The 90+ Study

in collaboration with

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

Biospecimens

Manual of Procedures

Version 1.0

September, 2020

TABLE OF CONTENTS

1.0 ABBREVIATIONS

AD	Alzheimer's Disease
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra-acetic Acid
GUID	Globally Unique Identifier
IATA	International Air Transport Association
IUGB	Indiana University Genetics Biobank
NCRAD	National Centralized Repository for Alzheimer's Disease and Related Dementias
PHI	Protected Health Information
UPS	United Parcel Service

2.0 PURPOSE

The collection of blood-based biofluids is an important part of the 90+ Study. The purpose of this manual is to provide study staff (PIs, study coordinators, phlebotomists) at the study site with instructions for collection and submission of blood-based biological samples for 90+ study visits. It includes instructions for blood-based biospecimen submission to NCRAD located in Indianapolis at Indiana University.

The following samples will be sent to NCRAD:

- 10ml Whole Blood EDTA tube (2)

OR

- Saliva (for DNA extraction), if not able to collect blood samples (**see Section 10.0**)

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

This manual also includes instructions for collection of saliva, labeling, storage prior to shipping, and shipping to NCRAD.

These procedures are relevant to all study personnel responsible for processing blood specimens being provided to NCRAD for the 90+ protocol.

3.0 NCRAD INFORMATION

3.1 NCRAD Contacts

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

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

Colleen Mitchell, Laboratory Manager
Phone: 317-278-9016
Email: mitchecm@iu.edu

General NCRAD Contact Information
Phone: 1-800-526-2839
Fax: 317-321-2003
Email: alzstudy@iu.edu
Website: www.ncrad.org
90+ Study Specific Webpage:
https://ncrad.org/resource_90plus.html

Kristi Wilmes, MS, CCRP Study Coordinator
Phone: 317-274-7546
Email: wilmesk@iu.edu

Sample Shipment Mailing address
NCRAD
Indiana University School of Medicine
351 West 10th Street
TK-217
Indianapolis, IN 46202

3.2 Hours of Operation

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

Ambient samples must be shipped **Monday-Thursday only**. For packaging and shipment details of ambient samples, please refer to Section 10.5 of this protocol.

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

3.3 Holiday Schedules

- Please note that courier services may observe a different set of holidays. Please be sure to verify shipping dates with your courier prior to any holiday.

3.4 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
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 Day

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. Should it be necessary to ship blood samples for DNA extraction to Indiana University during this period, please contact the Indiana University staff before December 20th by e-mailing alzstudy@iu.edu, so that they can arrange to have staff available to process incoming samples.

Please see: https://ncrad.org/holiday_closures.html for additional information.

4.0 GLOBALLY UNIQUE IDENTIFIER (GUID)

The GUID is a subject ID that allows researchers to share data specific to a study participant, without exposing personally identifiable information. A GUID is made up of random alpha-numeric characters and does not include any PHI in the identifier. By using GUIDs in your research data, the system can associate a single research participant's genetic, imaging, and clinical assessment data even if the data was collected at different locations or throughout different studies. No PHI will be sent to NCRAD, only the GUID.

To create a GUID follow these steps:

1. Create an account: https://bricsguid.nia.nih.gov/portal/jsp/login.jsp
2. Once you have an account, go to the GUID Tool – Create GUID
3. To open the 'Launch GUID Tool' you will need to have Java installed on your device
4. In order to generate a GUID, the following PHI is required (Appendix A):
   - Complete legal given (first) name of subject at birth
   - If the subject has a middle name
   - Complete legal family (last) name of subject at birth
   - Day of birth
   - Month of birth
   - Year of birth
   - Name of city/municipality in which subject was born
   - Country of birth

5.0 NCRAD LABORATORY COLLECTION

5.1 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 (21 gauge) and hub
- Microcentrifuge tube rack
- Sharps bin and lid

5.2 BIOSPECIMEN COLLECTION SCHEDULE

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

90+ Blood-Based Biomarker Collection Schedule:

	Baseline
10ml Whole Blood EDTA tube	X

Whole blood is collected in one type of tube (purple top 10 ml EDTA tube) for shipment to NCRAD. This tube is shipped ambient to NCRAD.

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/recommended_consent_language.html. A copy of the consent form for each subject should be kept on file by the site investigator.

5.3 BIOSPECIMEN COLLECTION CHART

5.3.1 Blood Collection

Sample Type	Tube Type	Number of Tubes Supplied in Kit	Aliquot Volume	Tubes to NCRAD	Ship
Whole blood for isolation of plasma & buffy coat	**EDTA (Purple-Top) Blood Collection Tube (10 ml)**	**2**	**N/A**	**2**	**Ambient**

If a sample is not obtained at a particular visit, this should be recorded in the notes section of the **Biological Sample and Shipment Notification Form (see** Appendix B**).** Submit a copy to NCRAD with a reason provided for the omission.

