Longitudinal Investigation for Enhancing Down Syndrome Research (LIFE-DSR) Study

of the

Down Syndrome – Clinical Trials Network

in collaboration with

The National Centralized Repository for Alzheimer's Disease and Related Dementias (NCRAD)

Biofluid Collection, Processing and Shipment Training Slides

Version 6.2

Contact Information

Questions?

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General NCRAD Contact Information

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Email: alzstudy@iu.edu

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Training Overview

- **Kit Request Module**
- **Main Study – Plasma and DNA**
  - Blood-Based Collection Schedule
  - Specimen Labels
  - Handling/Processing Study Specimens
  - Incomplete or Difficult Blood Draws
  - Sample Shipping
  - Sample Forms – Main Study
- **Addendum 1 Subset – RNA, PBMC and CSF** *- Ambient AND Frozen Substudy samples sent same day as collection*
  - Subset Collection Schedule
  - Specimen Labels
  - Handling/Processing Study Specimens
  - Sample Shipping
  - Sample Forms - Substudy
- **Creating Airbills/Scheduling Pickups via ShipExec**
- **NCRAD Website**
- **Questions?**

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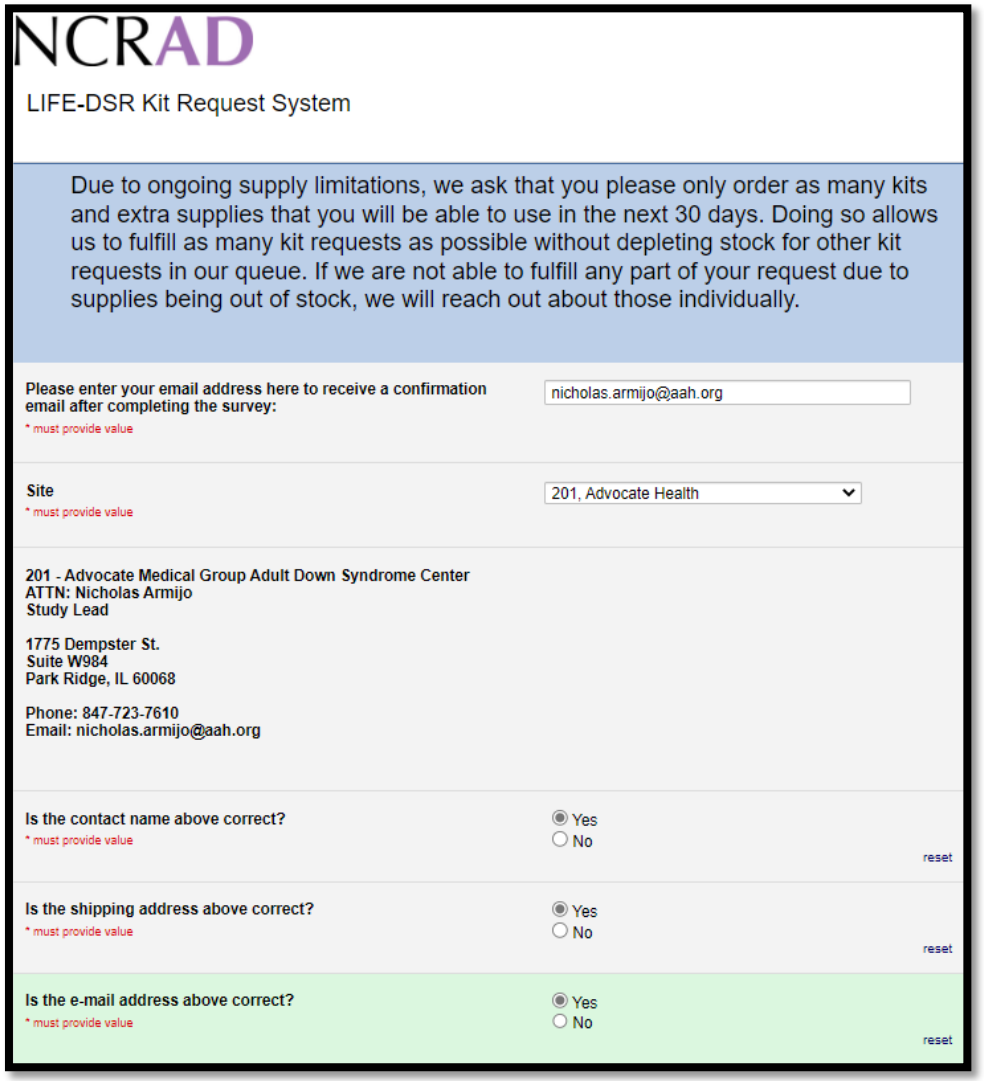
LIFE-DSR Kit Request Module

http://kits.iu.edu/lifedsr

LIFE-DSR Kit Request Module

If possible, only order what you will need in the next month



- Enter your email to receive a confirmation email after you submit your kit request.
- Choose your site from the drop-down list.
- The coordinator name and contact information will appear.
- Verify that this information is accurate. Correct if necessary.

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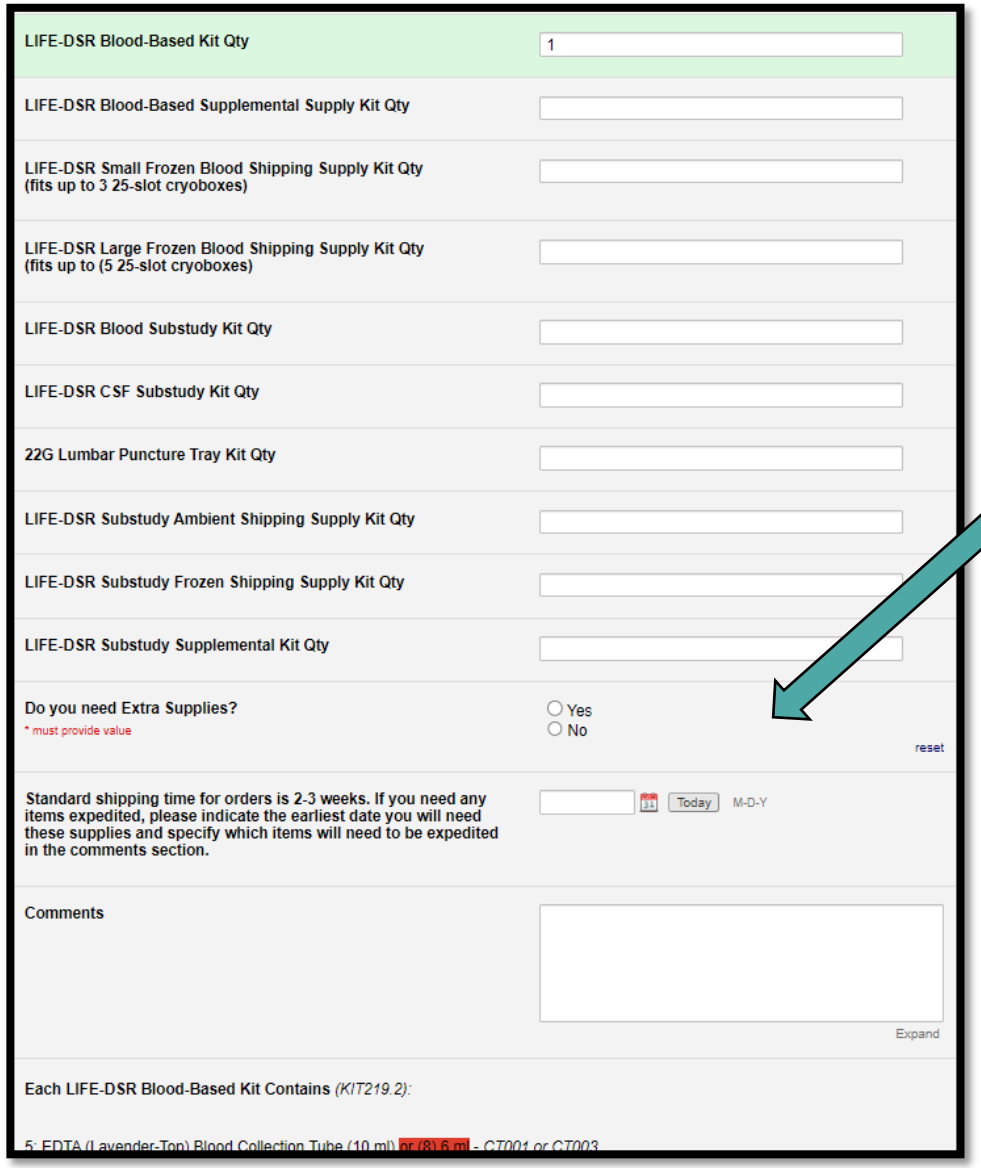
LIFE-DSR Kit Request Module



- Indicate the quantity needed of each kit
  - Once selected, kit components of the chosen kit will appear at the bottom of the screen
- You can order extra supplies individually by selecting “Yes” here.
- Please indicate the soonest date you will need the requested supplies
  - We typically return requests within 2-3 weeks from the order date.
- Click “Submit” to turn in your request.
- **Note: You can order more than one type of kit in a single kit request**

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LIFE-DSR Kit List

- **Main Study – Plasma and DNA**
  - LIFE-DSR Blood-Based Kit
  - LIFE-DSR Blood-Based Supplemental Kit
  - LIFE-DSR Small Frozen Blood Shipping Supply Kit
    - Fits up to (3) 25-slot cryoboxes
  - LIFE-DSR Large Frozen Blood Shipping Supply Kit
    - Fits up to (5) 25-slot cryoboxes
- **Addendum 1 Subset – RNA, PBMC and CSF**
  - LIFE-DSR Blood Substudy Kit
  - LIFE-DSR CSF Substudy Kit
  - 22G LP Tray Kit
  - LIFE-DSR Substudy Ambient Shipping Kit
  - LIFE-DSR Substudy Frozen Shipping Kit
  - LIFE-DSR Substudy Supplemental Kit

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Main Study

Plasma and DNA

Blood-Based Collection Schedule

Plasma and DNA

	Baseline	16M	32M
Plasma	X	X	X
DNA*	X	X	X

*Buffy coats used to extract DNA.

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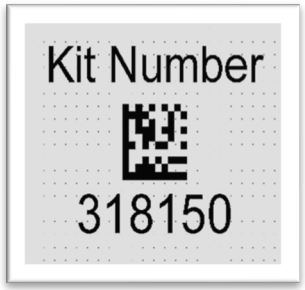
Specimen Labels

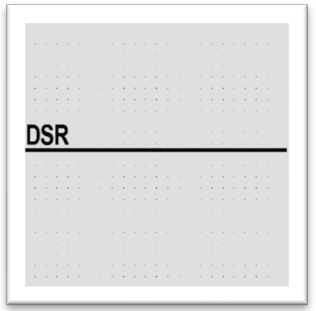
Provided by NCRAD

Three Label Types



Kit Number Labels

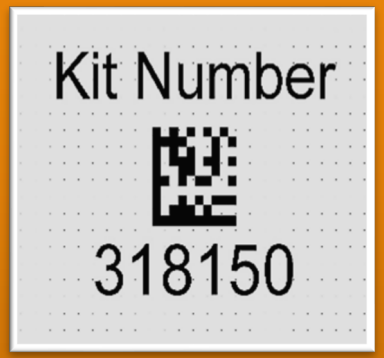
Collection and Aliquot Tube Labels



LIFE-DSR ID Labels

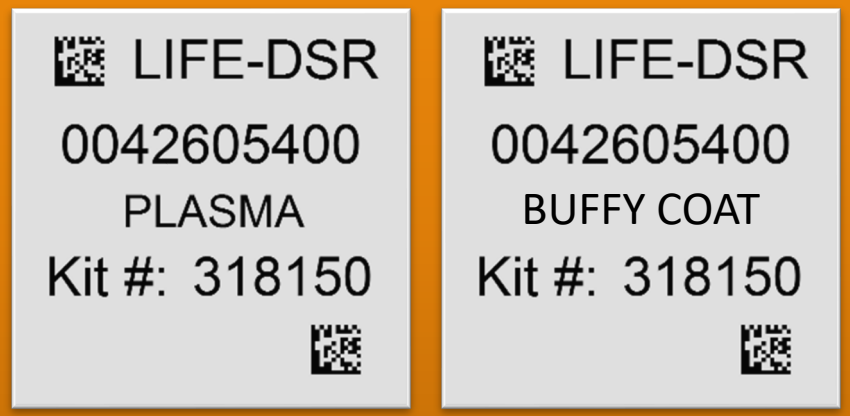
NCRAD

Kit Number Labels



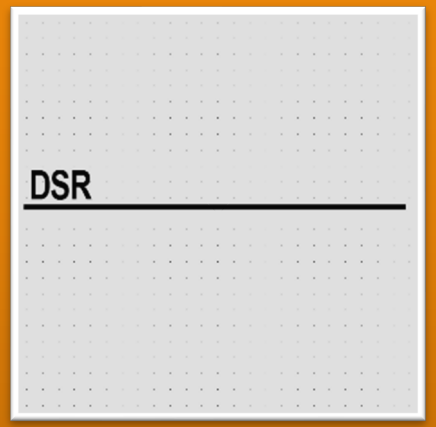
- Used to track patient samples and provide quality assurance – Will be placed on the following locations :
  1. Blood Sample and Shipment Notification Forms
  2. Outside cryobox that houses aliquot tubes during storage and shipment

Collection and Aliquot Tube Labels



- Collection and Aliquot Tube labels have 4 components:
  - Study name
  - 10-digit specimen number (assigned by NCRAD)
  - Specimen type
  - Kit number (assigned by NCRAD)
    - Unique to subject AND visit
- Will be placed on the following locations:
  - All collection and aliquot tubes
    - EDTA (Lavender-Top) Blood Collection Tube (10 mL)
    - Lavender Cap Plasma Aliquot Tubes
    - Clear Cap Buffy Coat Aliquot Tubes
    - Blue Cap Residual Plasma Aliquot Tube

LIFE-DSR ID Labels



- Subjects will be identified by their LIFE-DSR ID
- Sites will be responsible for handwriting this onto the provided labels
  - Must use fine point permanent marker
- Will be placed on the following locations:
  - All collection tubes
    - EDTA (Lavender-Top) Blood Collection Tube (10 mL)

EDTA Collection Tube Labels:

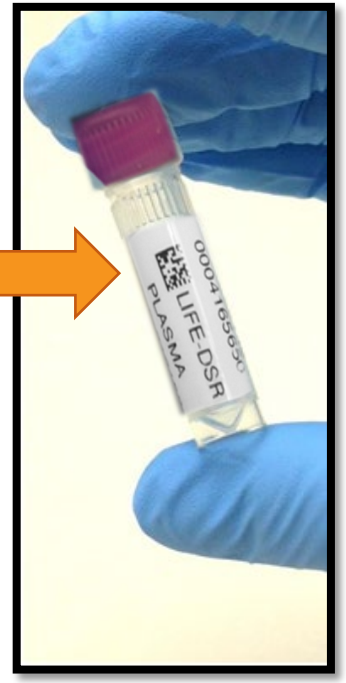
Collection Tube Label

LIFE-DSR ID Label

EDTA (Lavender-Top) Blood Collection Tube (10 mL)

Plasma and Buffy Coat Aliquot Tube Labels:

