# The Precision Aging Network: Healthy Minds for Life

**in collaboration with the**

# National Centralized Repository for Alzheimer's Disease and Related Dementias

National Centralized Repository for Alzheimer's Disease and Related Dementias

## Biospecimen Collection, Processing, and Shipment Manual of Procedures

**Version 09.2025**

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## 1.0 Abbreviations

| | |
|---|---|
| CSF | Cerebrospinal Fluid |
| DNA | Deoxyribonucleic Acid |
| EDTA | Ethylene Diamine Tetra-acetic Acid |
| IATA | International Air Transport Association |
| NCRAD | National Centralized Repository for Alzheimer's Disease and Related Dementias |
| PAN_HML | The Precision Aging Network: Healthy Minds for Life |
| RNA | Ribonucleic Acid |

## 2.0 Purpose

The collection of biofluids is an important part of The Precision Aging Network: Healthy Minds for Life (PAN_HML). 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 PAN_HML study visits. It includes instructions for biofluid submission to NCRAD located in Indianapolis at Indiana University.

*The following samples will be sent to NCRAD:*

- Plasma
- DNA
- RNA
- CSF

This manual includes instructions for sample aliquoting, labeling, storage prior to shipping, and shipping to NCRAD.

These procedures are relevant to all study personnel responsible for processing specimens being provided to NCRAD for the PAN_HML 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

**Diont'e Keys, BS, CCRP, Study Coordinator**
Phone: 317-278-1133
Email: dlkeys@iu.edu

**General NCRAD Contact Information**
Phone: 1-800-526-2839 or 317-278-8413
Fax: 317-321-2003
Email: alzstudy@iu.edu
Website: www.ncrad.org
PAN_HML Study Specific Webpage: kits.iu.edu/pan

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

## 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**.

Check weather report to make sure impending weather events (blizzards, hurricanes, etc.) will not affect 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 (observed) |
| July 4 | Independence Day (observed) |
| 1st Monday in September | Labor Day |
| 4th Thursday in November | Thanksgiving |
| 4th Friday in November | Friday after Thanksgiving |
| December 25 – January 1 | Winter Break |

Please note that between December 24th and January 2nd, NCRAD will be closed, and will re-open for normal operations on January 2nd. Biological specimens for submission to Indiana University should **NOT** be shipped to Indiana University after the second week of 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 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.

**Please see: https://ncrad.org/contact/holiday-closures for additional information.**

## 4.0 Laboratory Collection

### 4.1 Site Required Equipment

The following materials and equipment are necessary for the storing and shipping of specimens and are to be **supplied by the local site**:

- Dry ice pellets
- -80°C Freezer

# 5.0 Biofluid Collection

## 5.1 PAN_HML Collection Schedule:

| Specimen Type | Baseline | 2 Year Follow Up |
|---|---|---|
| Plasma | X | X |
| DNA | X | X |
| RNA | X | X |
| CSF* | X | X |

*CSF collection is optional.

Whole blood is collected in up to two different types of tubes (EDTA and PAXgene™). Collection tubes are provided by the site, not NCRAD. The whole blood is processed locally into plasma, DNA, and RNA; they are then aliquoted and frozen at the study site. Samples should be put into NCRAD-provided tubes prior to shipment.

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.

Frozen samples are to be submitted according to the shipping methods outlined in Section 9.0.

## 5.2 Biofluid Collection Charts

### *5.2.1 Biofluid Collection*

| Sample | Site Aliquot Amount | Site Tube | Site Processing | Amount Sent to NCRAD | Tube Sent to NCRAD | Shipment Temp | NCRAD Processing |
|---|---|---|---|---|---|---|---|
| DNA | 90uL - 200uL | LoBind 1.5mL Tube | Re-aliquot into NCRAD provided tubes. | 1 x 5ug aliquots | 0.75mL Micronic Tube | Frozen | Store |
| Plasma | 0.5mL | Sarstedt Cryovial | Re-aliquot into NCRAD provided tubes. | 3-5 x 0.5mL aliquots | 0.5mL Matrix Tube | Frozen | Store |
| RNA | 50 - 90uL | Non-Stick RNase-Free 1.5mL Tube | Re-aliquot & re-label using NCRAD provided tubes and labels | 2-6 x 500ng aliquots | 0.5mL Sarstedt Tube | Frozen | Store |
| CSF | 0.5mL | Sarstedt Cryovial | Re-aliquot into NCRAD provided tubes. | 10 x 0.5mL aliquots | 0.5mL Matrix Tube | Frozen | Store |