6.0 SPECIMEN COLLECTION KITS, SHIPPING KITS, AND SUPPLIES

NCRAD will provide: 1) Blood sample collection kits for research specimens to be stored at NCRAD and the Ambient Blood Shipment Supply Kit and 2) clinical lab supplies (with the exception of equipment supplies listed in Section 5.1). The provided materials include blood tubes, partially completed shipping labels to send materials to NCRAD and ambient shipping kits. Kit Number Labels, PTID Labels, and Collection Tube Labels will all be provided by NCRAD. Collection Tube 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 7.1.

6.1 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.*

90+ Blood Kit

Quantity	90+ Blood-Based Kit Components
2	EDTA (Purple-Top) Blood Collection Tube (10 ml)
2	Pre-printed Collection Tube Label
3	Pre-printed Kit Number Label
3	Label for handwritten PTID
1	Small IATA shipping box with insulated cooler

1	Small refrigerant pack
1	Aqui-Pak 6 tube absorbent pouch
1	UN3373 Biological Substance Category B label
1	List of contents card
1	UPS Clinic Pak

Individual Supplies

Quantities	Items Available upon request within the NCRAD kit module.
By Request	EDTA (Purple-Top) Blood Collection Tube (10 ml)
By Request	Label for handwritten PTID
By Request	Warning label packet
By Request	UN3373 label
By Request	Biohazard label

6.2 Kit Supply to 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 or supplies expire so you are prepared for study visits. Please go to http://kits.iu.edu/90+ to request additional kits and follow the prompts to request the desired supplies. Options include ordering a specific number of kits; we are also including the option of simply ordering the desired amount of extra supplies.

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

7.0 BLOOD COLLECTION PROCEDURES

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

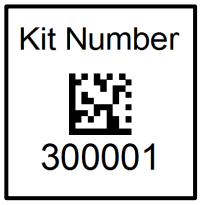
In order to ensure the highest quality samples are collected and stored, it is essential to follow the specific collection and shipment procedures detailed in the following pages. Please read the following instructions first before collecting any specimens. Have all your supplies and equipment out and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

SPECIFIC INSTRUCTIONS FOR COLLECTION OF EACH SAMPLE ARE DETAILED ON THE FOLLOWING PAGES.

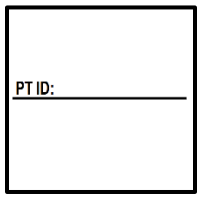
7.1 Labeling Samples

Label Type Summary

1. Kit Number Label
2. PTID Label
3. Collection Tube Label
4. Cryovial Label



The **Kit Number Labels** do not indicate a specimen type, but are affixed on the Biological Sample and Shipment Notification Forms and on each cryovial box.

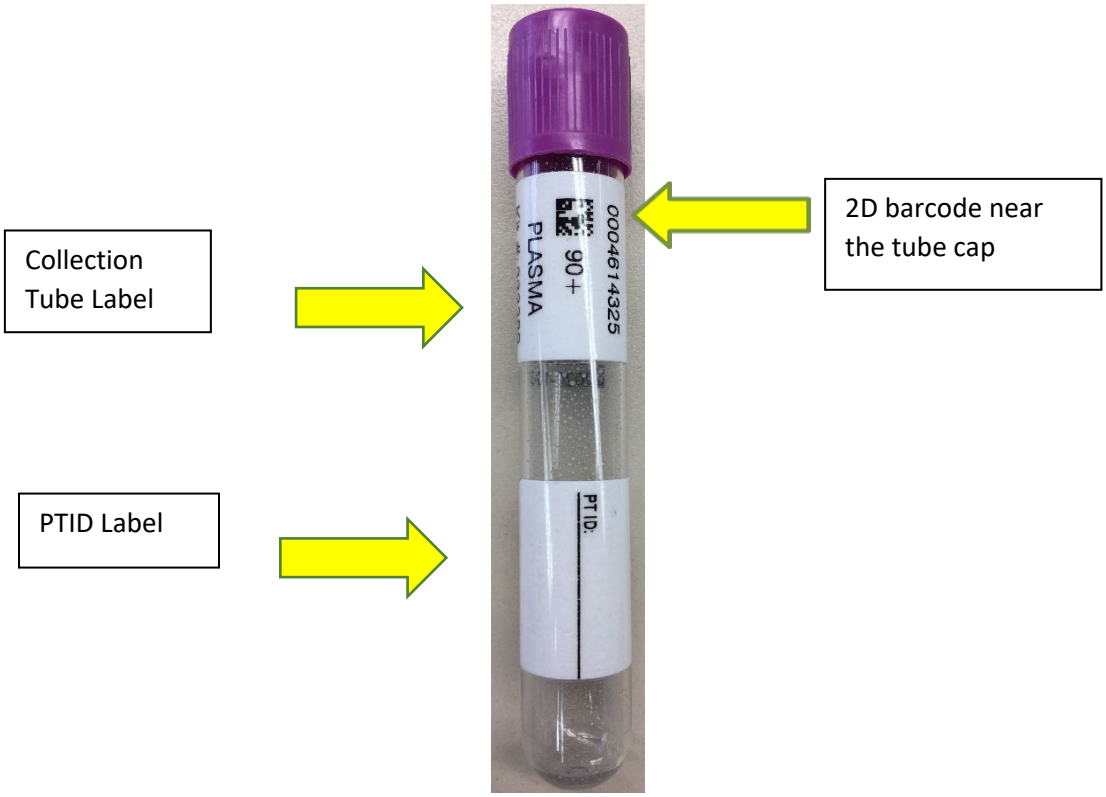


The **PTID Labels** are used to document the individual's unique PTID. Place one label on each blood collection tube.