Aliquot Tube Label



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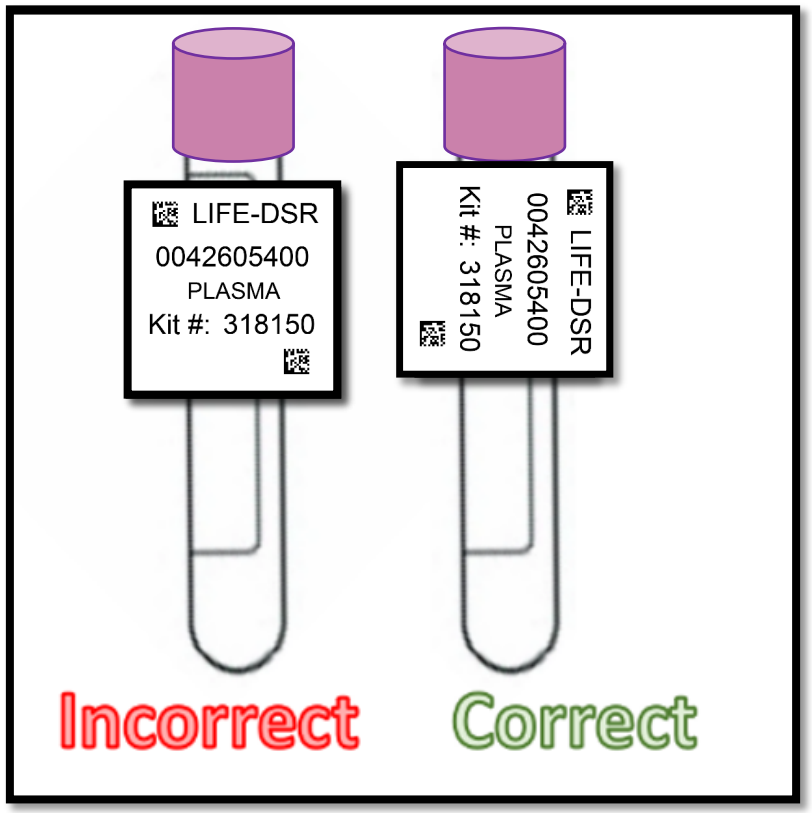
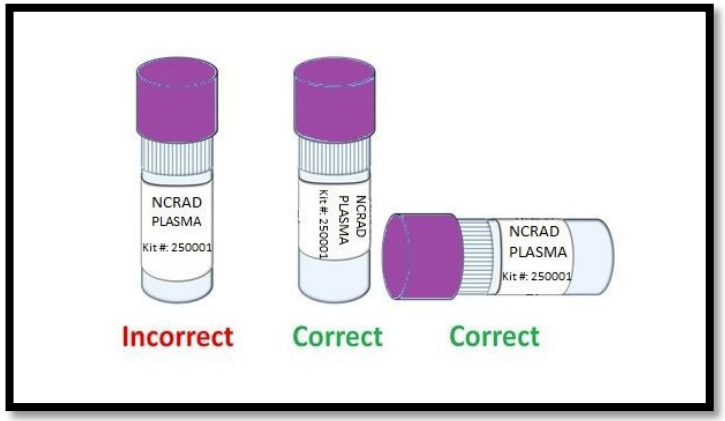
Properly Labeling Biologic Samples:

- 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





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Handling/Processing Study Specimens

Site Required Equipment

BLOOD COLLECTION/SAFETY EQUIPMENT

1) Personal Protective Equipment:
    1) lab coat, nitrile/latex gloves, safety glasses
2) Tourniquet
3) Alcohol Prep Pad
4) Gauze Pad
5) Bandage
6) Butterfly needles (21 gauge) and hub
7) Microcentrifuge tube rack
8) Sharps bin and lid

PROCESSING/STORAGE EQUIPMENT

1) Centrifuge capable of ≥ 2000 x g with refrigeration to 4°C
2) -80 ° C Freezer
3) Wet Ice Bucket
4) Wet Ice
5) Dry Ice - ~45 lbs.. per shipment

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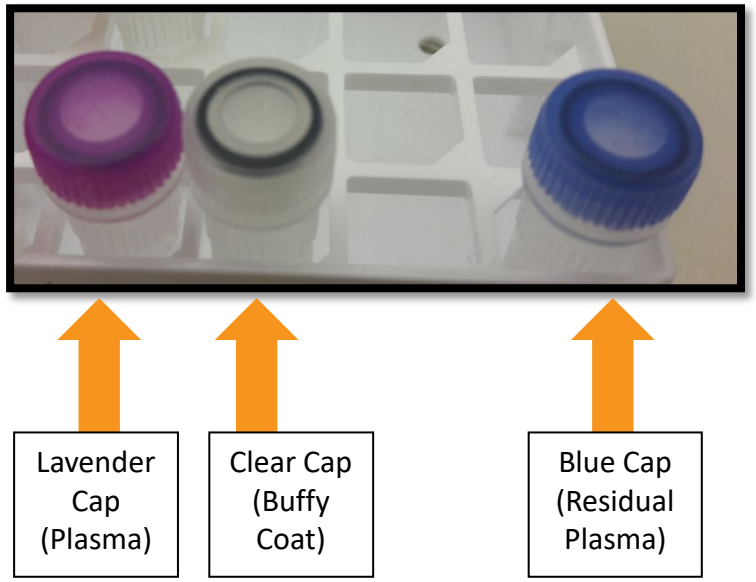
Plasma and Buffy Coat Collection Tubes

Tube Type	Number of Tubes Drawn	Tube Image
1. EDTA (Lavender-Top) Tube (10 ml)	X 5	

Aliquot Cap Colors

Cap Color	Sample Type
Lavender Cap	Plasma
Blue Cap	Plasma Residual (<1.5 mL) (Document Specimen Number and Volume of Residual Aliquot on Sample Form)
Clear Cap	Buffy Coat



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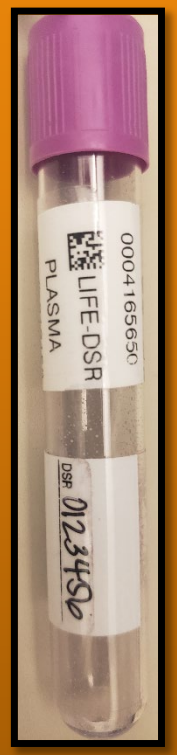
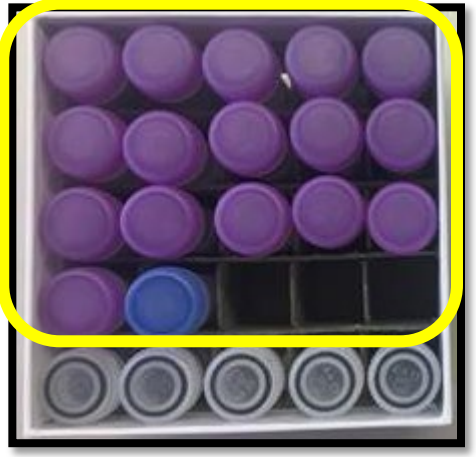
Plasma Collection

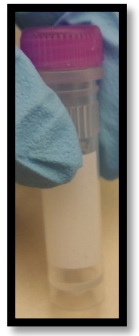


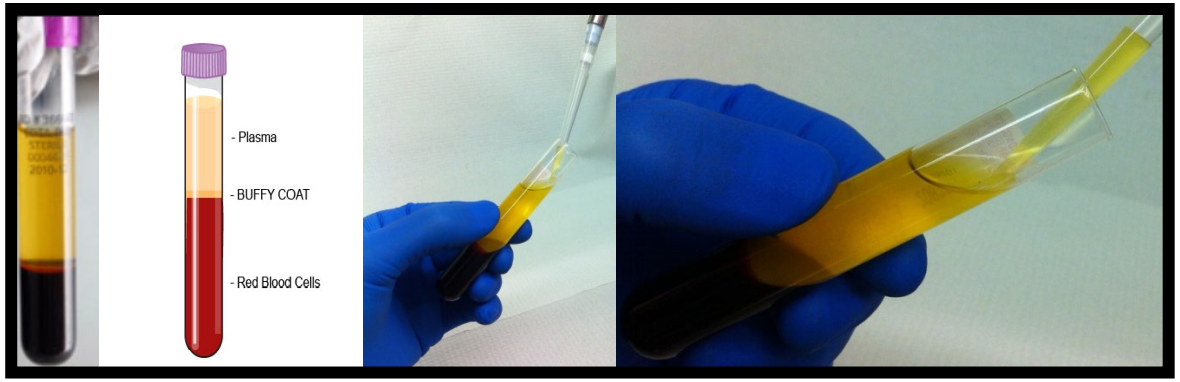


25 cell cryobox with 2 mL cryovials – sent to NCRAD

- **5 x EDTA (Lavender-Top) Blood Collection Tube (10 mL)**
  - **Create up to (17) 1.5 mL plasma aliquots**
    - If residual aliquot created, document specimen number and volume on sample form

Close up view of 2.0 ml Cryovial





NOTE: When pipetting plasma from the plasma tube into the 50 mL conical tube, be very careful to pipette the plasma top layer only, leaving the buffy coat and the red blood cell layers untouched.

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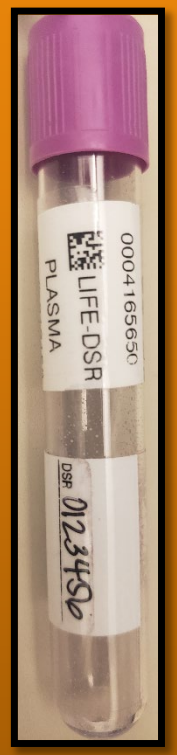
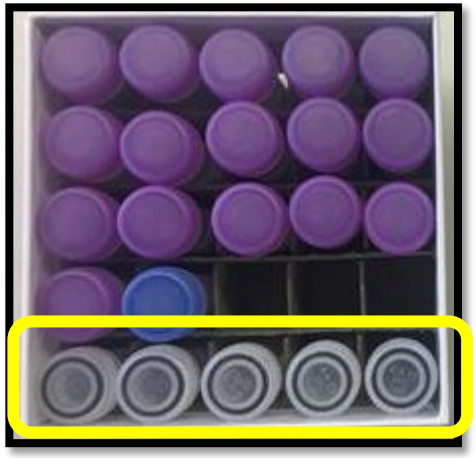
Buffy Coat Collection

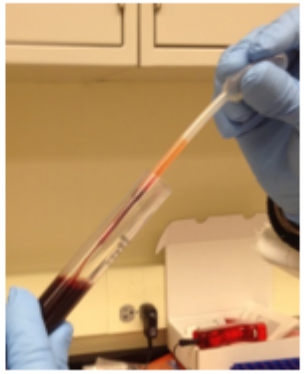


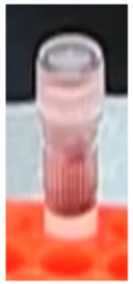


25 cell cryobox with 2 mL cryovials – sent to NCRAD

- **5 x EDTA (Lavender-Top) Blood Collection Tube (10 mL)**
  - **Create up to (5) 1 mL buffy coat aliquots**
    - Expected to have a reddish color from the RBCs.
    - Be sure to only place the buffy coat from one EDTA tube into each cryovial

Buffy Coat layer (mixed with RBCs)





Buffy Coat Aliquot (Please use CLEAR CAP

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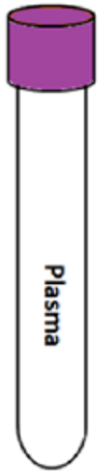
Plasma and Buffy Coat Preparation (10ml Lavender-Top Tube x 5)

Step One

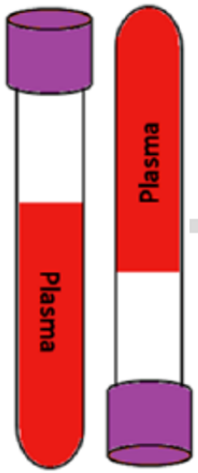


- Store tubes at room temperature.
- Label tubes with preprinted labels prior to blood draw.

Step Two

- Collect blood in EDTA Tubes 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.

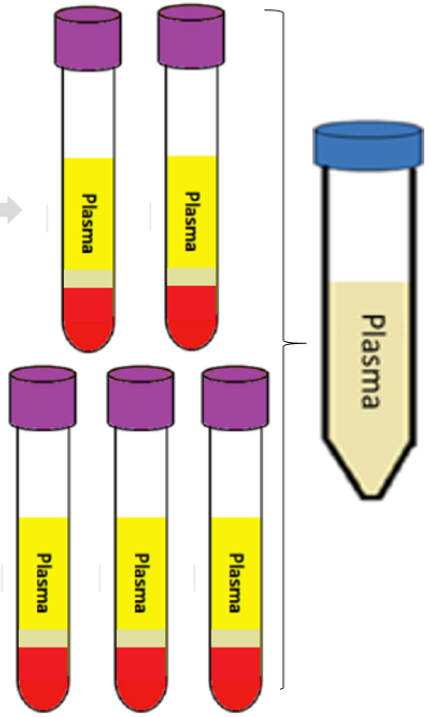
Step Four

- Place thoroughly mixed tubes on wet ice until centrifugation begins.

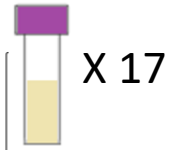
Step Five

- Centrifuge samples at 2000 x g at 4°C for 10 minutes.
- Samples need to be spun, aliquoted, and in the freezer within 1 hour from the time of collection.

Step Six

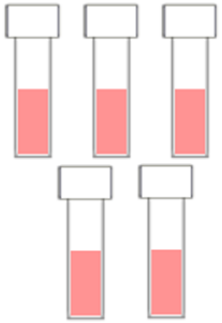


- Pool all plasma from the 5 EDTA tubes into a 50ml conical tube and invert gently 3 times to mix the plasma.



Step Seven

Adhere preprinted labels to the lavender cap cryovials.
Aliquot 1.5 ml into each cryovial tube.
If a residual aliquot is created, document specimen number and volume on Sample Notification Form.
Store plasma aliquots at -80°C until shipment.



Step Eight

- Adhere preprinted labels to the clear cap cryovials.
- Using a clean pipette tip, collect the buffy coats (may have residual plasma and some RBCs included).
- Transfer the buffy coats into the cryovial tubes.
- Store buffy coat aliquots at -80°C until shipment.

Important Note: Ensure all tubes are not expired prior to collection and processing of samples.

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Incomplete or Difficult Blood Draws

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

If challenges arise during the blood draw process, it is advised that the phlebotomist discontinue the draw. Attempt to process and submit any blood-based specimens that have already been collected to NCRAD. Document difficulties on the 'Biological Sample and Shipment Notification Form' prior to submission to NCRAD. Contact NCRAD and alert them of the challenging blood draw.

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

If the biofluids at a scheduled visit are not collected, contact the LIFE-DSR Study Team to alert them of the challenging blood draw or circumstances as to why biofluids were not collected:

Aisha Vanderhorst: avanderhorst@lumindidsc.org

Jill MacDougall: jmacdougall@lumindidsc.org

LuMind General Contact: lifedsr@lumindidsc.org

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Packaging and Shipping Samples

Plasma and DNA

Blood Sample Shipment Summary

Sample Type	Processing/ Aliquoting	Tubes to NCRAD	Ship
Whole blood (Lavender-Top EDTA) for isolation of plasma & buffy coat (for DNA extraction)	1.5 mL plasma aliquots per 2.0 mL cryovial (lavender cap); residual volume placed in 2.0 mL cryovial with blue cap	Up to 17	**Frozen**
	1 mL buffy coat aliquot per 2.0 mL cryovial (clear cap)	5	**Frozen**

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Frozen Shipping

Plasma and DNA

Notify NCRAD When Samples Ship:

1. **Notify NCRAD of shipment** by emailing NCRAD coordinators at: alzstudy@iu.edu

- Attach the following to the email:
  - Completed Biological Sample and Shipment Notification Form (Appendix B – also found on the NCRAD - LIFE-DSR Active Study Page).
  - If email is unavailable, please call NCRAD and do not ship until you've contacted and notified NCRAD coordinators about the shipment in advance.
  - Please include the tracking number in the body of the email.
  - **Place physical copy of the filled out Biological Sample and Shipment Notification Form (Appendix B) in your shipment.**

Frozen Shipment Packaging:

Place all frozen labeled aliquots of plasma and buffy coat in the cryovial cryoboxes.

