VCAD Manual of Procedures Update: Version 5.2025

Section	Change
Document Footer	Updated to “Version 5.2025”
6.2	Added Step 7 for storage of collection tubes if samples cannot be transferred same day.
Appendix A	Updated to include sections for additional documentation if samples cannot be transferred same day.
Document Footer	Updated to “Version 4.2025”
Throughout	Minor changes made to phrasing and wording. No alterations to procedures were made.
Section 3.2, Table 4.2.2 & Section 7.1	Riley Lab hours updated
Section 3.3	Winter Break holiday added
6.1.1	New Label examples added
Document Footer	The version date was updated for this amendment.
Throughout	Applied new template including change in colors, formats, and logo throughout.
Throughout	Updated NCRAD website links
Section 1.0	Added NCRAD acronym.
Section 2.0	Language on purpose.
Section 3.1, Section 3.2, Section 4.2 & Section 7.1.1	Update Riley Stat Lab room number to #2641
Section 3.3	Language on holiday observations.
Section 4.2	Renamed “Biospecimens sent to NCRAD”.
Section 5.2	Language on kit ordering and kit URL.
Section 6.2	Updated Step C to clarify “Release tourniquet as soon as blood starts to flow into last collection tube”.
Section 7.1	Renamed “Ambient Packaging Instructions”.
Section 7.1.1	Updated stat lab direct delivery instructions to accommodate for lab change.
Section 9.0	Added new Appendices section.

Virtual Reality and Computerized Cognitive Intervention for Mild Cognitive Impairment in Heart Failure (VCAD)

in collaboration with the

National Centralized Repository for Alzheimer's Disease and Related Dementias

Biospecimen Collection, Processing, and Shipment Manual of Procedures

Version 05.2025

Table of Contents

1.0 Abbreviations

AD	Alzheimer's Disease
EDTA	Ethylene Diamine Tetra-acetic Acid
IATA	International Air Transport Association
NCRAD	National Centralized Repository for Alzheimer's Disease and Related Dementias
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute
VCAD	Vita+Com Alzheimer's Disease

2.0 Purpose

The collection of biofluids is an important part of VCAD. The purpose of this manual is to provide study staff (PIs, study coordinators, phlebotomists) at the various study sites with instructions for collection and submission of biological samples for study visits. It includes instructions for biofluid submission to NCRAD located in Indianapolis at Indiana University.

Sites will collect and send the following samples to NCRAD:

- Whole Blood for Plasma and Buffy Coat

This manual includes instructions for the collection of blood, labeling and shipping to the Riley Stat Lab. These procedures are relevant to all study personnel responsible for processing specimens provided to NCRAD for the VCAD protocol.

3.0 NCRAD Information

3.1 NCRAD Contacts

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

Kristen Russ, PhD, IUBAL Director
Phone: 317-278-4707
Email: karuss@iu.edu

Clairisa Stayton, BS, IUBAL Study Coordinator
Phone: 317-278-1672
Email: cbstayto@iu.edu

General IUBAL Contact Information
Email: iubal@iu.edu
Website: www.ncrad.org

Riley Stat Lab
Tube Station #835 **or**
705 Riley Hospital Dr., Room RI 2641
Indianapolis, IN 46202
Phone: 317-278-3050

3.2 NCRAD Hours of Operation

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

Ambient samples are delivered to the Riley Stat Lab or tubed (tube station #835) **Monday – Friday between 8 AM – 4:30 PM.**

Riley Stat Lab Address:	705 Riley Hospital Drive Room #2641 Indianapolis, IN 46202 317-278-3050

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 possible, biological specimens for submission to Indiana University should **NOT** be collected and shipped to Indiana University after the second week in December. **Please see: https://ncrad.org/contact/holiday-closures for additional information.**

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

4.0 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
- Sharps bin and lid
- Wet Ice/Cold Packs

4.2 Biospecimens Sent to NCRAD

Samples are to be submitted according to the shipping methods outlined in Section 7.0. Guidelines for the processing, storage location, and timing of sample collection are listed in the following tables.

4.2.1 Biofluid Collection Schedule

Biospecimen	Baseline Visit
Plasma	X
Buffy Coat	X

Whole blood will be collected into (3) 10mL collection tubes (purple-top EDTA tubes). The purple top EDTA tubes will be sent to NCRAD's rapid processing lab 'Riley Stat Lab', located within Riley Hospital. Samples can be sent through the tubing station (#835) or hand delivered at 705 Riley Hospital Drive, Room #2641, Indianapolis, IN 46202. The Stat Lab staff associated with NCRAD will process into plasma fractions, aliquot, freeze, and retain them for long-term storage in the NCRAD Biobank.

