

ENIGMA-PAK

in collaboration with the

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



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

Version 04.2025



Biospecimen Collection, Processing, and Shipment Manual

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1.0 Abbreviations

AD	Alzheimer's Disease
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra-acetic Acid
GUID	Globally Unique Identifier
IATA	International Air Transport Association
NCRAD	National Centralized Repository for Alzheimer's Disease and Related Dementias
PHI	Protected Health Information
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute
UPS	United Parcel Service

2.0 Purpose

The collection of biofluids is an important part of ENIGMA-PAK. 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:

- Plasma
- Buffy Coat (DNA Extraction)

This manual includes instructions for collection of blood, storage prior to shipping and shipping to NCRAD. These procedures are relevant to all study personnel responsible for processing specimens provided to NCRAD for the ENIGMA-PAK protocol.



3.0 NCRAD Information

3.1 NCRAD Contacts

Tatiana Foroud, PhD, Core Leader

Phone: 317-274-2218

Kelley Faber, MS, CCRC, Senior Project Manager

Phone: 317-274-7360

Email: kelfaber@iu.edu

Mica Gosnell, MS, Clinical Research Coordinator

Phone: 317-274-7423

Email: gosnellm@iu.edu

General NCRAD Contact Information

Phone: 1-800-526-2839 or 317-278-8413

Email: alzstudy@iu.edu

Website: www.ncrad.org

Sample Shipment Mailing Address

NCRAD

Indiana University School of Medicine

351 W. 10th St. TK-317

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.

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
3 rd Monday in January	Martin Luther King, Jr Day
4 th Monday in May	Memorial Day
June 19	Juneteenth
July 4	Independence Day
1 st Monday in September	Labor Day
4 th Thursday in November	Thanksgiving
4 th Friday in November	Friday after Thanksgiving
December 25	Christmas Day
December 26-December 30	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. 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/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
- Microcentrifuge tube rack

- Sharps bin and lid
- Wet Ice Bucket
- Wet ice
- Pelleted dry 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 $\geq 2000 \times g$ with refrigeration to 4°C
- -80°C Freezer

In order to ship specimens, you must provide:

- Pelleted dry ice

4.2 Biospecimens Sent to NCRAD

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

4.2.1 Biofluid Collection Schedule

Sample Type	Visit
Plasma	X
Buffy Coat	X

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	Drawn At	Specimen Type	Aliquot Volume	Total Number of Aliquots	Shipping Temperature
2 EDTA (Purple-Top) Blood Collection Tubes (10 ml)	Each visit	Plasma	1.5 ml plasma aliquots	Up to 7	Frozen
	Each visit	Buffy Coat	~1.0 ml buffy coat aliquots	1	Frozen

5.0 Specimen Collection Kits, Shipping Kits, and Supplies

ENIGMA-PAK will provide: 1) Blood sample collection kits for research specimens to be stored at NCRAD and the Frozen Shipment Kit. The kits include blood tubes, pipettes, labels, boxes for plasma, buffy coat aliquots, as well shipping materials to send biospecimens to NCRAD.