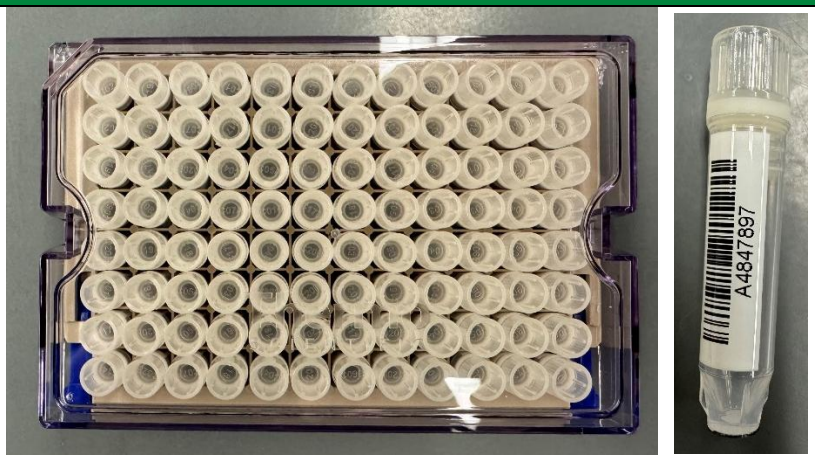
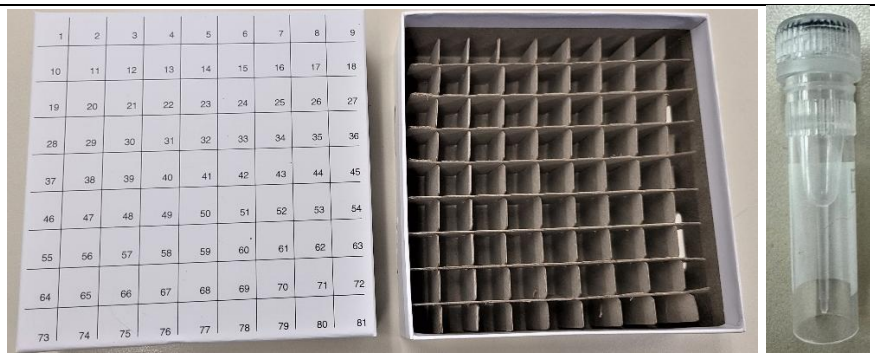
## 6.0 NCRAD Kits & Supplies

NCRAD will provide: 1) pre-barcoded tubes to re-aliquot DNA that will be stored at NCRAD; 2) pre-barcoded tubes to re-aliquot plasma that will be stored at NCRAD; 3) tubes and labels to re-aliquot and re-label RNA that will be stored at NCRAD; 4) pre-barcoded tubes to re-aliquot CSF that will be stored at NCRAD; 5) shipping supply kits; 6) individual supplies.

### 6.1 Supplies

Aliquot kits contain the following (for each participant visit) and provide the necessary supplies to aliquot samples from a given participant visit to send to NCRAD. 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.*

**Sample Aliquoting Supplies**

| Item | Image |
|---|---|
| 0.5mL Matrix Tube (for Plasma & CSF)<br><br>Sent in cases of 96 |   |
| 0.5mL Sarstedt Tube (for RNA) with labels<br><br>Sent in cases of 81 |  |

| Item | Image |
|---|---|
| 0.75mL Micronic Tube (for DNA)<br><br>Sent in cases of 96 |  |