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

Consent forms must specify that any biological samples and de-identified clinical data may be shared with academic and/or industry collaborators through NCRAD. Recommended consent language can be found on the NCRAD website at: https://ncrad.org/bank-samples/sample-management/recommended-consent-language. A copy of the consent form for each participant should be kept on file by the site investigator.

4.2.2 Biofluid Collection Charts

Collection Tube	Time	Specimen Type	Aliquot Volume	Shipping Destination	Shipping Temperature
3 EDTA (Purple-Top) Blood Collection Tubes (10mL)	8AM – 4:30PM	Whole Blood	3 x 10mL EDTA	705 Riley Hospital Drive Room #2641 Indianapolis, IN 46202	Wet Ice/Cold Packs

5.0 Specimen Collection Kits, Shipping Kits, and Supplies

NCRAD will provide blood sample collection kits for research specimens to be stored at NCRAD. The provided materials include blood tubes and biohazard bags for the transfer of samples to Riley. Kit number labels, PTID labels, and collection tube labels will all be provided by NCRAD. Details regarding the blood kits are found in this Manual of Procedures. Collection tube labels will be preprinted 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 6.0.

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. Please keep kit contents in original bag provided by NCRAD so supplies are not mixed together.*

Blood Kit

Quantity	VCAD Blood Kit Components
3	EDTA (purple-top) blood collection tube (10 ml)
2	Preprinted Kit Number Label
3	Preprinted Collection Tube Labels
4	Label for handwritten PID
1	Absorbent Sleeve
1	Ziploc bag – to place stat lab form and resealable biohazard bag with collected samples in prior to sending via tube station
1	Resealable biohazard bag – to place collected 10mL EDTA tubes in

5.2 Kit Supply to Study Sites

Each 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 or supplies expire so you are prepared for study visits. Please go to: kits.iu.edu/VCAD to request additional kits and follow the prompts to request the desired supplies.

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

6.0 Blood Collection and Processing Procedures

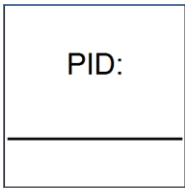
6.1 Labeling Samples

In order to ensure the highest quality samples are collected, it is essential to follow the specific collection 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.

6.1.1 Label Type Summary

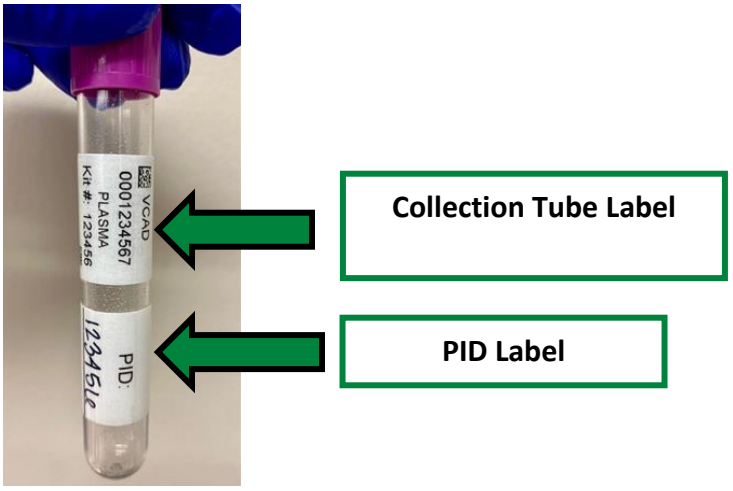
1. Kit Number Label
2. PID Label
3. Collection Tube and Aliquot Label

Kit Number Labels tie together all specimens collected from one participant at one visit. They should be place in designated location on the Blood Sample and Shipment Notification Forms.



PID Labels are use d to document the individual's unique PID. Place one label on each blood collection tube.

Place one **Collection Tube Label** on each blood collection tube.



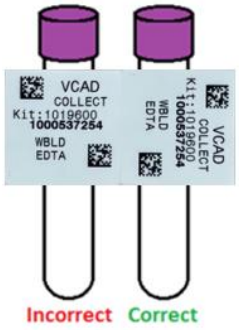
Labeled EDTA (Purple-Top) Blood Collection Tube

Each collection tube will contain two labels: the collection tube label and the PID Label. Be sure to place labels in the same configuration consistently among tubes, with the barcoded label near the top of the tube and the handwritten PID label near the bottom of the tube.