Blood Collection Kit & World Courier Shipping Boxes for Frozen Shipments



6.0 Blood Collection and Processing Procedures

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

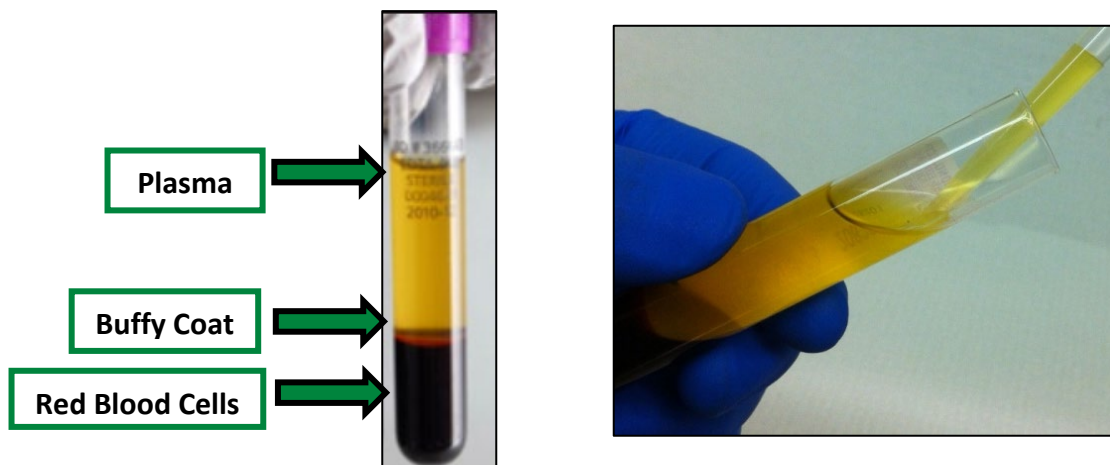
1. Store empty EDTA tubes at room temperature, 64°F - 77°F (18 °C – 25 °C) before use. Check expiration dates on all collection tubes before visit.
2. Set centrifuge to 4°C to pre-chill before use.
3. Using a blood collection set and a holder, collect blood into the **EDTA (Purple-Top) Blood Collection Tube (10 ml)** using your organizations'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. 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, place it on wet ice until centrifugation begins.

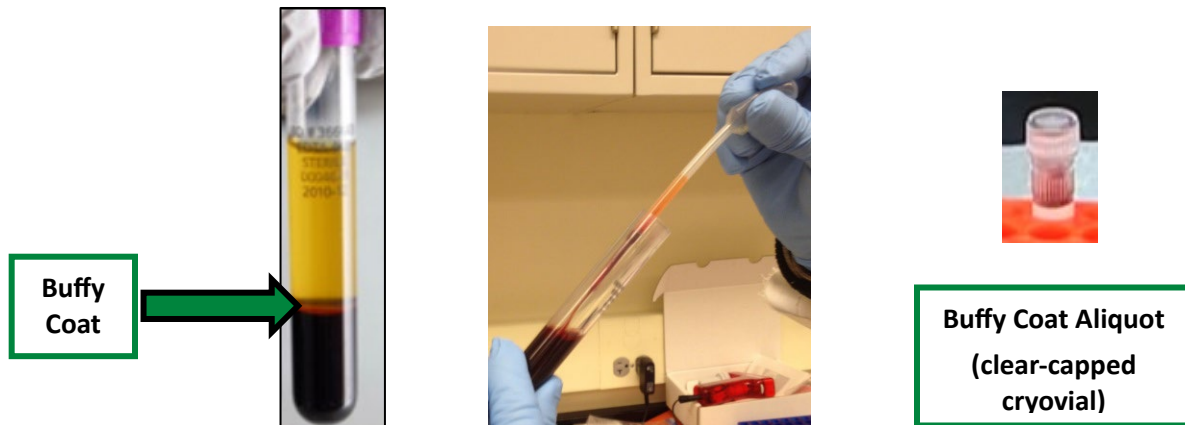


7. Centrifuge balanced tube for 10 minutes at 2000 x g at 4°C. **It is critical that the tube be centrifuged at the appropriate speed and temperature to ensure proper plasma separation (see worksheet in [Appendix A](#) to calculate equivalent RPM for spin at 2000 x g).**
 - a. While centrifuging, remember to record all times, temperatures and spin rates on the Biological Sample and Shipment Notification Form.
8. Record original volume drawn for each tube in spaces provided on the Biological Sample and Shipment Notification Form.
 - b. Plasma samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection.
9. Record time aliquoted on the Biological Sample and Shipment Notification Form.

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 red blood cells at the bottom of the collection tube.