Place kit number label(s) on cryoboxes

Place up to 17 plasma and 5 buffy coat cryovials per participant visit inside 25 cell cryobox. Label cryobox with kit number label and place inside biohazard bag. Seal biohazard bag according to the instructions on the bag. Ship to NCRAD frozen.

Frozen Shipment Packaging

- Place 2-3 inches of dry ice in the bottom of the Styrofoam shipping container, then insert the cryoboxes laying upright.
- Fully cover the cryoboxes with about 2 inches of dry ice in the provided shipper.
- Each Styrofoam shipper must contain about 45 lbs. (20 kg) of dry ice.
- Fill shipper to the top with dry ice!

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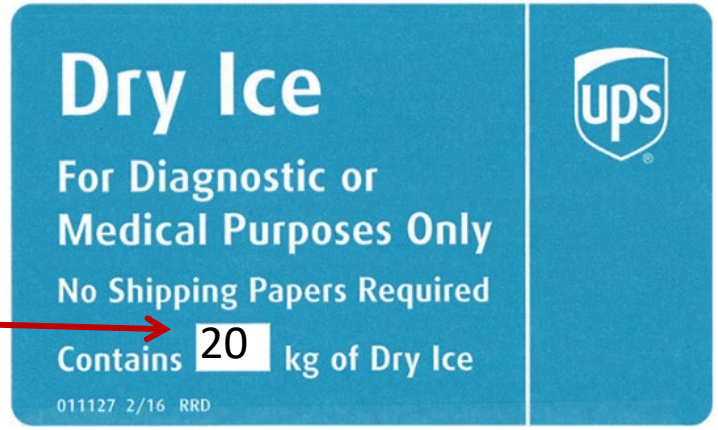
Frozen Shipping Dry Ice Requirements

- Apply all provided warning labels and the pre-printed UPS return airbill to the outside of package, taking care not to overlap labels.

Dry Ice label should not be covered with other stickers and must be completed, or the shipping carrier will reject/return your package!



Net weight of dry ice in **kg**

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Critical Frozen Shipping Instructions

1. Hold packaged samples in -80°C freezer until time of UPS pick-up/drop-off.

2. Frozen samples should be shipped via UPS Next Day Air

3. Frozen shipments should be sent Monday through Wednesday ONLY to avoid shipping delays on Thursday or Friday.

BE AWARE OF HOLIDAYS and current weather conditions!

4. Remember to complete the requisition forms and include a copy in your shipment: Biological Sample and Shipment Notification (Appendix B).

Batch Shipping

Batch shipping should be performed every (3) three months or when specimens from 5 participants accumulates, whichever is sooner.

- **If small shipper, batch ship up to 3 participants.**

*****Important Note for Frozen Shipments ONLY*****

Batch shipping main study specimens and subset study specimens together: If shipping main study specimens same day as a subset collection, the 25-slot cryoboxes holding plasma and buffy coats from main study can be batch shipped with the subset specimens (PAXgene™ tube, 15 mL conical holding Cryogenic vial of CSF cells, and 48-slot cryobox holding CSF aliquots). Ensure there is ~45 lbs.. of dry ice for large shippers and ~14 lbs.. for small shippers.

Large Frozen Shipper – fits 5 25-slot cryoboxes and ~45 lbs.. dry ice

Small Shipper – fits 3 25-slot cryoboxes and ~14 lbs.. dry ice

Shipping Regulations and Training

All study personnel responsible for shipping should be certified in biospecimen shipping.
It is the responsibility of each site to ensure that the appropriate training has been provided and conducted in regards to IATA shipping.

UN3373 Biological Substance, Category B Training

- Biological Substance, Category B are specimens being transported for “investigational purposes”
- Recommend: investigator sites document training of category B/dangerous goods
- We recommend establishing a record of your staff’s training and date of instruction
- The training records must be made available upon request by the appropriate national authority
  - Additional information from the Department of Transportation (DOT) can be found on their website http://hazmat.dot.gov

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NCRAD Forms

Appendix A: Rate of Centrifuge Worksheet

Appendix A
Rate of Centrifuge Worksheet

Please complete and return this form by fax or email to the NCRAD Project Manager if you have any questions regarding sample processing. The correct RPM will be sent back to you.

Submitter Information

Name: **Site:**

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:

$$RCF = \left(\frac{RPM}{1{,}000}\right)^2 \times r \times 1.118 \quad \Rightarrow \quad RPM = \sqrt{\frac{RCF}{r \times 1.118}} \times 1{,}000$$

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 centrifuge

Comments:

Please send this form to NCRAD Study Coordinator

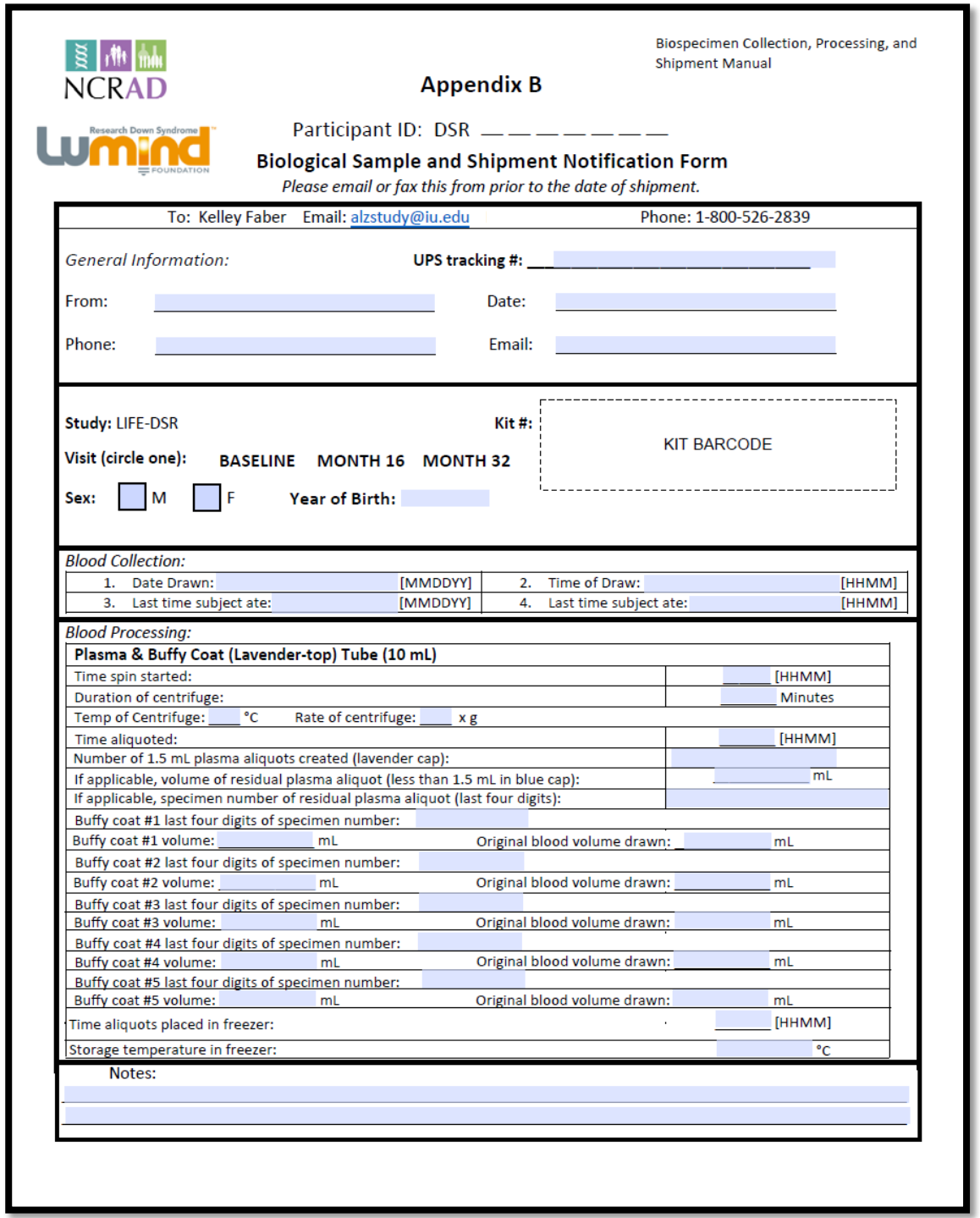
alzstudy@iu.edu

Note:

Use Rate of Centrifugation Worksheet to calculate RPM, if needed.

NCRAD

Appendix B: Biological Sample and Shipment Notification Form (link)



NCRAD

Biospecimen Collection, Processing, and Shipment Manual

Appendix B

Research Down Syndrome Lumind Foundation

Participant ID: DSR _ _ _ _ _ _ _ _

Biological Sample and Shipment Notification Form

Please email or fax this from prior to the date of shipment.

To: Kelley Faber Email: alzstudy@iu.edu Phone: 1-800-526-2839

General Information: **UPS tracking #:** _____

From: _____ Date: _____

Phone: _____ Email: _____

Study: LIFE-DSR **Kit #:** KIT BARCODE

Visit (circle one): **BASELINE** **MONTH 16** **MONTH 32**

Sex: ☐ M ☐ F **Year of Birth:** _____

Blood Collection:

1. Date Drawn:	[MMDDYY]	2. Time of Draw:	[HHMM]
3. Last time subject ate:	[MMDDYY]	4. Last time subject ate:	[HHMM]

Blood Processing:

Plasma & Buffy Coat (Lavender-top) Tube (10 mL)	
Time spin started:	[HHMM]
Duration of centrifuge:	Minutes
Temp of Centrifuge: °C Rate of centrifuge: x g	
Time aliquoted:	[HHMM]
Number of 1.5 mL plasma aliquots created (lavender cap):	
If applicable, volume of residual plasma aliquot (less than 1.5 mL in blue cap):	mL
If applicable, specimen number of residual plasma aliquot (last four digits):	
Buffy coat #1 last four digits of specimen number:	
Buffy coat #1 volume: mL Original blood volume drawn: mL	
Buffy coat #2 last four digits of specimen number:	
Buffy coat #2 volume: mL Original blood volume drawn: mL	
Buffy coat #3 last four digits of specimen number:	
Buffy coat #3 volume: mL Original blood volume drawn: mL	
Buffy coat #4 last four digits of specimen number:	
Buffy coat #4 volume: mL Original blood volume drawn: mL	
Buffy coat #5 last four digits of specimen number:	
Buffy coat #5 volume: mL Original blood volume drawn: mL	
Time aliquots placed in freezer:	[HHMM]
Storage temperature in freezer:	°C

Notes:

Note:

Please ensure Sample Notification Forms are filled out in their entirety. Complete during the participant study visit as samples are processed to guarantee accuracy.

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Addendum 1 Subset

RNA, PBMC, and CSF

Important Note

All Substudy samples should be collected Monday – Wednesday ONLY and shipped ON DAY OF COLLECTION

Substudy Collection Schedule
RNA, PBMC, and CSF

	Baseline/16M*	16M/32M*
RNA	X	X
PBMC	X	X
CSF	X	X

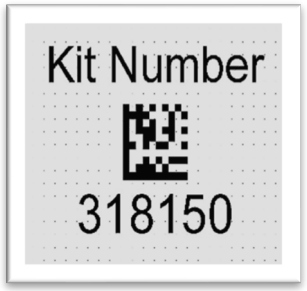
*Collection will be at 2 time-points – Either BL and M16 OR M16 and M32.

NCRAD

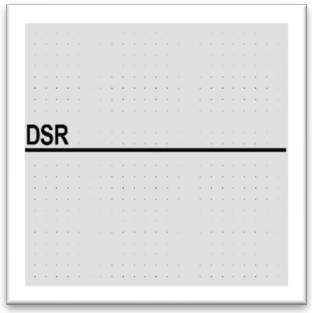
Specimen Labels

Provided by NCRAD

Four Label Types



Kit Number Labels



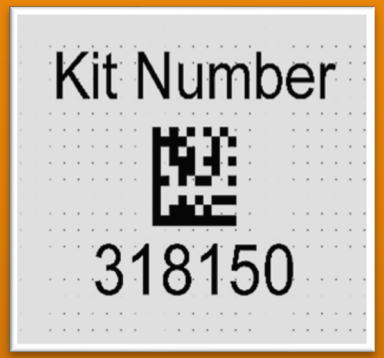
LIFE-DSR ID Labels

Collection Tube Labels

Cryovial Labels

NCRAD

Kit Number Labels

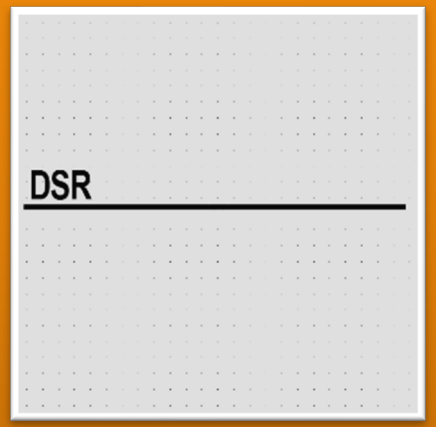


- Used to track patient samples and provide quality assurance – Will be placed on the following locations :
  1. Sample and Shipment Notification Forms
  2. Outside cryobox that houses aliquot tubes during storage and shipment

Collection Tube Labels

- Collection and Aliquot Tube labels have 4 components:
  - Study name
  - 10-digit specimen number (assigned by NCRAD)
  - Specimen type
  - Kit number (assigned by NCRAD)
    - Unique to subject AND visit
- Will be placed on the following locations:
  - All collection tubes and cryogenic vial (2 mL) containing CSF cells/pellet
    - PAXgene™ Blood Collection Tube (2.5 ml)
    - NaHep (Green-Top) Blood Collection Tube (10 ml)
    - Self-standing Cryogenic vial (2 mL)

LIFE-DSR ID Labels



- Subjects will be identified by their LIFE-DSR ID
- Sites will be responsible for handwriting this onto the provided labels
  - Must use fine point permanent marker
- Will be placed on the following locations:
  - All collection tubes
    - PAXgene™ Blood Collection Tube (2.5 ml)
    - NaHep (Green-Top) Blood Collection Tube (10 ml)
    - (1) Conical Tube (15 mL) - *Place Cryogenic vial containing CSF cells/pellet in labeled 15 mL conical for shipping.*

Cryovial Labels

- Only one label to be placed on each 2.0 mL cryovial
  - **CSF**
    - Smooth Orange Cap Cryovials (CSF)
    - Blue Cap Cryovial (CSF Residual)

PBMC and RNA Tube Labels:

Collection Tube Label

LIFE-DSR ID Label

NaHep (Green-Top) Blood Collection Tube (10 ml)

PAXgene™ Blood Collection Tube (2.5 ml)

CSF Cells/Pellet Tube and Conical Labels:

LIFE-DSR ID Label

Collection Tube Label

Self-standing Cryogenic vial (2 mL

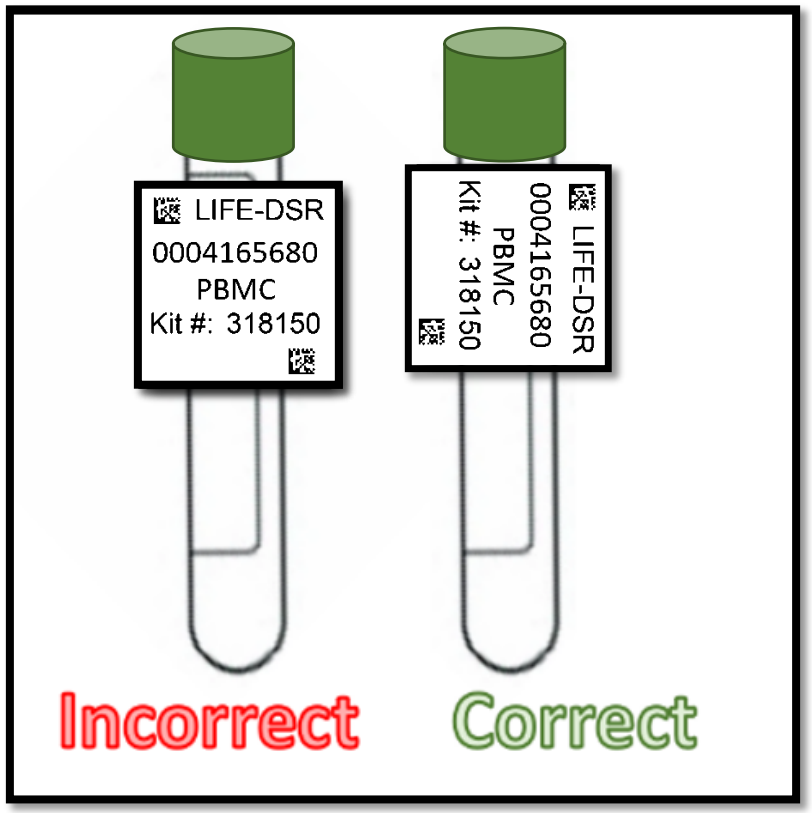
Conical Tube (15 mL)

