution

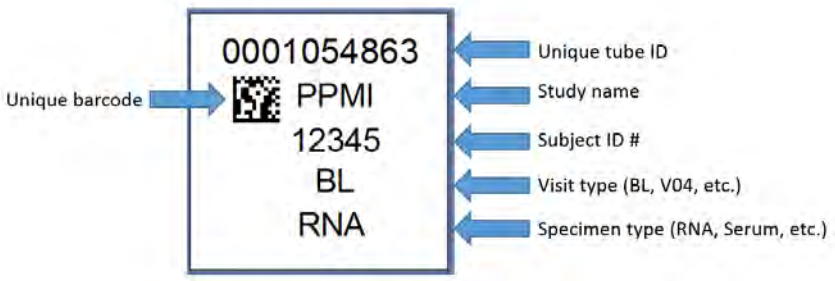
Each site will be responsible for ordering kits (labels included) for genetic pre-screening, SC, and BL visits using Indiana University's online kit ordering module (http://ppmi.iu.edu/kits). **Please note that this is a critical step, as there is no way to automatically distribute kits for new subjects.** After subject enrollment into PPMI (post BL), all kits (including study visit labels, PW labels, and ST visit labels, as applicable) for each subject's subsequent visits will be sent to the site automatically according to the subject's visit schedule. Kits will arrive at the site at least 15 days prior to the start of the 60 day study visit window. However, sites may still order kits and labels on demand through the kit ordering module in the event of an unscheduled visit, lost labels, etc.

3.5 Indiana University – Kit and Label Ordering on Demand

Sites will have the ability to request kits and labels on demand at any time.

Refer to the kit schedule diagram (section 3.1) to verify which kits are needed for a particular visit. In the electronic kit ordering module (http://ppmi.iu.edu/kits), select site number and name from the list provided, this will automatically fill with the site coordinator's contact and shipping information. Verify that this information is correct (and modify it if necessary), and select the kit types and/or labels needed. Provide the quantity of kits needed, as well as the PPMI IDs for the subjects for whom the kits will be used. Click "Submit" to send your request.

Study Labels



3.6 Covance – Initial Supply

Each site will be provided 6 initial screening kits and 2 unscheduled visit kits at start-up from Covance.

3.7 Covance – Resupply

Automatic Resupply: Covance 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 Covance. Please note that this service can result in extra kits being supplied to the sites to ensure appropriate kits quantities are on hand. Sites should actively monitor inventory and expiration dates of kits and shipping materials. Should additional supplies be needed, a minimum of 7 working days is required for kit resupply. If you are located in an extended delivery area your delivery may be longer. Please contact **Covance** if you have any questions.

4.0 Equipment Required at Clinical Sites

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

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

*****Important Note*****

In order to ensure the highest quality samples are collected, processed and stored, it is essential to follow the specific collection, processing and shipment procedures detailed in the following pages.

Please read the following instructions first before collecting any specimens. Have all your supplies and equipment out and prepared prior to drawing blood.

5.0. Blood Collection and Processing Procedures

Blood samples should be collected in the morning between 8am – 10am, preferably after the subject has fasted. If fasting is not feasible, the subject should follow the low fat diet (see Appendix J). Record the time of the subject's last meal (and whether the low fat diet was followed, if applicable) on the Laboratory Procedures Data Form (available from the CTCC).

5.1 Order of Blood Draws

Other than the Screening Visit when only the DNA sample and general clinical labs are collected, tubes should be filled in the following order:

1. 2 x 2.5 ml PAXgene™
2. 1 x 10 ml Plasma and Buffy Coat EDTA Purple Top
3. 1 x 6 ml Whole Blood EDTA Purple Top
4. 1 x 10 ml PBMC Sodium Heparin Green Top (when applicable)
5. 1 x 10 ml Serum Determination Red Top
6. General clinical lab tubes (REFER TO COVANCE LAB MANUAL) – SCREENING AND ANNUAL VISITS ONLY

5.2 Labeling Samples

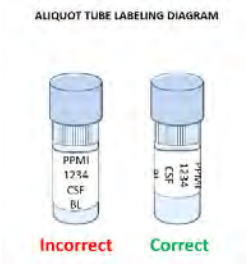
*****_Refer to Appendix I for a supplemental diagram of labeling samples_

In order to ensure the label adheres properly and remains on the tube:

- Place labels on **ALL** collection and aliquot tubes **BEFORE** sample collection, sample processing or freezing. This should help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.

- Place label **horizontally** on the tube (wrapped around sideways if the tube is upright) and **just below the ridges** of the aliquot tubes (see attached labeling diagram). There is enough space on the aliquot tube for the label to be placed without overlapping the ridges.

- Take a moment to ensure the label is **completely adhered** to each tube. It may be helpful to roll the tube between your fingers after applying the label.



5.3 Video List

The following training videos are available to assist you with the PPMI specimen processing, aliquoting, and shipping processes. The videos are available at (http://ppmi.iu.edu/videos).

5.3.1 Plasma and Buffy Coat Processing and Aliquoting
5.3.2 Serum Processing and Aliquoting
5.3.3 CSF Processing and Aliquoting
5.3.4 RNA Processing
5.3.5 Ambient Shipping
5.3.6 Frozen Shipping

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

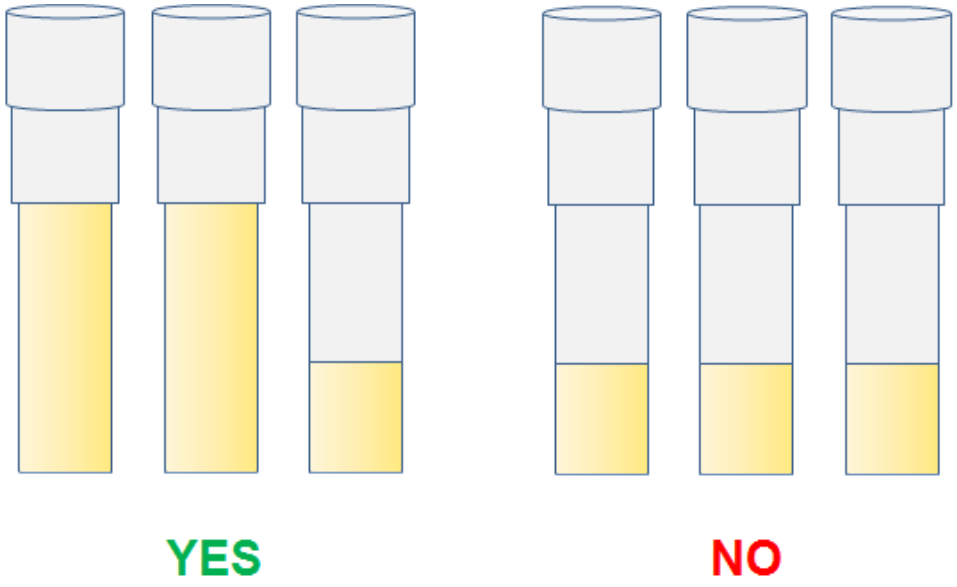
To assist in the preparation and aliquoting of samples, colored caps are used for the aliquot tubes. The chart below summarizes the correspondence between cap color and type of aliquot.

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

In order to ensure that the PPMI biorepositories receive a sufficient amount of the sample for processing and storage, and to avoid cracking of the tubes prior to shipment, each aliquot tube should ideally be filled to 1.5 milliliters (see picture, right) after processing is completed (refer to detailed processing instructions per sample type for average yield per sample 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 placed in the freezer, resulting in a loss of that sample.

If there is biologic material remaining that will not fill a subsequent aliquot tube to 1.5 ml, that remaining amount should still be kept and sent if a partially filled aliquot tube.

All material should be shipped to the appropriate PPMI biorepository. Aliquot the recommended maximum volume into as many aliquot tubes as will allow after processing the biospecimen sample. Fill as many tubes as possible with 1.5 ml of sample. For example, if 3.7 ml of sample is obtained, you should fill 2 cryovial tubes each with 1.5 ml, and one additional cryovial tube with the remaining .7 sample volume (see example below).

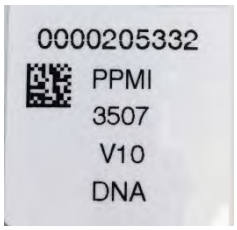


5.5 DNA– SCREENING VISIT ONLY*

DNA samples must be received at the PPMI Biorepository within *5 days* of collection. Samples not received within *5 days* of collection must be re-drawn at the site.

1. CRITICAL STEP: **Store empty Blood for DNA EDTA tube at room temperature, 64°F - 77°F (18°C to 25°C) before use.**

2. Place pre-printed "DNA" label on the 6 ml EDTA tube prior to blood draw (per Section 5.2).



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

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

4. Invert the tube gently 3 times.

5. Seal the EDTA tube in the ambient shipment kit.

6. Ship the sample back to the appropriate PPMI biorepository at <u>room temperature</u> according to kit instructions. If sample cannot be shipped the

same day as collected, hold at room temperature until shipping can be managed. Sample must be received at the appropriate PPMI biorepository within **5 days** of being collected.

7. Complete the DNA Sample data form and ensure timely data entry of data into the eClinical database.

5.6 PAXgene™ RNA

❖ See training video for blood draw for *RNA Processing:* http://ppmi.iu.edu/videos.

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

2. CRITICAL STEP: The **PAXgene™ Blood RNA Tubes should be the first tubes drawn** in the phlebotomy procedure (before CBC, plasma, etc.).

3. Place "RNA" label on the PAXgene™ RNA tubes prior to blood draw (per Section 5.2).

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

   The following techniques shall be used to prevent possible backflow:
   e. Place donor's arm in a downward position.
   f. Hold tube in a vertical position, below the donor's arm during blood collection.
   g. Release tourniquet as soon as blood starts to flow into tube.
   h. 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 in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The PAXgene™ Blood RNA Tube with its vacuum is designed to draw 2.5 ml of blood into the tube. Record time of draw on Laboratory Procedures data form.

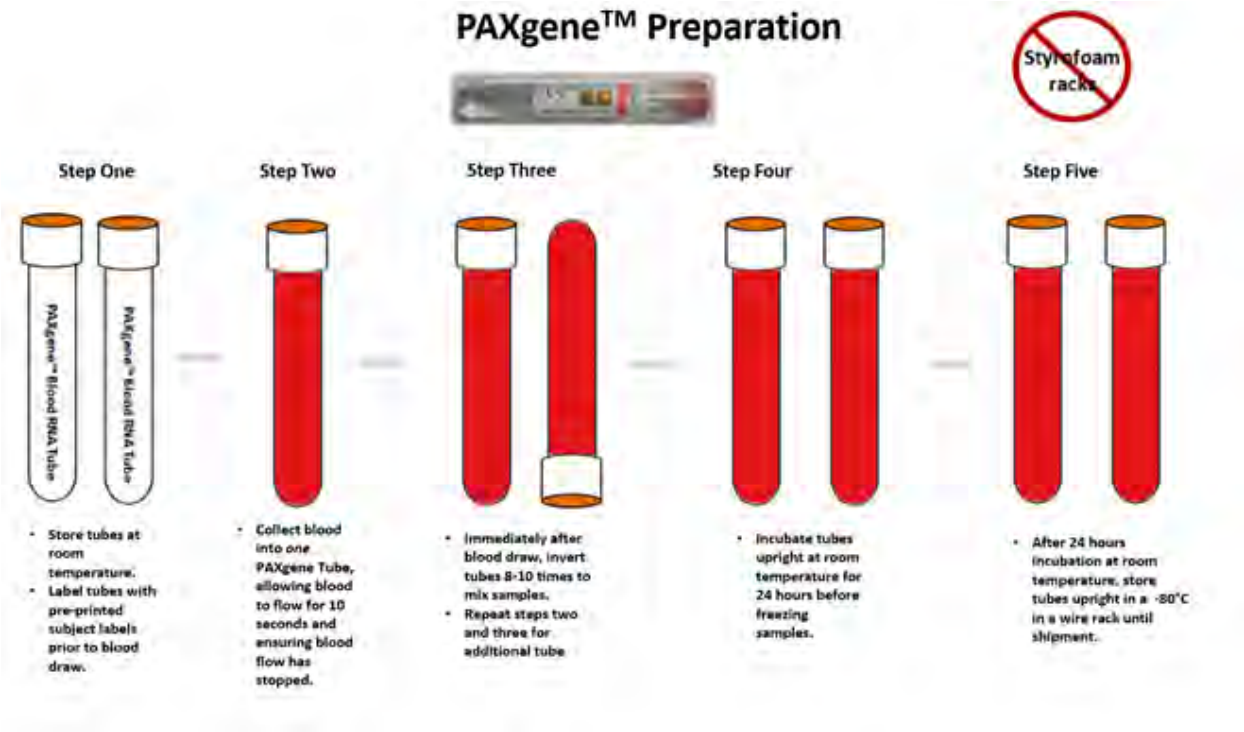
6. CRITICAL STEP: **Immediately after blood collection, gently invert/mix (180 degree turns) the PAXgene™ Blood RNA Tube 8-10 times.**

7. **REPEAT STEPS 4 TO 6** for the second PAXgene™ Blood RNA Tube to be collected.

8. CRITICAL STEP: Incubate the PAXgene™ Blood RNA Tubes **UPRIGHT** at room temperature, 64°F - 77°F (18°C to 25°C) **for 24 hours.** Record time and date of draw on the Laboratory Procedures data form.

   ❖ *If blood is drawn on a Friday and you are unable to return on Saturday to place tubes in the freezer, transfer the tubes as late as possible before leaving on Friday.* ***Samples must sit at room temperature for a minimum of 2 hours.***

9. After **24 hours** at room temperature, place the two PAXgene™ tubes **UPRIGHT** into a WIRE or PLASTIC type test tube rack (DO NOT use a solid Styrofoam test tube holder) and transfer into a **-80°C (minus eighty) freezer.** Keep the two **PAXgene™ Blood RNA Tubes at -80°C** until you ship on dry ice. Complete remainder of the Laboratory Procedures data form. Samples should be shipped within two weeks of collection, following the instructions in Appendix C or D.

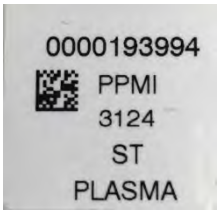

PAXgene™ Preparation
Styrofoam racks
Step One
Step Two
Step Three
Step Four
Step Five
PAXgene™ Blood RNA Tube
PAXgene™ Blood RNA Tube
• Store tubes at room temperature.
• Label tubes with pre-printed subject labels prior to blood draw.
• Collect blood into one PAXgene Tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.
• Immediately after blood draw, invert tubes 8-10 times to mix samples.
• Repeat steps two and three for additional tube
• Incubate tubes upright at room temperature for 24 hours before freezing samples.
• After 24 hours incubation at room temperature, store tubes upright in a -80°C in a wire rack until shipment.

5.7 Plasma/Buffy Coat

* See training video for blood processing for *Plasma and Buffy Coat Processing and Aliquoting:* http://ppmi.iu.edu/videos.

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

2. Place a pre-printed "PLASMA" label on the 10 ml EDTA tube prior to blood draw (per Section 5.2).



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

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

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

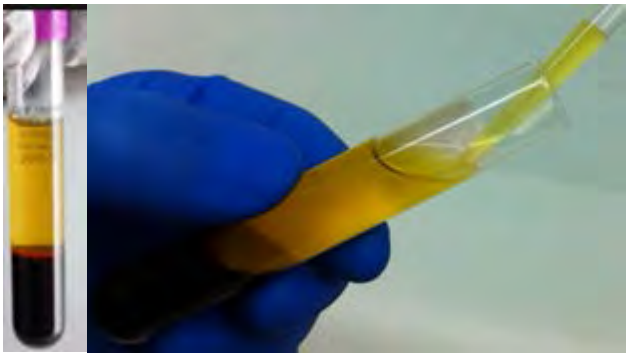
5. CRITICAL STEP: **Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tube 8-10 times.**

6. Within 30 minutes of plasma collection, centrifuge balanced tubes at 4°C for 15 minutes at 1500 x g. **It is critical that the tubes be centrifuged at the**

appropriate speed to ensure proper plasma separation. For assistance, see Appendix A.

- ❖ Equivalent rpm for spin at 1500 x g =
- ❖ While centrifuging, record the time of centrifuge start on the Laboratory Procedures data form.

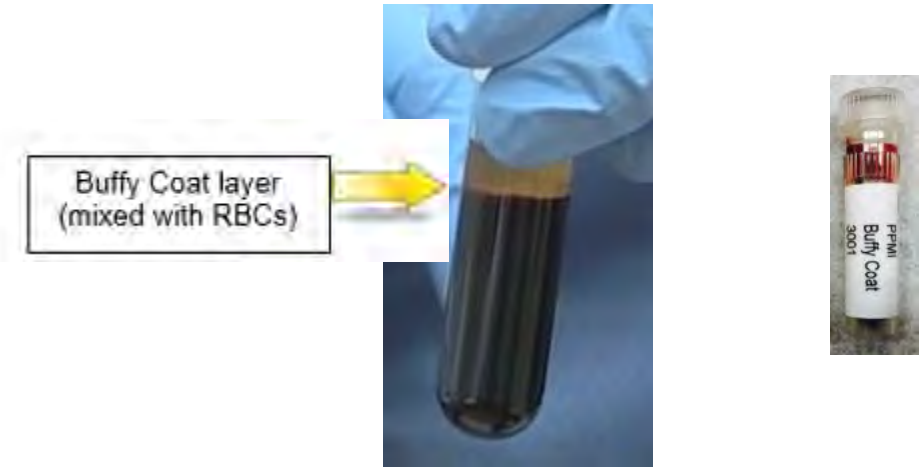
7. Place pre-printed "PLASMA" labels on the 15 ml centrifuge and 2 ml aliquot tubes. Remove the plasma, being careful not to agitate the packed blood cells at the bottom of the purple top tube, by tilting the tube and placing the pipette tip along the lower side of the tube wall without touching the pellet so that plasma is not contaminated by pellet material (see below). Using a disposable graduated transfer pipette, transfer plasma into the 15 ml centrifuge tube at room temperature, and mix gently by inverting 3-4 times.



8. Pipette at least 1.5 ml of plasma from the 15 ml centrifuge tube into each labeled 2 ml aliquot tube. The EDTA tube 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 pipet the plasma top layer only, leaving the buffy coat and the red blood cell layers untouched.

9. Place a pre-printed "BUFFY COAT" label on the 2 ml aliquot tube. Using a clean transfer pipette (micropipette preferred), transfer the buffy coat layer (middle layer) into an aliquot tube. Seal the aliquot tube with a clear cap. Residual plasma and RBCs may be collected during this isolation process.



10. Discard the used EDTA and 15 ml centrifuge tubes according to site guidelines for disposing of biomedical waste.

11. Within 60 minutes of plasma collection, freeze and store samples at **-80°C.** Samples should be frozen and stored **UPRIGHT**. A cryobox is provided for this purpose. Complete the remainder of the Laboratory Procedures data form and ensure timely entry of data into the eClinical database. Samples should be shipped within two weeks of collection, following the instructions in Appendix C or D.

APPENDIX F

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

Step One

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

Step Two

- Collect blood in Plasma Tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

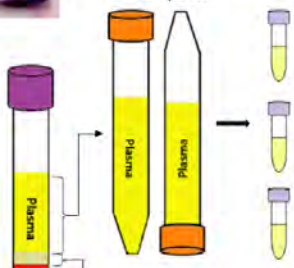
Step Three

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

Step Four

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

Step Five



- Label micro centrifuge tubes with preprinted PPMI labels.
- Using a clean transfer pipette, transfer all plasma into a 15 ml conical tube and mix gently by inverting 3-4 times.
- Aliquot 1.5 ml into each MCF tube.
- Store plasma aliquots at -80°C until shipment.

Step Six

- Label micro centrifuge tube with preprinted PPMI label.
- Using a clean transfer pipette, collect the buffy coat (may have residual plasma and some RBCs included).
- Transfer the buffy coat into the MCF tube.
- Store buffy coat aliquot at -80°C until shipment.

5.8 Whole Blood

1. CRITICAL STEP: **Store empty Whole Blood EDTA tubes at room temperature, 64°F - 77°F (18°C to 25°C) before use.**
2. Place a pre-printed "WBLD" label on the **6 ml EDTA tube** prior to blood draw (per section 5.2).

3. Using a blood collection set and a holder, collect whole blood into the 6 ml purple top whole blood tube using your institution's recommended procedure for standard venipuncture technique.