## Shipping Supply Kit

| Quantity | Frozen Shipping Kit Components for Blood-Based Biomarkers |
|---|---|
| 4 | Plastic Biohazard bag with absorbent sheet (Large) |
| 1 | Shipping box/Styrofoam container (Large) |
| 1 | UN3373 label |
| 1 | UPS Blue Dry Ice Sticker |
| 1 | Fragile Sticker |
| 1 | Shipping pouch |
| 1 | Resealable bag with kit type label |

## Individual Supplies

| Quantities | Items Available upon request within the NCRAD kit module |
|---|---|
| By Request | 81-slot cryobox (for storing/shipping RNA samples) |
| By Request | 0.5mL Sarstedt Tube (for RNA) |
| By Request | 0.75mL Micronic Tube (for DNA) |
| By Request | 0.5mL Matrix Tube (for CSF or Plasma) |
| By Request | UPS return airbill pouch |
| By Request | Shipping container for dry ice shipment (shipping and Styrofoam box) |
| By Request | Styrofoam shipping containers (11”x9”x8” 1 1/2” wall) |
| By Request | Plastic biohazard bag with absorbent sheet (large) |
| By Request | UN3373 label |
| By Request | UPS blue dry ice shipping label |
| By Request | Fragile label |
| By Request | Fine point permanent markers |

### 6.2 Kit Supply to Study Sites

Supplies should be ordered in bulk vis the kit request survey found here: https://redcap.uits.iu.edu/surveys/?s=48K3YP9RTEEXANDA

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

## 7.0 Blood Collection and Processing Procedures

Please refer to PAN_HML supplied procedures for blood collection and processing.

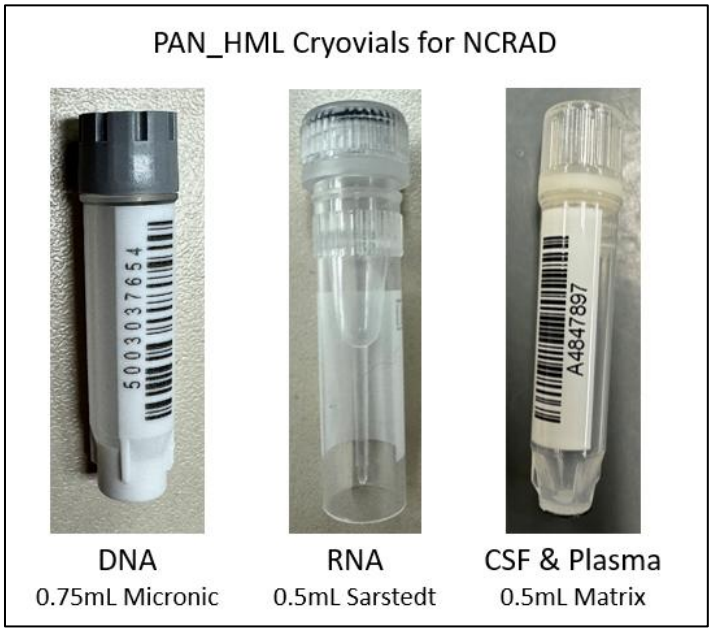
## 8.0 Sample Aliquoting Procedures

DNA, RNA, plasma, and CSF are aliquoted into cryovials provided by NCRAD.

### 8.1 Tube Types

Each sample type should be re-aliquoted into the appropriate tube. The chart below summarizes the association between tube type and sample type.