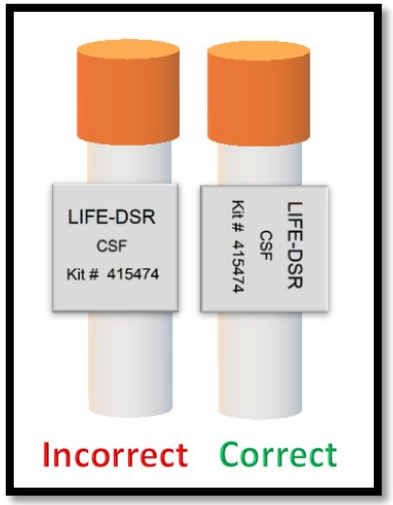
Self-standing Cryogenic vial will not fit in the 48-slot Cryobox due to the size of the tube. **Place Cryogenic vial containing CSF cells in labeled 15 mL conical for shipping.**

NCRAD

Properly Labeling Biologic Samples:

- Label all 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





NCRAD

Handling/Processing Study Specimens

Site Required Equipment

BLOOD COLLECTION/SAFETY EQUIPMENT

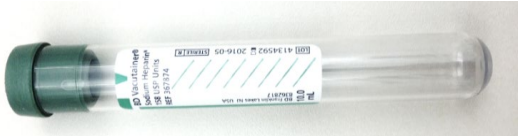
1) Personal Protective Equipment:
    1) lab coat, nitrile/latex gloves, safety glasses
2) Tourniquet
3) Alcohol Prep Pad
4) Gauze Pad
5) Bandage
6) Butterfly needles (21 gauge) and hub
7) Microcentrifuge tube rack
8) Sharps bin and lid

PROCESSING/STORAGE EQUIPMENT

1) Centrifuge capable of ≥ 350 x g with refrigeration to 4°C
2) -80 ° C Freezer
3) Wet Ice Bucket
4) Wet Ice
5) Dry Ice - ~10 lbs.. per shipment
6) CoolCell® - provided by NCRAD

NCRAD

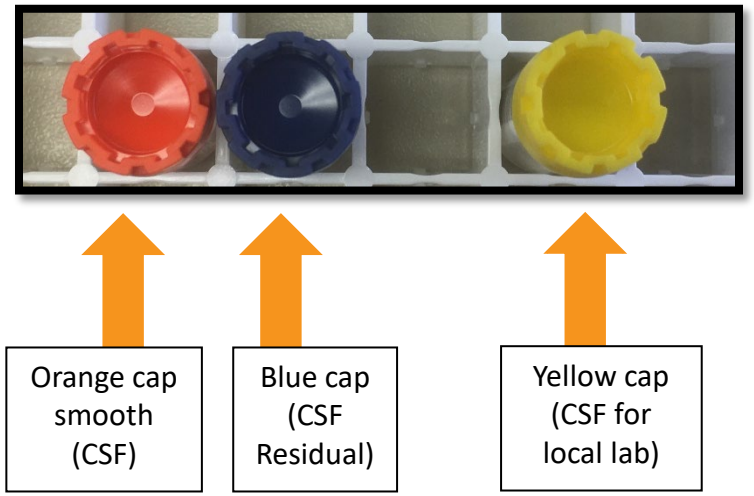
Blood and CSF Collection Tubes

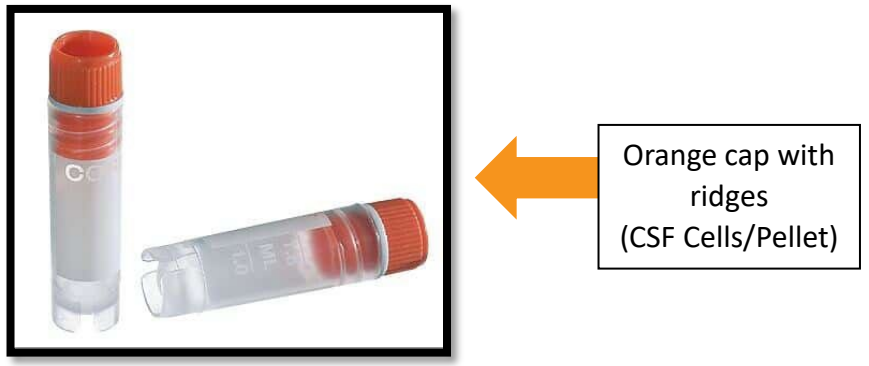
Tube Type	Number of Tubes Drawn	Tube Image
1. PAXgene™ Tube for RNA (2.5 mL)	X 1	
2. Sodium Heparin (Green-Top) Blood Collection Tube (10 mL)	X 5	
3. CSF Preparation (20 mL total) – Pellet/Cell Preparation	X 2	

NCRAD

Aliquot Cap Colors

Cap Color	Sample Type
Orange Cap, smooth	CSF
Blue Cap	CSF Residual (<1.5ml) (Document Specimen Number and Volume of Residual Aliquot on Sample Form)
Yellow Cap	CSF for local lab
Orange cap with ridges	CSF cells/pellet





NCRAD

RNA Collection

- 1 x PAXgene™ Blood Collection Tube (2.5 mL)
  - **Tube is to be shipped to NCRAD frozen, without processing at the collection site.**

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

All Substudy samples should be collected Monday – Wednesday ONLY and shipped ON DAY OF COLLECTION

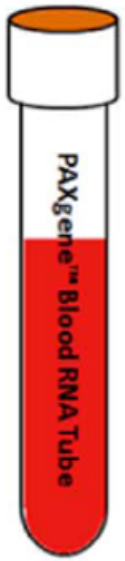
NCRAD

RNA Preparation (2.5ml PAXgene™ Tube)

Step One

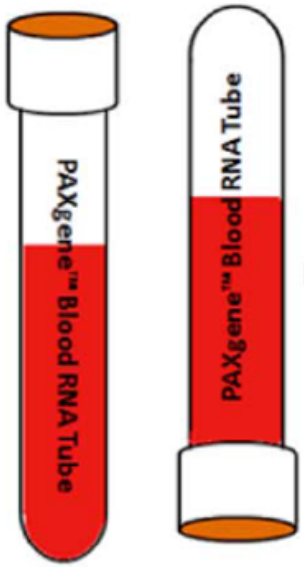
- Store tubes at room temperature.
- Label tubes with pre-printed labels prior to blood draw.

Step Two



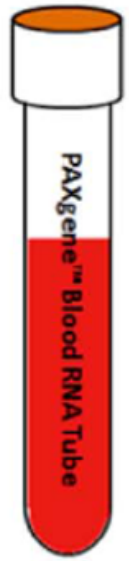
- Collect blood in PAXgene™ tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



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

Step Four



- Store tubes at -80°C in a wire rack until shipment.

- **Important Reminder:** All Substudy specimens are shipped same day of collection.

Important Note: Ensure all tubes are not expired prior to collection and processing of samples.

NCRAD

PBMC Collection

5 x Sodium Heparin (Green-Top) Blood Collection Tube (10 mL)

- **Tubes are to be shipped to NCRAD ambient on day of collection via UPS Next Day Air without further processing at the collection site.**

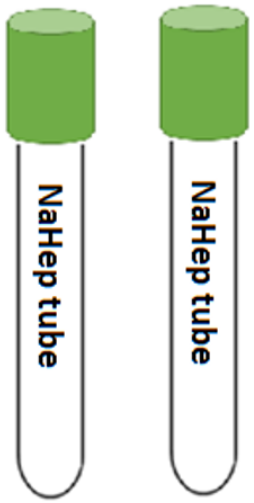
*****Important Note*****

All Substudy samples should be collected Monday – Wednesday ONLY and shipped ON DAY OF COLLECTION

NCRAD

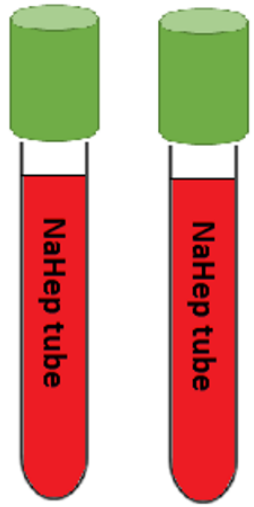
PBMC Preparation (10ml Sodium Heparin Tube x 5)

Step One



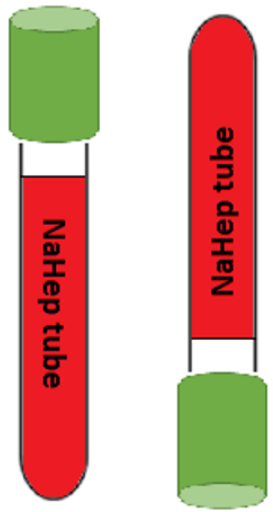
- Store tubes at room temperature.
- Label tubes with pre-printed labels prior to blood draw.

Step Two



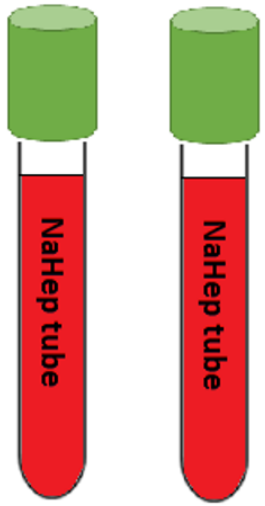
- Collect blood in Sodium Heparin Tubes allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

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>

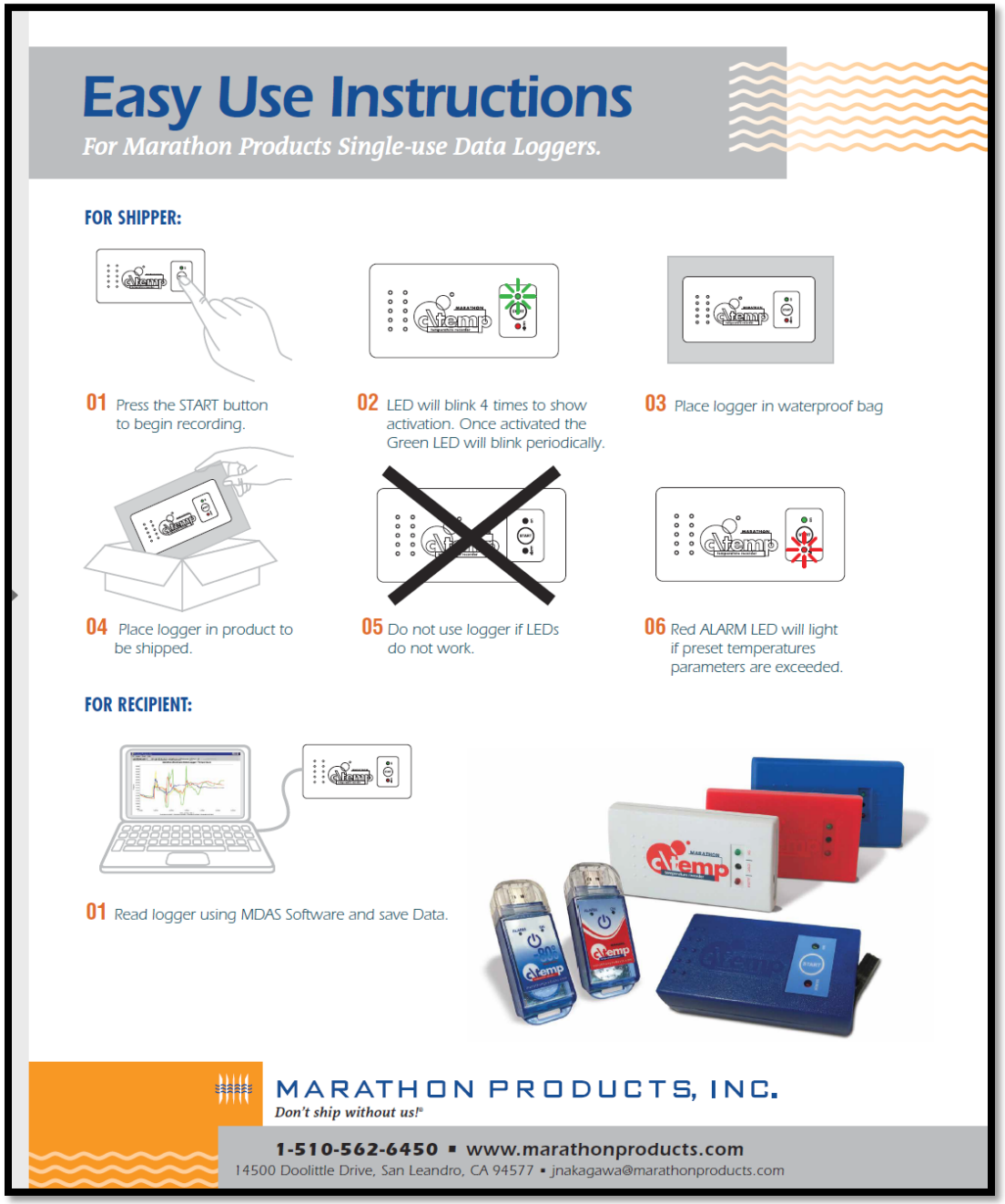
- **Important Reminder:** All Substudy specimens are shipped same day of collection.

Important Note: Ensure all tubes are not expired prior to collection and processing of samples.

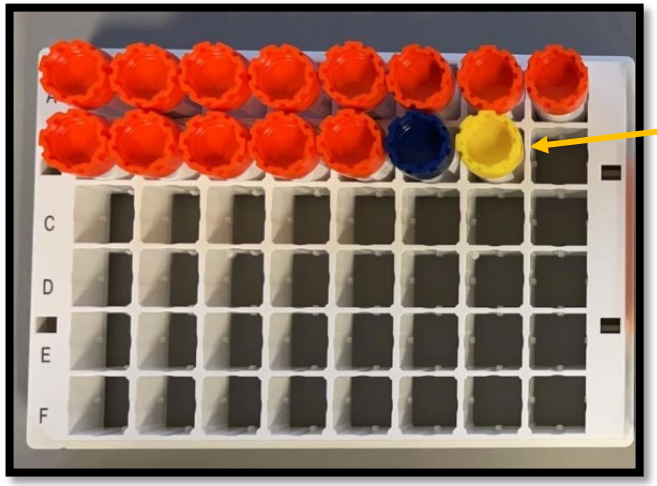
NCRAD

Temperature Monitor

- Seal the Sodium Heparin tubes in the ambient shipment kit. *There should be no more than 3 Sodium Heparin tubes in each ambient shipment kit.*
- Remember to add one (1) temperature monitor per ambient shipping kit. Temperature monitor will be placed in Styrofoam cooler with tubes. Please see directions below.



CSF Collection



48-slot cryobox with 2 mL cryovials

Cryogenic vial will not fit in the 48-slot Cryobox above due to the size of the tube.
Place Cryogenic vial containing CSF cells in labeled 15 mL conical for shipping.

Collect initial 1-2 mL of CSF into 15 mL conical. If not bloody, transfer 1-2 mL of CSF to yellow-cap cryovial and send to local lab for testing. **Do not send yellow cryovial to NCRAD.**

- **2 x 15 mL Sterile Conical Tubes for CSF and Cell/Pellet Preparation**
  - Create up to (14) 1.5 mL aliquots of CSF in orange-cap smooth cryovials
    - If residual aliquot created, aliquot into blue-cap cryovial and document specimen number and volume on sample form

*****Important Note*****
All Substudy samples should be collected Monday – Wednesday ONLY and shipped ON DAY OF COLLECTION

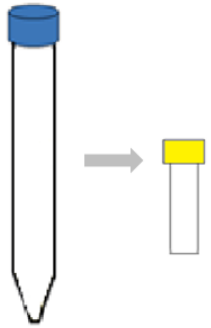
NCRAD

CSF Preparation (20 ml total) - Supernatant

Step One

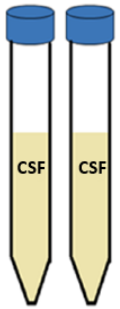
- Label tubes with pre-printed subject labels prior to collection.
- Pre-chill all cryovials on wet ice.

Step Two



- Collect initial 1-2ml (if bloody, collect CSF until cleared of blood) into 15 ml conical tube.
- If not bloody, transfer 1-2 ml into the yellow-cap cryovial.
- Send to local lab for testing.

Step Three



- Collect 20 ml total, including the 1-2 ml sent to the local lab.
- Collect sample into (2) 15 ml conical tubes.

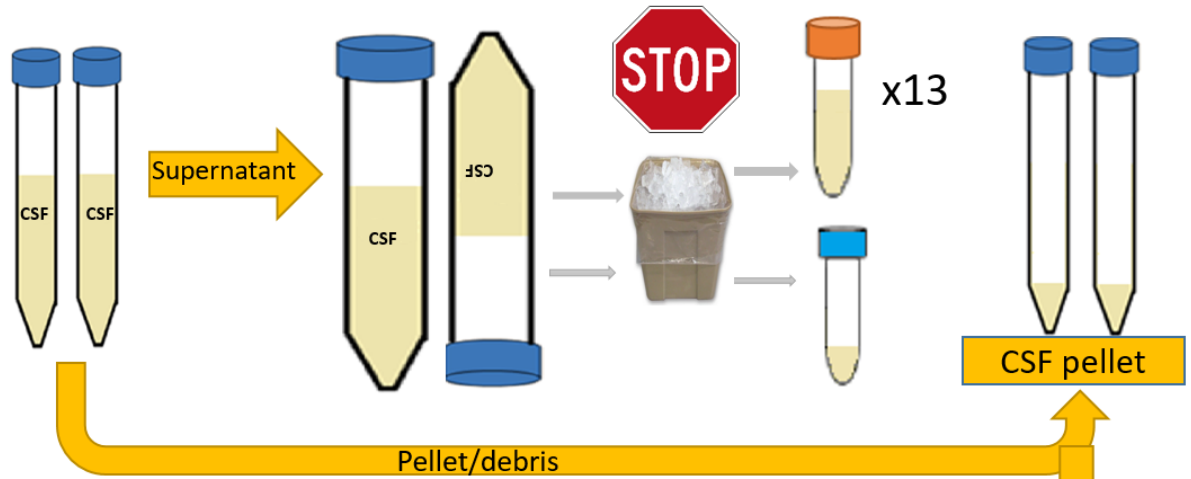
Step Four

- Place samples upright on wet ice until centrifugation begins.

Step Five

- Preferably within 15 minutes of collection, centrifuge samples at 4°C at 350 x g for 10 minutes.

Step Six



- Using a clean transfer pipette, transfer CSF from both 15 ml conical tubes into a 50 ml conical tube, leaving 100ul of CSF with pelleted cells at the bottom of each 15ml centrifuged conical tube.
- Gently invert the 50 ml conical tube 3-4 times to mix the sample. PLACE ON WET ICE.
- **STOP – TAKE CELL PELLETS AND SKIP TO STEP EIGHT. COMPLETE STEPS EIGHT THROUGH THIRTEEN BEFORE RETURNING TO STEPS SIX AND SEVEN.**
- Gently invert the 50 ml conical tube 3-4 times to mix the sample again.
- Aliquot 1.5 ml into the orange-cap cryovials.
- If a residual aliquot is created, aliquot into blue-cap cryovial. Document specimen number and volume on CSF Sample Notification Form.

Step Seven

- Within 2 hours of CSF collection, samples need to be spun, aliquoted and in the freezer. Store at -80°C until shipment. Record time of freezing on CSF Sample Notification Form.

- **Important Reminder:** All Substudy specimens are shipped same day of collection.

Important Note: Ensure all tubes are not expired prior to collection and processing of samples.

NCRAD

CSF Preparation (20 ml total) – Pellet/Cell Preparation

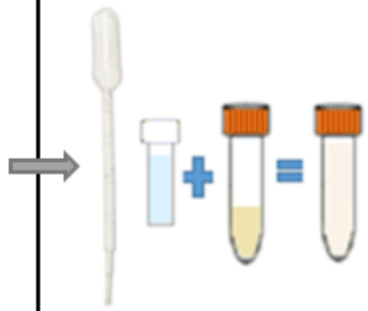
Step Eight

- **Remove an aliquot of CryoStor® CS10 Cryopreservation Medium (StemCell Technologies #07930) from the refrigerator and place on wet ice.**
- Place empty 2.0ml Cryogenic vial on wet ice.
- Ensure filler vials are at 4C. Either they can be stored in the refrigerator or they should be placed on ice for at least 20 min prior to use.

Step Nine

- Carefully resuspend the cell pellet in 1 mL of CryoStor® Medium by gently pipetting up and down near the pellet 5 times using the medium to wash the side of the pellet wall.
- Transfer all the cell suspension from the first 15 mL conical tube to the second 15 mL conical tube and resuspend the cell pellet the exact same way.
- Try not to touch the side of the conical with the pipette tip and avoid creating bubbles/foam during this process.

Step Ten



- Transfer all the cell suspension to the empty 2.0 mL cryogenic vial.
- Recap the vial.
- You should have approximately 1.2-1.4 mL of CryoStor®/CSF/cells in the cryogenic vial.

Step Eleven

- Place Cryogenic vial in a CoolCell well.
- A filler vial should be used to fill all empty spots.
- Fully seal the lid on CoolCell container.
- Place CoolCell container upright in -80°C freezer or dry ice locker.
- Ensure at least one inch of free space clearance around and under the CoolCell container.

Step Twelve

- **Document specimen number and volume of Cryogenic vial on the CSF Sample Notification Form.**
- **Leave samples in the CoolCell for at least 2 hours at -80°C before shipping on dry ice.**
- **Within 2 hours of CSF collection, samples need to be spun, aliquoted and in the freezer.**
- **Store at -80°C until shipment.**
- **Record time of freezing on CSF Sample Notification Form.**

**PLEASE RETURN TO STEP SIX TO COMPLETE CSF SUPERNATANT PROCESSING

- **Important Reminder:** All Substudy specimens are shipped same day of collection.

Important Note: Ensure all tubes are not expired prior to collection and processing of samples.

NCRAD

CSF Cell Isolation and Cryopreservation Demo

- CSF Cell Isolation and Cryopreservation Demo Video - YouTube

Packaging and Shipping Samples

RNA, PBMC, and CSF

Blood and CSF Sample Shipment Summary

Sample Type	Processing/ Aliquoting	Tubes to NCRAD	Ship
Whole blood for RNA extraction	N/A	1	Frozen / same day
Whole blood for PBMC	N/A	5	Ambient / same day
CSF Collection	1.5 mL CSF aliquots per 2.0 mL cryovial (orange cap smooth); residual volume placed in 2.0 mL cryovial with blue cap	Up to 14	Frozen / same day
	2.0 mL Cryogenic Vial with CSF Pellet/Cells (orange cap cryovial with ridges)	1	Frozen / same day

NCRAD

Frozen Shipping

RNA and CSF

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

All Substudy samples should be collected Monday – Wednesday ONLY and shipped ON DAY OF COLLECTION

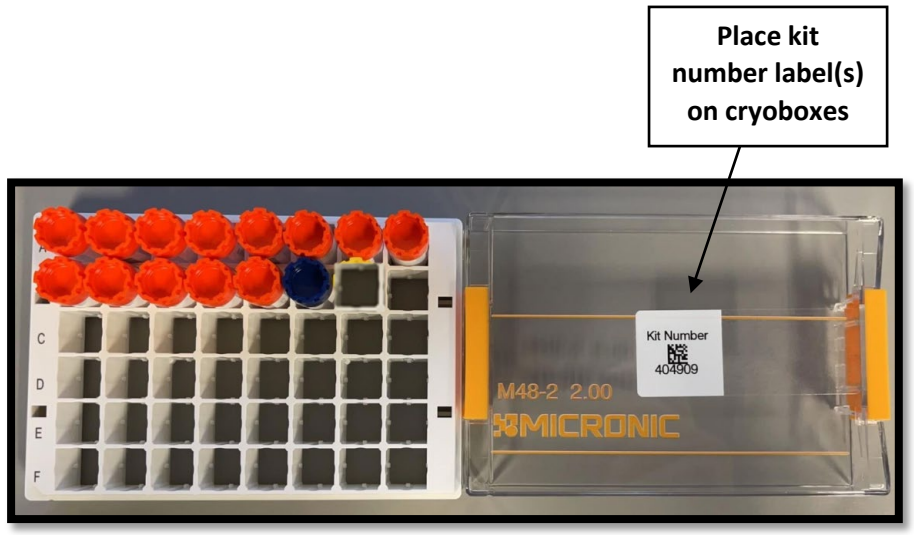
Notify NCRAD When Samples Ship:

1. **Notify NCRAD of shipment** by emailing NCRAD coordinators at: alzstudy@iu.edu

- Attach the following to the email:
  - Completed Biological Sample and Shipment Notification Form and CSF Sample and Shipment Notification Form (Appendix D and Appendix E– also found on the NCRAD - LIFE-DSR Active Study Page).
  - If email is unavailable, please call NCRAD and do not ship until you've contacted and notified NCRAD coordinators about the shipment in advance.
  - Please include the tracking number in the body of the email.
  - **Place physical copy of the filled out Biological Sample and Shipment Notification Form and CSF Sample and Shipment Notification Form (Appendix D and E) in your shipment.**

Frozen Shipment Packaging:

Place all frozen labeled aliquots of CSF and CSF cells/pellet in the cryovial cryoboxes.



Place up to 14 CSF cryovials per participant visit inside 48-slot cryobox. Ensure cryobox has kit number label on lid and then place cryobox inside biohazard bag. Place cryogenic vial containing CSF cells/pellet in labeled 15 mL conical. Place PAXgene™ tube and 15 mL conical holding cryogenic vial of CSF cells/pellet in provided bubble wrap tube sleeves, seal and place in biohazard bag. Seal biohazard bag according to the instructions on the bag. Ship to NCRAD frozen ON DAY OF COLLECTION.

Frozen Shipment Packaging

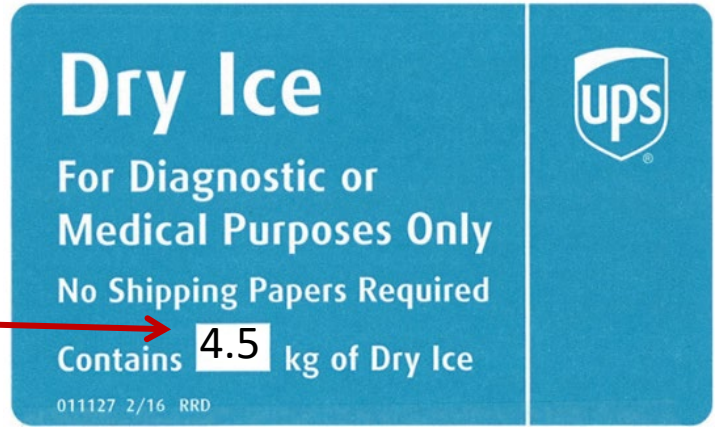
- Place 2-3 inches of dry ice in the bottom of the Styrofoam shipping container, then insert the cryoboxes laying upright.
- Fully cover the cryoboxes with about 2 inches of dry ice in the provided shipper.
- Each Styrofoam shipper must contain about 10 lbs. (4.5 kg) of dry ice.
- Fill shipper to the top with dry ice!

NCRAD

Frozen Shipping Dry Ice Requirements

- Apply all provided warning labels and the pre-printed UPS return airbill to the outside of package, taking care not to overlap labels.

Dry Ice label should not be covered with other stickers and must be completed, or the shipping carrier will reject/return your package!



Net weight of dry ice in **kg**

NCRAD

Critical Frozen Shipping Instructions

1. Hold packaged samples in -80°C freezer until time of UPS pick-up/drop-off.

2. Substudy samples should be shipped via UPS Next Day Air ON DAY OF COLLECTION!

3. Samples should be collected and sent on Monday through Wednesday ONLY!

BE AWARE OF HOLIDAYS and current weather conditions!

4. Remember to complete the requisition forms and include a copy in your shipment: Biological Sample and Shipment Notification Form - SubStudy and CSF Sample and Shipment Notification Form - SubStudy (Appendix D and E).

Batch shipping main study specimens and subset study specimens together:

Important Note for Frozen Shipments ONLY

Batch shipping main study specimens and subset study specimens together: If shipping main study specimens same day as a subset collection, the 25-slot cryoboxes holding plasma and buffy coats from main study can be batch shipped with the subset specimens (PAXgene™ tube, 15 mL conical holding Cryogenic vial of CSF cells, and 48-slot cryobox holding CSF aliquots). Ensure there is ~45 lbs.. of dry ice for large shippers and ~14 lbs.. for small shippers.

Large Frozen Shipper – fits 5 25-slot cryoboxes and ~45 lbs.. dry ice

Small Shipper – fits 3 25-slot cryoboxes and ~10 lbs.. dry ice

Ambient Shipping

PBMC

Notify NCRAD When Samples Ship:

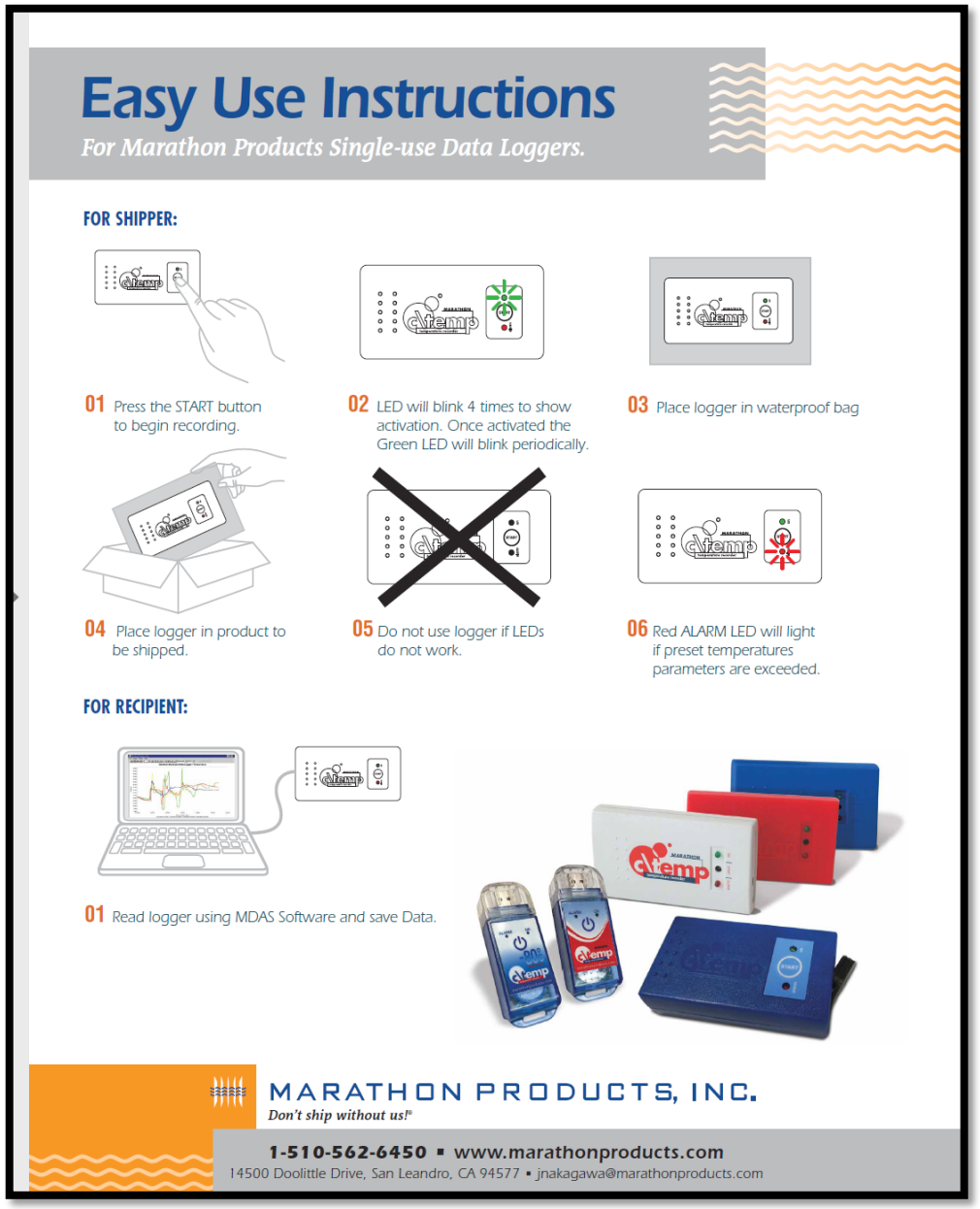
1. **Notify NCRAD of shipment** by emailing NCRAD coordinators at: alzstudy@iu.edu

- Attach the following to the email:
  - Completed Biological Sample and Shipment Notification Form (Appendix D– also found on the NCRAD - LIFE-DSR Active Study Page).
  - If email is unavailable, please call NCRAD and do not ship until you've contacted and notified NCRAD coordinators about the shipment in advance.
  - Please include the tracking number in the body of the email.
  - **Place physical copy of the filled out Biological Sample and Shipment Notification Form-SubStudy (Appendix D) in your shipment.**

Ambient Shipment Packaging:

Place refrigerant pack in the refrigerator, ~4°C, 24 hours prior to shipment. Place filled and labeled Sodium Heparin (Green-Top) Tubes (10 mL) within the slots in the absorbent pad provided, and place into the plastic biohazard bag with absorbent sheet. Ship (3) NaHep tubes in one ambient shipper and (2) tubes in the other (assuming all 5 tubes were collected). Seal the biohazard bag according to the directions on the bag. Place kit number label on the outside of the biohazard bag(s). Place refrigerant pack into the cooler on top of the filled biohazard bag.

Ambient Shipment Packaging (cont.):



Easy Use Instructions

For Marathon Products Single-use Data Loggers.

FOR SHIPPER:

01 Press the START button to begin recording.

02 LED will blink 4 times to show activation. Once activated the Green LED will blink periodically.

03 Place logger in waterproof bag

04 Place logger in product to be shipped.

05 Do not use logger if LEDs do not work.

06 Red ALARM LED will light if preset temperatures parameters are exceeded.

FOR RECIPIENT:

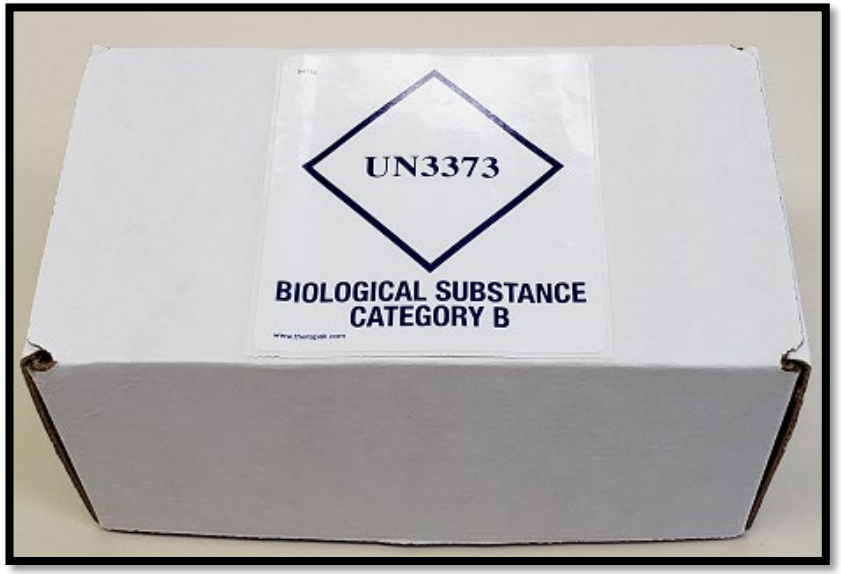
01 Read logger using MDAS Software and save Data.

MARATHON PRODUCTS, INC.

Don't ship without us!®

1-510-562-6450 ▪ www.marathonproducts.com

14500 Doolittle Drive, San Leandro, CA 94577 ▪ jnakagawa@marathonproducts.com



Place (1) temperature monitor in Styrofoam cooler with tubes. Place lid onto the cooler and place cooler into provided IATA Shipping Box. Place an extra copy of the emailed Biological Sample and Shipment Notification Form within the shipping box along with a list of contents form. Close the IATA Shipping Box and label the outside of the cardboard box with the enclosed UN3373 (Biological Category B) label. Place the closed, labeled shipping box within UPS Laboratory Pak. Seal the UPS Laboratory Pak.

Critical Ambient Shipping Instructions - Substudy

1. Substudy samples must be shipped the day of blood draw via UPS Next Day Air in Styrofoam cooler.

2. Substudy samples should be sent Monday through Wednesday ONLY.

BE AWARE OF HOLIDAYS and current weather conditions!

3. Remember to complete the requisition forms and include a copy in your shipment: Biological Sample and Shipment Notification Form - SubStudy (Appendix D).

4. Include no more than three tubes per shipping container and include only tubes from one participant.

5. Place (1) temperature monitor inside ambient shipper with samples. Directions on page 45-46 of the Manual of Procedures or slides 55-56.

Shipping Regulations and Training

All study personnel responsible for shipping should be certified in biospecimen shipping.
It is the responsibility of each site to ensure that the appropriate training has been provided and conducted in regards to IATA shipping.

UN3373 Biological Substance, Category B Training

- Biological Substance, Category B are specimens being transported for “investigational purposes”
- Recommend: investigator sites document training of category B/dangerous goods
- We recommend establishing a record of your staff’s training and date of instruction
- The training records must be made available upon request by the appropriate national authority
  - Additional information from the Department of Transportation (DOT) can be found on their website http://hazmat.dot.gov

NCRAD

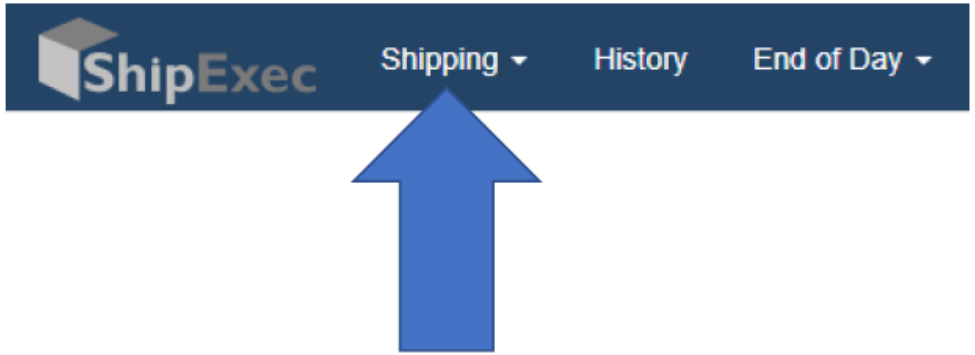
Creating Airbills/Scheduling Pickups

Frozen and Ambient Shipments

UPS ShipExex™ Thin Client Website

Log into the ShipExec Thin Client: https://kits.iu.edu/UPS

Click on the “Shipping” dropdown and click on “Shipping and Rating”

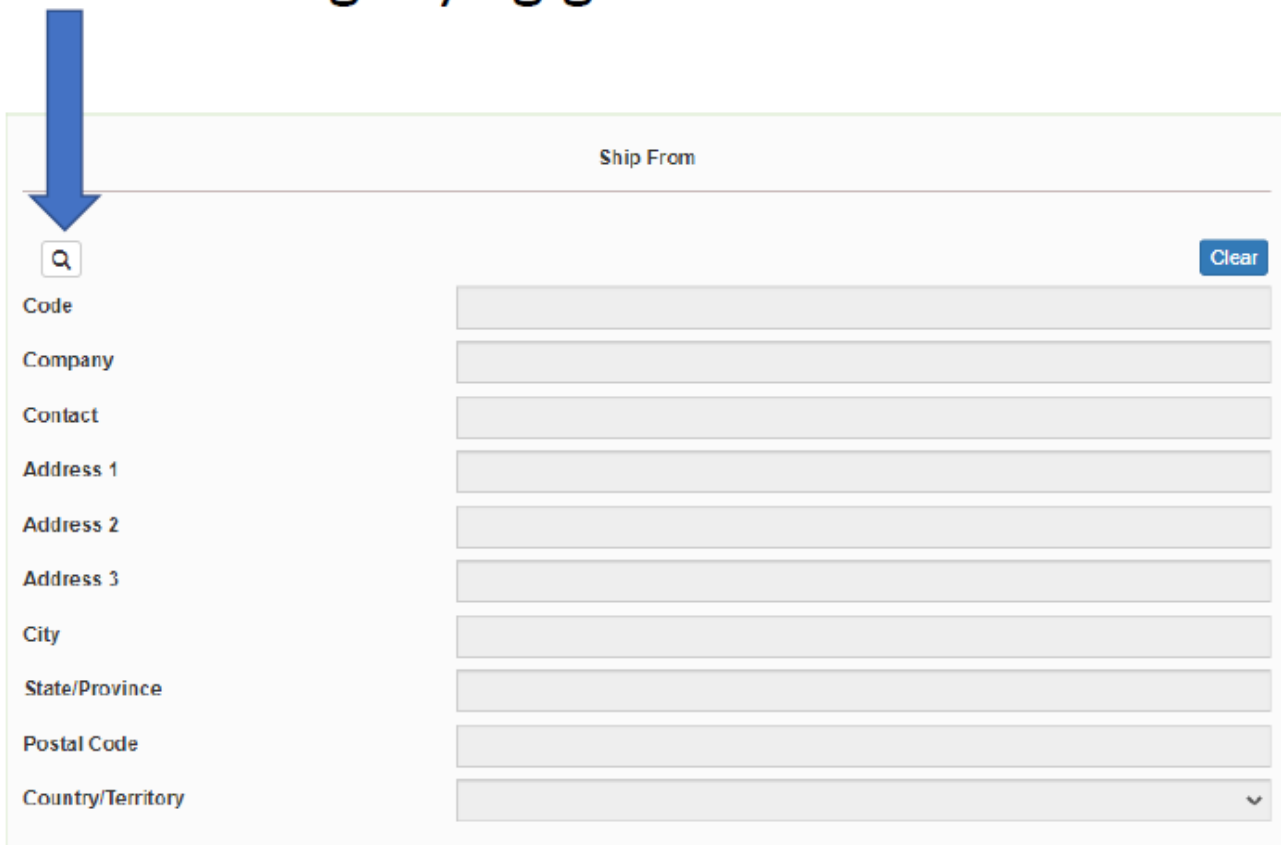


NCRAD

Finding Your Contact Information

- On the right side of the screen, choose the name of your study from the "Study Group" drop down menu
  - *This step <u>must</u> be done 1st*

- On the left side of the screen, Click on the magnifying glass icon



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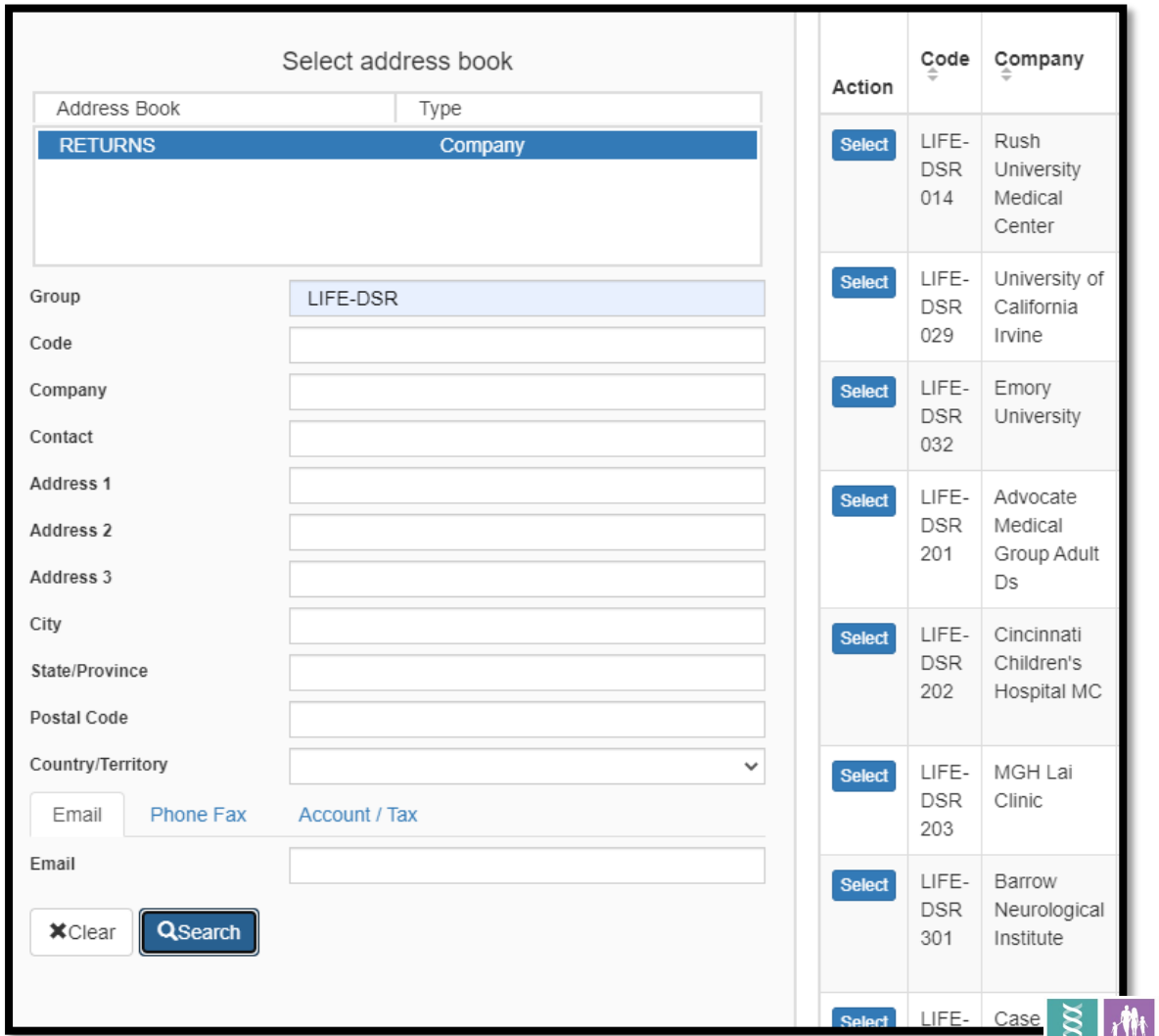
Finding Your Contact Information

- On the right side of the screen, a list of all the site addresses within the study you selected should populate
- User can filter the search for their address further by filling in the “Company”, “Contact”, or “Address 1” fields
- Hit “Search” when ready.
- Once you have found your site address, click on the “Select” button to the left of the address
- If any information needs to be updated, please reach out to the NCRAD Coordinator of your study



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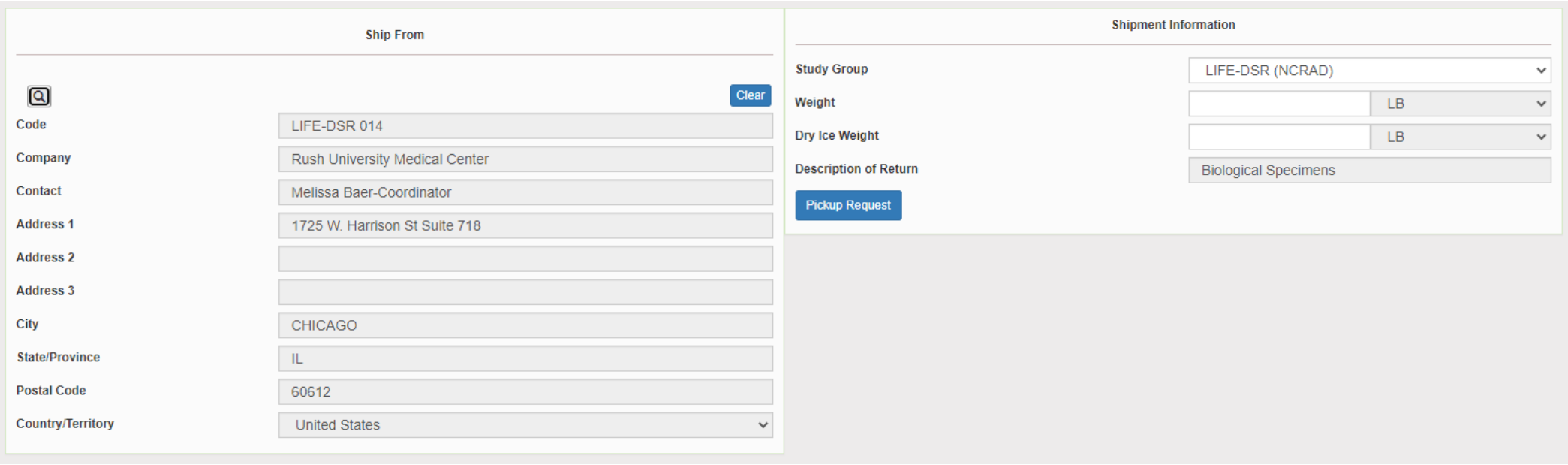
Verify Information

- Please verify that both the shipping information AND study reference are correct for this shipment



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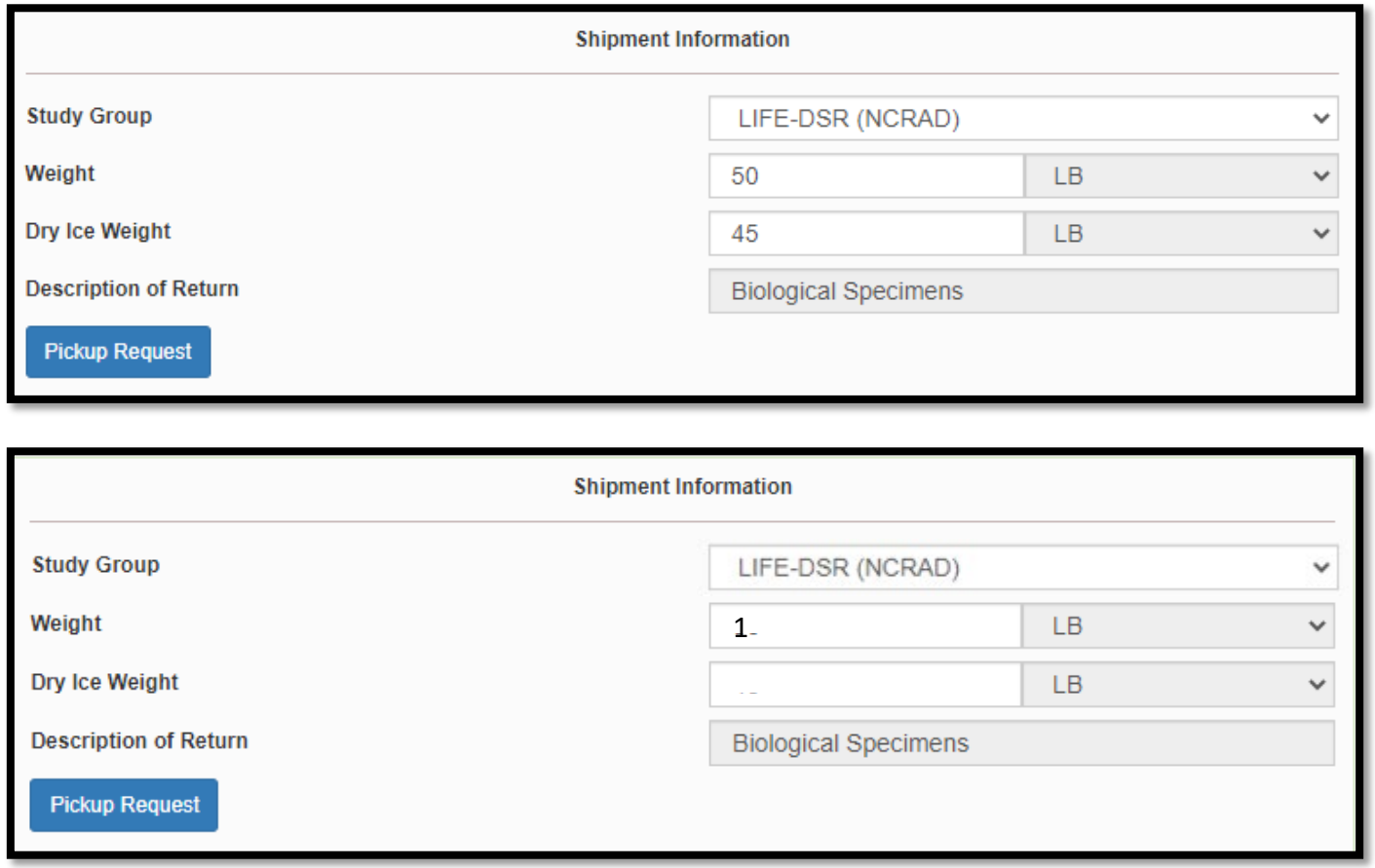
Entering Shipment Information

- **Frozen shipments**
  - Enter the total weight of your package in the “Weight” field
  - Enter the dry ice weight in the “Dry Ice Weight” field
  - The “Dry Ice Weight” field cannot be higher than the “Weight” field (will receive an error message)

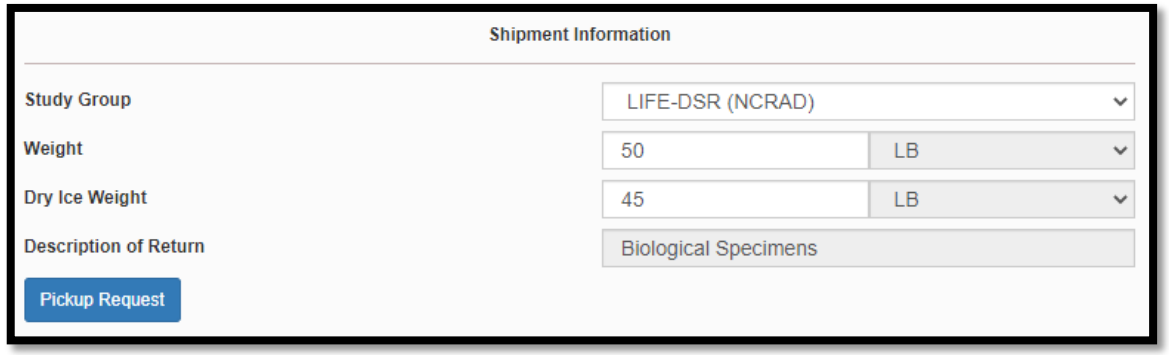
- **Ambient shipments**
  - Enter the total weight of your package in the “Weight” field and **leave the “Dry Ice Weight” field empty.**

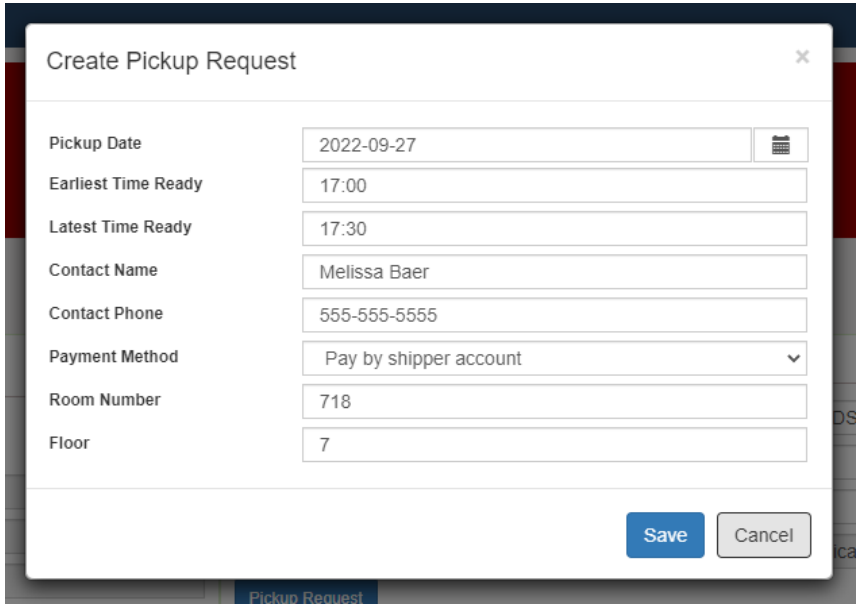


NCRAD

Need to request UPS Pickup?

- Click on the “Pickup Request” button
- Fill out all fields for the pickup request
- Enter in the “Earliest Time Ready” and “Latest Time Ready” in 24-hour format
  - Users must schedule pickup minimum 1 hour before “Earliest Time Ready”
- Choose a name and number that is the best to contact if the UPS driver has questions related to picking up your package
- Entering the Room Number and Floor will help the UPS driver locate your package
  - Room number field is free text
  - Floor field is numerical only
- Hit “Save” when done





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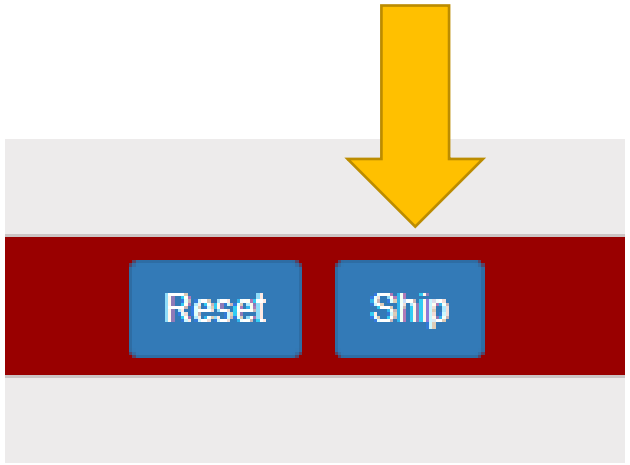
Shipping Packages

- If all fields in “Ship From” and “Shipment Information” fields are completed, and pickup request is completed (if necessary), click Ship in the bottom right corner of the page



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Accessing Airbill

Shipment Receipt

ShipExec™ Shipment Receipt

Transaction Date: Tuesday, December 8, 2020

Pickup No: 2929602E9CP

Address Information

Ship To:	Shipper:	Ship From:
John Smith	Iugb	Iugb
Indiana Unversity	Iu School Of Medicine	Iu School Of Medicine
980 W. Walnut Street	351 W 10Th St	351 W 10Th St
Indianapolis, IN 46202	Indianapolis, IN 46202	Indianapolis, IN 46202

Shipment Information

Service: UPS Next Day Air (UPS Adapter)

Package Information

Pkg No	Tracking No	Packaging Type	Actual Wt	Billable Wt	Insured Value
1	1Z976R8W8430841976	Customer Packaging	20.0	20	0.00

- Check Pickup Status by going to UPS.com, click on the Shipping, select Schedule a Pickup, and look on the right side of screen to click on "Pickup Request Status". Enter in the Pickup No. listed on receipt into PRN field and submit

Airbill

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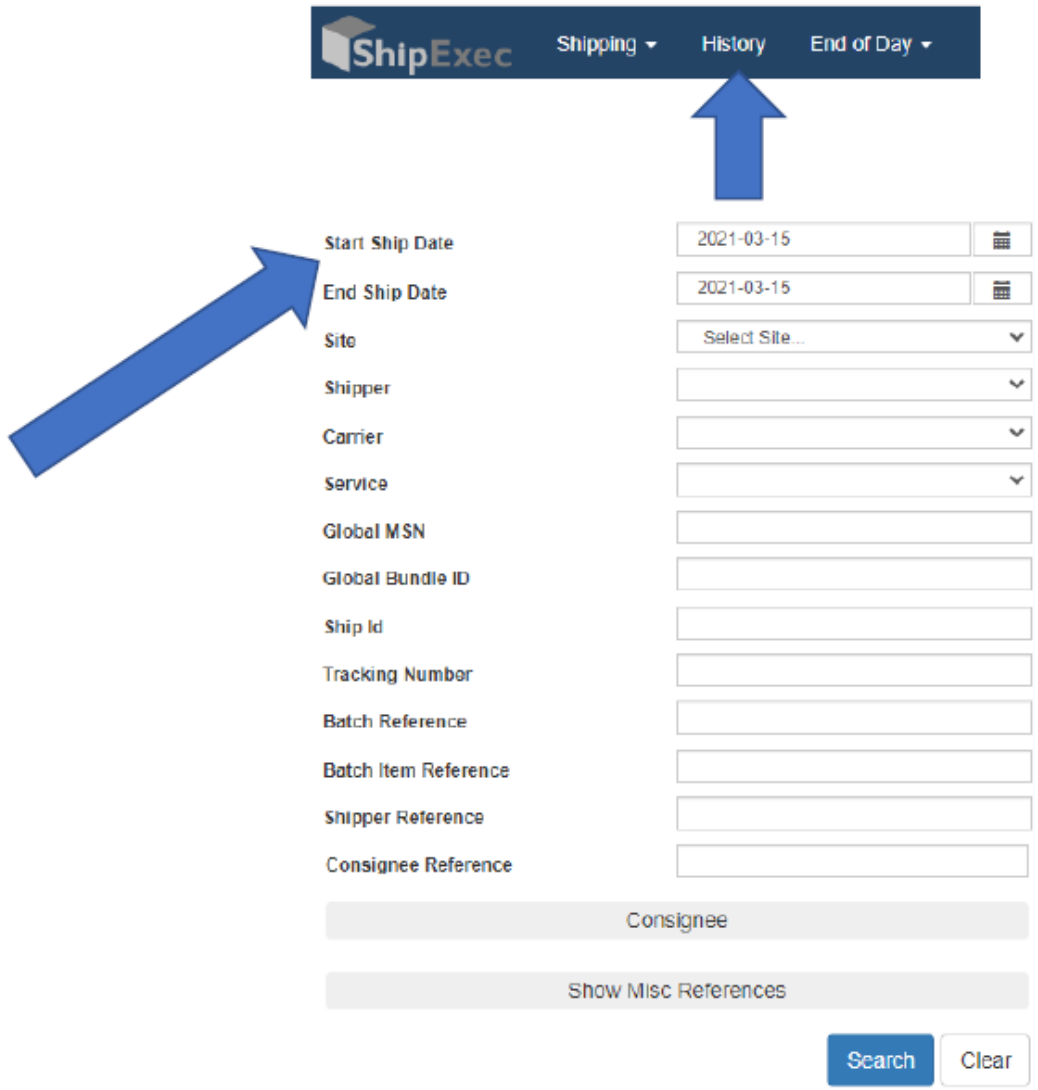
Accessing Airbill

- Print out the UPS air waybill
- Fold the UPS air waybill and slide it inside the plastic UPS sleeve (NCRAD will provide these in kit requests)
- Peel the back off the plastic UPS sleeve and stick the sleeve to your package, making sure it is laying as flat as possible along the surface of the package.

NCRAD

Reprint Airbills/Voiding Shipments

- To reprint airbill or void a shipment, click “History” at the top of the ShipExec Thin Client portal

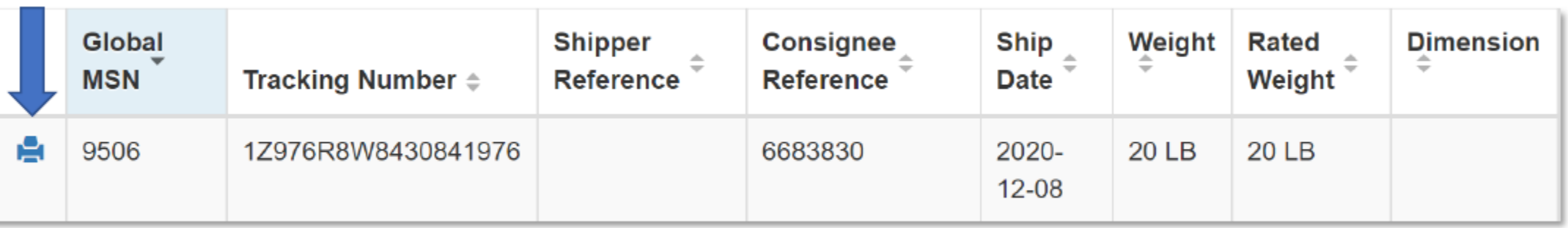


- If your shipment doesn’t automatically pop up, enter in the date of shipment and then click “Search”

NCRAD

Reprint Airbill

- Click the print icon to reprint airbill



Action	Global MSN	Tracking Number	Shipper Reference	Consignee Reference	Ship Date	Weight	Rated Weight	Dimension
	9506	1Z976R8W8430841976		6683830	2020-12-08	20 LB	20 LB	

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Void Shipment

- To void a shipment, click on the "X" symbol

Action	Global MSN	Tracking Number	Shipper Reference	Consignee Reference	Ship Date	Weight	Rated Weight	Dimension
	9506	1Z976R8W8430841976		6683830	2020-12-08	20 LB	20 LB	

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NCRAD SubStudy Forms

Appendix C: Rate of Centrifuge Worksheet

Appendix C
Rate of Centrifuge Worksheet

Please complete and return this form by fax or email to the NCRAD Project Manager if you have any questions regarding sample processing. The correct RPM will be sent back to you.

Submitter Information

Name: **Site:**
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:

$$RCF = \left(\frac{RPM}{1{,}000}\right)^2 \times r \times 1.118 \quad \Rightarrow \quad RPM = \sqrt{\frac{RCF}{r \times 1.118}} \times 1{,}000$$

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 centrifuge

Comments:

Please send this form to NCRAD Study Coordinator

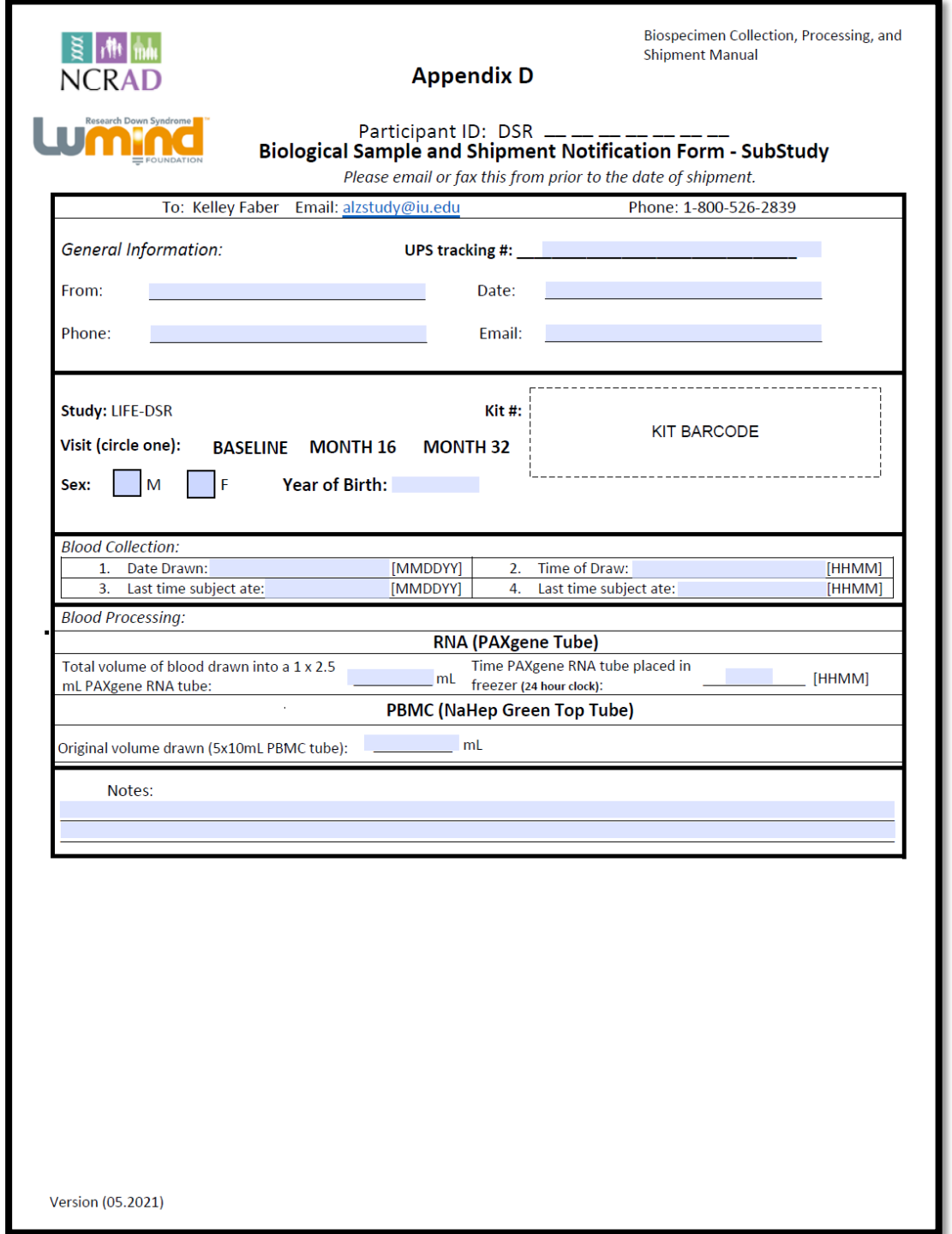
alzstudy@iu.edu

Note:

Use Rate of Centrifugation Worksheet to calculate RPM, if needed.

NCRAD

Appendix D: Biological Sample and Shipment Notification Form - SubStudy



Biospecimen Collection, Processing, and Shipment Manual

Appendix D

Participant ID: DSR __ __ __ __ __ __ __

Biological Sample and Shipment Notification Form - SubStudy

Please email or fax this from prior to the date of shipment.

To: Kelley Faber Email: alzstudy@iu.edu Phone: 1-800-526-2839

General Information: **UPS tracking #:** _____

From: _____ Date: _____

Phone: _____ Email: _____

Study: LIFE-DSR **Kit #:** KIT BARCODE

Visit (circle one): **BASELINE** **MONTH 16** **MONTH 32**

Sex: ☐ M ☐ F **Year of Birth:** _____

Blood Collection:

1. Date Drawn:	[MMDDYY]	2. Time of Draw:	[HHMM]
3. Last time subject ate:	[MMDDYY]	4. Last time subject ate:	[HHMM]

Blood Processing:

RNA (PAXgene Tube)

Total volume of blood drawn into a 1 x 2.5 mL PAXgene RNA tube: _____ mL Time PAXgene RNA tube placed in freezer **(24 hour clock):** _____ [HHMM]

PBMC (NaHep Green Top Tube)

Original volume drawn (5x10mL PBMC tube): _____ mL

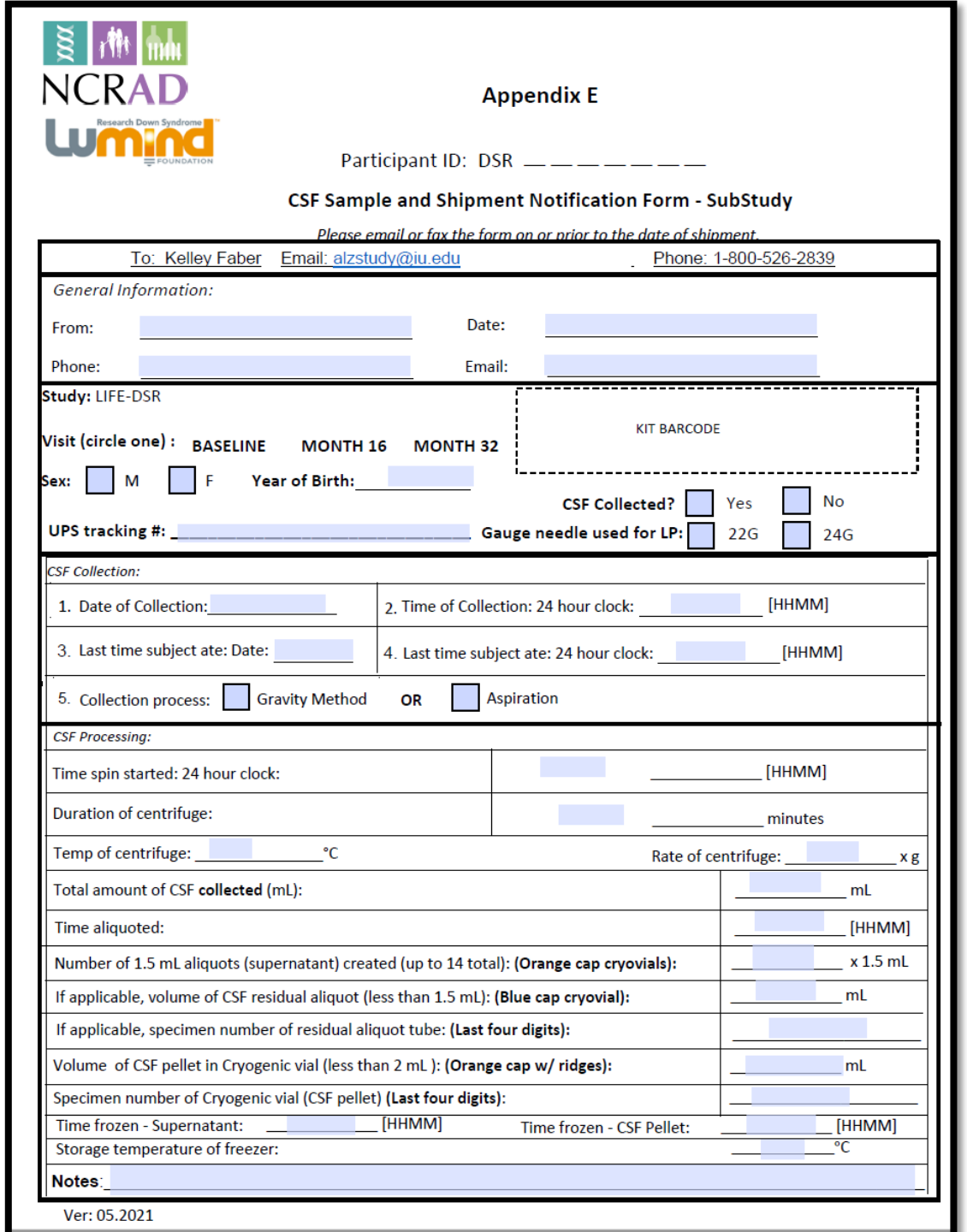
Notes:

Version (05.2021)

Note:

Please ensure Sample Notification Forms are filled out in their entirety. Complete during the participant study visit as samples are processed to guarantee accuracy.

Appendix E: CSF Sample and Shipment Notification Form - SubStudy



NCRAD

Research Down Syndrome Lumind Foundation

Appendix E

Participant ID: DSR _ _ _ _ _ _ _ _

CSF Sample and Shipment Notification Form - SubStudy

Please email or fax the form on or prior to the date of shipment.

To: Kelley Faber Email: alzstudy@iu.edu Phone: 1-800-526-2839

General Information:

From: _____ Date: _____

Phone: _____ Email: _____

Study: LIFE-DSR

KIT BARCODE

Visit (circle one) : **BASELINE** **MONTH 16** **MONTH 32**

Sex: ☐ M ☐ F **Year of Birth:** _____

CSF Collected? ☐ Yes ☐ No

UPS tracking #: _____ **Gauge needle used for LP:** ☐ 22G ☐ 24G

CSF Collection:

1. Date of Collection: _____	2. Time of Collection: 24 hour clock: _____ [HHMM]
3. Last time subject ate: Date: _____	4. Last time subject ate: 24 hour clock: _____ [HHMM]

5. Collection process: ☐ Gravity Method **OR** ☐ Aspiration

CSF Processing:

Time spin started: 24 hour clock:	_____ [HHMM]
Duration of centrifuge:	_____ minutes
Temp of centrifuge: _____ °C	Rate of centrifuge: _____ x g
Total amount of CSF **collected** (mL):	_____ mL
Time aliquoted:	_____ [HHMM]
Number of 1.5 mL aliquots (supernatant) created (up to 14 total): **(Orange cap cryovials):**	_____ x 1.5 mL
If applicable, volume of CSF residual aliquot (less than 1.5 mL): **(Blue cap cryovial):**	_____ mL
If applicable, specimen number of residual aliquot tube: **(Last four digits):**	_____
Volume of CSF pellet in Cryogenic vial (less than 2 mL): **(Orange cap w/ ridges):**	_____ mL
Specimen number of Cryogenic vial (CSF pellet) **(Last four digits)**:	_____
Time frozen - Supernatant: _____ [HHMM]	Time frozen - CSF Pellet: _____ [HHMM]
Storage temperature of freezer:	_____ °C

Notes: _____

Ver: 05.2021

Note:

Please ensure Sample Notification Forms are filled out in their entirety. Complete during the participant study visit as samples are processed to guarantee accuracy.

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NCRAD Website

NCRAD Website: Helpful Pages

NCRAD - LIFE-DSR Active Study Page

https://ncrad.org/holiday_closures.html

Holiday Closures

Date	Holiday
January 1	New Year's Day
3rd Monday in January	Martin Luther King, Jr Day
4th Monday in May	Memorial Day
June 19	Juneteenth (observed)
July 4	Independence Day (observed)
1st Monday in September	Labor Day
4th Thursday in November	Thanksgiving
4th Friday in November	Friday after Thanksgiving
December 25	Christmas

https://ncrad.org/shipping_address.html

Shipping Address

NCRAD
Indiana University School of Medicine
351 W. 10th St TK-217
Indianapolis, IN 46202

UPS Shipping Resources

To generate air waybills and schedule UPS pickups for shipments to NCRAD, please visit the UPS ShipExec™ Thin Client website.

For instructions on how to use the UPS ShipExec™ Thin Client website, please refer to the NCRAD UPS ShipExec™ Thin Client Guide.

Navigating UPS ShipExec™