The **Collection Tube Labels** for blood derivatives are placed on all collection tubes.

Important Note

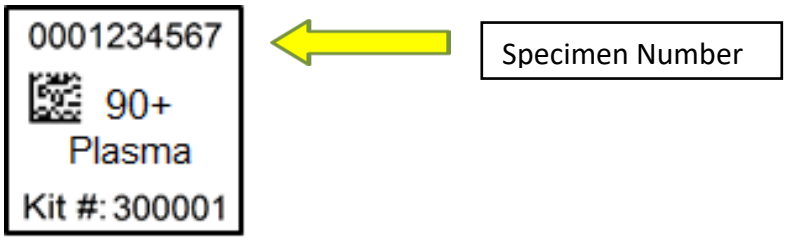
Each collection tube will contain two labels: the Collection Tube Label and the PTID Label. Be sure to place labels in the same configuration consistently among tubes, with the barcoded label near the top of the tube and the handwritten PTID label.



**EDTA (Purple-Top) Blood
Collection Tube (10 ml)**

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

- Place Collection Tube Labels on **ALL** 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.



- Using a fine point permanent marker, fill-in and place the PTID Labels on the collection tubes only (EDTA) **BEFORE** sample collection, processing, or freezing. 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 side of the label. Place this barcode toward the tube cap.

7.2 EDTA (Purple-Top) Blood Collection Tube (10 ml) for Plasma and Buffy Coat

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

1. Place completed PTID Label and pre-printed "**PLASMA**" Collection Tube Label on the purple-top EDTA tube.

2. Using a blood collection set and a holder, collect blood into the **EDTA (Purple-Top) Blood Collection Tube (10 ml)** using your institution's recommended procedure for standard venipuncture technique.

   The following techniques shall be used to prevent possible backflow:

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

3. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** 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.

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

DNA Preparation (10ml Purple Top Tube)

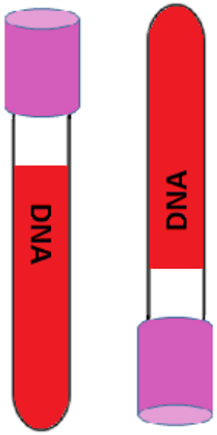
Step One

- Store tubes at room temperature.
- Label tubes 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

- Store tubes ambient until shipment
- Send within four days of extraction

8.0 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.

Situations may arise that prevent study coordinators from obtaining the total amount scheduled for biospecimens. In these situations, please follow the below steps:

1. *If the biospecimens at a scheduled visit **are partially** collected:*
   - 1.1. Document difficulties on the 'Biological Sample and Shipment Notification Form' prior to submission to NCRAD
     - 1.1.1. Indicate blood draw difficulties at the bottom of the 'Biological Sample and Shipment Notification Form' within the "Notes" section.
     - 1.1.2. Complete the 'Biological Sample and Shipment Notification Form' with tube volume approximations.
   - 1.2. Contact a NCRAD coordinator and alert them of the challenging blood draw.

2. *If the blood biospecimens at a scheduled visit **are not** collected:*
   - 2.1. See Section 10.0 Saliva Collection for instructions on how to collect saliva samples.

9.0 PACKAGING AND SHIPPING INSTRUCTIONS

ALL study personnel responsible for shipping should be certified in biospecimen shipping. If not available at your University, please contact NCRAD with questions and information regarding resources.

9.1 Ambient Packaging Instructions

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

For ambient EDTA (Purple-Top) Blood Collection Tube (2x10 ml) shipments, include no more than two tubes per shipping container and include only tubes from one participant. The ambient EDTA samples must be shipped the day of blood draw.

The labeled, unprocessed, EDTA tubes will be shipped to NCRAD as outlined below.

IMPORTANT!
AMBIENT SAMPLES MUST BE SHIPPED MONDAY-THURSDAY ONLY! **Do NOT draw blood for ambient shipments on Fridays!**

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

- The primary receptacle (EDTA tubes) must be leak proof and must not contain more than 10 ml total.
- The secondary packaging (foam box) must be leak proof.
- Absorbent material must be placed between the primary receptacle (EDTA tubes) and the secondary packaging (foam box). 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

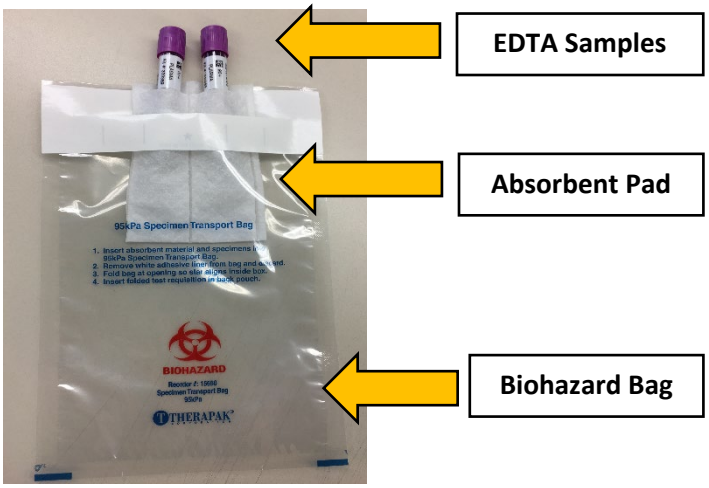
Ambient EDTA (Purple-Top) Blood Collection Tube (10 ml) shipments should be considered as Category B UN3373 and as such must be tripled packaged and compliant with the IATA Packing Instructions 650. *See the Latest Edition of the IATA Regulations for complete documentation.*

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.

1. Place refrigerant pack in the freezer 24 hours prior to shipment.
2. Contact UPS to confirm service is available and schedule package to be picked up.
3. Notify NCRAD of shipment by emailing NCRAD coordinators at: alzstudy@iu.edu

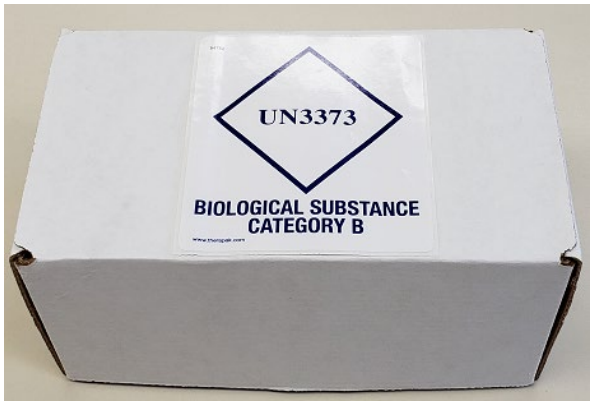
a. Complete and attach the Biological Sample and Shipment Notification Form to the email. (See Appendix B for an example of the form)

4. Place filled and labeled EDTA (purple-top) tubes within the slots in the absorbent pad provided, and place into the plastic biohazard bag with absorbent sheet.



5. Remove as much air as possible from the plastic biohazard bag and seal the bag according to the directions printed on the bag.
6. Place Kit Number Label on biohazard bag.
7. Place the refrigerant pack into the cooler on top of the filled biohazard bag.

8. Place the lid onto the cooler.
9. Place the cooler in the provided small IATA Shipping Box.
10. Place an extra copy of the emailed "Biological Sample and Shipment Notification Form" on top of the cooler lid along with a completed list of contents card.
11. Close shipping box. Label the outside of the cardboard box with the enclosed UN3373 (Biological Substance Category B) label.



12. Place the closed, labeled shipping box within a UPS Clinical Pak. **Seal the UPS Clinical Pak.**

13. Place prefilled UPS return airbill on the sealed UPS Clinical Pak.
14. Specimens should be sent to the below address via **UPS Next Day Air**. Ambient UPS shipments should be sent Monday through Thursday.

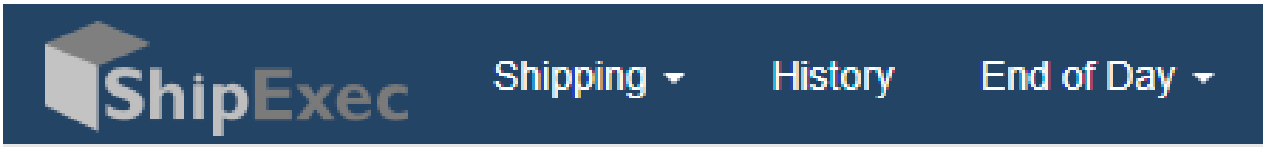
90+ at NCRAD
Indiana University School of Medicine
351 West 10th Street
TK-217
Indianapolis, IN 46202