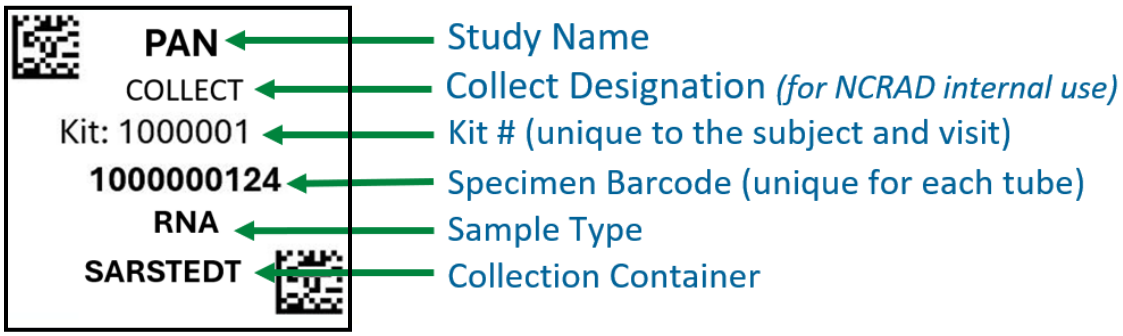
| Tube Type | Sample Type |
|---|---|
| 0.5 mL Sarstedt | RNA |
| 0.5 mL Matrix Tube | Plasma and CSF |
| 0.75 mL Micronic | DNA |



## 8.2 Cryovial Labels

The 0.75ml Micronic (for DNA) and 0.5ml Matrix (for CSF & plasma) will come pre-barcoded. Please ensure to link the barcode with the corresponding participant visit via the manifest (Appendix A).

The 0.5ml Sarstedt tube (for RNA) does not come pre-barcoded. A NCRAD-provided label should be placed on each RNA cryovial. The format of the sample label is shown in the following diagram.

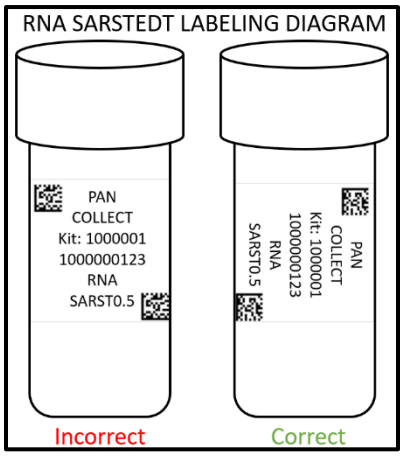



Labels will be provided in groups based on their kit number, a seven-digit number shown on line 3 of the label. **All RNA samples from a given participant visit should be receive labels that all have the same kit number.** Six (6) RNA labels will be provided per participant visit. Any extra labels should be thrown away. Kit numbers should have a 1 to 1 correlation with participant visit.

For example, if you only plan to send 3 x 500ng RNA aliquots for a given participant visit, you should use three cryovial labels with the same kit number and then throw the other three labels with that same kit number away.

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

- NCRAD considers blood and CSF draws to be separate "visits." All blood product aliquots for a given participant visit should have one kit number and all CSF aliquots should have another kit number.
- Place labels on **ALL** cryovials **BEFORE** aliquoting or freezing. This should help to ensure the label properly adheres to the cryovial before exposure to moisture or different temperatures.
- Place cryovials 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.
- The Collection and Aliquot Tube Labels contain two 2D barcodes on the upper left and lower right-hand sides of the label. Place the left-hand barcode toward the tube cap.




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

- 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.

- 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.

## 8.3 Filling Aliquot Tubes (DNA, RNA, 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 cryovial should be filled to the assigned volume with the respective biological material after processing is completed.

- DNA should be processed into 5ug aliquots.
  - *Minimum* amount of DNA needed to ship to NCRAD: 1 x 5ug aliquot at 50 ng/ul.
- RNA should be processed into 500ng aliquots.
  - *Minimum* amount of RNA needed to ship to NCRAD: 2 x aliquots at 500ng each.
  - Ideal/Maximum amount of RNA to ship to NCRAD: 6 x aliquots at 500ng each.
- Plasma should be processed into 0.5ml aliquots.
- CSF should be processed into 1.0ml aliquots.

Over-filled tubes may burst once placed in the freezer, resulting in a loss of that sample.