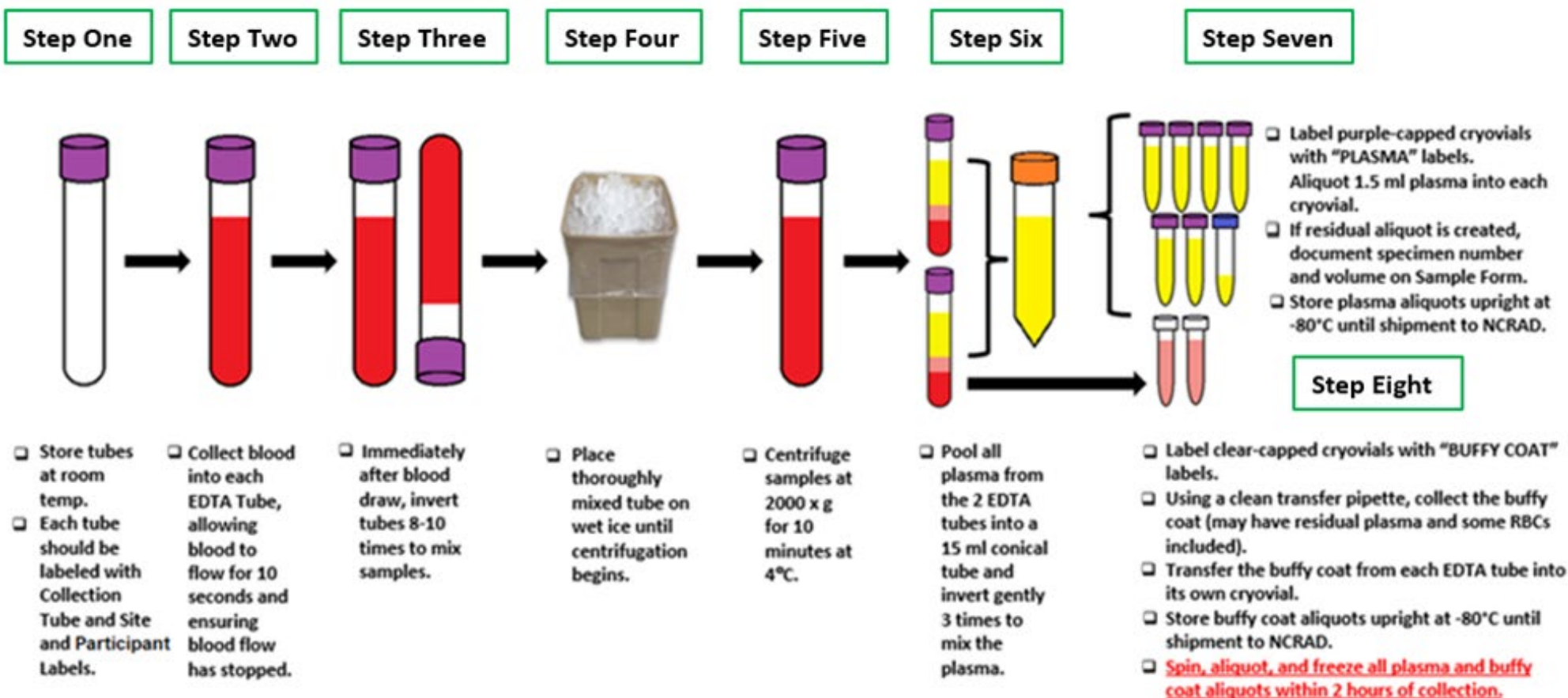


11. Each EDTA tube should yield, on average, 4-5 ml of plasma. Transfer plasma from both EDTA tubes into the 15 ml conical tube and gently invert 3 times. Aliquot 1.5 ml plasma per cryovial. Be sure to only place **plasma** in cryovials with purple caps and labeled with **PLASMA** labels. Place residual 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.**
12. 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 frozen and storage temperature of freezer on Biological Sample and Shipment Notification Form (Appendix B).
13. After plasma has been removed from the EDTA (Purple-Top) Blood Collection Tubes (10 ml), aliquot the buffy coat layer (in the top layer of cells, the buffy coat is mixed with RBCs-see figure) from one EDTA tube into a labeled, clear-capped cryovial using a micropipette. The buffy coat aliquot is expected to have a reddish color from the RBCs. Be sure to only place the buffy coat from one EDTA tube into each cryovial. Repeat this step for the second EDTA tube, placing this buffy coat into the second clear-capped cryovial.



14. Dispose of collection tube with red blood cell pellet according to your site's guidelines for disposing of biomedical waste.
15. Record the specimen number and volumes of the EDTA tubes and corresponding buffy coat samples on the Shipping Manifest.
16. 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 frozen and storage temperature of freezer on Biological Sample and Shipment Notification Form.

Plasma and Buffy Coat Preparation EDTA Purple-Top Tube (2 x 10 ml)



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.

7.1 Frozen 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.

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.

7.1.1 NCRAD Packaging Instructions – Frozen Shipments

1. World Courier provides all shipment materials (boxes, dry ice, etc).
2. If possible, hold packaged samples in -80°C freezer until time of pick-up/drop-off. If storage in a -80°C freezer until pick-up is not possible, package samples no more than 4 hours before the expected pick-up time.
3. Notify NCRAD of shipment by emailing NCRAD coordinators at alzstudy@iu.edu. Attach the following to the email:
 - a. Completed Sample Forms ([Appendix B](#)) to the email notification (email NCRAD coordinator prior to shipment to receive sample form).
 - a. If email is unavailable, please call NCRAD and do not ship until you've contacted and notified NCRAD coordinators about the shipment in advance.
 - b. Tracking number from courier



Biospecimen Collection, Processing, and Shipment Manual

4. Follow ENIGMA-PAK study and courier packaging instructions to ensure a sufficient amount of dry ice is packed in each shipper to ensure sample integrity upon arrival at NCRAD.

8.0 Appendices

Appendix A: Rate of Centrifuge Worksheet

Appendix B: NCRAD Blood Sample and Shipment Notification Form

Appendix C: ENIGMA PAK Shipping Manifest

Appendix D: Shipping Documentation



Biospecimen Collection, Processing, and Shipment Manual
Appendix A: Rate of Centrifuge Worksheet

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

Submitter Information

Name:

Site:

Submitter e-mail:

Centrifuge Information

Please answer the following questions about your centrifuge.

Centrifuge Type

Fixed Angle Rotor: ☐ Swing Bucket Rotor: ☐

Radius of Rotation (mm):

Determine the centrifuge's radius of rotation (in mm) by measuring distance from the center of the centrifuge spindle to the bottom of the device when inserted into the rotor (if measuring a swing bucket rotor, measure to the middle of the bucket).

Calculating RPM from G-Force:

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

RCF = Relative Centrifugal Force (G-Force)

RPM = Rotational Speed (revolutions per minute)

R = Centrifugal radius in mm = distance from the center of the turning axis to the bottom of centrifuge

Comments:

Please send this form to NCRAD Study Coordinator at alzstudy@iu.edu



Appendix B: NCRAD Blood Sample and Shipment Notification Form

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

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

From: _____ Tracking #: _____

Phone: _____ Email: _____

Study: ENIGMA-PAK

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]

Blood Processing:

Plasma and Buffy Coat (EDTA Tube)

EDTA #1 specimen number (Last four digits): _____	Original blood volume of EDTA #1: _____ mL
EDTA #2 specimen number (Last four digits): _____ ☐ N/A	Original blood volume of EDTA #2: _____ mL ☐ N/A
Time spin started: _____ [HHMM]	Duration of centrifuge: _____ mins
Temp of centrifuge: _____ °C	Rate of centrifuge: _____ RCF (x g)
Time aliquoted: _____ (HHMM)	Number of 1.5 mL aliquots created (purple cap): _____ ☐ N/A
Volume of residual plasma aliquot (less than 1.5 mL in blue cap): _____ mL ☐ N/A	Specimen number of residual plasma aliquot (Last four digits): _____ ☐ N/A
Buffy coat #1 specimen number (Last four digits): _____	Buffy coat #1 volume: _____ mL
Buffy coat #2 specimen number (Last four digits): _____ ☐ N/A	Buffy coat #2 volume: _____ mL ☐ N/A
Time aliquots frozen: _____ [HHMM]	Storage temperature of freezer: _____ °C