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

- Place Collection Tube Labels on **ALL** collection tubes **BEFORE** sample collection. This should help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.
- Using a fine point permanent marker, fill-in and place the PID Labels on the EDTA (purple-top) tubes **BEFORE** sample collection. These labels are placed on collection tubes in addition to the Collection Tube Label.
- The Collection Tube Labels contain a 2D barcode on the left-hand and bottom right-hand side of the label.
- Place label **horizontally** on the tube (wrapped around sideways if the tube is upright) with barcode toward the tube cap.

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. The following pictures show the correct direction of the labels on the collection tubes and cryovials.



Collection Tube Label Diagram

6.2 Whole Blood Collection with 10 ml EDTA (Purple-Top) Tube for Plasma and Buffy Coat – No Site Processing

Whole Blood Collection for Isolation of Plasma and Buffy Coat: three EDTA (Lavender-Top) Blood Collection Tubes (10 ml) (for processing of plasma aliquots and buffy coat aliquots).

1. Store empty EDTA tubes at room temperature, 64°F - 77°F (18 °C – 25 °C) before use. Check expiration dates on all collection tubes before visit.

2. Place completed PID Label and preprinted **PLASMA** Collection Tube Label on the purple-top EDTA tubes.

3. Using a blood collection set and a holder, collect blood into the **EDTA (Purple-Top) Blood Collection Tube (10 ml)** using your organization's recommended procedure for standard venipuncture technique. **The following techniques shall be used to prevent possible backflow:**
    a. Place participant's arm in a downward position.
    b. Hold tube in a vertical position, below the participant'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.

4. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.
    a. If complications arise during the blood draw, please note the difficulties on the 'Biological Sample and Shipment Notification Form'. Do not

attempt to draw an additional EDTA tube at this time. Process blood obtained in existing EDTA tube.

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

6. Immediately after inverting the EDTA tube, wrap the tubes in absorbent sheets and place them between cold packs in the lunch box until centrifugation begins.

7. If samples cannot be dropped off at Riley for processing on the same day, please store unprocessed collection tubes at 4°C for no more than 24 hours. When transporting these refrigerated tubes to Riley, ensure they are kept on wet ice to maintain a consistent temperature.

Plasma & Buffy Coat from EDTA Purple-Top Tube (3x10ml)

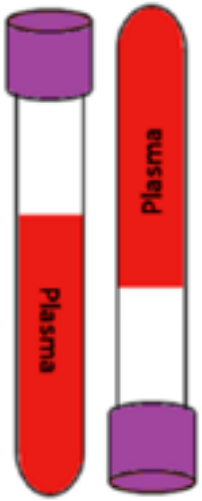
Step One

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

Step Two

- Collect blood in EDTA 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

- Place EDTA tubes in absorbent sheets between cold packs.
- EDTA tubes need to be placed on cold packs and delivered to the Stat Lab within 4 hours of collection.

7.0 Packaging & Shipping Instructions

ALL study personnel responsible for shipping should be certified in biospecimen shipping. If you have difficulty finding biospecimen shipping training, please notify a NCRAD coordinator.

In addition to tracking and reconciliation of samples, the condition and number 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 frozen samples are packed with sufficient amounts of pelleted dry ice to avoid thawing in the shipment process.

7.1 Ambient Packaging Instructions

AMBIENT SAMPLES MUST BE DELIVERED to RILEY STAT LAB MONDAY-FRIDAY BETWEEN 8AM – 4:30PM ONLY!

1. Place the filled and labeled ambient EDTA tubes into the resealable plastic biohazard bag with the absorbent sheet.

2. Place the resealable plastic biohazard bag inside of the Ziploc bag.

3. Place the stat lab form (Appendix A) inside of the Ziploc bag along with resealable biohazard bag. Send the entire kit to the Riley Stat Lab using tube station #835 or walk the samples directly to the stat lab

7.1.1 Direct Delivery to Stat Lab Instructions

1. Enter Riley Hospital through the front entrance.

2. Take the main green elevators to the 2nd floor.

3. Exit the elevator and turn left.

4. Walk through the doors towards the front desk, turn left, and then right.

5. Walk all the way down the hall, passing through a set of doors.

6. The stat lab room is Room 2641.

7. Ring the doorbell and the staff member will let you in the lab.

8. Please let them know that you are dropping off for the VCAD study.

8.0 Data Queries and Reconciliation

Sample and Shipment Notification forms must be completed on the day that samples are collected because they include information that will be used to reconcile sample collection and receipt, as well as information essential to future analyses.