### 8.4 Storing Filled Aliquot Tubes (DNA, RNA, Plasma, and CSF)

- **DNA**: place the filled 0.75ml Micronic tubes into the Micronic tube rack.
  - Ensure that all DNA samples for a given participant visit are placed in the same Micronic tube rack.
  - Place on dry ice pellets and transfer to -80°C Freezer when possible.
  - Store all samples at -80°C until shipped to NCRAD on dry ice pellets.
- **RNA**: place the filled 0.5ml Sarstedt tubes into the 81-cell cryobox.
  - Ensure that all RNA samples for a given participant visit are placed in the same cryobox.
  - Place on dry ice pellets and transfer to -80°C Freezer when possible.
  - Store all samples at -80°C until shipped to NCRAD on dry ice pellets.
- **Plasma**: place the filled 0.5ml Matrix tubes into the Matrix tube rack.
  - Ensure that all plasma samples for a given participant visit are placed in the same Matrix tube rack.
  - Place on dry ice pellets and transfer to -80°C Freezer when possible.
  - Store all samples at -80°C until shipped to NCRAD on dry ice pellets.
- **CSF**: place the filled 0.5ml Matrix tubes into the Matrix tube rack. Place on dry ice pellets and transfer to -80°C Freezer when possible. Store all samples at -80°C until shipped to NCRAD on dry ice pellets.
  - Ensure that all CSF samples for a given participant visit are placed in the same Matrix tube rack.
  - Place on dry ice pellets and transfer to -80°C Freezer when possible.
  - Store all samples at -80°C until shipped to NCRAD on dry ice pellets.

## 9.0 Shipping

**ALL** study personnel responsible for shipping should be certified in biofluid shipping (i.e. IATA certification). The ATRI Clinical Monitor will review training and certification through the study. If not available at your institution, please contact NCRAD with questions and information regarding resources.

| Sample Type | Aliquot Size | Aliquots per Visit | Max Aliquots per Box | Ship |
|---|---|---|---|---|
| **DNA** | 5ug | 1 | 96 | Frozen |
| **RNA** | 500ng | 2-6 | 81 | Frozen |
| **Plasma** | 0.5ml | 2-5 | 96 | Frozen |
| **CSF** | 1.0ml | 2-5 | 96 | Frozen |

1. The Large Frozen Shipper (17x17x17”) can fit ~45lbs of pelleted dry ice and 4 of any combination of the following:
   a. 81-slot cryobox
   b. 96-slot Matrix rack
   c. 96-slot Micronic rack

2. Hold samples in -80°C freezer until it is time to package the specimens on pelleted dry ice for shipment.

3. On the day of scheduled pick-up, begin packaging specimens on pelleted dry ice **~1 hour before the courier arrives**.

4. Ensure that all of a given sample type for a given participant visit are placed in the same cryobox/rack.
   a. It is OK if there are a few empty slots in the cryobox/rack. Please arrange samples so that all of one sample type from a participant visit are in the same box, placed next to each other.
   b. Rows should be filled in a left-to-right and top-to-bottom manner.

5. Place one filled cryobox (or rack) in each clear plastic biohazard bag (do NOT remove the absorbent material found in the bag). Seal biohazard bag according to the instructions on the bag.

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

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

8. Fully cover the cryoboxes and tubes with approximately 2 inches of dry ice pellets.

9. The inner Styrofoam shipping container must contain approximately 30-45lbs (or 21kg) of pelleted dry ice. The dry ice pellets should entirely fill the inner box to ensure the frozen state of the specimens.

10. Replace the lid on the Styrofoam carton. Place the completed Manifest (Appendix A) 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.

11. Complete the FedEx Dry Ice Label
    a. Net weight of 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.
    c. FedEx may reject or return your package if the Dry Ice Label is incomplete or unreadable.

12. Ensure that the outside of the package is labeled with all applicable labels, including, but not limited to: warning labels, a dry ice label, and the return label. Ensure that all labels are readable and do not overlap.

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

14. Notify NCRAD of shipment by emailing NCRAD coordinators at: alzstudy@iu.edu & cc Diont'e Keys.

15. Attach the following to the email:
    a. Completed Manifest (Appendix A).
    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.