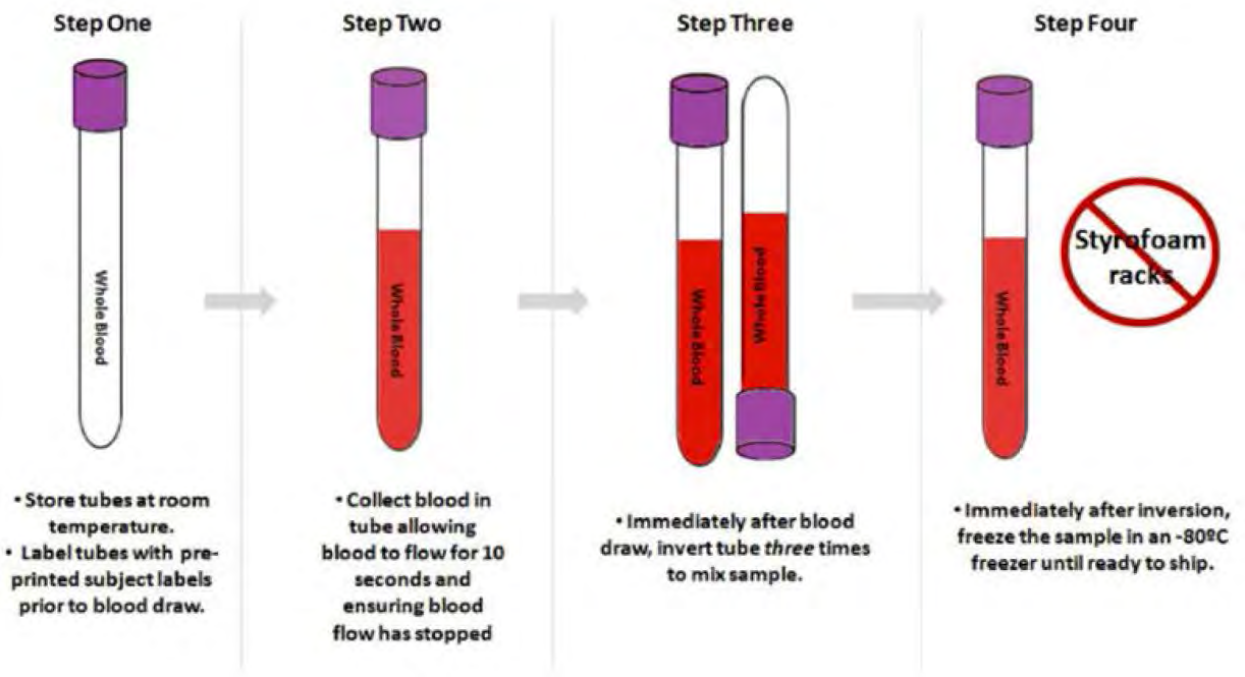
   The following techniques shall be used to prevent possible backflow:

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

4. Invert the tube gently 3 times.

5. Transfer the tube immediately to a **-80°C Freezer.** Complete the Whole Blood Sample data form and ensure timely entry of data into the eClinical database. The sample should be frozen and stored **UPRIGHT** in a WIRE or PLASTIC type test tube rack (DO NOT use a solid Styrofoam test tube holder). Samples should be shipped within two weeks of collection, following the instructions in Appendix C or D.

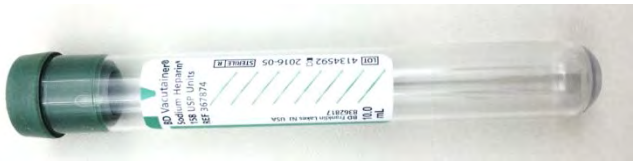
Whole Blood Preparation (6 mL Purple Top Tube)



5.9 Peripheral Blood Mononuclear Cells (PBMC)

Once drawn, Sodium Heparin tubes MUST be shipped to the appropriate PPMI biorepository on the day of collection via FedEx/DHL overnight delivery. This is to ensure the specimen has the most viable cells available at extraction.

These samples should only be collected Monday-Thursday. Please DO NOT collect these samples on Fridays.



1. CRITICAL STEP: **Store empty Sodium Heparin tube at room temperature, 64°F - 77°F (18°C to 25°C) before use.**
2. Place pre-printed "PBMC" label on the 10 ml Sodium Heparin tube prior to blood draw (per Section 5.2).

3. Using a blood collection set and a holder, collect blood into the 10 ml Sodium Heparin tube using your institution's recommended procedure for standard venipuncture technique.

   The following techniques shall be used to prevent possible backflow:

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

4. Immediately after blood collection, gently invert the tube 8-10 times to mix sample.

5. Seal the Sodium Heparin tube in the ambient shipment kit.

6. Ship the unprocessed tube ambient to the appropriate PPMI biorepository. **Sample must be shipped the same day as collection. Sample must be received at the appropriate PPMI biorepository the following day after collection. Do NOT draw or ship ambient samples on Friday. Only Monday-Thursday collection and same day shipping.**

Complete the PBMC Sample data form and ensure timely data entry of data into the eClinical database.

PBMC Preparation (10ml Green-Top Tube)

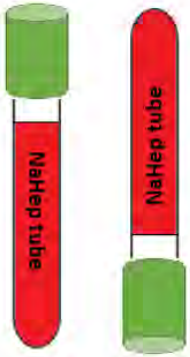
Step One

- Store tubes at room temperature.
- Label tubes with Site/Subject ID and Collection/ Cryovial Labels prior to blood draw.

Step Two

- Collect blood in Sodium Heparin Tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



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

Step Four

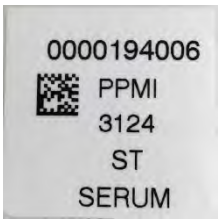
- Store tubes at room temperature until shipment.
- Ship ambient same day as blood draw

5.10 Serum Determination

** See training video for blood processing for *Serum Processing and Aliquoting:* http://ppmi.iu.edu/videos.

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

2. Place a pre-printed "SERUM" label on the 10 ml Serum tube prior to blood draw (per Section 5.2)



3. Using a blood collection set and a holder, collect blood into the **serum determination (red top)** tube using your institution's recommended procedure for standard venipuncture technique.

   The following techniques shall be used to prevent possible backflow:

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

4. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube. Record the time of draw on the Laboratory Procedures data form.

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

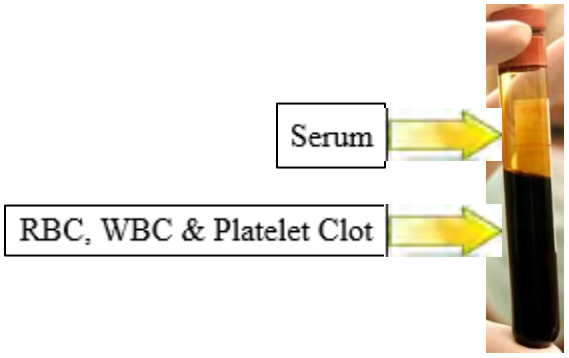
degree turns) the serum determination tube 8-10 times.

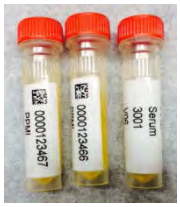
6. CRITICAL STEP: **Allow blood to clot at room temperature for at least 15 minutes.**

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

   - ❖ Equivalent rpm for spin at 1500 x g =
   - ❖ While centrifuging, record the time of centrifuge start on the Laboratory Procedures data form.

8. Place pre-printed "SERUM" labels on the 15 ml centrifuge and 2 ml aliquot tubes. Remove the serum, being careful not to disturb the clot at the bottom of the tube by tilting the tube and placing the disposable graduated pipette tip along the lower side of the tube wall without touching the pellet. Using a disposable graduated transfer pipette, transfer all blood serum (top layer) into the 15 ml centrifuge tube at room temperature, and mix gently by inverting 3-4 times.



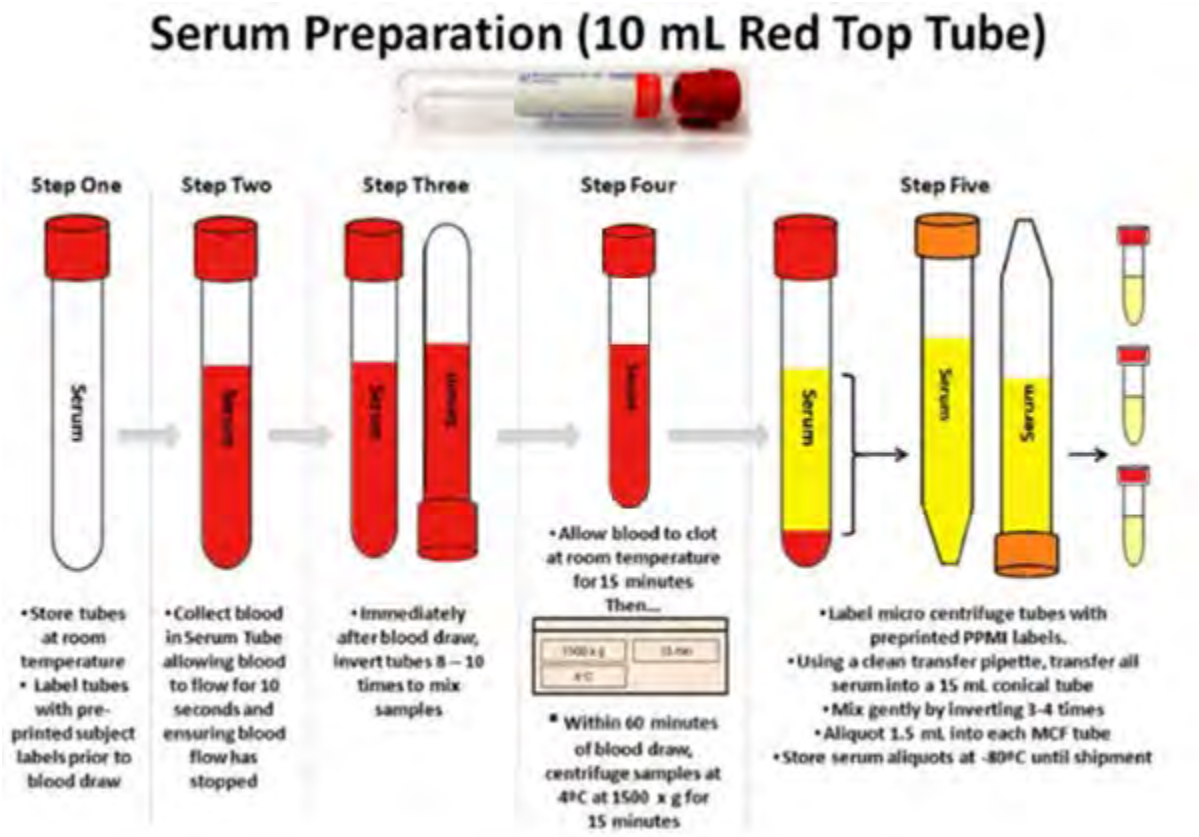


NOTE: When pipetting serum from the serum tube into the 15 ml centrifuge tube, be very careful to pipet the serum top layer only, leaving the red blood cell layer untouched.

9. Pipette at least 1.5 ml of serum from the 15 ml centrifuge tube into each labeled 2 ml aliquot tube. The serum tube 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.

10. Discard the used red-top and 15 ml centrifuge tubes according to site guidelines for disposing of biomedical waste.

11. Within 60 minutes of serum collection, freeze and store samples at **-80°C.** Samples should be frozen and stored **UPRIGHT**. A cryobox is provided for this purpose. Complete the remainder of the Laboratory Procedures data form and ensure timely entry of data into the eClinical database. Samples should be shipped within two weeks of collection, following the instructions in Appendix C or D.



5.11 General Clinical Labs

General clinical labs (Covance kits) should be drawn after all research labs have been collected. Blood for the general labs are drawn only at Screening and annual follow up visits. Please refer to the Covance Laboratory Manual for detailed instructions on collection and shipment of blood samples to Covance.

6.0 Urine Collection and Processing Procedures

1. Label one urine collection cup prior to urine collection with a pre-printed "URINE" label. Ask study subject to collect a urine specimen in the collection cup. Urine should be collected midstream and should remain as sterile as possible.

2. Label one 15 mml centrifuge tube with a pre-printed "URINE" label. Pour enough of the urine specimen to at least half-fill the tube and cap with the screw-cap.

3. Within 30 minutes of collection, centrifuge the tube at 4°C. Spin for 15 minutes to remove sediment and cells at 2500 x g. For assistance, see Appendix A.

   - Equivalent rpm for spin at 2500 x g =
   - While centrifuging, record the time of centrifuge start on the Biomarker Lab data form.

4. Place a pre-printed "URINE" label on a fresh 15 ml conical tube (orange top). Using a clean transfer pipette, carefully transfer supernatant from the 15 ml processing centrifuge tube into the new 15 ml conical tube labeled "URINE", then firmly cap with the orange screw cap. Discard the original processing tube.

5. After the urine aliquot has been transferred to the conical tube and capped, place the labeled tube upright in dry ice and allow the sample to freeze completely.

6. Within 60 minutes of urine collection, freeze and store samples at **-80°C.** Samples should be frozen and stored **UPRIGHT**. Complete the remainder of the Laboratory Procedures data form and ensure timely entry of data into the eClinical database. Samples should be shipped within two weeks of collection, following the instructions in Appendix C or D.

7.0 Cerebrospinal Fluid Collection

CSF should be collected in the morning between 8 am – 10 am, preferably fasted. If fasting is not feasible, the low fat diet should be followed (***see Appendix J***). Record time of last meal (and whether low fat diet followed, if applicable) on the Lumbar Puncture data form.

7.1 Lumbar Puncture Supplies

The lumbar puncture tray contains the following items, which will be used to perform lumbar puncture. Check the dates of expiration: these reflect the expiration date of the lidocaine, if included. Supplies for collection and shipment of CSF are sent to sites in a separate kit from Indiana University.

7.1.1 Lumbar Puncture Tray Components

Quantity	Lumbar Puncture Tray Kit Component
1	Pencil point spinal needle, 24g x 3.5”
1	Introducer needle, 1 mm x 30 mm
1	Hypodermic needle, 22G x 1.5”
1	Plastic syringe, (3 ml, luer lock) with 25G x 5/8” needle attached
4	Polypropylene syringe (6 ml, luer lock)
1	Needle stick pad
1	Adhesive bandage
1	Drape, fenestrated, 2 tabs, paper, 18” x 26”
2	Towel, 13.5” x 18”
6	Gauze pad, 2” x 2”
3	Sponge stick applicator
1	Lidocaine 1%, 5 ml **(US TRAYS ONLY)**
1	Povidone-Iodine Topical Solution, 0.75 oz

7.2 Setting Up the 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.

7.3 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.

7.4 Tips for Clinicians Performing Lumbar Puncture

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

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

2. Use of a Sprotte 24g atraumatic spinal needle and careful technique are optimal for reducing post-LP headache risk. A pencil point spinal needle such as Spinocan 22g or 24g may also be used.

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

4. Increasing fluid intake immediately after LP is helpful.

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

7.5 Post-LP Care Instructions

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

7.5.1 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.
  - Tylenol should be used for symptomatic relief. If a subject cannot tolerate Tylenol, ibuprofen should be used. Avoid aspirin. If these do not relieve the headache, Tylenol with codeine or an equivalent could be considered.

7.5.2 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.

7.6 Detailed Lumbar Puncture Procedure

CSF is processed at Room Temperature [64^{0}F - 77^{0}F (18°C to 25°C)].
Also, a portion of the CSF must be sent to your clinical lab and analyzed within 4 hours of collection.

* See training video for *CSF Processing and Aliquoting:* http://ppmi.iu.edu/videos

1. Place a pre-printed "CSF" label on the 15 ml centrifuge tubes used for processing. Place pre-printed "CSF" labels on the 2 ml cryovial aliquot tubes (per section 5.2). Prepare at least 10 aliquot tubes based on the collection of 15-20 mls of CSF.

2. Place aliquot tubes on wet ice prior to the procedure so they are pre-cooled.
3. Perform lumbar puncture using the atraumatic technique.
4. Collect CSF into syringes (if a noticeably blood tap, discard the first 1-2 mls). After the LP has begun and fluid is being collected, take the first 1-2 mls of CSF from the first syringe and place in the CSF labs tube (PURPLE TOP), and send it 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

5. Collect an additional 15-20 mls of CSF and transfer to 15 ml conical polypropylene tubes at room temperature. Mix gently by inverting 3-4 times. Record the time of draw (once collection is complete) on the Lumbar Puncture data form.
6. Within 15 minutes of collection, spin the remaining CSF sample down at 2000 x g for 10 minutes at room temperature, 64°F – 77°F (18°C to 25°C). For assistance, see Appendix A.

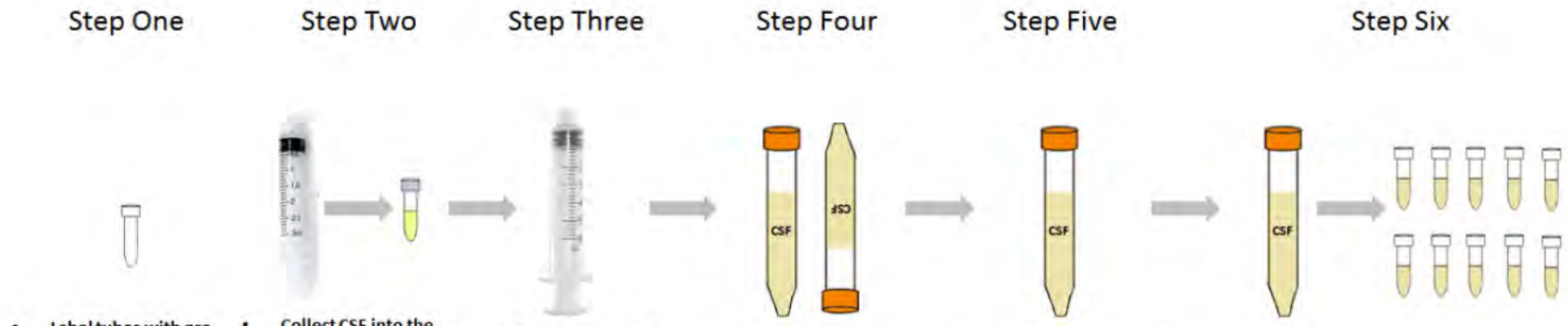
   ❖ Equivalent rpm for spin at 2000 x g =

- ❖ While centrifuging, record the time of centrifuge start on the Laboratory Procedures data form.

7. Pipette (micropipette preferred) at least 1.5 ml of supernatant directly into pre-cooled polypropylene CSF collection 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.

8. Within 60 minutes of CSF collection, freeze aliquots immediately on dry ice and then store at **-80°C** or ship on dry ice in a shipping container. Samples should be frozen and stored **UPRIGHT**. A cryobox is provided for this purpose. Complete the remainder of the Laboratory Procedures data form and ensure timely entry of data into the eClinical database. Samples should be shipped within two weeks of collection, following the instructions in Appendix C or D.

CSF Preparation (15-20 ml total)



- Label tubes with pre-printed subject labels prior to collection.
- Pre-chill all cryovials on wet ice.

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

- Collect CSF into the 6 ml luer lock syringe.
- Collect 15-20 ml total, including the 1-2 ml sent to the local lab.

- Immediately after collection, invert tubes 3-4 times in conical tubes to mix samples.

- Within 15 minutes of collection, centrifuge samples at RT at 2000 x g for 10 minutes.

- Aliquot 1.5 ml into clear cap cryovials, leaving the pellet in the bottom of the 15 ml conical tubes.
- Store CSF aliquots at -80°C until shipment.

8.0 Packaging and Shipping Instructions

8.1 US Sites and Australia

Please refer to **Appendix C** for detailed shipping instructions regarding:

- PPMI Ambient Shipping Instructions/Domestic
- PPMI Frozen Shipping Instructions/Domestic

8.2 European Sites

Please refer to **Appendix D** for detailed shipping instructions regarding

- PPMI Ambient Shipping Instructions/Europe
- PPMI Frozen Shipping Instructions/Europe

*****Important Notes*****

Include only one subject visit set of samples per shipping carton in order to have room for a sufficient amount of dry ice to keep samples frozen up to 24 hours unless otherwise noted by Indiana University/BioRep/Israel.

Ship all frozen samples Monday through Wednesday ONLY! Ambient whole blood sample for DNA may be shipped on Thursday. BE AWARE OF HOLIDAYS!!

Remember to complete the Sample Record Summary and Shipment Notification Form (Appendix B), include a copy in your shipment AND notify Indiana University/BioRep/Israel IN ADVANCE to confirm the shipment.

Ambient (DNA) samples must be shipped within FIVE DAYS of collection.

Ambient (PBMC) samples must be shipped the same day as collection.

Frozen samples must be shipped within TWO WEEKS of collection.

9.0 Sample Quality Checks and Feedback to Sites

In addition to tracking and reconciliation of samples, the condition and amount of samples received is tracked by Indiana University/BioRep/Israel for each sample type received. Sites are responsible to ensure the requested amounts of each fluid are collected to the best of their ability and that samples are packed well with sufficient amounts of dry ice to avoid thawing in the shipment process. Indiana University and BioRep will complete a Non-Conformance Report (**Appendix K**) should there be any issues with a shipment and will provide this feedback to the site. Issues of concern that may impact collection, processing, or future analyses of the samples will be addressed by the PPMI Steering Committee and communicated to sites.

10. Data Queries and Reconciliation

The Laboratory Procedures and Lumbar Puncture data forms must be completed on the day that samples are collected, since they capture information related to the details of the sample collection and processing. These forms include information that will be used to reconcile sample collection and receipt, as well as information essential to future analyses. All data should be recorded on the DNA Sample, Laboratory Procedures, and LP worksheets within 14 days of the subject visit per protocol.

Indiana University will be collaborating with the Laboratory of Neuro Imaging (LONI) to reconcile information captured in the EDC database compared to samples received and logged in at Indiana University. Information that appears incorrect in the EDC database will be queried and additional discrepancies that may be unrelated to data entry will be resolved with sites in a separate follow-up communication.

Data queries or discrepancies with samples shipped versus received at Indiana University/BioRep/Israel may result from:

- Missing samples at Indiana University/BioRep/Israel
- Incorrect samples collected and shipped to Indiana University/BioRep/Israel
- Damaged or incorrectly prepared samples
- Unlabeled samples, samples labeled with incomplete information, or mislabeled samples
- Discrepant information documented on the Sample Record Summary and Shipment Notification Form and logged in at Indiana University compared to information entered into EDC.

APPENDIX A

Rate of Centrifugation Worksheet

Please complete and return this form by fax or e-mail to the PPMI Project Manager if you have any questions regarding sample processing. The correct RPM will be sent back to you. Make note of this in your PPMI Biologics Manual.

Submitter Information

Name: Site #: 001

Submitter e-mail:

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).

Calculating RPM from G-force:

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

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 the centrifuge

Comments:

Please send this form to Renee Wilson, PPMI Project Manager

585-273-4242 (Fax)

renee.wilson@chet.rochester.edu

APPENDIX B

PPMI Sample Record Summary and Shipment Notification Form

Please complete this form electronically (i.e., typed). All fields are required.

Site #: 001 **Site Name:** University of Rochester **Principal Investigator:**

Coordinator: **Telephone:** **e-mail:**

Instructions: **Ship frozen samples Monday – Wednesday ONLY.** This form must be completed for all biorepository sample shipments. Notify Indiana University or BioRep (e-mail preferred) prior to shipment using the contact information below. Place a copy of the completed form in the shipment box and also file a copy in the site study binder. The site will be contacted if any issues with the samples/form are noted upon receipt.

Completed by Submitter/Site List Subject ID that corresponds to pre-printed labels List only one Specimen Type per row.							**Completed by Biorepository**
Subject ID #	**Specimen Type** (RNA, DNA, WB, Urine, CSF, Plasma, Serum, BC, PBMC)	**Visit Type** (BL, V01, ST, etc.)	**# of Tubes**	**Gender**	**Date of Draw**	**Tube Volume** (if less than standard)	**Notation of problems**
Total number of tubes:							

Courier (check one): □**FedEx** □**FedEx Priority Alert Plus** □**DHL** □**Other (specify):**

Date shipped:

Tracking #:

IMPORTANT! **BEFORE SHIPPING, EMAIL (PREFERRED) OR FAX A COPY OF THE COMPLETED FORM TO:**	
European sites only - BioRep PPMI@biorep.it Fax: +39 02 58018471 Ph: +39 02 58014369	**US Sites – Indiana University** PPMIBio@iu.edu **Fax: 317-278-1100** **Ph: 317-274-5744**

APPENDIX C

PPMI Ambient Shipping Instructions – Domestic

PPMI US SITES: AMBIENT SAMPLE PACKAGING AND SHIPMENT TO INDIANA UNIVERSITY

Samples Shipped Ambient:

- DNA Purple Top 6ml EDTA Blood Tube

IMPORTANT!
SCREENING SAMPLES MAY BE SHIPPED MONDAY-THURSDAY ONLY! **Up to four screening samples may be shipped in a single package.**

1. Contact FedEx to confirm service is available and schedule package to be picked up.

2. Notify Indiana University of shipment by e-mailing PPMIBio@iu.edu (preferred) or faxing (317-278-1100) a copy of the completed Sample Record Summary and Shipment Notification Form (Appendix B).

3. Insert up to four purple top EDTA tubes in the tube sleeve. Place the sleeve into the canister and close securely. Wrap the canister in the enclosed bubble wrap and place into the cardboard box.

4. Apply the UN3373 label to the outside of the cardboard box.

5. Place the cardboard box and the completed Sample Record Summary and Shipment Notification Form in the FedEx Clinical Pak, making sure the UN3373 label is visible through the Clinical Pak, and seal according to the instructions on the envelope.

6. Complete the “From” portion of the provided FedEx air waybill by filling in your name, address, and phone number. FedEx is likely to reject or return your shipment without this information.

7. Apply the completed FedEx air waybill to the outside of package and arrange for FedEx pick up.

8. Ship the sample(s) to Indiana University on the day of collection. If the sample(s) cannot be shipped the same day as collected, hold at room temperature until shipping can be arranged. Sample(s) must be received at Indiana University within 5 days of collection.

APPENDIX C

PPMI Ambient Shipping Instructions – Domestic

PPMI US SITES: AMBIENT SAMPLE PACKAGING AND SHIPMENT TO INDIANA UNIVERSITY

Samples Shipped Ambient:

- PBMC Green Top 10ml Sodium Heparin Blood Tube

IMPORTANT!
PBMC SAMPLES MUST BE COLLECTED AND SHIPPED MONDAY-THURSDAY ONLY!

1. Contact FedEx to confirm service is available and schedule package to be picked up.

2. Notify Indiana University of shipment by e-mailing PPMIBio@iu.edu (preferred) or faxing (317-278-1100) a copy of the completed Sample Record Summary and Shipment Notification Form (Appendix B).

3. Insert the green top Sodium Heparin tube in the tube sleeve. Place the sleeve into the canister and close securely. Wrap the canister in the enclosed bubble wrap and place into the cardboard box.

4. Apply the UN3373 label to the outside of the cardboard box.

5. Place the cardboard box and the completed Sample Record Summary and Shipment Notification Form in the FedEx Clinical Pak, making sure the UN3373 label is visible through the Clinical Pak, and seal according to the instructions on the envelope.

6. Complete the “From” portion of the provided FedEx air waybill by filling in your name, address, and phone number. FedEx is likely to reject or return your shipment without this information.

7. Apply the completed FedEx air waybill to the outside of package and arrange for FedEx pick up.

8. Sample collection and shipment **only** Monday – Thursday with same day shipment. Do NOT draw or ship ambient samples on Friday. Ship the sample(s) to Indiana University on the day of collection. Sample must be received at Indiana University the day after collection.

APPENDIX C

PPMI Frozen Shipping Instructions – Domestic

PPMI US SITES: FROZEN SAMPLE PACKAGING/SHIPMENT TO INDIANA UNIVERSITY

Samples Shipped on Dry Ice:

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

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

1. Contact FedEx to confirm service is available and schedule package to be picked up.

2. Notify Indiana University of shipment by e-mailing PPMIBio@iu.edu (preferred) or faxing (317-278-1100) a copy of the completed Sample Record Summary and Shipment Notification Form (Appendix B).

3. Place all frozen 2ml aliquots 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. Insert this into the white Tyvek biohazard envelope and seal according to the instructions on the envelope.

5. Insert frozen EDTA, PAXgene™, and urine tubes into the bubble wrap pouch provided. In the event that the tubes cracked or broke during previous shipments, one is advised to package the bubble wrapped tubes with additional padding.

6. Place bubble-wrapped tubes into the 2nd clear plastic biohazard bag with the absorbent sheet and seal according to the instructions on the bag. Insert this into the 2nd white Tyvek biohazard envelope and seal according to the instructions on the envelope.

APPENDIX C

PPMI Frozen Shipping Instructions – Domestic

7. Place both envelopes upright and side-by-side in the provided Styrofoam-lined shipping carton, as shown below:

8. Fill the remaining space in the shipping carton with approximately 10 lbs of dry ice, ensuring ice surrounds both envelopes and reaches the top of the carton, as shown below:

9. 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!
Complete the required fields on the FedEx air waybill and Class 9 Dry Ice label, or FedEx may reject or return your package.

10. Complete the FedEx air waybill with the following information:
    a. Section 1, “From”: fill in your name, address, and phone number
    b. Section 6, “Special Handling and Delivery Signature Options”: under “Does this shipment contain dangerous goods?” check the boxes for “Yes, Shipper’s Declaration not required” and “Dry Ice”. Enter the number of packages (1) x the net weight of the dry ice in kg.

11. Complete the Class 9 UN 1845 Dry Ice label (black and white diamond) with the following information:
    a. Your name and return address
    b. Net weight of dry ice in kg
    c. Consignee name and address: PPMI Biorepository, IU School of Medicine, Walther Hall R3-C102, 980 W. Walnut St., Indianapolis, IN 46202
    d. Do not cover any part of this label with other stickers, including pre-printed address labels.

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

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

APPENDIX C

PPMI Bulk Frozen Shipping Instructions – Domestic

PPMI US SITES: FROZEN SAMPLE BULK PACKAGING/SHIPMENT TO INDIANA UNIVERSITY

Samples Shipped on Dry Ice:

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

IMPORTANT!
DO NOT SHIP MORE THAN ONE BASELINE VISIT PER PACKAGE. **Up to FOUR sets of samples may be shipped in a single package.** **Pack no more than 2 cryoboxes and 2 bubble wrap pouches per package.** **FROZEN SAMPLES MAY BE SHIPPED MONDAY-WEDNESDAY ONLY!**

1. Contact FedEx to confirm service is available and schedule package to be picked up.

2. Notify Indiana University of shipment by e-mailing PPMIBio@iu.edu (preferred) or faxing (317-278-1100) a copy of the completed Sample Record Summary and Shipment Notification Form (Appendix B).

3. Place all frozen 2ml aliquots in the provided cardboard cryoboxes. Label the outside of the cryoboxes with the subject ID and visit number.

4. Place the cryoboxes into a single clear plastic biohazard bag with the absorbent sheet and seal according to the instructions on the bag. Insert this into the white Tyvek biohazard envelope and seal according to the instructions on the envelope.

5. Insert all frozen EDTA, PAXgene™, and urine tubes into the bubble wrap pouches provided. In the event that the tubes cracked or broke during previous shipments, one is advised to package the bubble wrapped tubes with additional padding.

6. Place bubble-wrapped tubes into a single clear plastic biohazard bag with the absorbent sheet and seal according to the instructions on the bag. Insert this into a 2nd white Tyvek biohazard envelope and seal according to the instructions on the envelope.

APPENDIX C
PPMI Bulk Frozen Shipping Instructions – Domestic

7. Place both envelopes upright and side-by-side in the provided Styrofoam-lined shipping carton, as shown below:

8. Fill the remaining space in the shipping carton with approximately 10 lbs of dry ice, ensuring ice surrounds both envelopes and reaches the top of the carton, as shown below:

9. 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!
Bulk packages are to be shipped via FedEx Priority Alert Plus Service! **Packages must be labeled with a pink "PA" sticker and a Priority Alert Plus FedEx air waybill. If the FedEx air waybill and Class 9 Dry Ice label are not completed, your package may be rejected or returned.**

10. Complete the FedEx air waybill with the following information:
    a. Section 1, “From”: fill in your name, address, and phone number
    b. Section 6, “Special Handling and Delivery Signature Options”: under “Does this shipment contain dangerous goods?” check the boxes for “Yes, Shipper’s Declaration not required” and “Dry Ice”. Enter the number of packages (1) x the net weight of the dry ice in kg.

11. Complete the Class 9 UN 1845 Dry Ice label (black and white diamond) with the following information:
    a. Your name and return address
    b. Net weight of dry ice in kg
    c. Consignee name and address: PPMI Biorepository, IU School of Medicine, Walther Hall R3-C102, 980 W. Walnut St., Indianapolis, IN 46202
    d. Do not cover any part of this label with other stickers, including pre-printed address labels.

12. Apply the completed FedEx air waybill, pink “PA” sticker, and all other provided warning labels to the outside of the package, taking care not to overlap labels.

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

APPENDIX D

PPMI Ambient Shipping Instructions – Europe

PPMI EUROPEAN SITES: AMBIENT SAMPLE PACKAGING/SHIPMENT TO BIOREP

Samples Shipped Ambient:

- DNA Purple Top 6ml EDTA Blood Tube

IMPORTANT!
SCREENING SAMPLES MAY BE SHIPPED MONDAY-THURSDAY ONLY! **Up to three screening samples may be shipped in a single package.**

1. Contact DHL to confirm service is available and schedule package to be picked up.

2. Notify BioRep of shipment by e-mailing PPMI@biorep.it (preferred) or faxing (+39 02 58018471) a copy of the completed Sample Record Summary and Shipment Notification Form (Appendix B).

3. Insert up to four purple top EDTA tubes in the tube sleeve. Place the sleeve into the canister and close securely. Wrap the canister in the enclosed bubble wrap and place into the cardboard box.

4. Apply the UN3373 label to the outside of the cardboard box.

5. Place the cardboard box and the completed Sample Record Summary and Shipment Notification Form in the DHL Clinical Pak, making sure the UN3373 label is visible through the Clinical Pak, and seal according to the instructions on the envelope.