Data queries or discrepancies with samples shipped and received at NCRAD may result from:

- Missing samples
- Incorrect samples collected and shipped
- Damaged or incorrectly prepared samples
- Unlabeled samples, samples labeled with incomplete information, or mislabeled samples
- Discrepant information documented on the Blood Sample and Shipment Notification Form compared to the information entered in the OnCore database
- Samples processed outside of the two-hour processing window
- Use of an incorrect Processing Form

9.0 Appendices

Appendix A: Blood Sample and Shipment Notification Form

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:_____

Email: _____

Phone:_____

KIT BARCODE

Study: VCAD ☐ Baseline Visit

Participant ID: _____

Sex: ☐M ☐F **Year of Birth:** _____

Blood Collection:

Date of Draw: _____ [MMDDYY]	Time of Draw: _____ [HHMM]
Date Participant Last Ate: _____ [MMDDYY]	Time Participant Last Ate: _____ [HHMM]

Time EDTA Tubes Placed on Cold Pack: _____ [HHMM]

Expected # of Collection Containers	# of Tubes Collected	Notes/Deviations
(3) 10mL EDTA Tubes		

The section is only filled out if samples are not transferred to Riley on the same day as collection

Date Samples Placed in Fridge: _____[MMDDYY]	Time Samples Placed in Fridge: _____ [HHMM]
Temperature Reading of Fridge: _____	
Date Samples Transferred to Riley:_____ [MMDDYY	Time Samples Transferred to Riley:_____[HHMM]

Appendix B: Stat Lab Processing Sheet

VCAD	IUGB STAT LAB PROCESSING SHEET			COLLECTION DATE:
Protocol: MMGE-NIA-NCRAD-VCAD ARM: CASE: FORMAT: KIT NUMBER	VERSION:2 LAST UPDATED: 4/12/2023 EFFECTIVE DATE: 4/6/2023	VISIT:	KIT#:	COLLECTION TIME: TIME REC'D:

FINISH ALL PROCESSING WITHIN TWO (2) HOURS OF DRAW TIME UNLESS OTHERWISE NOTED.

TUBE TYPE/ QUANTITY	BARCODES	CENTRIFUGE	HEMOLOGY /TURBIDITY SCALE 0-3	INSTRUCTIONS/ # SAMPLES	ACTUAL # ALIQUOTS (µL)	TIME FROZEN
1 x 10 mL EDTA (Purple)	_____ BARCODE _____-_____ CHILD ALIQUOTS _____ Buffy Coat	TEMP: 4°C SPEED:2000 x G TIME: 10 MINUTES START TIME:		1) POOL ALL PLASMA REGARDLESS OF HEMOLYSIS 2) VOLUME: 3 x 500uL 3) VOLUME: 7 x 200uL 4) REMAINING ALIQUOTS x 1500uL 5) KEEP RESIDUAL 6) TUBES: SARSTEDT TUBES 7) CAPS: PURPLE 8) STORE: 1 FULL ALIQUOT IN BU, REST IN MAIN	_____ x 500 uL ALIQUOTS _____ x 200 uL ALIQUOTS _____ x 1500 uL ALIQUOTS 1 x _____ uL RESIDUAL	DRY ICE/-80°C
				1) KEEP ALL BUFFY COATS 2) CAPS: CLEAR 3) Store: 1 into BU, rest in Main	____ x 750uL (BC)	DRY ICE/-80°C
1 x 10 mL EDTA (Purple)	_____ Barcode _____ Buffy Coat	TEMP: 4°C SPEED:2000 x G TIME: 10 MINUTES START TIME:		SEE ABOVE INSTRUCTIONS**	_____ x 750 uL Buffy Coat	DRY ICE/-80°C
1 x 10 mL EDTA (Purple)	_____ Barcode _____ Buffy Coat	TEMP: 4°C SPEED:2000 x G TIME: 10 MINUTES START TIME:		SEE ABOVE INSTRUCTIONS**	_____ x 750 uL Buffy Coat	DRY ICE/-80°C

NOTES: **In Oncore, add the time documented under "Time EDTA Tubes Placed on Cold Packs" on paperwork into the "Preparation Date/Time" slot for each parent tube.**

If samples arrive frozen or have a hemolysis above a 3, please contact Clairisa Stayton and Kelley Faber ASAP.

PROCESSED BY / DATE	ENTERED DATA / DATE:	LABELED BY / DATE:	STORED / DATE:	QC'D BY / DATE: