Veri-T Manual of Procedures Update: Version 04.2025

Section	Change Log Item
Document Footer	The version date was updated for this amendment.
Throughout Document	Minor changes made to phrasing and wording. No alterations to procedures were made.
6.1	New label examples updated from last revision, now including ‘COLLECT’ and ‘ALIQUOT’

Veri-T (Dr. Peter Ljubenkov) – A R01 Grant Funded Initiative Utilizing ALLFTD infrastructure, protocols, and procedures

in collaboration with the

National Centralized Repository for Alzheimer's Disease and Related Dementias

Biospecimen Collection, Processing, and Shipment Manual

Version (4.2025)

Table of Contents

1.0 Abbreviations

Veri-T	A phase 1 Placebo-Controlled Trial of Verdiperstat in Semantic Variant Primary Progressive Aphasia Due to Underlying FTLD-TDP
CITI	Collaborative Institutional Training Initiative
CSF	Cerebrospinal Fluid
EDTA	Ethylene Diamine Tetra-acetic Acid
ET	Early Termination
IATA	International Air Transport Association
IUGB	Indiana University Genetics Biobank
LP	Lumbar Puncture
NCRAD	National Centralized Repository for Alzheimer's Disease and Related Dementias
PD	Pharmacodynamic
PK	Pharmacokinetic
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute

2.0 Purpose

The purpose of this manual is to provide Veri-T staff (PIs, study coordinators, and the sample collection and processing teams) at the various study sites with instructions for collection and submission of biological samples for Veri-T study visits. It includes instructions for biospecimen submission to the National Centralized Repository for Alzheimer's disease and Related Dementias (NCRAD) located at Indiana University. The following samples may be collected at each study visit:

- Plasma
- CSF

This manual includes instructions for collection of blood and CSF, fractionation of blood from collection tubes, aliquoting, labeling, storage prior to shipping, and shipping to NCRAD.

These procedures are relevant to all study personnel responsible for processing blood specimens to be submitted to NCRAD for the Veri-T protocol.

3.0 NCRAD Information

3.1 NCRAD Contacts

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

Kelley Faber, MS, CCRC, Project Manager
Phone: 317-274-7360
Email: kelfaber@iu.edu

Corinne Kim, MA, CCRC, Study Coordinator
Phone: 317-274-7417
Email: kimcor@iu.edu

Jazmyn Dickinson, Study Coordinator
Email: jazdicki@iu.edu

General NCRAD Contact Information
Phone: 1-800-526-2839
Fax: 317-321-2003
Email: alzstudy@iu.edu
Website: www.ncrad.org
Veri-T Study Specific Webpage: https://ncrad.org/coordinate-studies/veri-t

Sample Shipment Mailing Address
Veri-T at NCRAD
Indiana University School of Medicine
351 W. 10th St TK-217
Indianapolis, IN 46202
Phone: 1-800-526-2839

3.2 NCRAD Hours of Operation

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

Frozen samples must be shipped **Monday-Wednesday only**.

For packing and shipment details of frozen samples, please refer to Section 8.0 of this protocol.

Check the weather report to make sure impending weather events (blizzards, hurricanes, etc.) will not impact the shipping or delivery of the samples.

3.3 NCRAD Holiday Observations

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
July 4	Independence Day
1st Monday in September	Labor Day
4th Thursday in November	Thanksgiving
4th Friday in November	Friday after Thanksgiving
December 25	Christmas Day
December 26-31	Winter Break

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 after the second week in December.

See: https://ncrad.org/holiday_closures.html for additional information.

- Courier services may observe a different set of holidays. Verify shipping dates with your courier prior to any holiday.
- **Weekend/holiday delivery must be arranged in advance with NCRAD staff.**

4.0 Veri-T Laboratory Collection

4.1 Site Required Equipment

The following materials and equipment are necessary for the processing of specimens at the collection site and are to be **supplied by the local site**:

- Personal Protective Equipment: lab coat, nitrile/latex gloves, safety glasses
- Tourniquet
- Alcohol Prep Pad
- Gauze Pad
- Bandage
- Butterfly needles and hub
- Microcentrifuge tube rack
- Sharps bin and lid
- Wet ice bucket
- Wet ice

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

- Centrifuge capable of ≥ 1500 rcf (1500 x g) with refrigeration to 4°C for plasma

- Centrifuge capable of ≥ 2000 rcf (2000 x g) at room temperature for CSF
- -80°C Freezer

In order to batch-ship frozen specimens, you must provide:

- Pelleted dry ice (approximately 30-45 lbs per shipment)

4.2 Biospecimens Sent to NCRAD

Frozen samples are to be submitted according to the shipping methods outlined in Section 8.1. Guidelines for the processing, storage location, and timing of sample collection are listed by site in the tables below.

Biospecimens collected include whole blood and CSF. Please refer to the below table for the biospecimen schedule for both the pre-dose and the post-dose collection schedule.

	Screening	Day 1	Week 12		Week 24		ET
		Pre-Dose	Pre-Dose	Post-Dose	Pre-Dose	Post-Dose	
Plasma PK		x	x	x	x	x	x
Plasma PD		x		x		x	x
CSF	x					x	x

Whole blood will be collected into three lavender-top collection tubes. The lavender top EDTA tubes are processed locally into plasma fractions, aliquoted, frozen at the study site, and then shipped to NCRAD.

CSF will be aliquoted locally, frozen at the study site, and then shipped to NCRAD.

Frozen samples (plasma and CSF) are to be submitted according to the shipping methods outlined in Section 8.1. Guidelines for the processing, storage location, and timing of sample collection are listed in the tables below.

The **Blood Sample and Shipment Notification Form** (see Appendix B) is completed for every visit**. The CSF Sample and Shipment Notification Form** (see Appendix C) is completed for the Screening and last visit (Week 24 or ET). Be sure to complete all required fields on the **Biological Sample and Shipment Notification Form and CSF Sample and Shipment Notification Form.** In addition, samples not collected need to be recorded in the notes section on the Sample forms. Submit a copy to NCRAD with a reason provided for the omission.

4.3 Informed Consent

Consent forms must specify that any biological samples and de-identified clinical data may be shared with academic and/or industry collaborators through NCRAD. A copy of the consent form for each participant should be kept on file by the site investigator.

4.4 Biospecimen Collection Charts

4.4.1 Blood Collection

Participants will have blood collected at Day 1, Week 12, and Week 24. Participants who leave the study before week 24 visit will have an ET blood collection. If a sample is not obtained at a particular visit, this should be recorded in the notes section of the **Blood Sample and Shipment Notification Form (see Appendix B).** Submit a copy to NCRAD with a reason provided for the omission.

* Please refer to the table in Section 4.2 for another view of the specimen collection schedule

Visit	Dose	Collection Tube	Specimen Type	Cryovial Cap Color	Aliquot Volume	Number of Aliquots Sent to NCRAD	Shipping Temperature
Day 1	**Pre-Dose**	**EDTA (Lavender-Top) Blood Collection Tube (10 ml)**	Plasma PK	Purple	1.5 ml plasma aliquots	Up to 8	Frozen
	Pre-Dose	**EDTA (Lavender-Top) Blood Collection Tube (10 ml)**	Plasma PD	Purple	1.5 ml plasma aliquots	Up to 4	Frozen
Week 12 and Week 24	**Pre-Dose**	**EDTA (Lavender-Top) Blood Collection Tube (10 ml)**	Plasma PK	Purple	1.5 ml plasma aliquots	Up to 4	Frozen
	Post-Dose	**EDTA (Lavender-Top) Blood Collection Tube (10 ml)**	Plasma PD	Purple	1.5 ml plasma aliquots	Up to 4	Frozen
	Post-Dose	**EDTA (Lavender-Top) Blood Collection Tube (10 ml)**	Plasma PK	Purple	1.5 ml plasma aliquots	Up to 4	Frozen
ET	**ET**	**EDTA (Lavender-Top) Blood Collection Tube (10 ml)**	Plasma PK	Purple	1.5 ml plasma aliquots	Up to 8	Frozen
		EDTA (Lavender-Top) Blood Collection Tube (10 ml)	Plasma PD	Purple	1.5 ml plasma aliquots	Up to 4	Frozen