15. Use UPS tracking to ensure the delivery occurs as scheduled and is received by NCRAD.

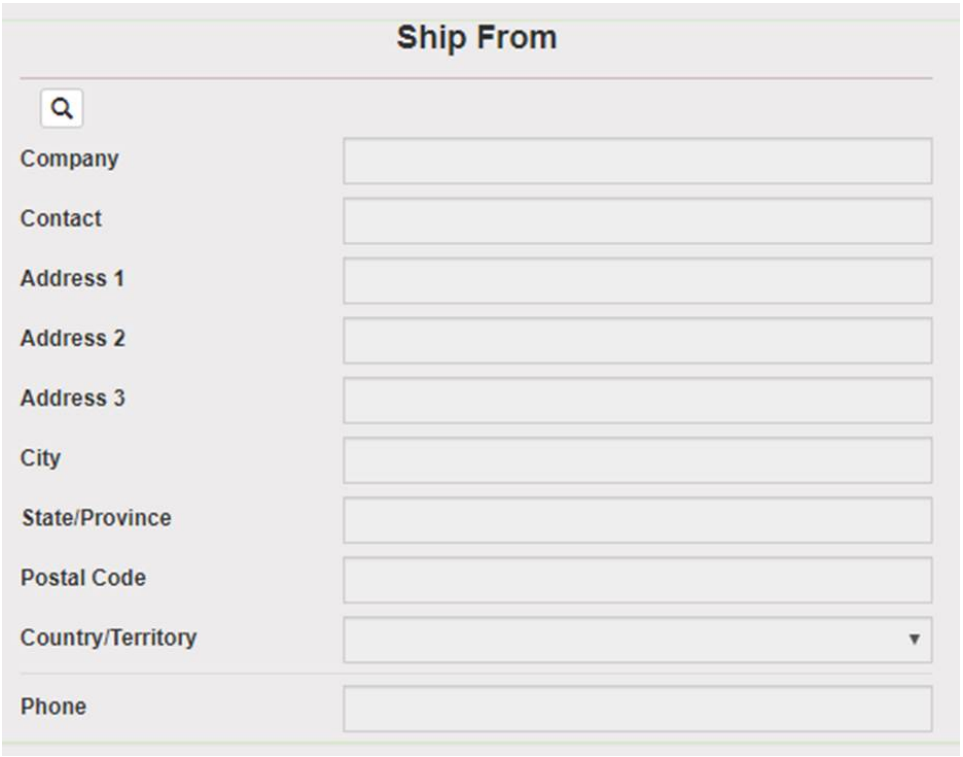
In addition to tracking and reconciliation of samples, the condition and amount of samples received are tracked by NCRAD for each sample type. Investigators and clinical coordinators for each project are responsible to ensure the requested amounts of each fluid are collected to the best of their ability.

9.2. Ambient 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



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.

1. 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.
2. Enter Package Information

a. Ambient shipments
    i. Enter the total weight of your package in the “Weight” field and leave the “Dry Ice Weight” field empty.
b. 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.
c. Click Ship in the bottom right of the page when complete.

3. 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.
4. Fold airbill, and place inside plastic UPS sleeve.
5. Peel the back off of the UPS sleeve, and stick the sleeve to the package.
6. 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.

10.0 SALIVA COLLECTION

10.1 Saliva Specimens sent to NCRAD

Saliva will be collected in an Oragene Saliva Collection Tube. After collection, these samples are then shipped to NCRAD.

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

Ambient samples are to be submitted according to the shipping methods outlined in Section 10.5. Guidelines for the timing of sample collection and storage of samples are detailed in the tables below.

10.2 Biospecimen Collection Chart

Sample Type	Tube Type	Number of Tubes Supplied in Kit	Tubes to NCRAD	Volume	Ship
Saliva for isolation of buffy coat (for DNA extraction)	**Saliva Kit (DNA Genotek) OGR-500**	**1**	**1**	**2.0 ml of Saliva collected in each 4.0 ml tube**	**Ambient**

If a sample is not obtained at a particular visit, this should be recorded in the notes section of the **Biological Sample and Shipment Notification Form (see** Appendix B**).** Submit a copy to NCRAD with a reason provided for the omission.

Saliva collection should occur **only** after waiting **30 minutes after the subject has last ingested any substance**.

If the subject did not wait 30 minutes before providing a sample, this should be noted in the field provided on the **Biological Sample and Shipment Notification Form (see Appendix B)**.

10.3 Specimen Collection Kits, Shipping Kits, and Supplies

Saliva collection kits as well as shipment supplies will be provided by the NCRAD Biorepository. These materials include items listed below. Kit Number Labels, PTID Labels, and Collection Tube Labels will all be provided by NCRAD. Collection 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 10.5

10.3.1 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.*

90+ Saliva Kit

Quantity	90+ Saliva Collection Kit Components
1	Oragene Saliva Collection Kit
2	Collection Tube Labels
1	PTID Label
1	Small Saliva Biohazard Bag
1	Large Resealable Bag

90+ Saliva Shipping Kit

Quantity	90+ Saliva Collection Kit Components
1	Saliva Shipping Box (holds 36 saliva specimens)
1	Large Biohazard Bag
1	250ml Absorbent Sheet
1	Large Resealable Bag
1	Human Exempt Specimen Label
1	UPS ClinPak

Individual Supplies

Quantity	90+ Saliva Collection Kit Components
By Request	Oragene Saliva Collection Kit
By Request	PTID Label
By Request	Small Saliva Biohazard Bag
By Request	Large Resealable Bag
By Request	250ml Absorbent Sheet
By Request	Human Exempt Specimen Label
By Request	UPS ClinPak

10.3.2 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: http://kits.iu.edu/90+ to request additional kits and follow the prompts to request the desired supplies. Options include ordering a specific number of kits; we are also including the option of simply ordering the desired amount of extra supplies.

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

10.4 Saliva Collection and Processing Procedures

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

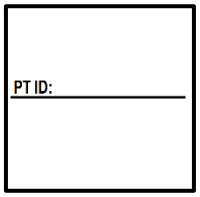
In order to ensure the highest quality samples are collected, processed, and stored, it is essential to follow the specific collection, processing, and shipment procedures detailed in the following pages. Please read the following instructions before collecting any specimens. A minimum of 30 minutes must elapse after ingesting any substance, chewing gum, or smoking prior to drawing saliva. Have all supplies and equipment out and prepared prior to saliva collection.

10.4.1 Labeling Samples

Label Type Summary

1. PTID Label
2. Collection Tube Label

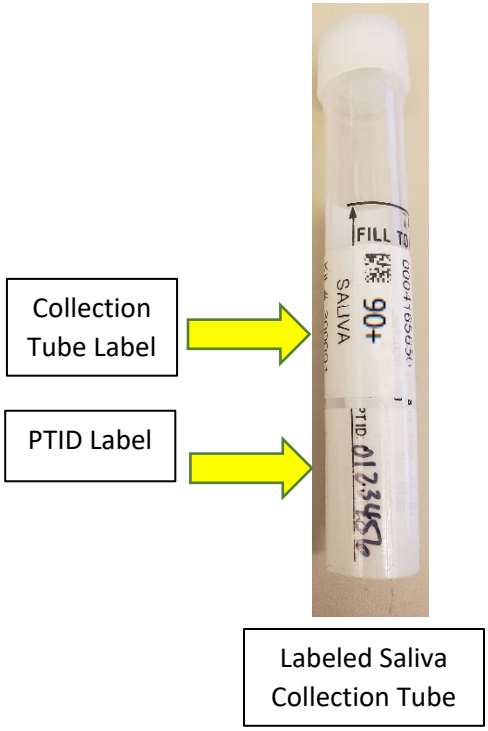
Each kit is supplied with labels for specimens destined for the NCRAD Biorepository.



The **PTID Labels** are used to document the individual's unique PTID. Place one label on the saliva collection tube.

Place one **Collection Tube Label** on the saliva collection tube. The second, matching **Collection Tube Label** should be affixed to the subject's corresponding **Biological Sample and Shipment Notification Form (see Appendix B**).

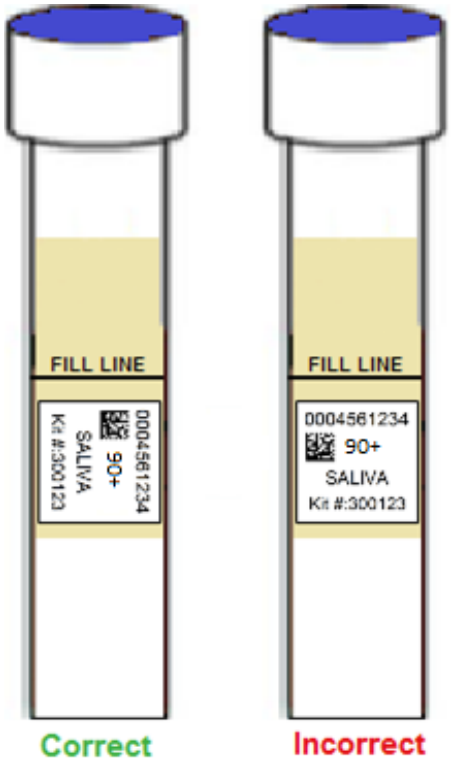
Important Note
Each collection tube will contain two labels: the Collection Tube Label and the PTID Label. Be sure to place labels in the same configuration, with the Collection Tube Label near the top of the tube and the PTID label at the bottom of the tube.



To ensure the label adheres properly and remains on the tube, please follow these instructions:

- Using a fine point permanent marker, fill in and place the PTID Label on the Oragene collection tube BEFORE sample collection.
- Collection Tube Labels contain a 2D barcode on the left hand side of the label. Affix the label so this barcode is oriented toward the tube cap.
- Place label **horizontally** on the tube (wrapped around sideways if the tube is upright).
- Be sure not to cover the "Fill To" line.
- Take a moment to ensure the label is **completely adhered** to the tube. It may be helpful to roll the tube between your fingers after applying the label.

