

Schizophrenia Spectrum Biomarkers Consortium

BIOSPECIMEN COLLECTION & PROCESSING

Overview

- 1. Specimen uniformity and quality
- 2. Site Equipment
- 3. Procedures
 - Kit Contents and Ordering
 - Sample Labelling
 - Sample Collection and Processing
 - Shipping Samples
 - Non-Conformance
- 4. Contact Information

Specimen Uniformity and Quality

I WILL REPEAT THESE WORDS A LOT. YOU MAY WANT TO START A TALLY.

Biorepositories at Indiana University







M NCRAD



PARKINSON'S **PROGRESSION** MARKERS INITIATIVE

Play a Part in Parkinson's Research







NCAA•DOD Grand Alliance CARE Consortium

Specimen Standardization and Quality

Most biomarkers are sensitive to *time* and *temperature*

- Standardization of processing across sites is key
- Specimens must be processed within 2 hours of collection
- Reference the SSBC Biomarker Specimen Collection, Processing, and Shipment Manual as needed
- Do not replace or supplement any kit components without first receiving approval from Indiana

Questions? Email ssbc@iu.edu

Site Equipment

Sites will need to supply the following items:

- Gloves
- Alcohol wipes
- Butterfly needles
- Tourniquet
- Gauze pads
- Bandages
- Microcentrifuge tube rack
- Sharps bin and lid

- Crushed ice
- Pipettes and pipette tips
- Centrifuge capable of maintaining 4°C
- -80°C Freezer
- Dry ice
- CryoStor® freezing media

Procedures

MAINTAINING SPECIMEN UNIFORMITY AND QUALITY

Subject ID: Assigning SSBC Subject ID

- Each site will assign SSBC Subject ID from list of unique, site-specific IDs provided by Broad Institute
- SSBC Subject ID will be patient identifier throughout participation in study
- Batch of barcoded SSBC Subject ID labels will be sent to each site with initial kit shipment
- Replacements and additional SSBC Subject ID label sets can be requested from IUGB via the Kit Request Module https://kits.iu.edu/ssbc

- Affix labels to:
 - Local site documents
 - Specimen kits
 - Biological specimens sent to local analysis labs
 - Do NOT place on specimens sent to IUGB!
- SSBC Subject ID should also be entered into IUGB Sample Submission form when shipping to IUGB

Subject ID: Requesting the GUID

- Globally Unique Identifier (GUID) will be the "master" subject ID
- Random alpha-numeric string
- To request a GUID for a subject, you must set up an account with the NIMH Data Archive

https://nda.nih.gov/s/guid/nda-guid.html

In order to generate a GUID, the following PHI is required:

- Complete legal given (first) name of subject at birth
- o If the subject has a middle name
- Complete legal family (last) name of subject at birth
- Day of birth
- Month of birth
- Year of birth
- Name of city/municipality in which subject was born
- Country of birth

Kit Contents and Ordering

- All sites will be sent a Supplemental Kit with their first kit shipment
 - Contains extra blood collection tubes, processing supplies, and LP needles
 - May be used to replace items in study visit kits
- Study Visit Kits should be ordered as soon as visits are planned
 - Contains collection, processing, and shipping supplies specific to each visit
 - Include barcoded labels
 - The supplies/labels in each study visit kit are intended for that visit only

Kit Contents and Ordering – REDCap Survey

http://kits.iu.edu/ssbc

Order kits online through the Kit Request Module for:

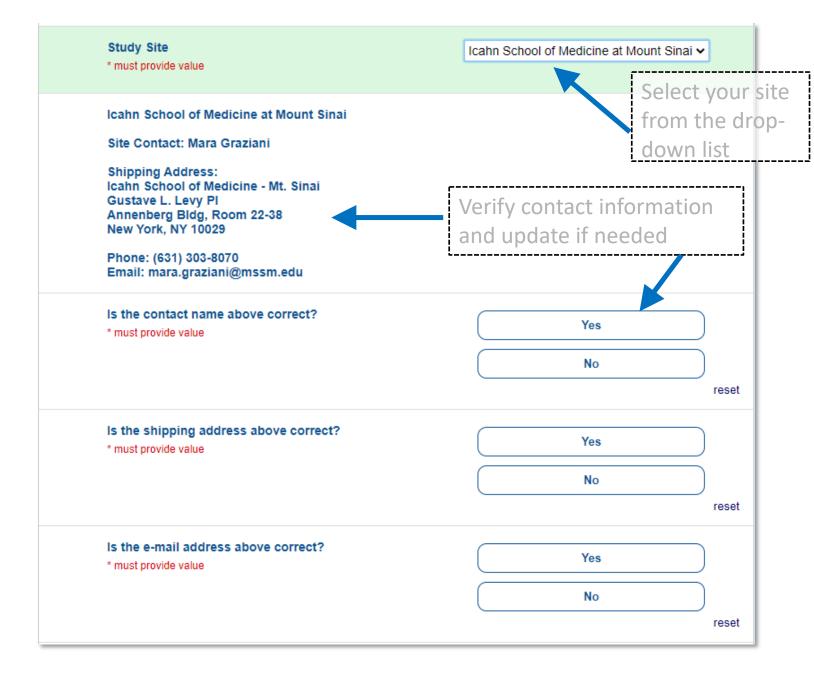
- Baseline Blood & CSF kits (includes PBMC collection)
- Follow-Up Blood & CSF kits (No PBMC)
- Subject Labels
- Extra Supplies

Please provide as much notice as possible when ordering kits and/or supplies.



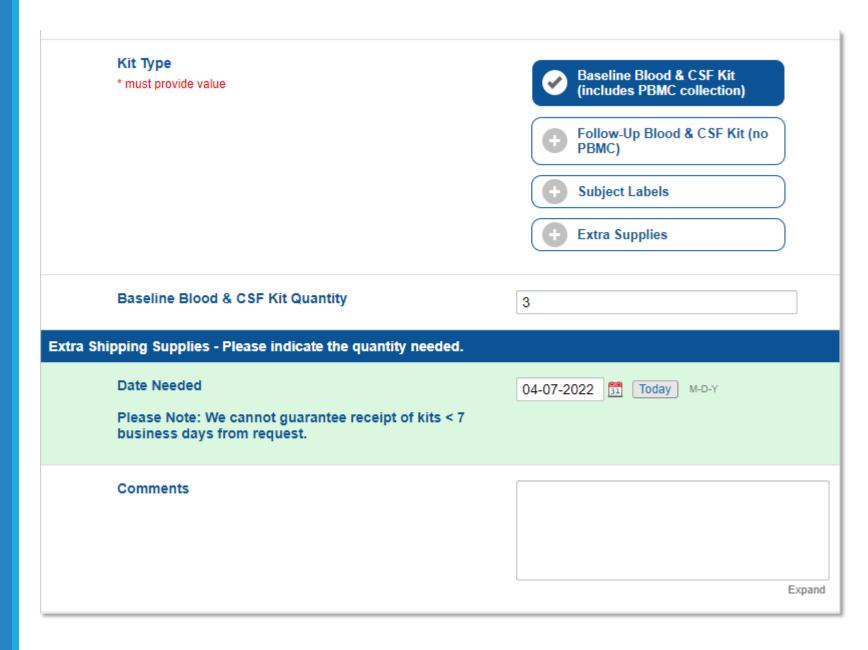
Kit Contents and Ordering: Confirm Site Info