6. Apply the DHL air waybill to the outside of package and arrange for DHL pick up.

7. Ship the sample(s) to BioRep on the day of collection. If the sample(s) cannot be shipped the same day as collected, hold at room temperature until shipping can be arranged. Sample(s) must be received at BioRep within 5 days of collection.

APPENDIX D

PPMI Ambient Shipping Instructions – Europe

PPMI EUROPEAN SITES: AMBIENT SAMPLE PACKAGING/SHIPMENT TO BIOREP

Samples Shipped Ambient:

- PBMC Green Top 10 ml Sodium Heparin Blood Tube

IMPORTANT!
PBMC SAMPLES MUST BE COLLECTED AND SHIPPED MONDAY-THURSDAY ONLY!

8. Contact DHL to confirm service is available and schedule package to be picked up.

9. Notify BioRep of shipment by e-mailing PPMI@biorep.it (preferred) or faxing (+39 02 58018471) a copy of the completed Sample Record Summary and Shipment Notification Form (Appendix B).

10. Insert the green top Sodium Heparin tube in the tube sleeve. Place the sleeve into the canister and close securely. Wrap the canister in the enclosed bubble wrap and place into the cardboard box.

11. Apply the UN3373 label to the outside of the cardboard box.

12. Place the cardboard box and the completed Sample Record Summary and Shipment Notification Form in the DHL Clinical Pak, making sure the UN3373 label is visible through the Clinical Pak, and seal according to the instructions on the envelope.

13. Apply the DHL air waybill to the outside of package and arrange for DHL pick up.

14. Sample collection and shipment only Monday – Thursday with same day shipment. Do NOT draw or ship ambient samples on Friday. Ship the sample(s) to BioRep on the day of collection. Sample must be received at BioRep the day after collection.

APPENDIX D

PPMI Frozen Shipping Instructions – Europe

PPMI EUROPEAN SITES: FROZEN SAMPLE PACKAGING/SHIPMENT TO BIOREP

Samples Shipped Ambient:

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

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

1. Contact DHL to confirm service is available and schedule package to be picked up.

2. Notify BioRep of shipment by e-mailing 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 all frozen 2ml aliquots in the provided cardboard cryobox. Label the outside of the cryobox with the subject ID and visit number.

4. Place the cryobox in a clear plastic biohazard with the absorbent sheet and seal according to the instructions on the bag. Insert this into the white Tyvek biohazard envelope and seal according to the instructions on the envelope.

5. Insert frozen EDTA, PAXgene™, and urine tubes into the provided bubble wrap pouch. In the event that tubes cracked or broke during previous shipments, one is advised to package the bubble wrapped tubes with additional padding.

6. Place the bubble-wrapped tubes into the 2nd clear plastic biohazard bag with the absorbent sheet and seal according to the instructions on the bag. Insert this into the 2nd white Tyvek biohazard envelope and seal according to the instructions on the envelope.

APPENDIX D

PPMI Frozen Shipping Instructions – Europe

7. Place both envelopes upright and side-by-side in the provided Styrofoam-lined shipping carton, as shown below:

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

9. 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.

10. Complete the Class 9 UN 1845 Dry Ice label (black and white diamond) with the following information:
    a. Your name and return address
    b. Net weight of dry ice in kg
    c. Consignee name and address: BioRep Srl, c/o DIBIT2 Palazzina San Michele, Via Olgettina 60, 20132, Milano - Italy
    d. Do not cover any part of this label with other stickers, including pre-printed address labels.

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

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

APPENDIX D

PPMI Bulk Frozen Shipping Instructions – Europe

PPMI US SITES: FROZEN SAMPLE BULK PACKAGING/SHIPMENT TO BIOREP

Samples Shipped on Dry Ice:

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

IMPORTANT!
BULK SHIPMENTS MUST BE SCHEDULED 5-7 DAYS IN ADVANCE.

1. Contact BioRep (PPMI@biorep.it) 5-7 days in advance to schedule a shipment. Provide the following information:
    - The number of samples you wish to ship
    - Your name, complete address, and phone number
    - Preferred shipping date

2. BioRep will contact a private courier and provide all necessary documentation for your shipment and coordinate a pick-up date and time with you.

3. Package samples using materials provided in kits received from Indiana University.

    3.1. Place all frozen 2ml aliquot tubes in the provided cardboard cryobox. You may place more than one subject-visit in a cryobox, provided that all vials are correctly labeled.
    3.2. Insert frozen EDTA, PAXgene™, and urine tubes into the provided bubble wrap pouch. You may place more than one subject-visit in a bubble wrap pouch, provided that all tubes are correctly labeled. In the event that tubes cracked or broke during previous shipments, one is advised to package the bubble-wrapped tubes with additional padding.
    3.3. Place cryoboxes, wrapped tubes, and the completed Sample Record Summary and Shipment Notification Form in a clear plastic biohazard bag with the absorbent sheet, being careful not to overfill the bag. Seal according to the directions printed on the bag.
    3.4. Store bagged samples at -80°C until the courier arrives for pick-up.

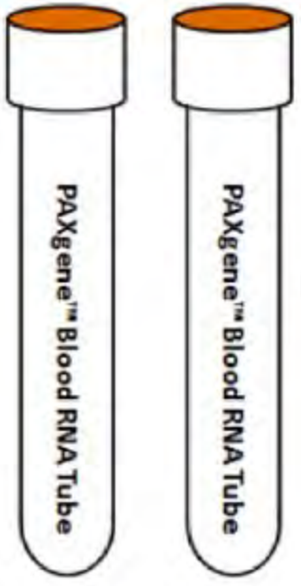
4. The courier will provide dry ice and shipping cartons at the time of pick-up.

5. Notify BioRep of the shipment the day the samples are shipped by e-mailing PPMI@biorep.it a copy of the completed Sample Record Summary and Shipment Notification Form (Appendix B). If faxing the form (+39 02 58018471), e-mail BioRep the subject IDs and visit numbers of the samples on the form that was faxed.

APPENDIX E

PAXgene™ Tube Preparation

Step One

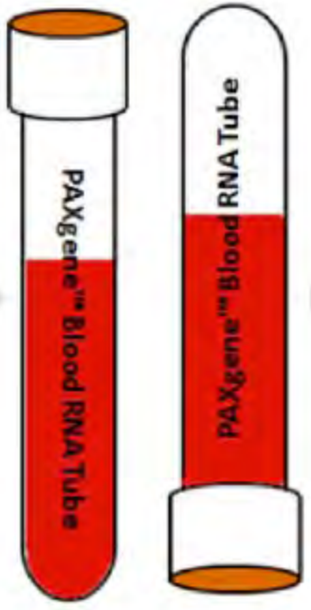


- Store tubes at room temperature.
- Label tubes with pre-printed subject labels prior to blood draw.

Step Two

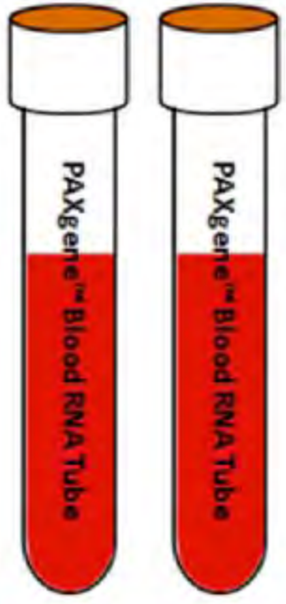
- Collect blood into *one* PAXgene tube, allowing blood to flow 10 seconds and ensuring blood has stopped flowing each time.

Step Three



- Immediately after blood draw, invert tube gently 8 – 10 times to mix samples. Repeat Steps Two and Three for the second tube.

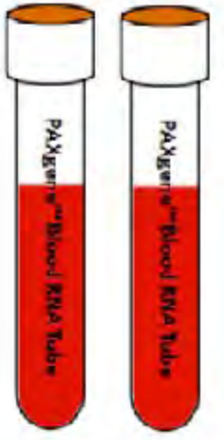
Step Four



- Incubate tubes upright at room, temperature for 24 hours before freezing samples.

Step Five

- After 24 hour incubation at room temperature, store tubes at – 80°C until shipment.



APPENDIX F

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

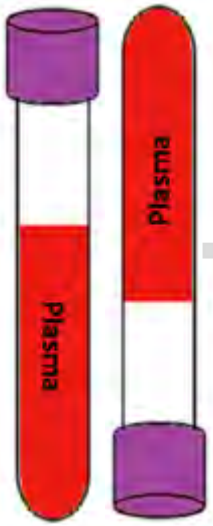
Step One

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

Step Two

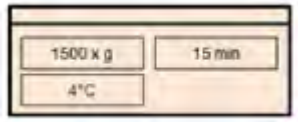
- Collect blood in Plasma Tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



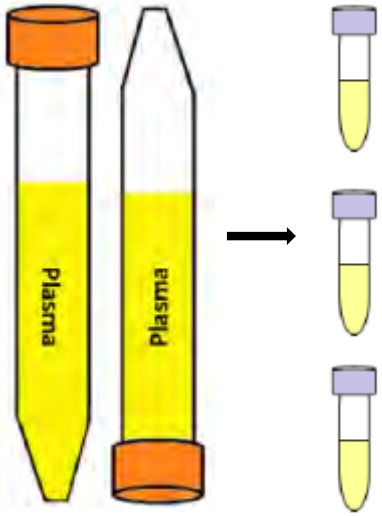
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Four



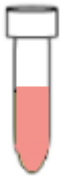
- Within 30 minutes of blood draw, centrifuge samples at 4°C at 1500 x g for 15 minutes.

Step Five



- Label micro centrifuge tubes with preprinted PPMI labels.
- Using a clean transfer pipette, transfer all plasma into a 15 ml conical tube and mix gently by inverting 3-4 times.
- Aliquot 1.5 ml into each MCF tube.
- Store plasma aliquots at -80°C until shipment.

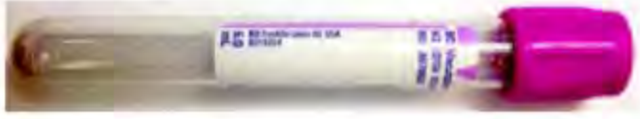
Step Six



- Label micro centrifuge tube with preprinted PPMI label.
- Using a clean transfer pipette, collect the buffy coat (may have residual plasma and some RBCs included).
- Transfer the buffy coat into the MCF tube.
- Store buffy coat aliquot at -80°C until shipment.