Saliva Tube Labeling



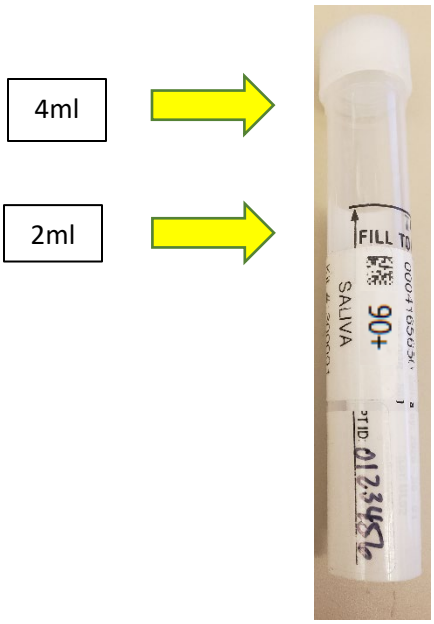
10.4.2 Saliva Collection Video

The following training video is available to assist you with the saliva collection: http://www.dnagenotek.com/ROW/support/ciOG500.html

10.4.3 Saliva Collection Tube Maximum Volume

In order to ensure that the NCRAD Biorepository receives a sufficient amount of sample for processing and storage, the saliva collection tube should be filled to the assigned volume. Over-filled tubes may leak during shipment, resulting in a loss of sample. Volume should be recorded by the site on the Saliva Sample Form.

Saliva Tube Volume



10.4.4 Saliva Collection

1. Do NOT remove the plastic film from the lid of the container. Spit directly into the funnel at the top of the tube until the amount of liquid saliva (not including bubbles) reaches the fill line shown. The saliva tube has a false bottom, so you will only need to provide 2 ml of saliva to reach the fill line. Do NOT fill above the line.

Please Note: Most people take between 2 and 5 minutes to deliver a saliva sample. If the subject finds it difficult to produce a sample, instruct them to relax and rub their cheeks gently for 30 seconds to generate saliva.

2. After collection, hold the tube upright. Unscrew the funnel from the tube. Pick up the small cap for the tube. Use the small cap to close the tube tightly. Discard the funnel.

3. Shake the capped tube for 5 seconds. Complete the **Biological Sample and Shipment Notification Form (see Appendix B).** Place sample into the

provided biohazard bag. Peel the protective wrapping off to seal the bag. Roll packaging around the tube.

4. Place sample into the provided Bulk Saliva Shipping Box.

Oragene Saliva Collection Kit Contents and Warnings

Intended Use: This product is designed for the safe collection of human saliva samples.
Contents: The funnel lid contains 2 mL of Oragene • DNA liquid. The solution should be clear and colorless.
Warnings: Do not ingest the Oragene • DNA liquid. Wash with water if the Oragene • DNA liquid comes in contact with eyes or skin. Small cap, choking hazard.
Storage: Store at room temperature 15-30˚C (59-86˚F).

10.5 Packaging and Shipping Instructions

Saliva specimens being shipped to the NCRAD Biorepository should be considered as Exempt Human Specimens and as such must be packaged and compliant with IATA Packing Instructions. *See the Latest Edition of the IATA Regulations for complete documentation.*

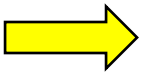
***** Packing and Labeling Guidelines *****

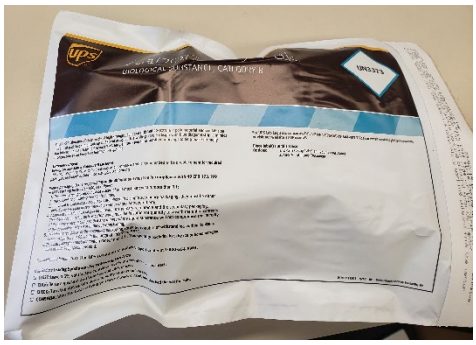
- The primary receptacle (saliva tube) must be leak proof and in total must not contain more than 1 liter of fluid.
- The secondary packaging (bulk shipping box and biohazard bag) must be leak proof and, if multiple saliva tubes are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent direct contact with adjacent saliva tubes.
- Absorbent material must be placed between the primary receptacle (within the biohazard bag) and the secondary packaging. 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 included in shipment 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 "Exempt Human Specimen"

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 to the email notification.
  (See Appendix B for an example of the NCRAD sample form)
- If email is unavailable please call NCRAD and do not ship until you've contacted and notified NCRAD coordinators about the shipment in advance.

2. Ensure all ambient, labeled saliva samples, are appropriately packaged (see below).

UPS ClinPak 

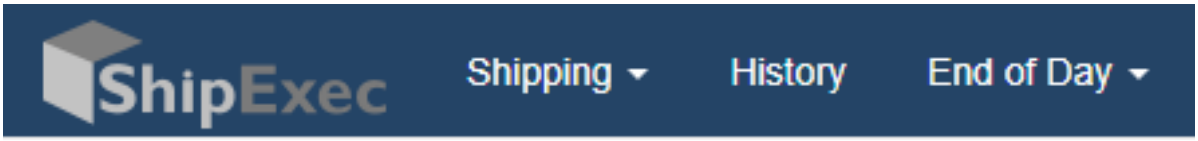


3. Place the completed Biological Sample and Shipment Notification Forms in the ClinPak.
4. Apply all provided warning labels and the pre-printed UPS return label to the outside of package, taking care not to overlap labels.
5. Use UPS tracking to ensure the delivery occurs as scheduled and is received 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.