SSBC Kit Request Module



Kit Contents and Ordering: Kit Types

SSBC Kit Request Module



Kit Contents and Ordering: Kit Breakdown

SSBC Kit Request Module

Comments

Expand

Each Complete Blood & CSF Kit includes:

- 1 Set of protective bubble pouches for tubes
- 1 Cryobox
- 21 Cryogenic vials (2 ml) with clear caps
- 3 Cryogenic vials (2 ml) with red caps
- 4 Cryogenic vials (2 ml) with purple caps
- 3 Cryogenic vials (2 ml) with orange caps
- 3 Cryogenic vials (2 ml) with blue caps
- 1 Cryogenic vial (4 ml) with orange cap
- 2 Sterile screw-top centrifuge tubes (15 ml)
- 2 Screw-top centrifuge tubes (15 ml)
- 1 Shipping container for dry ice shipments
- 2 95 kPa biohazard bag with absorbent sheet
- 2 PAXgene™ tube (2.5 ml)
- 1 Purple-top EDTA tube (10 ml)
- 1 Red-top serum tube (10 ml)
- 2 Green-top sodium heparin tube (10 ml)
- 1 Gold-top SST tube (5 ml)
- 3 Transfer pipette
- 2 Shipping label packets
- 2 Shipping instruction sheets
- 1 Ambient shipping box with coldpack and biohazard bag
- 1 Ambient shipping overpack
- 1 Lumbar puncture tray
- 1 Medication transfer filter straw

Kit contents of selected kit will appear at the bottom of the page

Submit

Kit Type

* must provide value

	Baseline Blood & CSF Kit
T	(includes PBMC collection)



Subject Labels
-

	Entes Consilies
-	Extra Supplies

ood Collection and Processing Supplie	s - Please indicat	e the quantity nee	ded.	
	2	5	10	20
EDTA tube, 10 mL (glass)	\circ	\circ	\circ	C
Serum tube, 10 mL (glass)	0	0	0	С
PAXgene™ RNA tube, 2.5 mL	0	0	0	С
NaHep tube, 10 mL	0	0	0	С
SST tube, 5mL	0	0	0	С
Purple-capped cryotube, 2 mL	0	0	0	С
Red-capped cryotube, 2 mL	0	0	0	С
Blue-capped cryotube, 2 mL	0	0	0	С
Clear-capped cryotube, 2 mL	0	0	0	С
Orange-capped cryotube, 2 mL (Corning)	0	0	0	С
Disposable pipettes, 3mL	0	0	0	С
Orange-cap Conical tube, 15 mL	0	0	0	С
Conical tube, 50 mL	0	0	0	С
Orange-capped cryotube, 4mL	0	0	0	С

Extra CSF Collection Supplies - Please indic	ate the quantity needed.			
	1	2	3	
22G LP Tray	0	0	C	eset
22G LP Needle w/ introducer, 3.5"	0	0	0	eset
22G LP Needle w/ introducer, 4.75"	0	0	0	eset
Medication transfer filter straw	0	0	0	eset
Sterile Conical tube,15mL	0	0	0	eset
Extra Shipping Supplies - Please indicate the	e quantity needed.		Te	set
	1	2	3	
Ambient shipping kit	0	0	O	eset
Frozen shipping box (small)	0	0	0	eset
95kPa biohazard bag w/ absorbent sheet (large)	0	0	0	eset
UN3373 Category B labels	0	0	0	eset
UN1875 Dry Ice label	0	0	0	eset
Clear UPS Label Sleeves	0	0	0	eset
Frozen shipping box (XS) - for UPenr NMDA samples	n ()	0	0	eset
Date Needed		04-07-2022 🛅 Today	1-D-Y	odl .
Please Note: We cannot guarantee business days from request.	e receipt of kits < 7			
Comments				
			Ехра	ind
	Submit			

Kit Contents and Ordering: Blood Kits

Blood Kit (frozen):



PBMC supplies:



Kit Contents and Ordering: CSF Kits

CSF: LP Tray:

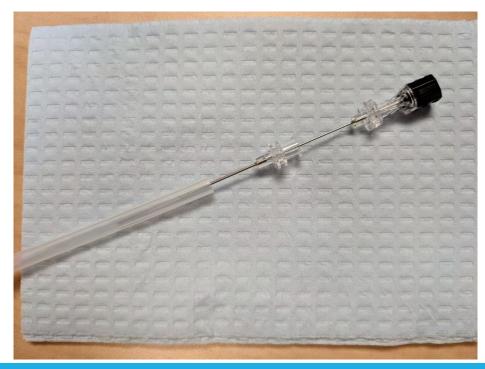




22G Needle with Introducer

These needles an be ordered from the Extra Supplies option of the SSBC Kit Request Survey







Kit Contents and Ordering: NMDA Kit

UPenn anti-NMDA Shipping Kit:



Collection Volumes

Total blood and CSF volumes

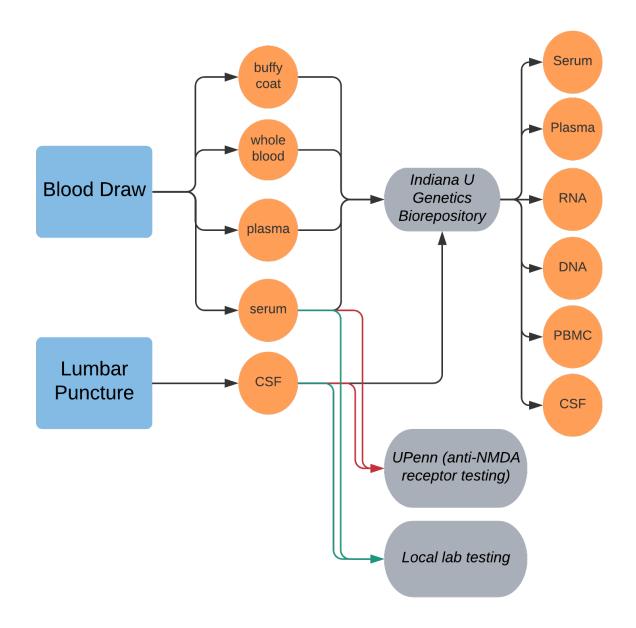
Sample Type	Amount
Whole Blood for Clinical Labs	5 ml
Whole Blood for RNA	5 ml
Whole Blood for Plasma and Buffy Coat	10 ml
Whole Blood for Serum	10 ml
Whole Blood for PBMCs (OPTIONAL)	20 ml
Cerebrospinal Fluid	13-15 ml

Maximum blood volume: 50 ml (approx. 8 teaspoons)

Maximum CSF volume: 15 ml (approx. 3 teaspoons)

Sample Flow

"Wait, where are these samples going?"



Sample Labelling: Example Labels

Labels are provided by Indiana University

- Please check that all samples are properly labelled to ensure correct identification by IU
- If do not have enough labels to complete a visit, please contact IU immediately
- Labelling the tubes during processing prevents sample mix-ups

Kit ID

125

987654

0001234567 SSBC 987654 CSF

CSF NMDA

###
987654

Sample Labelling: Label Placement

Please...