APPENDIX G

Whole Blood Preparation (6 mL Purple Top Tube)



Step One

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

Step Two

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

Step Three

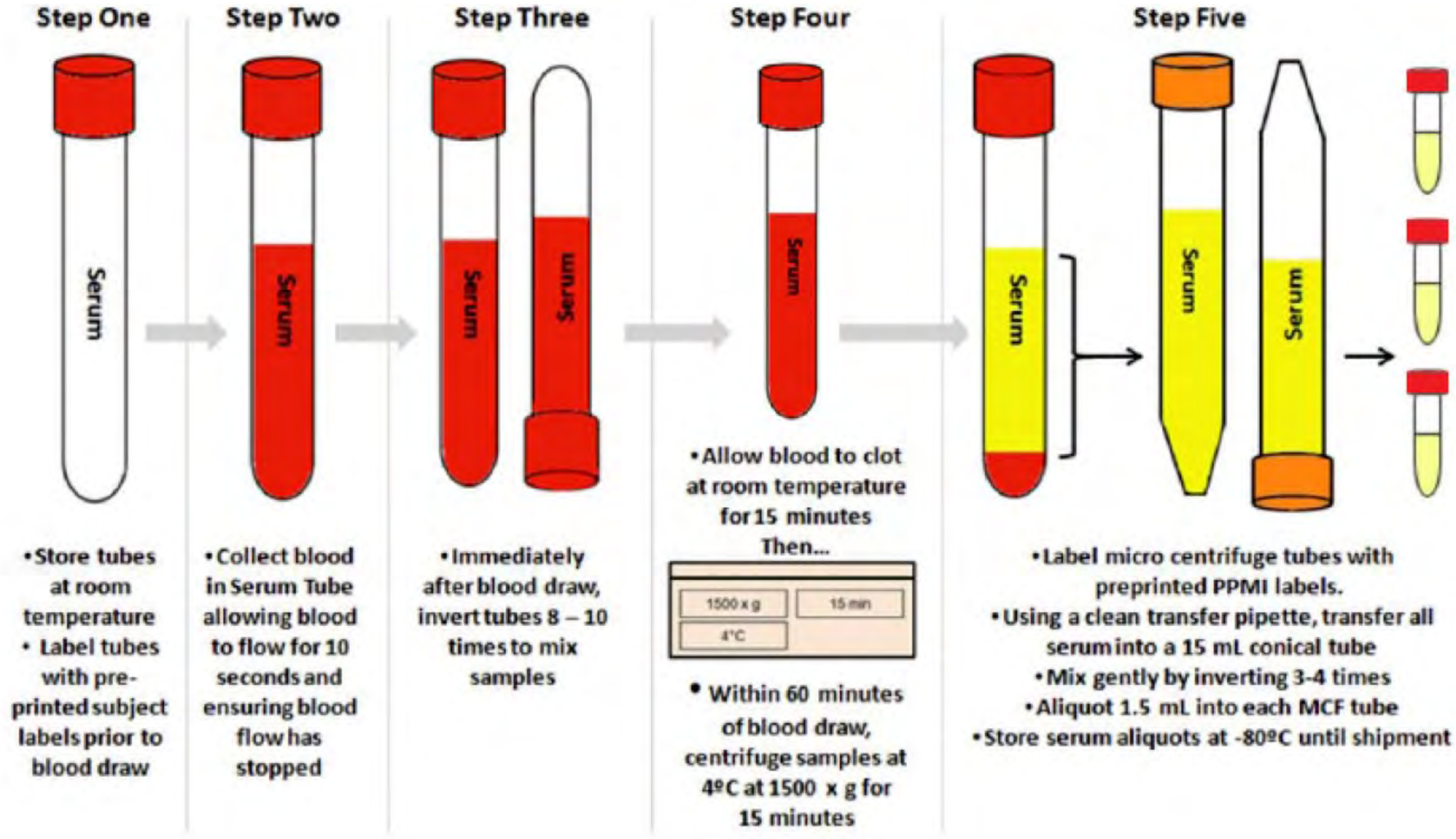
- Immediately after blood draw, invert tube *three* times to mix sample.

Step Four

- Immediately after inversion, freeze the sample in an -80ºC freezer until ready to ship.

APPENDIX H

Serum Preparation (10 mL Red Top Tube)



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

- Collect blood in Serum Tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped

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

- Allow blood to clot at room temperature for 15 minutes

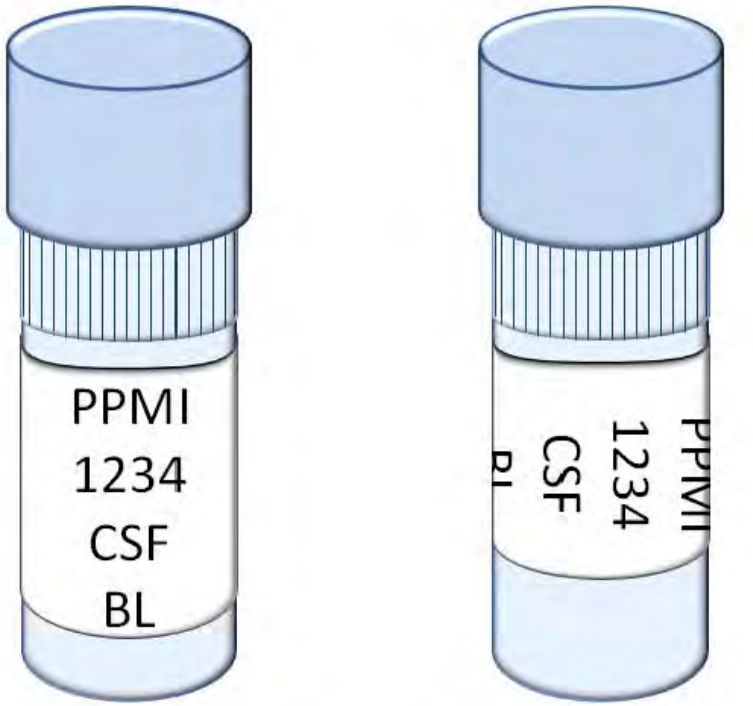
Then...

- Within 60 minutes of blood draw, centrifuge samples at 4ºC at 1500 x g for 15 minutes

- Label micro centrifuge tubes with preprinted PPMI labels.
- Using a clean transfer pipette, transfer all serum into a 15 mL conical tube
- Mix gently by inverting 3-4 times
- Aliquot 1.5 mL into each MCF tube
- Store serum aliquots at -80ºC until shipment

APPENDIX I

ALIQUOT TUBE LABELING DIAGRAM



Incorrect **Correct**

APPENDIX J

Low-Fat Diet Menu Suggestions

Due to the interference of lipid content in blood specimens collected for biomarker evaluation in the PPMI study, it is **strongly advised that 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 subject should be on a low-fat diet for at least 8 hours prior to blood collection.

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

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

APPENDIX J

Low-Fat Diet Menu Suggestions

Foods to avoid prior to blood collection:

Avoid: All fats and nuts such as:

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

Avoid: All milk and dairy products such as:

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

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

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

APPENDIX K

PPMI Sample Submission Non-Conformance Report

Repository Name: ☐ Site #: 001

Subject ID: ☐ Visit Type: ☐

Received Date: ☐ Received By: ☐

Submission Type: ☐ Ambient ☐ Frozen

Shipping Issues Noted:

- ☐ Shipment notification not received, incomplete, or inaccurate
- ☐ Submission form not included in package, incomplete, or inaccurate
- ☐ Samples shipped for weekend or holiday delivery
- ☐ Samples improperly packaged
- ☐ Samples received damaged
- ☐ Frozen submission received thawed
- ☐ Other ☐

Sample Collection Issues Noted:

- ☐ Submitted in non-standard tube(s)
- ☐ Unlabeled or mislabeled tube(s)
- ☐ Low volume received
- ☐ Sample discolored
- ☐ Other ☐

Details/Comments:

☐

APPENDIX L

CSF Preparation (15-20 ml total)

Step One

- Label tubes with pre-printed subject labels prior to collection.
- Pre-chill all cryovials on wet ice.

Step Two

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

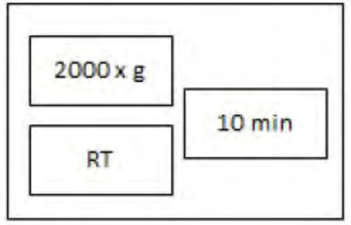
Step Three

- Collect CSF into the 6 ml luer lock syringe.
- Collect 15-20 ml total, including the 1-2 ml sent to the local lab.

Step Four

- Immediately after collection, invert tubes 3-4 times in conical tubes to mix samples.

Step Five



- Within 15 minutes of collection, centrifuge samples at RT at 2000 x g for 10 minutes.

Step Six

- Aliquot 1.5 ml into clear cap cryovials, leaving the pellet in the bottom of the 15 ml conical tubes.
- Store CSF aliquots at -80°C until shipment.

APPENDIX M - Blood Collection for Induced Pluripotent Stem Cells (iPSCs) – Unscheduled Visit

iPSC blood collections will be obtained at a select group of PPMI study sites, at an unscheduled visit. Kits for this collection will be provided by IU and all filled vacutainer tubes must be sent to Cellular Dynamics International (CDI) on the same day of collection. Please do not collect these samples on Fridays.

1.0 Kit Components – Blood Tubes

Item Type	Tube Color	Purpose
1 – 4 ml Lithium Heparin tube	Light Green	DNA extraction
1 – 4 ml Serum Separation Tube (SST)	Gold	Infectious disease testing
4 – 8 ml Cell Preparation Tube (CPT)	Blue	Reprogramming

Each site will also be provided with a supplemental supply of tubes to be used in the case of a faulty tube or issues with the blood draw. Extra shipping materials will be provided as well.

2.0 Kit Components – Shipping Materials

Insulated resealable pouch
Biohazard Bag
Absorbent tube shuttle
Rigid cardboard shipper
Gel coolant packs – **REFRIGERATE AT 4^{o}C UPON RECEIPT**
FedEx Airbill
FedEx ClinPak

3.0 Order of Blood Draws

1. 1 x 4 ml Lithium Heparin tube (light green)
2. 1 x 4 ml Serum Separation tube (gold)
3. 4 x 8 ml Cell Preparation tubes (blue)

Please store all vacutainer tubes at room temperature until ready to perform blood collection. Use the tubes by the printed expiration date.

4.0 Equipment required at sites

Alcohol Prep Pads	Butterfly Needles
Bandages	Tube Racks
Tourniquets	Sharps Bin and Lid
Gloves	Crushed ice
Gauze Pads	Centrifuge with Fixed Angle and **Swinging Bucket Rotor***

***If a swinging bucket rotor is not available on-site, please notify IU so that an appropriate centrifuge can be supplied by CDI.

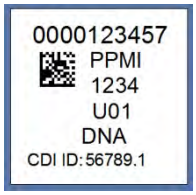
5.0 Label format

Labels will be provided by IU for each tube to be collected. **Figure 1** demonstrates the label format.

Figure 1: Labels are pre-printed with:
Unique tube ID (10 digits) and barcode
Study name (PPMI)
Subject ID number (4 or 5 digits)
Visit number (U01, U02, etc.)
Specimen type (DNA, PBMC, or Serum)
CDI ID number (5 digits, plus .1)

6.0 Collection of Blood for DNA Extraction – Lithium Heparin Tube (Light Green)

1. Place **DNA** label on the Lithium Heparin tube prior to blood draw.



2. Using a blood collection set and a holder, collect blood into the tube using your institution's recommended procedure for standard venipuncture technique.

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

3. Allow at least 10 seconds for a complete blood draw to take place in the tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 4 ml of blood into the tube. Record the time of draw on the iPSC Blood Sample data form.