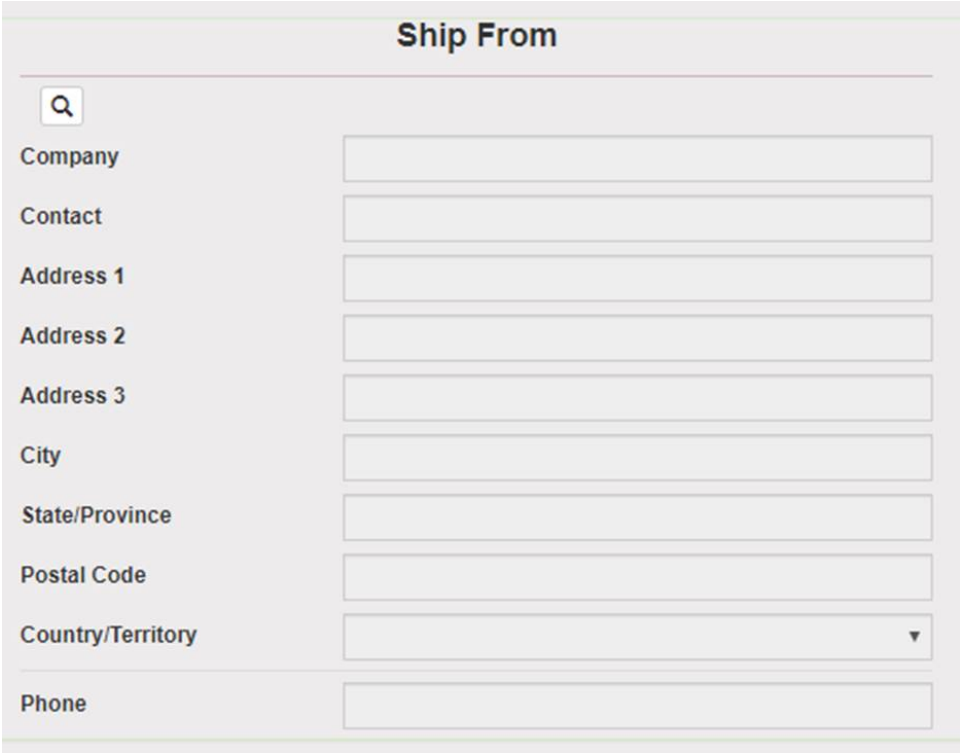
In addition to the tracking and reconciliation of samples, the condition and amount of samples received are recorded for each sample type. Investigators and clinical coordinators at each site are responsible for ensuring the requested amounts of each fluid are collected to the best of their ability and that samples are packed correctly.

10.5.1. Saliva 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



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



   c. Search by Company (site), Contact (name), or Address 1 (first line of your site’s street address). Click Search.
   d. 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. Ambient shipments
      i. Enter the total weight of your package in the “Weight” field and leave the “Dry Ice Weight” field empty.
   b. 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.
   c. 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.

11.0 DATA QUERIES AND SAMPLE RECONCILIATION

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

NCRAD will collaborate with the data team to reconcile information captured in the study database compared to samples received and logged at NCRAD. Additional discrepancies may be sent directly to the site staff to reconcile.

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

- 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 and logged at NCRAD compared to information entered into the study database.

12.0 APPENDICES LIST

Appendix A: GUID Demographics Form

Please be certain to collect the following demographic information to generate a Global Unique Identifier. **Do NOT** return this information to NCRAD. Only send the GUID to NCRAD.

1. Compete legal given (first) name of subject at birth: _____
2. Complete additional (middle) name or names at birth: _____
3. Complete legal family (last) name of subject at birth: _____
4. Suffix: _____
5. Date of Birth: _____
6. Name of city/municipality in which subject was born: _____
7. Country of birth: _____

Appendix B: Sample and Shipment Notification Form

NCRAD Biological Sample Form

90+ Study

Please complete this form when sending blood to NCRAD. The form can be completed on your computer and submitted electronically by an email attachment or can be completed by hand and faxed. Use the Tab key to move to the next field. The contact information for emailing or faxing the form is in the box below.

Please email or fax the form as soon as possible after the blood is drawn. NCRAD would like to receive this form before the blood arrives.

To:	Kelley Faber	FAX: 1-317-321-2003	Email: alzstudy@iu.edu
Phone:	1-800-526-2839	Phone: 1-317-274-7546	

From:	_____	Site:	_____
Phone:	_____	Fax:	_____
Email:	_____	Date:	_____

Subject #: _____

Gender: _____

Sample Type Included in Shipment:

☐ **Blood in Purple top EDTA Tube** ☐ **Saliva in Oragene Collection Kit**

Date sample collected: _____

UPS tracking #: _____

For internal NCRAD use only, do not complete.

Specimen Barcodes:

Volume(ml): Purple _____

Volume(ml): Purple _____

Volume(ml): Saliva _____