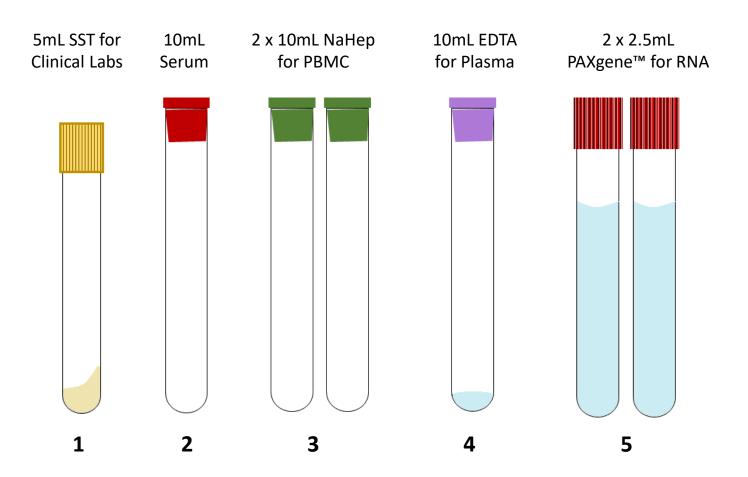
- Label all collection and aliquot tubes before cooling, collecting, processing, or freezing samples
- Label only 1 subject's tubes at a time to avoid mix-ups
- Wrap the label around the tube horizontally label position is important for all tube types
- Make sure the label is completely adhered by rolling between your fingers



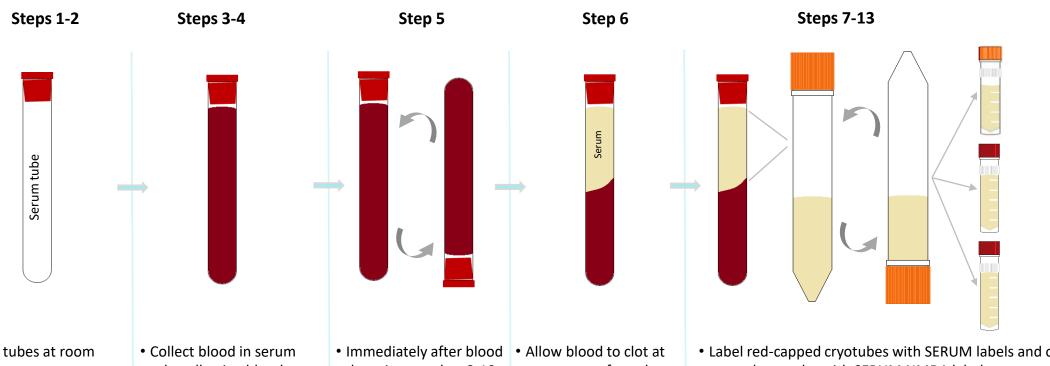


Sample Collection and Processing

Blood Tube Draw Order

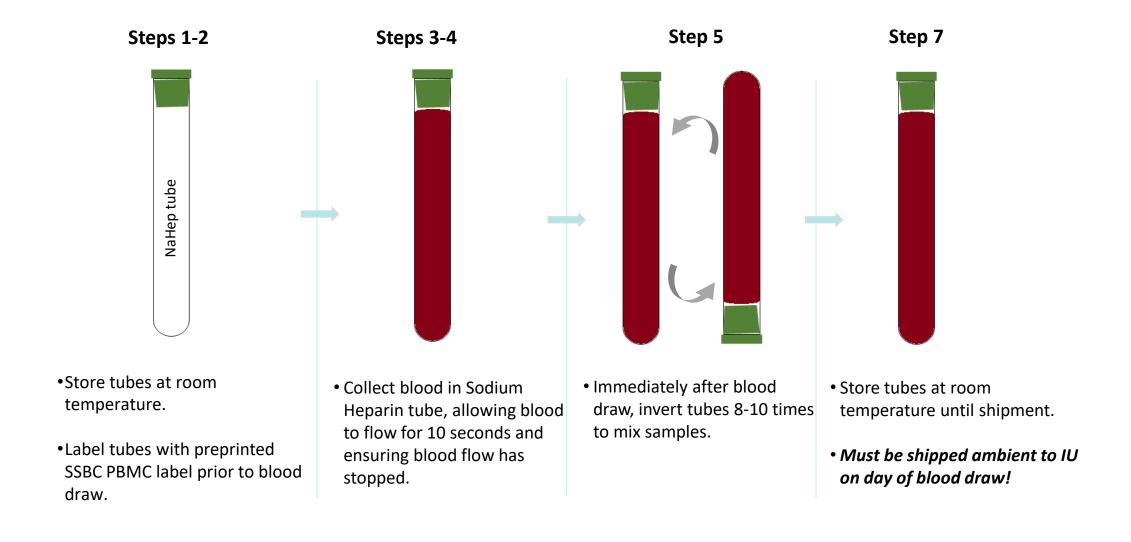


Sample Collection and Processing: Serum



- Store tubes at room temperature.
- Label tubes with preprinted SSBC serum label prior to blood draw.
- tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.
- draw, invert tubes 8-10 times to mix samples.
- room temp. for at least 15 minutes
- Within 60 minutes of blood draw, centrifuge sample at <u>4°C</u> at <u>1500 x</u> g for 15 minutes.
- Label red-capped cryotubes with SERUM labels and orangecapped cryotube with SERUM NMDA labels.
- Using a clean transfer pipet, transfer all serum into a 15 ml conical tube and mix gently by inverting 3-4 times.
- Aliquot 1 ml of serum into the orange-capped SERUM NMDA labelled tube. Aliquot 1.5 ml into each SERUM cryotube.
- If a residual aliquot (<1.5 ml) is created, place a blue cap on this aliquot.
- Store serum aliquots upright at -80°C until shipment.

Sample Collection and Processing: PBMCs



Labeling Ambient NaHep

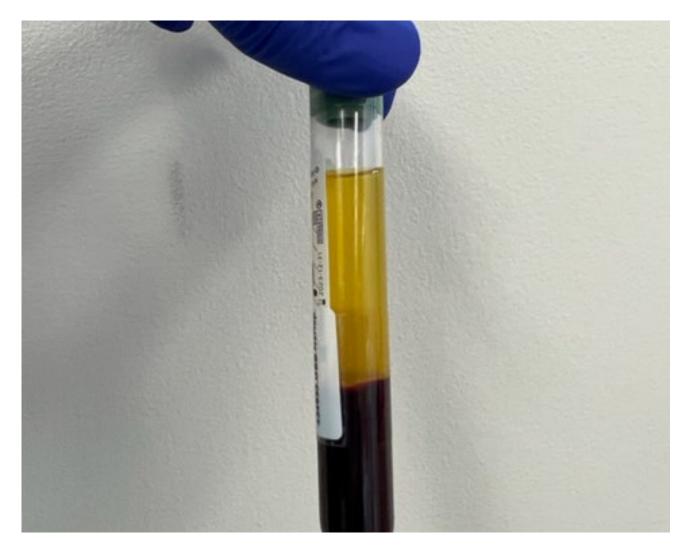
Position

Position the label so that one edge of the OnCore label starts at the back edge of the label with the PTID. Place label toward the bottom of the tube.

Attach

Attach the OnCore label to allow a viewing window down the length of the tube.

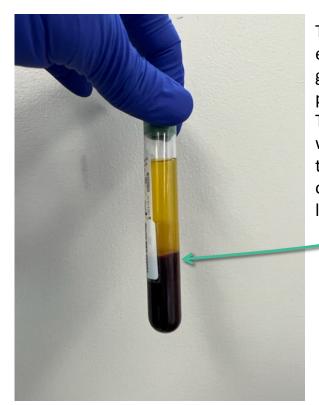
Avoid Stopper If label must be placed near the top of the tube, do not allow label to come in contact with the stopper.



Correct label placement allows a clear view of the buffy coat.

Labeling Ambient NaHep

Good label placement



This is an example of good label placement. The new label was started at the back edge of the existing label.

Poor label placement



This label was applied in a poor position. The window to view the buffy coat is not visible. The processing tech would need to remove the label to see and collect the buffy coat.

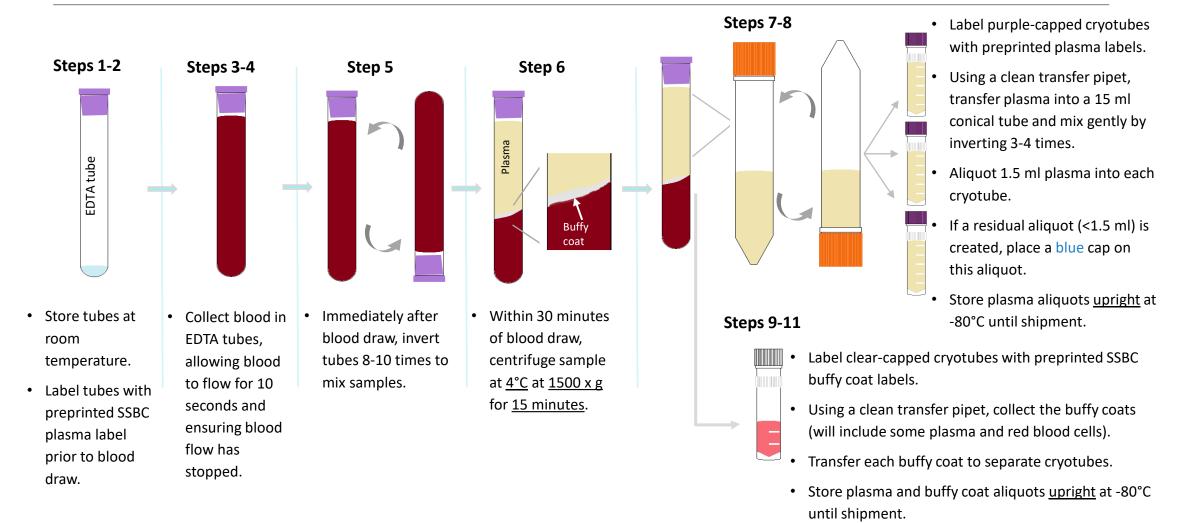
Labeling Ambient NaHep

Correct label placement will allow the processing lab to more easily see and collect the buffy coat layer after centrifugation.

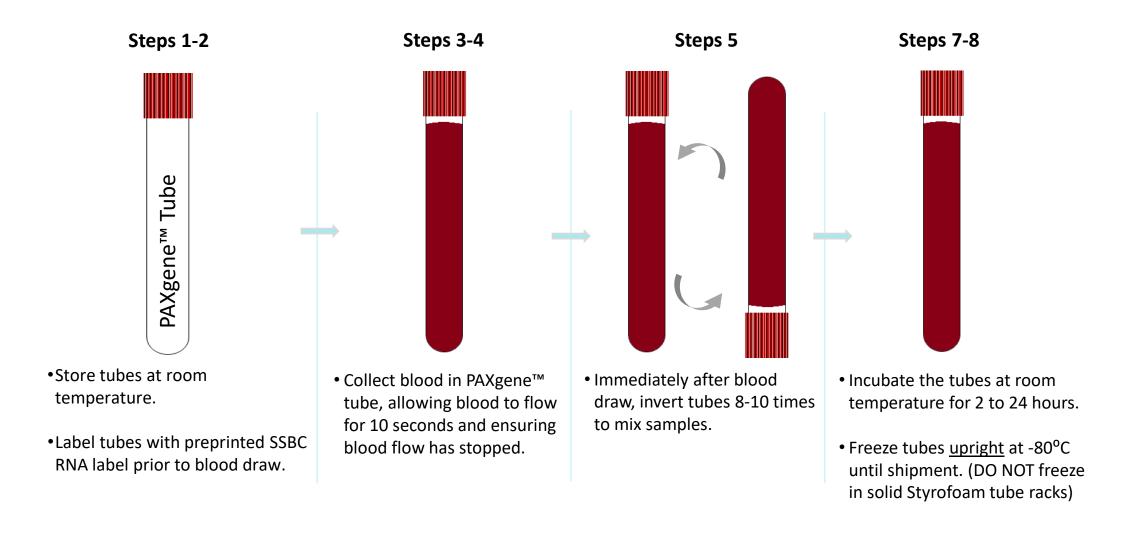


Correct label placement allows a clear view of the buffy coat.

Sample Collection and Processing: Plasma

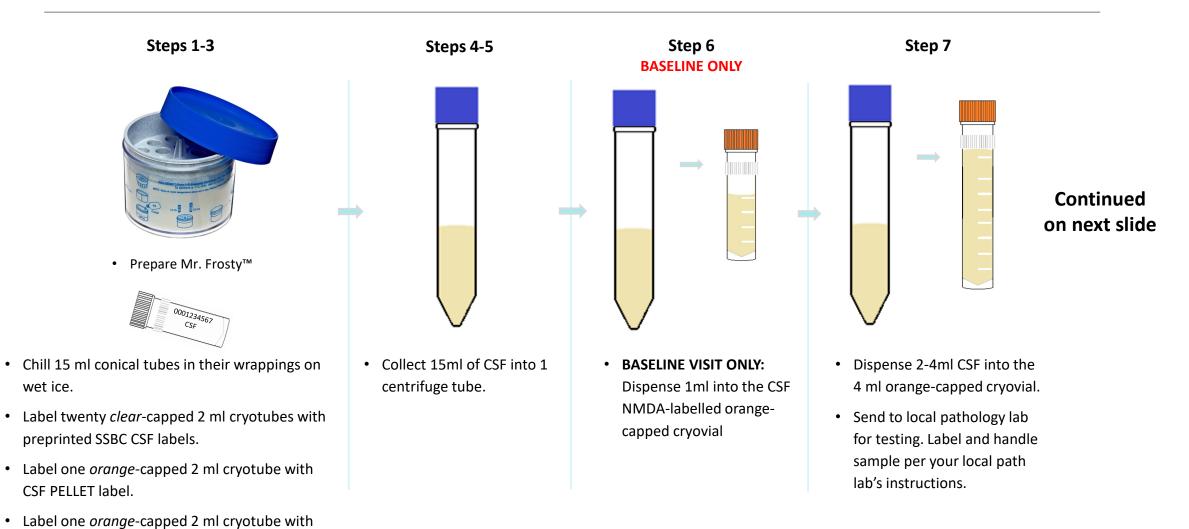


Sample Collection and Processing: Whole blood RNA

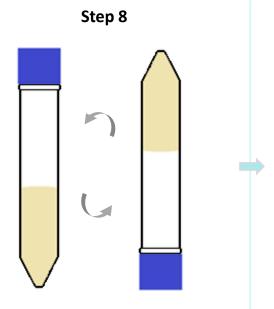


Sample Collection and Processing: CSF

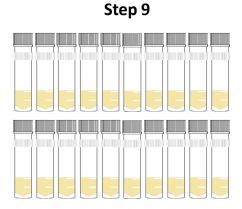
CSF NMDA label.



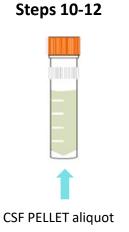
Sample Collection and Processing: CSF (cont'd)



• Gently invert the remaining 10 ml of CSF in the centrifuge tube 3-4 times to mix the sample.



- Within 30 minutes of collection, centrifuge samples at 300 x g for 10 minutes at 4° C.
- Aliquot 500 ul of supernatant directly into each of the prepared cryotubes, being careful not to disturb the pellet at the bottom of the conical tube.
- Leave 500 ul of CSF in the conical tube.



- Add 500 ul CryoStor® to 500 ul of CSF and cell pellet in the 15 ml conical tube.
- Resuspend pellet using pipetting technique.
- Transfer the resuspended CSF pellet to the pre-labeled orange cryotube.
- Within 60 minutes of CSF collection, freeze CSF aliquots **upright** in rack or cryobox at -80° C.
- Place pellet aliquot in the prepared Mr. Frosty™ and store at -80° C overnight.

Sample Collection and Processing: Aliquots

Filling biomarker serum, plasma, and CSF aliquots:

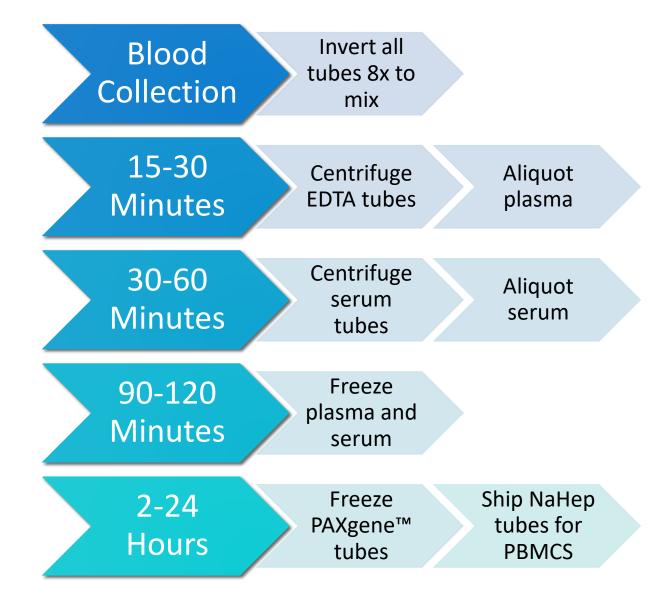
- Ensure that you are using the correct vials
 - Red cap = serum
 - Purple cap = plasma
 - Clear cap = CSF and buffy coat
 - Blue cap = residuals
 - Orange = CSF pellet, anti-NMDA samples
- Fill as many cryovials as possible to 1.5 ml (plasma & serum) or 0.5 ml (CSF)
- Over-filled vials may burst in freezer!
- Ship ALL material to IU, even if final vial is less than standard volume





Sample Collection and Processing: Timeline

Timeline for blood processing



Sample Collection and Processing: Issue #1

Troubleshooting Blood Collection

Issue #1: Tube with little/no vacuum

- Always check expiration date on the tube before beginning blood draw and discard expired tubes
- Store tubes at "room temperature" extreme temperature can affect vacuum
- Keep extra vacutainer tubes from supplemental kit nearby during blood draw to replace "bad" tubes
- If this is a frequent occurrence, report tube type and lot number to IU.

Sample Collection and Processing: Issue #2

Troubleshooting Blood Collection

Issue #2: Hemolyzed serum and/or plasma caused by incorrect collection

Cause: Blood Collection Methods	Corrective Action
Improper venipuncture site	Draw from median cubital, basalic, and cephalic veins from antecubital region of arm
Prolonged tourniquet use	Tourniquet should be released after no more than 1 min, excessive fist clenching should be avoided
Not allowing alcohol to dry on skin before venipuncture	Without touching, allow the venipuncture site to air dry
Use of too large/small bore needle resulting in excess force applied to blood	Avoid using too small/large needle. Needle size dependent on the subject's physical characteristics & amount of blood to be drawn. Most commonly used sizes are 19 – 23.
Pulling/pushing plunger too fast while drawing/transferring blood	Avoid drawing the syringe plunger too forcefully when collecting blood
Ensure all blood collection assemblies	are fitted securely, to avoid frothing

For more information, visit: http://www.bd.com/vacutainer/pdfs/techtalk/TechTalk_Jan2004_VS7167.pdf

Sample Collection and Processing: Issue #2 continued

Troubleshooting Blood Collection

Issue #2: Hemolyzed serum and/or plasma caused by incorrect processing

Cause: Sample Processing Methods	Corrective Actions
Vigorous mixing/shaking	Gently invert blood collection tube when mixing additive with specimen, follow guidelines in Biologics Manual regarding number of times to invert each type of tube
Not allowing serum to clot for recommended time	Serum tubes without clot activator should be allowed to clot for 60 min in a vertical position
Exposure to excessive heat or cold	Keep samples at ambient temperature until processing
Prolonged contact of serum/plasma with cells	Do not store uncentrifuged samples beyond recommended time

For more information, visit: http://www.bd.com/vacutainer/pdfs/techtalk/TechTalk_Jan2004_VS7167.pdf

Sample Collection and Processing: REDCap Survey



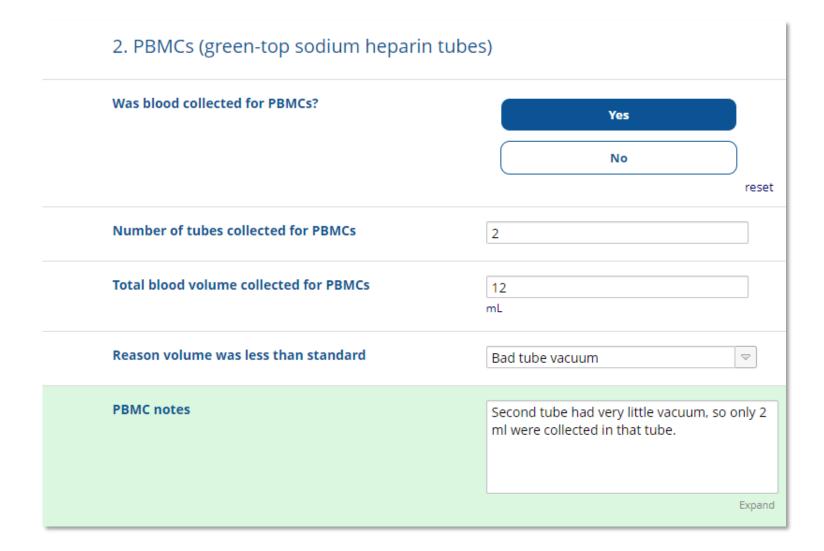
Sample Collection and Processing: Serum Data

	Page 2 or 3
Blood Collection and Processing	
Date of blood collection	06-01-2020 Today M-D-Y
Time of blood collection	09:00 Now H:M Use 24 Hour clock.
Patient's fasting status at time of blood collection	Fasted
1. SERUM (red-top serum tube)	
Was blood collected and processed for SERUM?	No reset
Blood volume collected for SERUM	10 mL
Time of SERUM tube centrifugation	09:30 Now H:M Use 24 Hour clock.
Rate of SERUM tube centrifugation	1500 × g
Duration of SERUM tube centrifugation	15 minutes
Temperature of SERUM tube centrifugation	4 degrees Celsius
Total volume of SERUM collected	4.5 mL

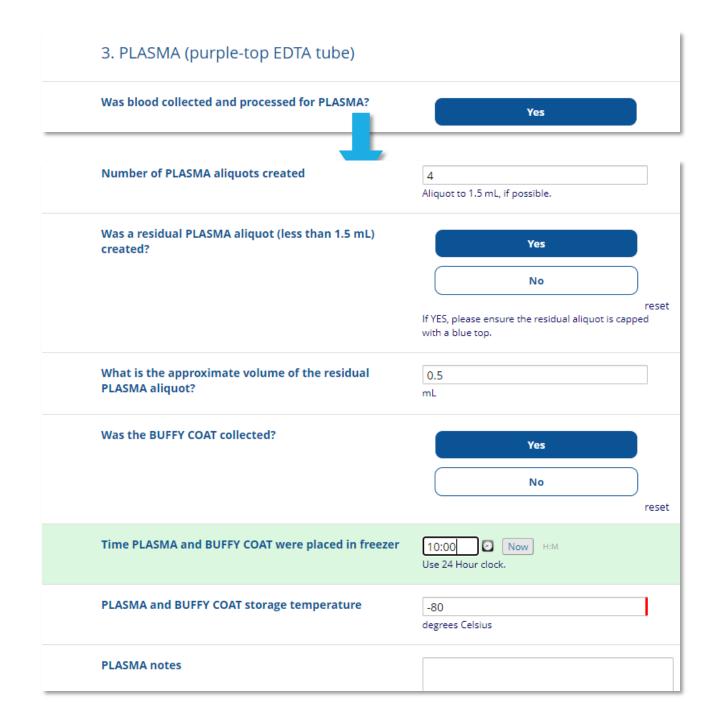
Sample Collection and Processing: Serum Data con't

Total volume of SERUM collected	4.5 mL
Number of SERUM aliquots created	Aliquot to 1.5 mL, if possible.
Was a residual SERUM aliquot (less than 1.5 mL) created?	No reset If YES, please ensure the residual aliquot is capped with a blue top.
Time SERUM was placed in freezer	10:00 Now H:M Use 24 Hour clock.
SERUM storage temperature	-80 degrees Celsius
SERUM notes	
	Expand

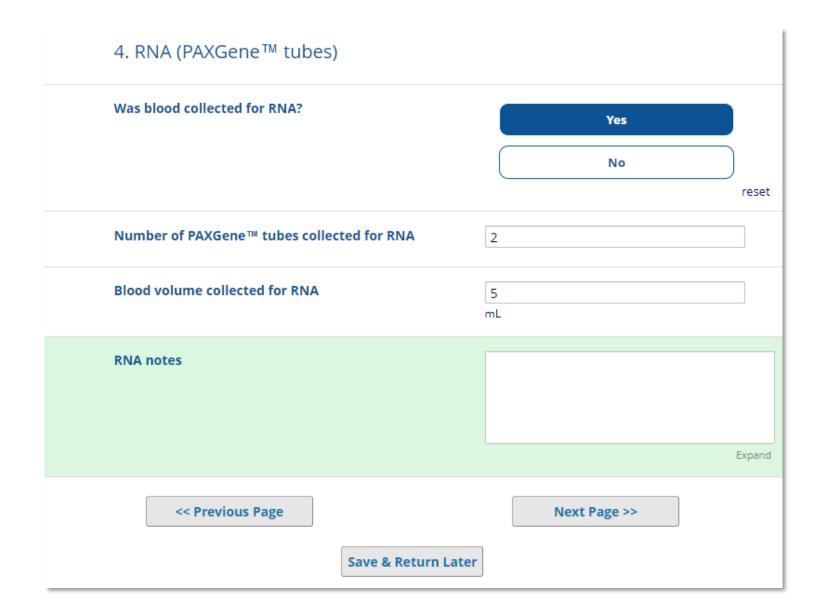
Sample Collection and Processing: PBMC Data



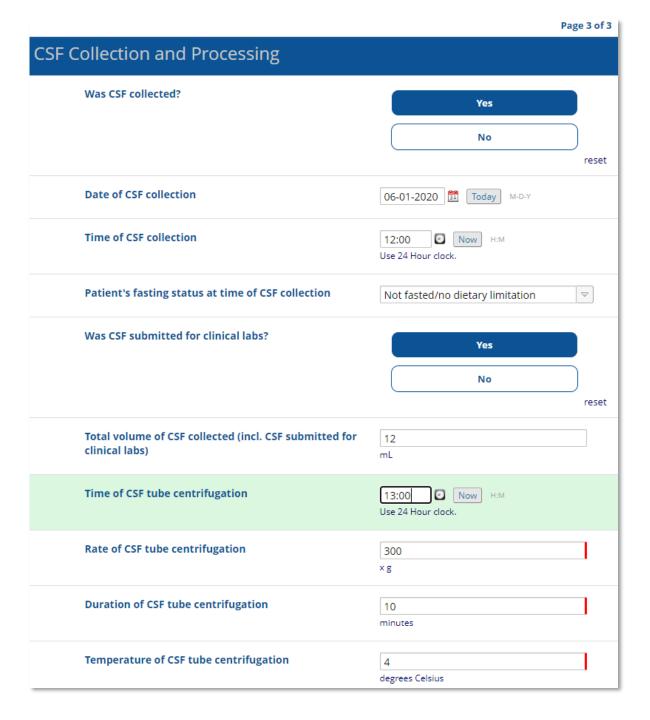
Sample Collection and Processing: Plasma Data



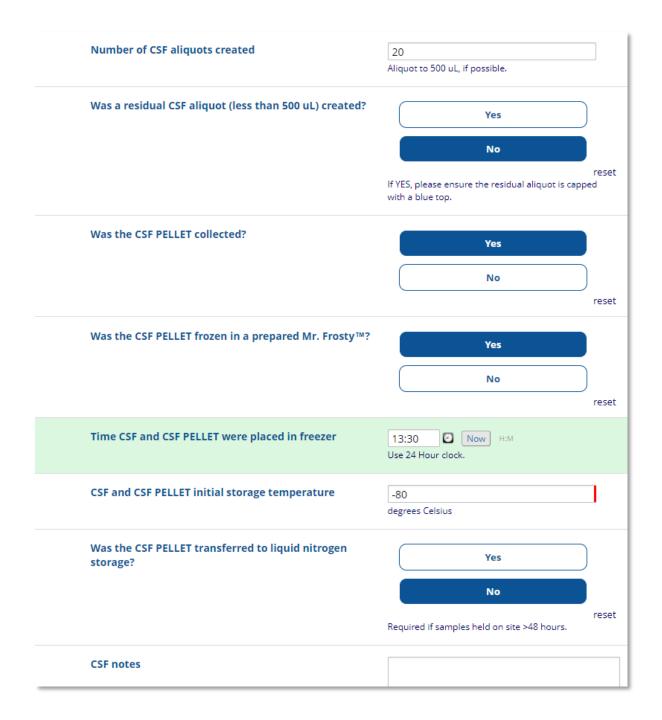
Sample Collection and Processing: RNA Data



Sample Collection and Processing: CSF Data



Sample Collection and Processing: CSF Data con't



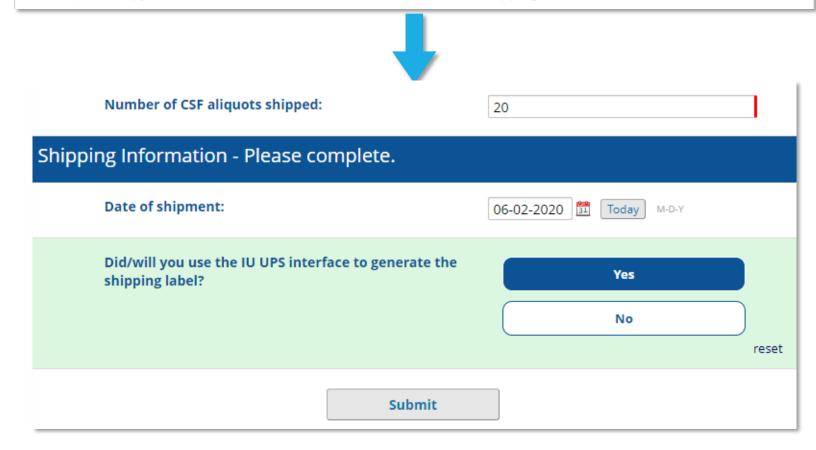
Shipping Samples: Shipping Manifests

A copy of the Shipping Manifest should be included in every shipment to Indiana University

SSBC Frozen Shipping Manifest

Please verify/update the information below. When you click the "Submit" button below, a PDF copy of the Frozen Shipping Manifest will be emailed to you for Subject MS123456.

Please print a copy of that document and include it in the Kit #987654 shipping container.



Shipping Samples: Frozen Shipping Manifest

A copy of the Shipping Manifest should be included in every shipment to Indiana University

fidential		Page 1
SSBC Frozen Shipping Manifest	:	rage 1
Please verify/update the information below. When you click the Shipping Manifest will be emailed to you for Subject [subj_id].	"Submit" button below, a PDF copy of th	e Frozen
Please print a copy of that document and include it in the Kit #	[kit_num] shipping container.	
Study Site:		
Subject ID:		
Visit:		
IU Kit Number:		
Date of blood collection:		
SERUM		
Number of SERUM aliquots shipped:		
Volume of residual SERUM aliquot:	(mL)	
PLASMA		
Number of PLASMA aliquots shipped:		
Volume of residual PLASMA aliquot:	(mL)	
RNA		
Number of PAXGene™ tubes shipped		
CSF		
Date of CSF collection:		
Number of CSF aliquots shipped:		
Number of CSF CELL PELLET shipped:		

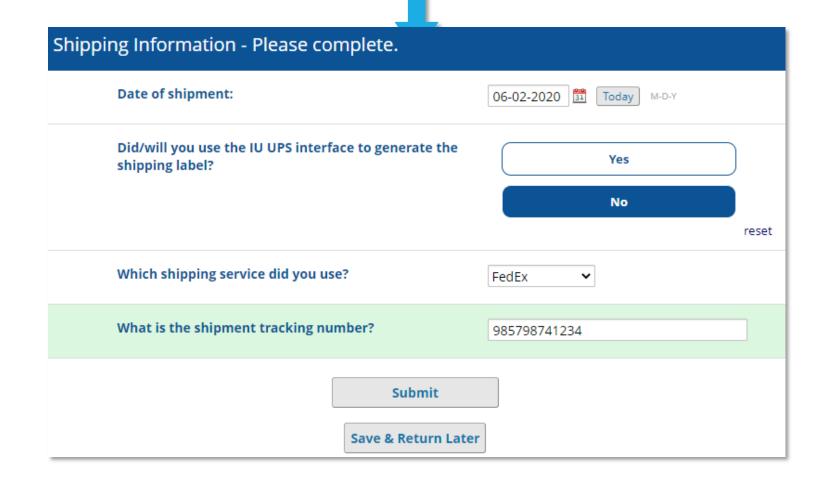
Shipping Samples: Ambient Shipping Manifest

A copy of the Shipping Manifest should be included in every shipment to Indiana University

SSBC Ambient Shipping Manifest

Please verify/update the information below. When you click the "Submit" button below, a PDF copy of the Ambient Shipping Manifest will be emailed to you for Subject MS123456. Please print a copy of that document and include it in the shipping container with Kit #987654.

If you did NOT collect PBMCs, this form should be blank.



Shipping Samples: Ambient Shipping Manifests

A copy of the Shipping Manifest should be included in every shipment to Indiana University Confidential

Page 1

SSBC Ambient Shipping Manifest

Please verify/update the information below. When you click the "Submit" button below, a PDF copy of the Ambient Shipping Manifest will be emailed to you for Subject [subj_id]. Please print a copy of that document and include it in the shipping container with Kit #[kit_num].

If you did NOT collect PBMCs, this form should be blank.

Because blood for PBMCs was not collected from this subje	ct, please skip this form.	
Study Site:		
Subject ID:		
Visit:		
IU Kit Number:		
Number of sodium heparin tubes collected for PBMC extraction:		
Total volume of blood collected for PBMC extraction:		
	(mL)	
Date of collection:		
Time of collection:		
	(24-hour clock)	
Shipping Information - Please complete.		
Date of shipment:		
Did/will you use the IU UPS interface to generate the shipping label?	○ Yes ○ No	
Which shipping service did you use?	○ UPS ○ FedEx ○ World Courier ○ Other	
What is the shipment tracking number?		

Shipping Samples: NMDAR Shipping Manifest

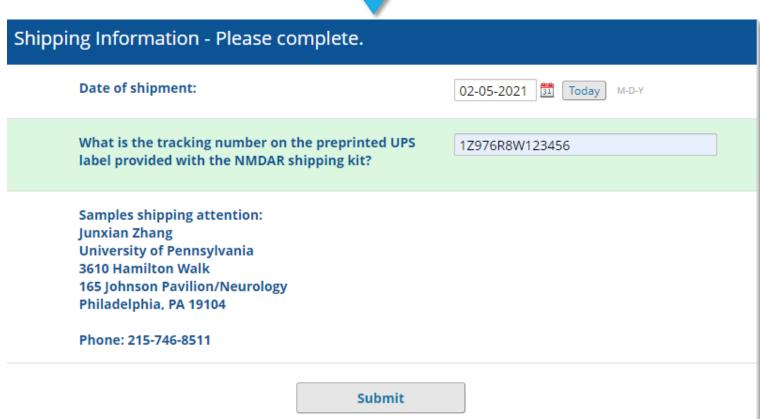
A copy of the NMDAR Shipping Manifest should be included in every shipment to UPenn

SSBC NMDAR Shipping Manifest

Please verify/update the information below. When you click the "Submit" button below, a PDF copy of the NMDAR Shipping Manifest will be emailed to you for Subject MS123456.

Please print a copy of that document and include it in the UPENN Kit #987654 shipping container.





Shipping Samples: NMDAR Shipping Manifests

A copy of the NMDAR Shipping Manifest should be included in every shipment to UPenn

SSBC NMDAR Shipping Manife	e st	
Please verify/update the information below. When you click the "Submit" button below, a PDF copy of the NMDAR Shipping Manifest will be emailed to you for Subject [subj_id].		
Please print a copy of that document and include it in the UP	ENN Kit #[kit_num] shipping container.	
Study Site:		
Email address of site contact:		
Subject ID:		
Visit:		
IU Kit Number:		
SERUM		
Number of 1 ml SERUM aliquots for anti-NMDAR testing:		
Date of serum collection:		
bate of Seram Confection.		
CSF		
Number of 1 ml CSF aliquots for anti-NMDAR testing:		
Date of CSF collection:		
Shipping Information - Please complete.		
Date of shipment:		
What is the tracking number on the preprinted UPS label provided with the NMDAR shipping kit?		
Estimated date and time of hand delivery (UPENN site only):		
Please call the laboratory (215-746-8511) or text Junxian (mobile: 610-368-2425) and Eric (mobile: 215-200-7646) to let them know you are on your way.		
Samples shipping to: Junxian Zhang University of Pennsylvania 3610 Hamilton Walk 165 Johnson Pavilion/Neurology		
165 JOHNSON PAVIIION/NEUROIOUV		

Shipping Samples: Ambient

Packing and Shipping Ambient Samples

- Whole blood for PBMCs ships at ambient temperature
- Ship ambient samples only Monday through Thursday
- Ship on same day of collection
- Only ship one subject's specimens in each shipping carton (unless otherwise instructed)

Shipping Ambient Samples

Packing and Shipping Ambient Samples









Shipping Frozen Samples: Tips

Packing and Shipping Frozen Samples

- Biomarker serum, plasma, buffy coats, CSF, and whole blood for RNA all ship frozen
- Ship frozen samples on dry ice
- Frozen samples should be shipped only Monday through Thursday
- Only ship one subject's specimens in each shipping carton
- Always fill carton to top with dry ice
- Do not pack shipment until the day of pickup



Shipping Frozen Samples

Special instructions for CSF cell pellet:

- Ship within two days of collection*
 - CSF pellet must be frozen in Mr. Frosty™ / -80° C freezer overnight and shipped to
 IU the next day
- If frozen samples collected on Thursday, CSF pellet must be frozen in Mr. Frosty™ / -80° C freezer overnight and then placed in LN2 tank until shipment following Monday

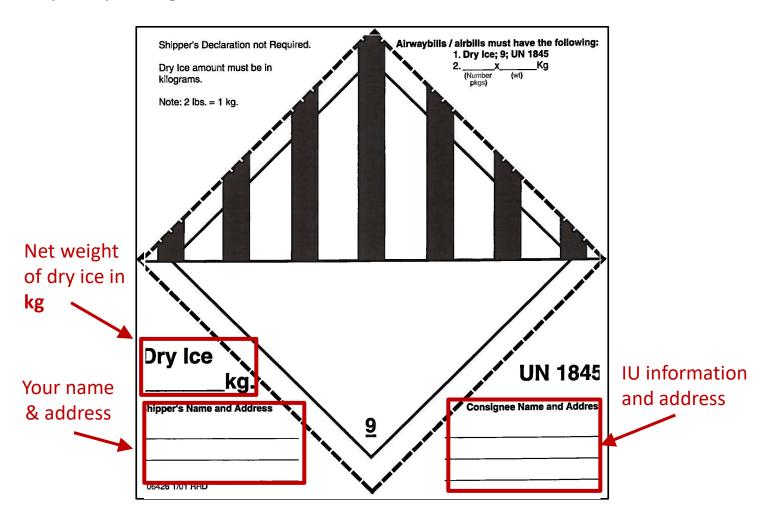
*CSF pellet must be moved to LN2 if not shipped within 48 hours



Shipping Samples

Packing and Shipping Frozen Samples

Class 9 Dry Ice Label should not be covered with other stickers and must be completed, or UPS will reject/return your package!



Shipping Samples: Frozen

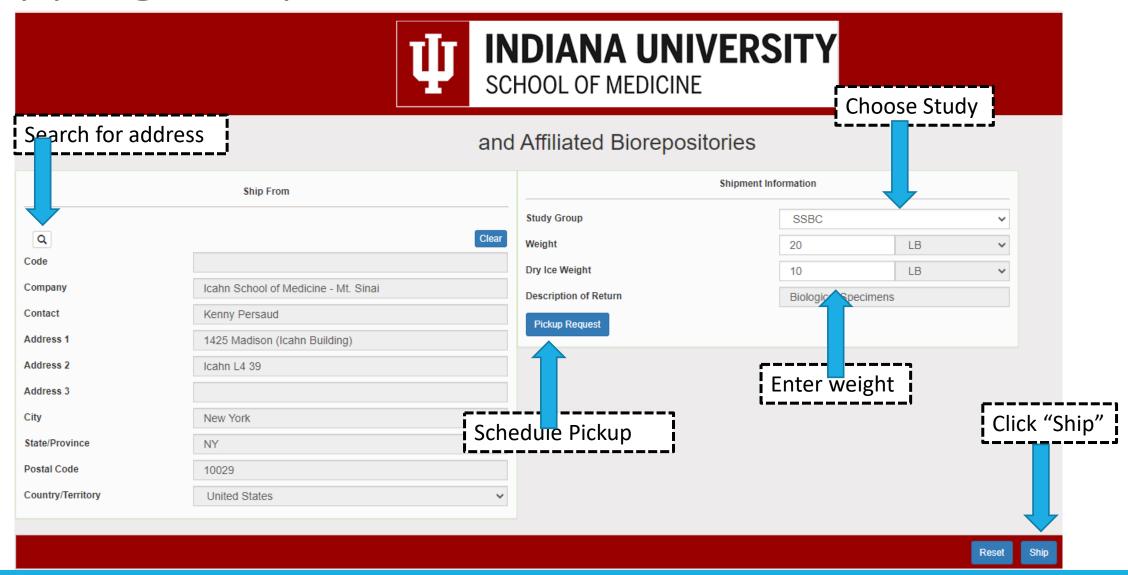
Packing and Shipping Frozen Samples







Shipping Samples – UPS: https://kits.iu.edu/UPS



Shipping Samples via UPS

IU UPS ShipExec Shipping Portal

- Print out UPS air waybill
- Fold and insert UPS air waybill into clear plastic sleeve on package

JOHN SMITH INDIANA UNIVERSITY 410 WEST 10TH STREET INDIANAPOLIS IN 46202 2 LBS

1 OF 1

RS

SHIP TO:

SCHOOL OF MEDICINE 317-278-2694 INDIANA UNIVERSITY TK 217 351 W 10TH ST

INDIANAPOLIS IN 46202



IN 461 9-01



UPS NEXT DAY AIR

TRACKING #: 1Z 976 R8W 84 3985 8595

1



BILLING: P/P
DESC: Biological Specimens
RETURN SERVICE

Reference No.1: 4087277

OL 20.03.09 NV45 83.0A 12/



Non-Conformance

Non-conformance to standard procedures may reduce the utility of the biospecimens:

- Not processing serum/plasma within 2 hours of collection allows for breakdown of certain proteins and small molecules
- Delayed shipping of PBMC samples leads to extraction failures
- Over/under centrifuging changes plasma, serum, CSF composition



Non-Conformance Reporting

IU will send non-conformance report for any specimen submission that does not meet requirements

- A copy of each report should be filed in the study binder
- Confirm procedures are being followed per the Biologics Manual
- Track issues over time to determine where changes may be needed

	sues Noted:
	notification not received, incomplete, or inaccurate
Submissi	on form not included in package, incomplete, or inaccurate
Samples	shipped for weekend or holiday delivery
Samples	improperly packaged
Samples	received damaged
Frozen su	ubmission received thawed
Other	
	d in non-standard tube(s)
Unlabeled	d or mislabeled tube(s)
Low volui	me received
Sample d	iscolored
Other	
Details/Com	nments:

Non-Conformance Reporting con't

Most common non-conformance issues:

- Shipment notification not sent
- Samples shipped for weekend/holiday delivery
- Sample form incomplete/inaccurate
- Low volume
- Unlabeled or mislabeled tube(s)
- Sample hemolysis



Contacts

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