4. CRITICAL STEP: **Immediately after blood collection, gently invert/mix (180 degree turns) the Lithium Heparin tube 8-10 times. Record the number of inversions on the iPSC Blood Sample data form.**

5. CRITICAL STEP: **Maintain the tube on ice until ready to package for shipment.**

6. Once all tubes have been collected and packaged for shipment, record the date of shipment, whether cold gel packs were used for shipping, and the CDI ID number (found on the labels) on the iPSC Blood Sample data form and ensure timely data entry into the eClinical database.

Blood for DNA Preparation (4 ml Light Green Top Tube)

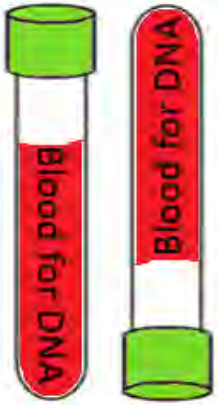
Step One

- Store tube at room temperature.
- Label tube with pre-printed subject label prior to blood draw.

Step Two

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

Step Three



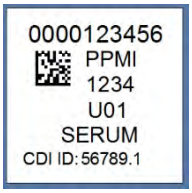
- Immediately after blood draw, invert tube *eight to ten* times to mix sample.

Step Four

- Maintain the tube on ice until ready to package for shipment.

7.0 Collection of Blood for Infectious Disease Testing – Serum Separation Tube (Gold)

1. Place a **SERUM** label on the Serum Separation tube prior to blood draw.



2. Using a blood collection set and a holder, collect blood into the tube using your institution's recommended procedure for standard venipuncture technique.

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

3. Allow at least 10 seconds for a complete blood draw to take place in the tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 4 ml of blood into the tube. Record the time of draw on the iPSC Blood Sample data form.

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

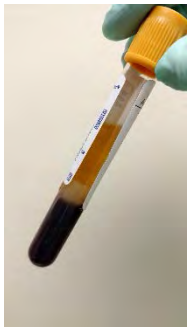
5. CRITICAL STEP: Incubate the Serum Separation Tube **UPRIGHT** at room temperature, 64°F - 77°F (18°C to 25°C) **for at least 30 minutes and no more than 120 minutes.**

6. After incubation at room temperature, centrifuge the balanced tube at room temperature, using the guidelines below for time, at 1100 - 1300 x g. **It is critical that the tubes be centrifuged at the appropriate speed to ensure proper plasma separation.** For assistance, see Appendix A. Record the information about the time, rate, duration, and temperature of centrifugation on the iPSC Blood Sample data form.

Duration (min)	
Swinging Bucket Rotor	**Fixed Angle Rotor**
10	15

7. Inspect the tube to ensure that proper blood separation was achieved (see Figure 2). Blood in SST tubes separates into phases upon centrifugation. If the blood did not separate into phases, ensure that the rotor is balanced and centrifuge the tube once more.

Figure 2: Proper Blood Separation of SST Tube



8. CRITICAL STEP: **Maintain the tube on ice until ready to package for shipment.**

9. Once all tubes have been collected and packaged for shipment, record the date of shipment, whether cold gel packs were used for shipping, and the CDI ID number (found on the labels) on the iPSC Blood Sample data form and ensure timely data entry into the eClinical database.

Blood for Infectious Disease Testing (4 ml Gold Top Tube)

Step One

- Store tube at room temperature.
- Label tube with pre-printed subject label prior to blood draw.

Step Two

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

Step Three

- Immediately after blood draw, invert tube *five* times to mix sample.

Step Four

- Incubate the tube UPRIGHT at room temperature for 30-120 minutes to allow the blood to clot.

Step Five

- Centrifuge the balanced tube at RT at 1100-1300 x g using the guidelines below:

Duration (min)	
Swinging Bucket Rotor	Fixed Angle Rotor
10	15

Step Six

- Ensure proper separation of the tube after centrifuging.
- If the blood did not separate into phases, ensure that the rotor is balanced and centrifuge the tube once more.
- Maintain the tube on ice until ready to package for shipment.

8.0 Collection of Blood for Reprogramming Cell Preparation Tube (Blue)

1. Place a **PBMC** label on the Cell Preparation tubes prior to blood draw.

2. Using a blood collection set and a holder, collect blood into the tubes using your institution's recommended procedure for standard venipuncture technique.

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

3. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** Each tube with its vacuum is designed to draw 8 ml of blood into the tube. Record the number of tubes collected and the time of draw on the iPSC Blood Sample data form.

4. CRITICAL STEP: **Immediately after blood collection, gently invert/mix (180 degree turns) the Cell Preparation tubes 5 times.**

5. **REPEAT STEPS 2 TO 4** for the remaining 3 Cell Preparation Tubes to be collected.

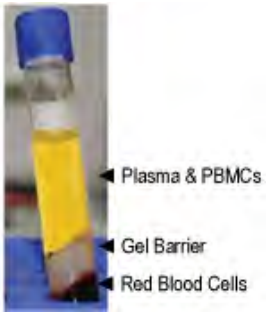
6. CRITICAL STEP: Incubate the Cell Preparation Tubes **UPRIGHT** at room temperature, 64°F - 77°F (18°C to 25°C) **for at least 30 minutes and no more than 120 minutes.**

7. After incubation at room temperature, centrifuge the balanced tube at room temperature, using the guidelines below for time, at 1500 - 1800 x g. **It is critical that the tubes be centrifuged at the appropriate speed to ensure proper plasma separation.** For assistance, see Appendix A. Record information about the time, rate, duration, and temperature of centrifugation on the iPSC Blood Sample data form.

Duration (min)	
Swinging Bucket Rotor	**Fixed Angle Rotor**
20	10

8. Inspect the tubes to ensure that proper blood separation was achieved (see Figure 3). Blood in Cell Preparation Tubes separates into phases upon centrifugation. If the blood did not separate into phases, ensure that the rotor is balanced and centrifuge the tube once more; however, be aware that there will be a reduction in the number of peripheral blood mononuclear cells (PBMCs) isolated. If the blood still does not separate, collect a fresh sample from the same donor.

Figure 3: Proper Blood Separation of Cell Preparation Tubes



9. CRITICAL STEP: **Maintain the tubes on ice until ready to package for shipment.**

10. Once all tubes have been collected and packaged for shipment, record the date of shipment, whether cold gel packs were used for shipping, and the CDI ID number (found on the labels) on the iPSC Blood Sample data form and ensure timely data entry into the eClinical database.

Blood for Reprogramming (4 x 8 ml Blue Top Tubes)

Step One

- Store tube at room temperature.
- Label tube with pre-printed subject label prior to blood draw.

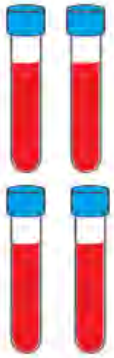
Step Two

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

Step Three

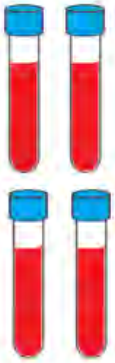
- Immediately after blood draw, invert tube *five* times to mix sample. Repeat Steps Two and Three for the remaining 3 tubes.

Step Four



- Incubate the tubes UPRIGHT at room temperature for 30-120 minutes.

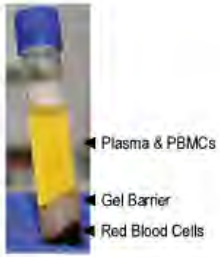
Step Five



- Centrifuge the balanced tubes at RT at 1500 to 1800 x g using the guidelines below:

Duration (min)	
Swinging Bucket Rotor	Fixed Angle Rotor
20	10

Step Six



- Ensure proper separation of the tubes after centrifuging.
- If the blood did not separate into phases, ensure that the rotor is balanced and centrifuge the tubes once more.
- Maintain the tubes on ice until ready to package for shipment.

9.0 Shipping Blood Samples to CDI

Package fresh blood samples on the same day as blood collection and ship by FedEx, using the Airbill provided, for overnight delivery to CDI. All samples must arrive on Monday through Thursday of any given week. Please do not collect or ship these samples on Fridays.

1. Equilibrate the 2 gel coolant packs at 4°C overnight. **Note: Maintain the gel coolant packs at 4°C until ready to use. Do not freeze the gel coolant packs.**

2. Place the Vacutainer tubes into the absorbent tube shuttle.

3. Place the absorbent tube shuttle, with tubes inside, into the biohazard bag. Seal the bag according to the directions printed on the bag.

4. Place the gel coolant packs around the bag as indicated below.

5. Place the biohazard bag, with the gel coolant packs around it, into the insulated resealable pouch. Seal the pouch.

6. Complete the shipping manifest and insert into the rigid cardboard shipper.

7. Place the insulated resealable pouch into the rigid cardboard shipper. Close the shipper by inserting the tabs into the slots.

8. Affix the UN3373 and biohazard labels to the outside of the rigid cardboard shipper.

9. Affix the FedEx Airbill to the outside of the FedEx ClinPak where indicated.

10. Complete the list of contents as applicable and place this and the rigid cardboard shipper into the FedEx ClinPak.

11. Ship by FedEx for overnight delivery using the supplied shipping labels to:

    Cellular Dynamics International
    c/o AllCells (CDI PPMI project)
    1301 Harbor Bay Parkway, Suite 200,
    Alameda, CA 94502

12. Send an email with the FedEx tracking number to ppmi-cdi@allcells.com to enable CDI to track and prepare for delivery of the shipment. Also copy the PPMI Biorepository at IU at ppmibio@iu.edu.

APPENDIX N

PPMI iPSC Collection Sample Manifest

Please complete this form electronically (i.e., typed). All fields are required.

Site #: 001 **Site Name:** University of Pennsylvania **Principal Investigator:**

Coordinator: **e-mail:**

Instructions: **Ship samples on the day of collection, Monday – Thursday ONLY.** This form must be completed for all sample shipments. Notify CDI and Indiana University by e-mail prior to shipment using the contact information below. Place a copy of the completed form in the shipment box and file a copy in the site study binder. The site will be contacted if any issues with the samples/form are noted upon receipt.

Completed by Submitter/Site List PPMI and CDI ID that correspond to pre-printed labels. List only one tube type per row.						
PPMI ID #	**CDI ID #**	**Tube Type (Li Hep, SST, or CPT)**	**# of Tubes**	**Tube Volume (if less than standard)**	**Date of Draw**	**Gender (M/F)**
Total number of tubes:						

Date shipped: **FedEx tracking #:**

IMPORTANT!

BEFORE SHIPPING, EMAIL A COPY OF THE COMPLETED FORM TO:

CDI: ppmi-cdi@allcells.com

IU: ppmibio@iu.edu

PLEASE REMEMBER TO SHIP THESE SAMPLES TO CDI, NOT TO IU!

APPENDIX O

PBMC Preparation (10ml Green-Top Tube)

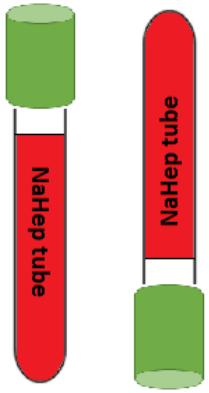
Step One

- Store tubes at room temperature.
- Label tubes with pre-printed subject label prior to blood draw.

Step Two

- Collect blood in Sodium Heparin Tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



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

Step Four

- Store tubes at room temperature until shipment.
- <u>Ship ambient same day as blood draw</u>