4.4.2 Cerebrospinal Fluid

CSF is collected at screening and at week 24 (or at ET visit if participant leaves study earlier). If a CSF sample *is or is not* obtained at a particular visit, this should be recorded in the CSF section of the **Biological Sample and Shipment Notification Form (see Appendix B).**

Visit	Collection Tube	Specimen Type	Cryovial Cap Color	Aliquot Volume	Number of Aliquots Sent to NCRAD	Shipping Temperature
Screening	**50 ml screw top centrifuge tubes with blue caps**	**CSF**	Clear	0.5 ml CSF aliquots	5	Frozen
			Orange & Blue	1.5 ml CSF aliquots	Up to 14	Frozen
Week 24 or ET	**50 ml screw top centrifuge tubes with blue caps**	**CSF**	Clear	0.5 ml CSF aliquots	5	Frozen
			Orange & Blue	1.5 ml CSF aliquots	Up to 14	Frozen

5.0 Specimen Collection Kits, Shipping Kits and Supplies

There are 8 types of Veri-T kits:

- Veri-T Day 1 Pre-Dose Blood Kit
- Veri-T Weeks 12 or 24 Pre-Dose Blood Kit
- Veri-T Weeks 12 or 24 Post-Dose Blood Kit
- Veri-T Early Termination Blood Kit
- Veri-T CSF Kit
- Veri-T Frozen Shipping Supply Kit
- Veri-T Supplemental Supply Kit

Research specimen collection kits as well as clinical lab supplies (except pelleted dry ice and equipment supplies listed above in Section 4.1) will be provided by NCRAD. These materials include blood tubes, Lumbar Puncture trays (when applicable), boxes for plasma/CSF aliquots storage and shipment, as well as partially completed shipping labels to send materials to NCRAD. Barcoded kit labels, site and participant ID labels, collection tube labels, and aliquot tube labels will all be provided by NCRAD. Collection tube labels and aliquot tube labels will be pre-printed with study information specific to the type of sample being drawn. Ensure that all tubes are properly labeled during processing and at the time of shipment according to Section 6.1.

5.1 NCRAD Specimen Collection Kit Contents

Collection kits contain the following (for each participant) and provide the necessary supplies to collect samples from a given participant. Do not replace or supplement any of the tubes or kit components provided with your own supplies unless you have received approval from the NCRAD Study team to do so. *Please store all kits at room temperature until use.*

Veri-T Day 1 Pre-Dose Blood Kit

Quantity	Veri-T Day 1 Pre-Dose Blood Kit Components
3	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
9	Cryovial tube (2 ml) with lavender cap
3	Cryovial tube (2 ml) with blue cap
2	Disposable graduated transfer pipette
15	Pre-printed labels for blood collection and aliquot tubes
3*	Pre-printed labels with kit number
3**	Labels for handwritten Participant ID
1	Cryovial tube box (holds up to 25 cryovials)
1	Day 1 Pre-Dose Kit Label (placed on kit bag)

***1 label frozen shipment form, 1 label for cryobox, 1 label for biohazard bag,**

****3 Participant ID labels used for blood collection tubes**

Veri-T Week 12/24 Pre-Dose Blood Kit

Quantity	Veri-T Week 12/24 Pre-Dose Blood Kit Components
1	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
3	Cryovial tube (2 ml) with lavender cap
1	Cryovial tube (2 ml) with blue cap
1	Disposable graduated transfer pipette
5	Pre-printed labels for blood collection and aliquot tubes
3*	Pre-printed labels with kit number
2**	Labels for handwritten Participant ID
1	Cryovial tube box (holds up to 25 cryovials)
1	Pre-Dose Kit Label (placed on kit bag)

***1 label frozen shipment form, 1 label for cryobox, 1 label for biohazard bag**

****1 participant ID label used for blood collection tube, 1 extra label**

Veri-T Week 12/24 Post-Dose Blood Kit

Quantity	Veri-T Week 12/24 Post-Dose Blood Kit Components
2	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
6	Cryovial tube (2 ml) with lavender cap
2	Cryovial tube (2 ml) with blue cap
2	Disposable graduated transfer pipette
10	Pre-printed labels for blood collection and aliquot tubes
3*	Pre-printed labels with kit number
3**	Labels for handwritten Participant ID
1	Post-Dose Kit Label (placed on kit bag)

***1 label frozen shipment form, 1 label for cryobox, 1 label for biohazard bag**

****2 participant ID labels used for blood collection tubes, 1 extra label**

Veri-T Early Termination Blood Kit

Quantity	Veri-T Early Termination Blood Kit Components
3	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
9	Cryovial tube (2 ml) with lavender cap
3	Cryovial tube (2 ml) with blue cap
2	Disposable graduated transfer pipette
15	Pre-printed labels for blood collection and aliquot tubes
3*	Pre-printed labels with kit number
3**	Labels for handwritten Participant ID
1	Cryovial tube box (holds up to 25 cryovials)
1	Early Termination Kit Label (placed on kit bag)

***1 label frozen shipment form, 1 label for cryobox, 1 label for biohazard bag,**

****3 participant ID labels used for blood collection tubes, 1 extra label**

Veri-T CSF Kit

Quantity	Veri-T CSF Kit Components
14	Cryovial tube (2 ml) with orange cap
5	Cryovial tube (2 ml) with clear cap
1	Cryovial tube (2 ml) with blue cap
1	Lumbar Puncture tray with 24G Sprotte needle
2	Screw-top individually wrapped conical tube with blue cap (50 ml)
4	Disposable graduated transfer pipette
1	Cryovial tube box (holds up to 25 cryovials)
19	Pre-printed labels for CSF collection and aliquot tubes
4*	Pre-printed labels with kit number

***1 label frozen shipment form, 1 label for cryobox, 1 label for biohazard bag, 1 extra label**

Shipping Supply Kit

Quantity	Frozen Shipping Kit Components
8	Small plastic Biohazard bag with absorbent sheets
1	Shipping box/Styrofoam container
1	Warning label packet with dry ice sticker

Veri-T Supplemental Supply Kit

Quantity	Veri-T Supplemental Kit Components
3	Resealable plastic kit bags
5	Cryovial tube box (holds up to 25 cryovials)
5	Small plastic Biohazard bag with absorbent sheet
10	Cryovial tube (2 ml) with blue cap
50	Cryovial tube (2 ml) with lavender cap
1	Needle – Sprotte Needle w/ Introducer 24G x 90mm, 1mm x 30mm
5	Screw-top individually wrapped conical tube with blue cap (50 ml)
10	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
5	Disposable graduated transfer pipette
5	Warning label packet

We realize there may be instances where additional supplies are needed; therefore, one supplemental kit will be provided with the initial kit shipment. Replacement supplemental kits can be requested on the kit web site. In addition, individual supplies can be requested. The following table lists all individual supplies that can be requested and the quantities at which they are available.

Individual Supplies*

Quantities	Item
5 or 10	Microcentrifuge tube box (holds up to 25 cryovials)
10, 25	Cryovial tube (2 ml) with blue cap
10, 25	Cryovial tube (2 ml) with orange cap
25, 50	Cryovial tube (2 ml) with lavender cap
25, 50	Cryovial tube (2 ml) with clear cap
1, 2, 3	Shipping container for dry ice shipment (shipping and Styrofoam box)
1, 5	Needle – Sprotte Needle w/ Introducer 24G x 90mm, 1mm x 30mm
5, 10	Screw-top individually wrapped conical tube with blue cap (50 ml)
5, 10	Plastic biohazard bag
5, 10, 15	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
30, 60	Disposable graduated transfer pipette
5, 10	Warning label packet
By Request	Participant ID label

*Available upon request on the kit web-site

5.2 Kit Supply at Study Sites

Each individual site will be responsible for ordering and maintaining a steady supply of kits from NCRAD. We advise sites to keep a supply of each kit type available. Be sure to check your supplies and order additional materials before you run out so you are prepared for study visits; it can take up to 3 weeks for requested kits/supplies to be delivered to you. Please go to: http://kits.iu.edu/verit to request additional kits and follow the prompts to request the desired supplies.

Options include ordering specific number of kits or individual supplies.

Please allow **THREE weeks** for kit orders to be processed and delivered.

6.0 Blood Collection and Processing Procedures

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

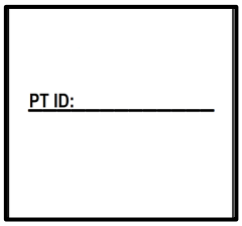
In order to ensure the highest quality samples are collected, processed, and stored, it is essential to follow the specific collection, processing, and shipment procedures detailed in the following pages. Please read the following instructions first before collecting any specimens. Have all your supplies and equipment out and prepared prior to drawing blood.

6.1 Labeling Samples for NCRAD

Label Type Summary

1. Kit Number Label
2. Participant ID Label
3. Collection and Aliquot Tube Labels

Kit Number Labels link all specimens collected from a single participant at one visit. They should be placed in the designated location on the Sample and Shipment Notification Form (either Blood or CSF). **There will be a different kit number for the Blood Kit and the CSF Kit. There will also be different kit numbers for the Pre-Dose and Post-Dose blood kits.** The Kit Number Label will also need to be placed on the corresponding patient's cryovial box which can be used for storage and is required for sending as a frozen BATCH shipment to NCRAD. For visits that included pre-dose and post-dose kits, both kit number labels will need to be placed on the cryovial box.



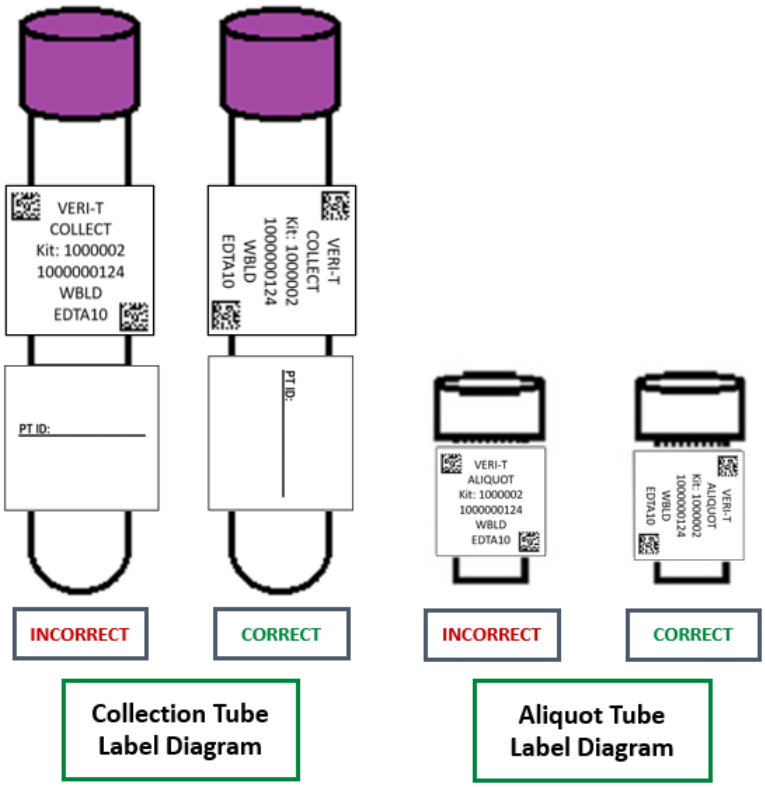
Participant ID Labels are used to document the individual's unique Participant ID. They are placed on each of the collection tubes (EDTA). Veri-T site staff will write-in the participant ID.

Place the **COLLECT Labels** on the collection tube. Each collection tube will contain two labels: the **Participant ID Label** and the **Collection Tube Label**.

Note: The Collection and Aliquot Tube Labels intended for the CSF tubes will contain a kit number differing from the patient's other cycle specimens.

Place the **ALIQUOT Labels** on the aliquot tube.

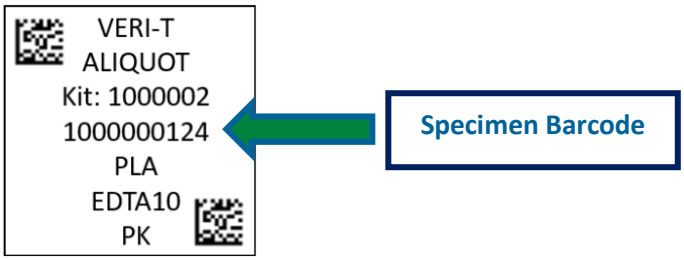
Note: Aliquot Tube Labels intended for the CSF tubes will contain a kit number differing from the patient's other cycle specimens.



In order to ensure the label adheres properly and remains on the tube, please follow these instructions:

- Place specimen 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 cryovials in cryovial box in numerical order based on the specimen number, located at the top of the label. This ensures that no aliquot is misplaced or lost during the shipment process (see depiction below).

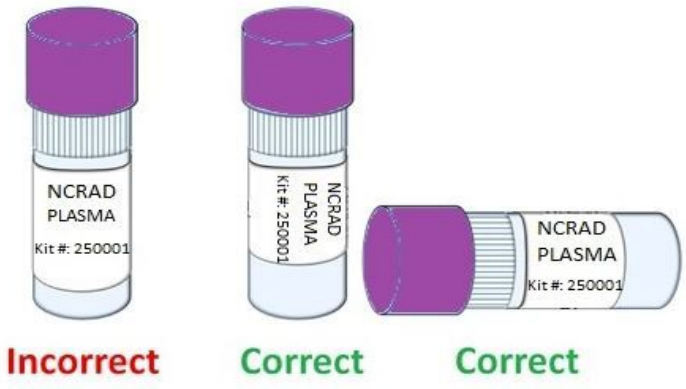


- Using a fine point permanent marker, fill-in and then place the Participant ID labels on the collection tubes only (EDTA). For best adhesion results place the label on the tubes **BEFORE** sample collection, processing, or freezing. **DO NOT** place Participant ID labels on any NCRAD cryovials.

- There will be 2 types of labels on all collection tubes: Participant ID Labels and Collection and Aliquot Tube Label

- The Collection and Aliquot Tube Labels contain a 2D barcode on the left-hand side of the label. **Place this barcode toward the tube cap.**

- Always place the label **horizontally** on the aliquot tube (wrapped around sideways if the tube is upright) and **just below the ridges** of the aliquot tubes (see attached labeling diagram).



- 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.

6.2 Video List

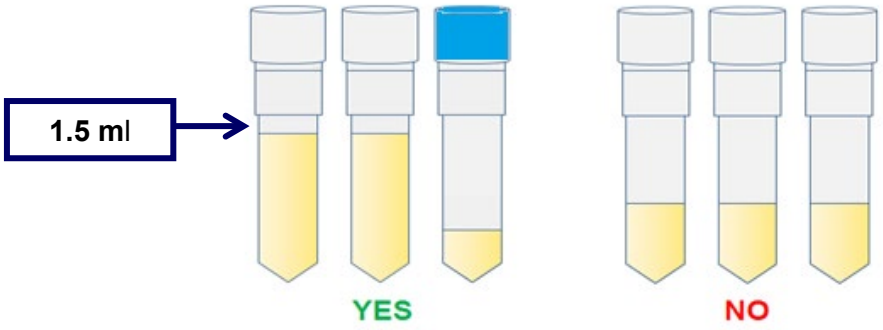
- NCRAD offers training videos that are available to assist you with the specimen processing, aliquoting, and shipping processes. The videos are available at https://ncrad.org/resource_Veri-T.html.
  - Frozen Shipping
  - Plasma Processing and Aliquoting

- CSF Processing and Aliquoting
- Veri-T MOP Training

6.3 Filling Aliquot Tubes (Plasma and CSF)

In order to ensure that NCRAD receives a sufficient amount of sample for processing and storage, and to avoid cracking of the tubes prior to shipment, each aliquot tube should be filled to the assigned volume after processing is completed (refer to detailed processing instructions for average yield per sample). Over-filled tubes may burst once placed in the freezer, resulting in a loss of sample.

Aliquot the remaining biologic material into a blue-cap residual tube and ship to NCRAD. Ship *all* material to NCRAD. Fill as many aliquot tubes as possible. For example, if 2.7 ml of a plasma sample is obtained, fill 1 cryovial tube with 1.5 ml, and one additional cryovial tube with the remaining 1.2 ml.



Please note: It is critical for the integrity of future studies using these samples that study staff note if an aliquot tube contains a residual volume (anything under 1.5 ml). Please highlight that the aliquot contains a small volume by utilizing the blue cryovial cap provided in each kit. Please record the last four digits of the residual aliquot on the Biological Sample and Notification Form.
If there are any unused cryovials, please do not send the empty cryovials to NCRAD. These unused cryovials (ensure labels are removed) can be saved as part of a supplemental supply at your site or the cryovials can be disposed of per your site's requirements.

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

Cap Color	Sample Type
Lavender	Plasma PD or Plasma PK
Clear	CSF Aliquots (0.5 ml)
Orange	CSF Aliquots (1.5 ml)

Blue	Residual Aliquot (Plasma or CSF)

6.4 EDTA (Lavender-Top) Blood Collection Tube (10 ml) for Plasma (PD and PK)

Whole Blood Collection for Isolation of Plasma: one EDTA (Lavender-Top) Blood Collection Tubes (10 ml) (for processing of plasma aliquots) will be collected for Pre-Dose Plasma-PK, Post-Dose Plasma-PK and Plasma PD.

1. Store empty EDTA tubes at room temperature, 64°F - 77°F (18 °C – 25 °C) before use.

2. Set centrifuge 4°C to pre-chill before use. Please note that the centrifuge could take 30 minutes to chill completely.

3. Place completed Participant ID and pre-printed "**PLASMA**" (for Plasma PD) and "**PLASMA PK**" (for plasma PK) *collection tube labels* on the EDTA (Lavender-Top) Blood Collection Tubes (10 ml). Place pre-printed "**PLASMA**" and "**PLASMA PK**" *aliquot labels* on the 2 ml cryovial tubes with lavender caps.

4. Please ensure that aliquots are kept in numerical order (by specimen number) throughout the aliquoting and shipping process, from left to right.

5. Using a blood collection set and a holder, collect blood into the **10 ml EDTA 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 last collection tube.
   d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.
6. 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 each tube.

7. Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tube 8-10 times.

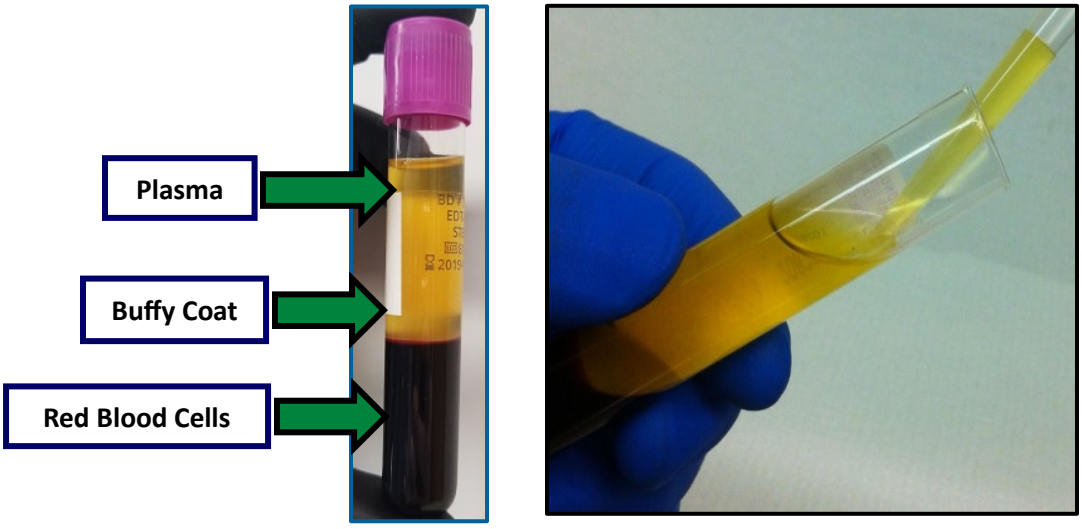
8. Immediately after inverting the EDTA tube, place it on wet ice until centrifugation begins.

9. Centrifuge balanced tubes for 15 minutes at 1500 RCF (x g) at 4°C. **It is critical that the tubes be centrifuged at the appropriate speed and temperature to ensure proper plasma separation (see worksheet in Appendix A to calculate RPM in your particular rotor).**

    a. Equivalent rpm for spin at 1500 x g
    b. While centrifuging, remember to record all times, temperatures and spin rates on the Blood Sample and Shipment Notification Form.
    c. Record time aliquoted on the Blood Sample Shipment and Notification Form.
    d. Plasma samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection.

10. Remove the plasma by tilting the tube and placing the pipette tip along the lower side of the wall, being careful not to agitate the packed blood cells at the bottom of the collection tube.

11. Each EDTA tube should yield, on average, 4-5 ml of plasma. Aliquot 1.5 ml plasma per cryovial. **Be sure to only place plasma (PD) in cryovials with purple caps and labeled with "PLASMA" labels and place only plasma PK in cryovials with purple caps labeled "PLASMA PK."** Place residual plasma (<1.5 ml) in the blue-capped cryovial. **If a residual aliquot (<1.5 ml) is created, document the specimen number and volume on the Biological Sample and Shipment Notification Form.**

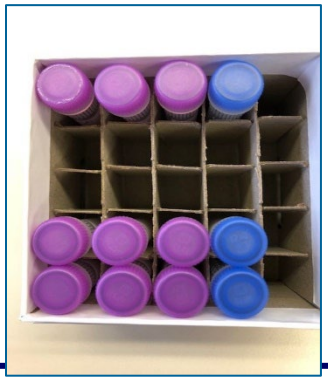


NOTE: When pipetting plasma from the plasma tube into the cryovials, be very careful to pipette the plasma top layer only, leaving the buffy coat and the red blood cell layers untouched.

12. Dispose of collection tube with red blood cell pellet according to your site's guidelines for disposing of biomedical waste.

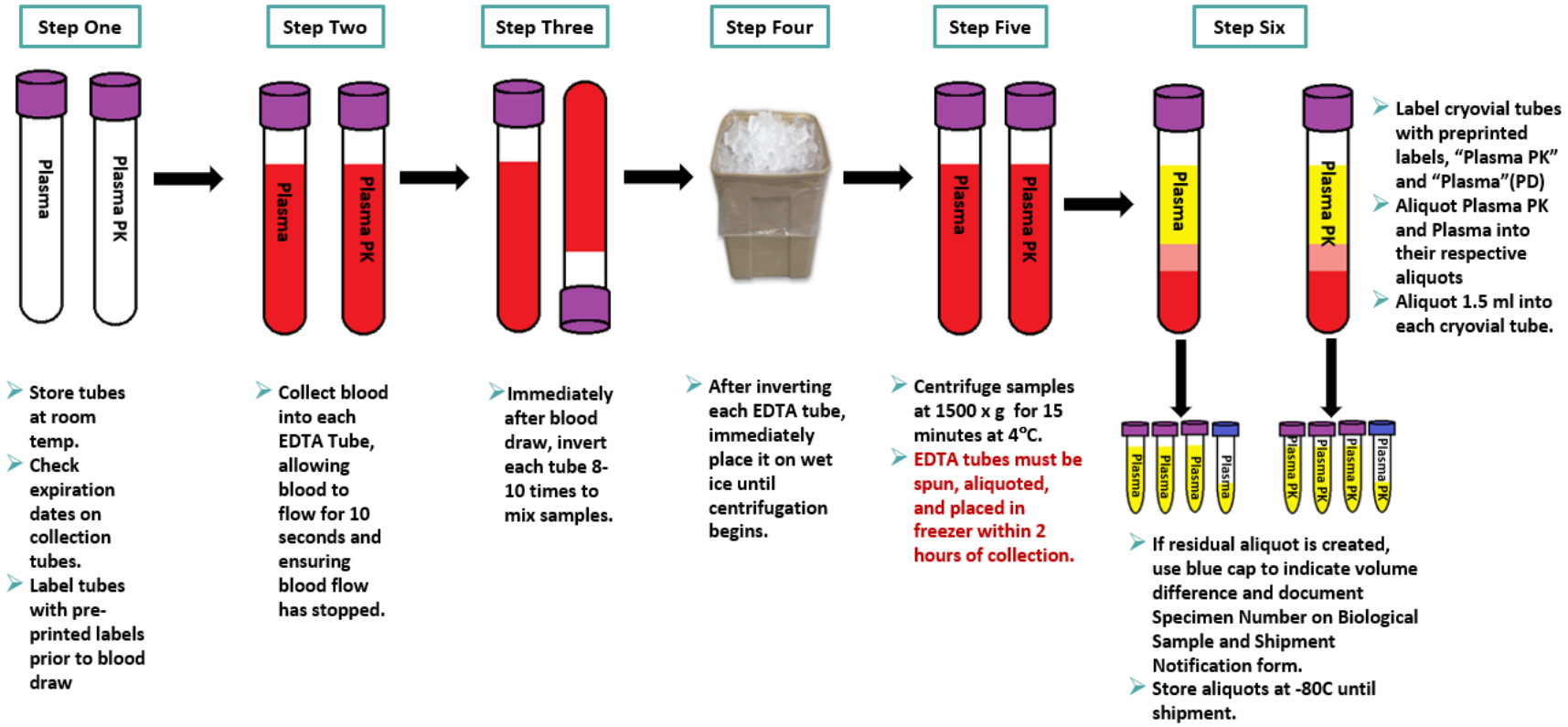
13. Record the specimen number and volumes of the EDTA tubes on the Biological Sample Shipment and Notification Form.

14. Place the labeled cryovials in the 25 cell cryobox and place on pelleted dry ice. **Transfer to -80°C Freezer when possible.** Store all samples at -80°C until shipped to NCRAD on pelleted dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample and Shipment Notification Form.



Plasma Aliquots from Pre-Dose and Post-Dose Visits

Plasma Preparation (10 ml EDTA Purple-Top Tube)



7.0 Cerebrospinal Fluid Collection

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

CSF should ideally be collected in the morning between 8am – 10am, preferably fasted. Record the time of last meal.

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. Supplies for collection and shipment of CSF are sent to sites in a separate kit from NCRAD.

7.1.1 Lumbar Puncture Tray Components

Quantity	Lumbar Puncture Tray Kit Components
1	Sprotte needle, 24G x 90mm
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
1	Povidone-Iodine Topical Solution, 0.75 oz

Sterile, individually packaged 50ml conical tubes are available to sites completing the Lumbar Puncture through the use of the gravitational method. Because not all sites are utilizing this method, the sterile conical tubes must be requested separately from the kit. They are located within the Individual Supply list of the kit request module (Please see Section 5.1).

Do not send blue cap conical tubes used to collect CSF to NCRAD. Discard according to local guidelines. Collection and processing site staff may write on this conical tube for their own reference.

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 participant.

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

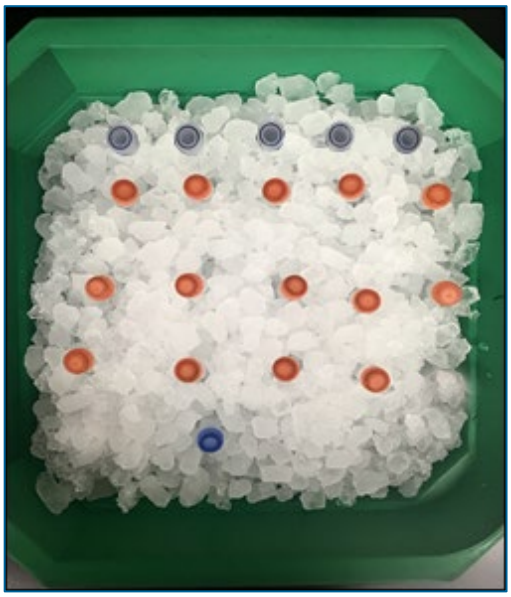
1. Keep in mind that there is usually a lot of staff in the room during an LP, and a big part of assisting with the LP is keeping the field sterile—keeping people away from it and reminding them to be careful around it. If anyone touches the inside of the paper wrapper or any part of the contents of the kit, throw away the kit away and start over. If there is any 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 Detailed Lumbar Puncture Procedure

* See training video for CSF Processing and Aliquoting: https://ncrad.org/resource_Veri-T.html

Place the "**CSF"** Collection and Aliquot Tube Labels on the aliquot tubes (per Section 6.1). Prepare the 19 aliquot tubes provided by NCRAD based on the collection of ≤25 mls of CSF. Additional tubes may be necessary; these tubes may be retrieved from the Veri-T Supplemental kit provided to each site.

1. Place aliquot tubes on wet ice prior to the procedure so they are pre-cooled (See below):



2. Perform lumbar puncture.

3. Collect CSF into syringes or sterile conical tube (if a noticeably bloody 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 (ORANGE 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.
        1. Cell count
        2. Total protein
        3. Glucose
    - *NOTE: Sample must be analyzed within 4 hours of collection.*

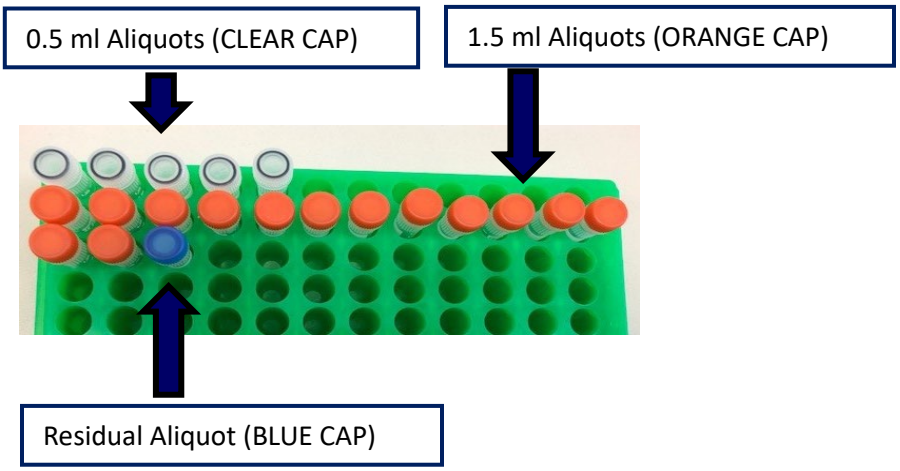
4. Collect an additional 23 mls of CSF and transfer to 50 ml conical polypropylene tubes at room temperature. Mix gently by inverting 3-4 times. Record the time of draw (once collection is complete) on the CSF Sample and Shipment Notification Form.