16. Specimens should be sent to the below address so that they arrive the following day. Frozen shipments should be sent **Monday through Wednesday** to avoid shipping delays on Thursday or Friday.

    NCRAD
    351 West 10th Street
    TK-217
    Indianapolis, IN 46202
    Phone: 1-800-526-2839

17. All shipments will be sent through FedEx. Please use FedEx tracking services, to ensure the delivery occurs as scheduled and is received by NCRAD. Please notify NCRAD by email (alzstudy@iu.edu) and cc the coordinator, Diont'e Keys (dlkeys@iu.edu), that a

shipment has been sent and include the tracking number in your email. NCRAD will monitor the tracking number that is provided in the email.

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

## 9.1 Packaging Instructions

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.

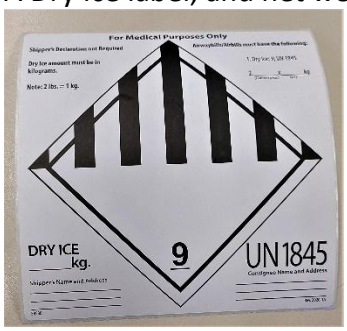
IMPORTANT
Frozen samples **MUST** be shipped Monday-Wednesday only

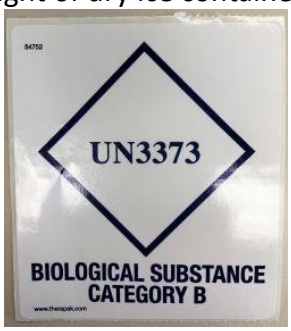
Specimens being shipped to NCRAD should be considered as Category B UN3373 specimens and as such must be tripled packaged and compliant with IATA Packing Instructions 650. *See the Latest Edition of the IATA Regulations for complete documentation.*

**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 (within the cryovial box containing the frozen cryovials) 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 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

- A Dry Ice label, and net weight of dry ice contained




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.

## 10.0 Data Queries and Sample Reconciliation

One manifest should be completed per sample type in each shipment.

## 11.0 Appendices List

Appendix A: Manifest

## Appendix A

**PAN_HML Specimens**

Please complete this form when sending Samples to NCRAD. The contact information for emailing the form is in the box below. NCRAD would like to receive this form BEFORE the samples arrive.

To: Kelley Faber

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

From:_____ Site:_____

Phone: _____ Fax:_____

Email:_____ Date:_____

| Participant ID | Kit Number | Sex | Year of Birth | Collection Date | Specimen Barcode | Specimen Type* | Quantity | Quantity UoM | Collection Container** | Hemolyzed | Turbid | Freeze/Thaw Cycle*** | Box | Position |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | 1 | 1 |
| | | | | | | | | | | | | | 1 | 2 |
| | | | | | | | | | | | | | 1 | 3 |
| | | | | | | | | | | | | | 1 | 4 |
| | | | | | | | | | | | | | 1 | 5 |
| | | | | | | | | | | | | | 1 | 6 |
| | | | | | | | | | | | | | 1 | 7 |
| | | | | | | | | | | | | | 1 | 8 |
| | | | | | | | | | | | | | 1 | 9 |
| | | | | | | | | | | | | | 1 | 10 |
| | | | | | | | | | | | | | 1 | 11 |
| | | | | | | | | | | | | | 1 | 12 |
| | | | | | | | | | | | | | 1 | 13 |
| | | | | | | | | | | | | | 1 | 14 |
| | | | | | | | | | | | | | 1 | 15 |
| | | | | | | | | | | | | | 1 | 16 |
| | | | | | | | | | | | | | 1 | 17 |
| | | | | | | | | | | | | | 1 | 18 |
| | | | | | | | | | | | | | 1 | 19 |
| | | | | | | | | | | | | | 1 | 20 |
| | | | | | | | | | | | | | 1 | 21 |
| | | | | | | | | | | | | | 1 | 22 |
| | | | | | | | | | | | | | 1 | 23 |