5. 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

6. Using a clean transfer pipette, transfer all CSF into a second 50ml conical tube leaving the pellet in the bottom. Mix the second tube gently by inverting 3-4 times. Pipette (micropipette preferred) 0.5ml of supernatant directly into 5 pre-cooled clear capped polypropylene CSF collection aliquot tubes. Next pipette, 1.5 ml of supernatant directly into pre-cooled orange capped polypropylene CSF collection aliquot tubes. This will yield, on average, 13 orange capped aliquot tubes per

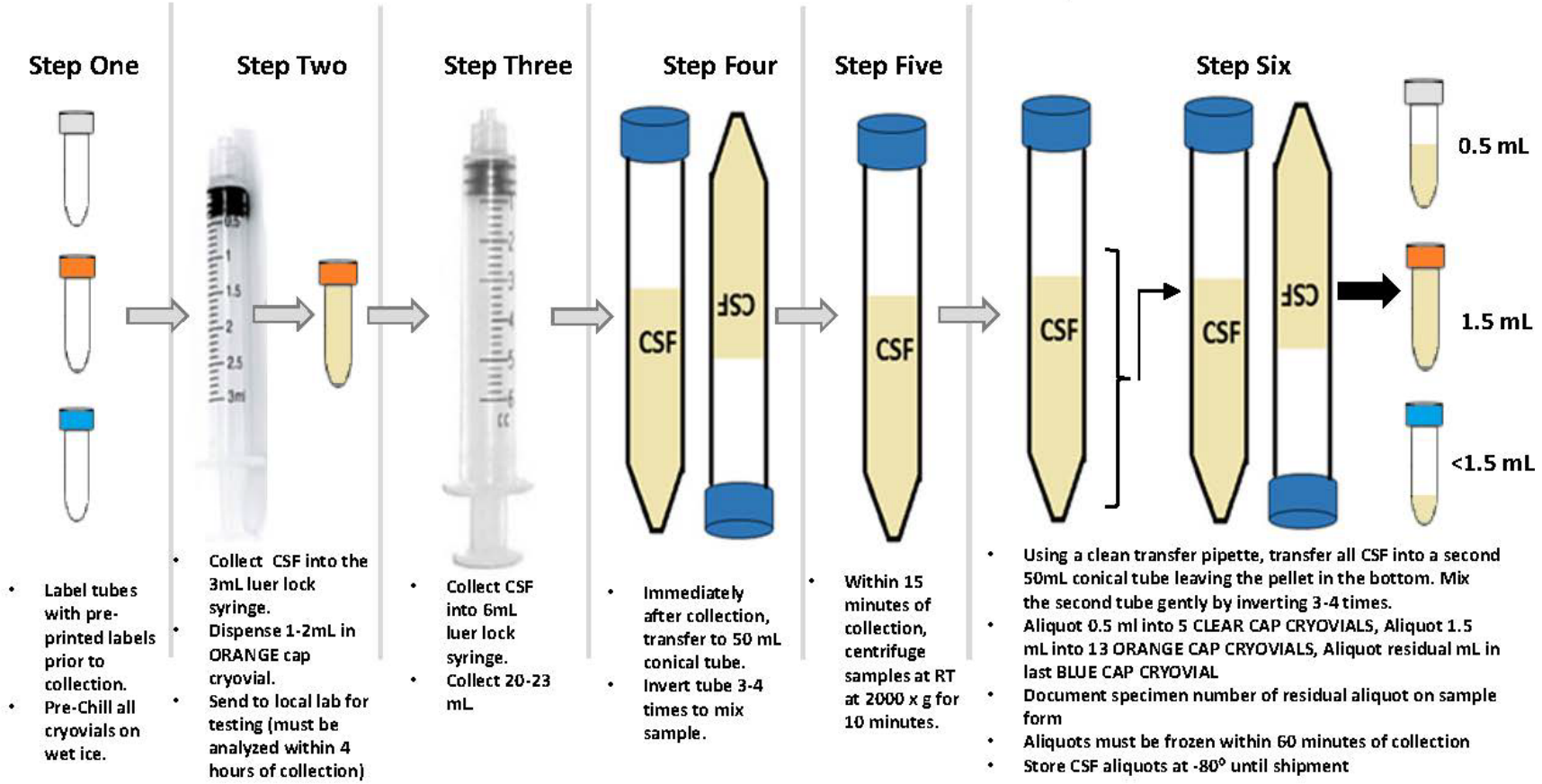
participant and 1 blue capped residual aliquot. (Use more aliquot tubes if needed do not discard any CSF). Seal each aliquot tube with correct cap color. If there is residual amount of CSF remaining (<1.5 ml), please utilize a BLUE cap cryovial to indicate that this aliquot has low volume.

- If at least 20 mls are able to be sent to NCRAD, the remaining 3 mls can be kept locally. If there is no local repository, NCRAD will accept all aliquots.



7. Within 60 minutes of CSF collection, freeze aliquots immediately on pelleted dry ice and then store at **-80°C** or ship on pelleted dry ice in a shipping container. Complete the remainder of the Laboratory Procedures data form and ensure timely entry of data into the UCSF database.

CSF Preparation (20-25 mL in Syringes)



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

- Collect CSF into the 3mL luer lock syringe.
- Dispense 1-2mL in ORANGE cap cryovial.
- Send to local lab for testing (must be analyzed within 4 hours of collection)

- Collect CSF into 6mL luer lock syringe.
- Collect 20-23 mL

- Immediately after collection, transfer to 50 mL conical tube.
- Invert tube 3-4 times to mix sample.

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

- Using a clean transfer pipette, transfer all CSF into a second 50mL conical tube leaving the pellet in the bottom. Mix the second tube gently by inverting 3-4 times.
- Aliquot 0.5 ml into 5 CLEAR CAP CRYOVIALS, Aliquot 1.5 mL into 13 ORANGE CAP CRYOVIALS, Aliquot residual mL in last BLUE CAP CRYOVIAL
- Document specimen number of residual aliquot on sample form
- Aliquots must be frozen within 60 minutes of collection
- Store CSF aliquots at -80° until shipment

8.0 Packaging & Shipping Instructions

ALL study personnel responsible for shipping should be certified in biospecimen shipping. If not available at your university, training and certification is available through the CITI training site (Course titled "Shipping and Transport of Regulated Biological Materials" at https://www.citiprogram.org/).

Guidelines for the processing, storage location, and timing of sample collection are listed in Section 4.4.

8.1 Frozen Packaging Instructions

IMPORTANT!
FROZEN SAMPLES MUST BE SHIPPED MONDAY-WEDNESDAY ONLY!

The most important issue for shipping is to maintain the temperature of the samples. The frozen samples must never thaw; not even the outside of the tubes should be allowed to defrost. This is best accomplished by making sure the Styrofoam container is filled completely with pelleted dry ice.

Large Frozen Shipper:

** 45 lbs of dry ice pellets

AND

- Fits up to 8 x 25-cell cryoboxes

Specimens being shipped to NCRAD should be considered as Category B UN3373 specimens and as such must be tripled packaged and compliant with IATA Packing Instructions 650. *See the Latest Edition of the IATA Regulations for complete documentation.*

Triple packaging consists of a primary receptacle(s), a secondary packaging, and a rigid outer packaging. The primary receptacles must be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured, or leak their contents into the secondary packaging. Secondary packaging must be secured in outer packaging with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging.

***** Packing and Labeling Guidelines *****

- The primary receptacle (frozen cryovials) must be leak-proof and must not contain more than 1L total.
- The secondary packaging (biohazard bag) must be leak-proof, and if multiple blood tubes are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent direct contact with adjacent blood tubes.
- Absorbent material must be placed between the primary receptacle (cryovial box containing the frozen cryovials) and the secondary packaging (biohazard bag). The absorbent material should be of sufficient quantity in order to absorb the entire contents of the specimens being shipped. Examples of absorbent material are paper towels, absorbent pads, cotton balls, or cellulose wadding.
- A shipping manifest of specimens being shipped must be included between the secondary and outer packaging.
- The outer shipping container must display the following labels:
  - ✓ Sender's name and address
  - ✓ Recipient's name and address
  - ✓ Responsible Person
  - ✓ The words "Biological Substance, Category B"
  - ✓ UN3373
  - ✓ Class 9 label including UN 1845, and net weight of pelleted dry ice contained

1. If possible, hold packaged samples in -80°C freezer until time of UPS pick-up/drop-off. If storage in a -80°C freezer until UPS pick-up is not possible, package samples no more than 4 hours before the expected pick-up time.
2. Notify NCRAD of shipment by emailing NCRAD coordinators at: alzstudy@iu.edu. Attach the following to the email:
   a. Completed Biological Sample and Shipment Notification Form to the email notification. (See Appendix B and/or Appendix C for the NCRAD sample forms)
   b. If email is unavailable please call NCRAD and do not ship until you've contacted and notified NCRAD coordinators about the shipment in advance.

3. Place all frozen labeled 1.5 ml aliquots of pre-dose and post-dose plasma PK as well as the plasma PD from one day in the one 25-slot cryobox. Please leave two empty rows between the pre-dose and post-dose samples.

    a. Each cryobox holds up to 25 cryovials and there will be a maximum of 12 cryovials (9 lavender-capped and 3 blue-capped residual) per blood draw (see next page).

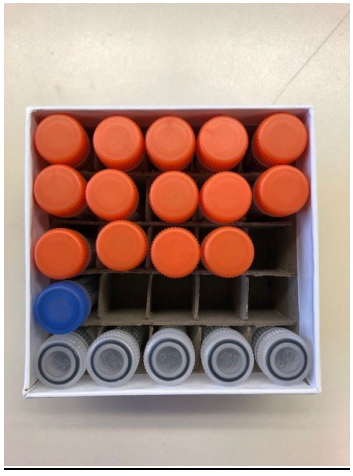
4. If CSF is drawn at that time point, include the CSF aliquots in a separate 25-count cryobox in the same batch shipment (see next page).

5. A batch shipment should contain all of the specimens from the same patient, per time point. For example, if blood and CSF were collected at the same time point for a participant, two 25-count cryoboxes should be included in the same batch shipment. They will be placed in separate small biohazard bags.

- **Batch shipping should be performed quarterly or when 8 cryoboxes of samples have been obtained.**

    - Example #1: 4 participants with blood AND CSF collected (8 total cryoboxes for 4 participants)
    - Example #2: 8 participants with ONLY blood collected (8 total cryoboxes for 8 participants)
    - Example #3: 2 participants with blood AND CSF collected (4 total cryoboxes for 2 participants) and 4 participants with ONLY blood collected (4 total cryoboxes for 4 participants)

25-Slot Cryoboxes Containing Biospecimens from One Blood kit (left) and One CSF kit (right)



Blood kit (left) and CSF kit (right) Prepped for Batch Shipping

***Note: Blood and CSF kits will have different kit numbers, but the same Participant IDs. Also, the pre-dose and post-dose samples will have different kit numbers.**

5. Label the outside of each cryobox with the kit number label (shown above). Please place the cryoboxes containing blood derivatives in one small biohazard bag. Label the outside of the small biohazard bag with a kit number label as well (shown above).

6. **If collected, place the cryoboxes containing CSF derivatives for the patient in a separate small biohazard bag.** Label the outside of this second small biohazard bag with the CSF kit label.

7. As the cryoboxes are placed in the small clear plastic biohazard bag, do NOT remove the absorbent material found in the bag. Seal according to the instructions on the bag. The kit number label should have been placed on each cardboard cryobox prior to inserting into the biohazard bag. A kit number label should also have been placed on the outside of the biohazard bag. Please note that if you are shipping blood and CSF samples for 1 participant, the kit numbers will be different on each small biohazard bag. The pre and post dose kit numbers will also be different from each other.

Cryoboxes placed in clear biohazard bags

Packaged blood kit:
One 25-count cryobox with plasma and plasma PK aliquots

2 kit numbers, 1 for pre-dose kit and 1 for post-dose

Packaged CSF kit:

One 25-count cryobox with CSF and residual aliquots

8. Place approximately 2-3 inches of pelleted dry ice in the bottom of the Styrofoam shipping container.

9. Place the biohazard bags into the provided Styrofoam-lined shipping container on top of the pelleted dry ice. Please ensure that cryoboxes are placed so the cryovials are upright in the shipping container.

10. Fully cover the biohazard bags containing the cryoboxes with approximately 2 inches of pelleted dry ice.

11. The inner Styrofoam shipping container must contain approximately 45 lbs (or 20kg) of pelleted dry ice. The pelleted dry ice should entirely fill the inner box and be placed on top of the biohazard bags to ensure the frozen state of the specimens.

12. Replace the lid on the Styrofoam carton. Place the completed Biological Sample and Shipment Notification Form in the package on top of the Styrofoam lid for each patient specimen, and close and seal the outer cardboard shipping carton with packing tape.
    a. Attach provided UPS label for packages.

13. Complete the UPS Dry Ice Label
    a. Net weight of pelleted dry ice in kg (must match amount on the airbill)
    b. Do not cover any part of this label with other stickers, including pre-printed address labels.

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

IMPORTANT!
Ensure UPS address label is attached and UPS Dry Ice label is filled out, or UPS may reject or return your package.

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

16. Specimens should be sent to the below address via **UPS Next Day Air**. Frozen shipments should be sent Monday through Wednesday to avoid shipping delays on Thursday or Friday. UPS does not replenish dry ice if shipments are delayed or held over during the weekend.

Veri-T at NCRAD
Indiana University School of Medicine
351 W. 10th St TK-217
Indianapolis, IN 46202
Phone: 1-800-526-2839

17. Use UPS tracking to ensure the delivery occurs as scheduled and is received by NCRAD. Please notify NCRAD by email (alzstudy@iu.edu) that a shipment has been sent and include the UPS tracking number in your email.

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

For frozen shipments, include no more than eight cryovial boxes (separated by patient within biohazard bags) per shipping container in order to have room for a sufficient amount of pelleted dry ice to keep samples frozen up to 24 hours.

The labeled, processed, aliquoted, and frozen cryovials of plasma and CSF must be shipped to NCRAD as outlined above.

SHIP ALL FROZEN SAMPLES MONDAY - WEDNESDAY ONLY!
BE AWARE OF HOLIDAYS!!
BE AWARE OF INCIPIENT INCLEMENT WEATHER THAT MAY DELAY SHIPMENT/DELIVERY OF SAMPLES

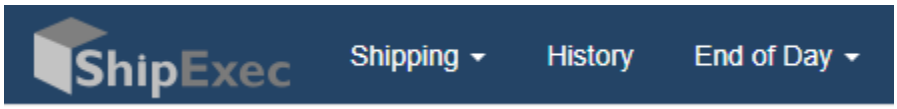
Remember to complete the Biological Sample and Shipment Notification (Appendix B and C (if applicable)), include a copy in your shipment AND notify the NCRAD Study Coordinator by email at alzstudy@iu.edu (include UPS tracking number in email) IN ADVANCE to confirm the shipment.

In addition to tracking and reconciliation of samples, the condition and amount of samples received are tracked by NCRAD for each sample type. Investigators and clinical coordinators for each project are responsible to ensure the requested amounts of each fluid are collected to the best of their ability and that samples are packed with sufficient amounts of pelleted dry ice to avoid thawing in the shipment process.

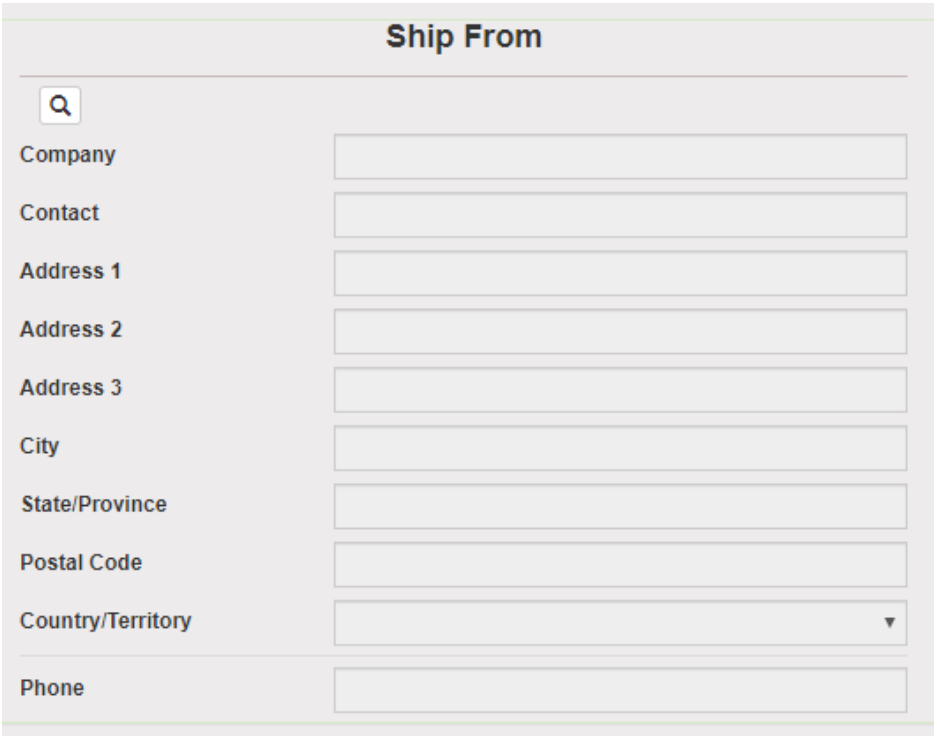
8.2 Frozen Shipping Instructions

1. Log into the ShipExec Thin Client at kits.iu.edu/UPS.
    a. If a new user or contact needs access, please reach out to your study contact for access.

2. Click “Shipping” at the top of the page and click on “Shipping and Rating”



3. Click on the magnifying glass icon in the “Ship From” section to search for your shipping address.



   a. Search by Company (site), Contact (name), or Address 1 (first line of your site’s street address). Click Search.
   b. Click Select to the left of the correct contact information.

4. Verify that both the shipping information AND study reference are correct for this shipment.
   a. If wrong study contact or study reference, click Reset in the bottom right of the screen to research for the correct information.

5. Enter Package Information
   a. Frozen shipments
      i. Enter the total weight of your package in the “Weight” field.
      ii. Enter the dry ice weight in the “Dry Ice Weight” field.
      iii. If the “Dry Ice Weight” field is higher than the “Weight” field, you will receive an error message and need to reenter these values.
   b. Click Ship in the bottom right of the page when complete.

6. Print the airbill that is automatically downloaded.
    a. To reprint airbill, click History at the top left of the page.
    b. Click Detailed Report from the dropdown menu on the right side of the page.
    c. Enter tracking number if known. Otherwise, search by ship date. Click Search.
    d. Click print icon on right side of the tracking number line.

7. Fold airbill, and place inside plastic UPS sleeve.

8. Peel the back off of the UPS sleeve, and stick the sleeve to the package.

9. A UPS Pickup is automatically scheduled at the address you are shipping from, and the pickup is charged to NCRAD.
    a. If shipment occurs too late in the day for an automatic UPS pickup, you will receive an email stating that the pickup could not be scheduled, and you will need to make other arrangements.

9.0 Appendices

Appendix A: Rate of Centrifuge Worksheet

Please complete and return this form by email to NCRAD if you have any questions regarding sample processing. The correct RPM will be sent back to you. Make note of this in your Veri-T Biologics Manual.

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 at
alzstudy@iu.edu

Appendix B: Blood Sample and Shipment Notification

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

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

From:_____ **UPS tracking #:**_____

Phone:_____ **Email:**_____

Study: Veri-T **Site #:**_____ **Participant ID:**_____ **Sex:** ☐ M ☐ F **Year of Birth:** _____

Visit:

Dose	Timepoint			
☐ Pre-Dose	☐ Day 1	☐ Week 12	☐ Week 24	☐ ET
☐ Post-Dose				

KIT BARCODE

Blood Collection:

Date of Draw: _____ [MMDDYY]	Time of Draw: _____ [HHMM]
Date participant last ate: _____ [MMDDYY]	Time participant last ate: _____ [HHMM]

Blood Processing:

Plasma PK (Lavender-Top) Tube (10 mL)	
Original Volume EDTA Drawn (could be up to 2 * 10mL):	_____ mL
Time spin started:	_____ [HHMM]
Duration of centrifugation:	_____ Minutes
Temp of centrifuge: ____ °C Rate of centrifuge: _____ x g	
Time aliquoted:	_____ [HHMM]
Number of 1.5 mL plasma aliquots created (lavender cap, up to 9):	_____
If applicable, volume of residual plasma aliquot (less than 1.5 mL in blue cap):	_____ mL
If applicable, last four digits of residual plasma aliquot barcode:	_____
Time plasma aliquots frozen:	_____ [HHMM]
Plasma PD (Lavender-Top) Tube (10 mL)	
Original Volume EDTA Drawn (could be up to 2 * 10mL):	_____ mL
Time spin started:	_____ [HHMM]
Duration of centrifugation:	_____ Minutes
Temp of centrifuge: ____ °C Rate of centrifuge: _____ x g	
Time aliquoted:	_____ [HHMM]
Number of 1.5 mL plasma aliquots created (lavender cap, up to 9):	_____
If applicable, volume of residual plasma aliquot (less than 1.5 mL in blue cap):	_____ mL
If applicable, last four digits of residual plasma aliquot barcode:	_____
Time plasma aliquots frozen:	_____ [HHMM]
NOTES: _____	

Appendix C: CSF Sample and Shipment Notification Form

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

To: Kelley Faber Email: alzstudy@iu.edu FAX: 317-278-1100 Phone: 1-800-526-2839

From:_____ **UPS tracking #:**_____

Phone:_____ **Email:**_____

Site #:_____

Study: Veri-T

Visit: ☐ Screening ☐ Week 24 ☐ Early Termination

Patient ID: _____

Kit #: KIT BARCODE

Sex: ☐ M ☐ F **Year of Birth:** _____

CSF Collection:

1. Date of Draw: _____ [MMDDYY]	2. Time of Draw: _____ [HHMM]
3. Date subject last ate: _____ [MMDDYY]	4. Time subject last ate: _____ [HHMM]

Collection Process: ☐ Gravitational **OR** ☐ Pull

CSF Processing:

Time spin started:	_____ [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 CSF aliquots created (up to 15 total): **(Orange cap cryovials):**	_____
If applicable, volume of residual CSF aliquot (less than 1.5 mL): **(Blue cap cryovials):**	_____ mL
If applicable, specimen number of residual aliquot tube: **(Last four digits)**	_____
Time frozen:	_____ [HHMM]
Storage temperature of freezer:	_____°C
NOTES: _____	