Notes:

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Appendix D: Shipping Documents

CUSTOMS INVOICE

Date: 28th September 2024

Shipper: Dr. XXXXXXXX,
Assistant Professor, Department of Paediatrics & Child Health
Attn: Ms. Aneeta Hotwani
Manager Infectious Diseases
Department of Pediatrics and Child Health
The Aga Khan University
Stadium Road Karachi 74800, Pakistan
Tel: 92 21 3486 xxx/92 21 34864990
E-mail: xxxxxxxx@aku.edu / aneeta.hotwani@aku.edu

Consignee Dr. xxxxxxxxxxxx
c/o Dr. xxxxxx
Address: Building : _____ Room #: _____
Street #: _____
City : _____ Country: _____
Phone: _____
Email: _____
Alternative contact info:
Phone #: _____ Fax #: _____
Mobile #: _____

Contents : The materials being shipped are Human Blood samples.
Biological Substance, Category B UN 3373
Type of sample : Human whole Milk samples Quantity : xxx vials in xx Boxes
Packed on dry Ice 01 Box

- This Shipment contains material obtained from humans that has not been inoculated with or exposed to any livestock or poultry disease agent.
 - The material is not tissue culture Origin.
 - The Materials is not zoonotic.
 - The material is not from Non-human primates.
- For Research analysis only. The specimen is for Laboratory testing only.

For Customs Purpose Only \$10

Mr. Abdul Khaliq Pesnani
Consultant, Imports & Special Assignment
CNIC# 42000-0500070-7
CELL# 0300-8224185
Khaliq.pesnani@aku.edu



Biospecimen Collection, Processing, and Shipment Manual
CDC Declaration

Shipper: Dr. XXXXXXXX,
Assistant Professor, Department of Paediatrics & Child Health
Attn: Ms. Aneeta Hotwani
Manager Infectious Diseases
Department of Pediatrics and Child Health
The Aga Khan University
Stadium Road Karachi 74800, Pakistan
Tel: 92 21 3486 xxx/92 21 34864990
E-mail: xxxxxxxx@aku.edu / aneeta.hotwani@aku.edu

Consignee Dr. xxxxxxxxxxxx
c/o Dr. xxxxxx
Address: Building : _____ Room #: _____
Street #: _____
City : _____ Country: _____
Phone: _____
Email: _____
Alternative contact info:
Phone #: _____ Fax #: _____
Mobile #: _____

Contents: The materials being shipped are Human whole Milk samples.
Biological Substance, Category B UN 3373
Type of sample: Human whole Milk samples Quantity : 267 vials in 04 Boxes
Packed on dry Ice 01 Box

- This Shipment contains material obtained from humans that has not been inoculated with or exposed to any livestock or poultry disease agent.
- The material is not tissue culture Origin.
- The material is not zoonotic.
- The material is not from Non-human primates.

For Research analysis only. The specimen is for Laboratory testing only.

For Customs Purpose Only \$10

Mr. Abdul Khaliq Pesnani
Consultant, Imports & Special Assignment
CNIC# 42000-0500070-7
CELL# 0300-8224185
Khaliq.pesnani@aku.edu
Date: 28th September 2024

UNDERTAKING

This is to certify that shipment does not contain any unauthorized explosives, narcotics and firearms or hazardous material; I consent to a search of this shipment.

I am aware that this endorsement containing original signatures, along with other shipping documents will be retained on file until the shipment is delivered.

Name of Cargo Agent: BNS Logistics (Pvt) Ltd.
 Airway Bill No:
 Number of Cartons: 1 Box Dry Ice Shipment
 Weight:
 Commodities : Human whole Milk samples Quantity : 267 vials in 04 Boxes
 Packed on Dry Ice.
 01 Box Dry Ice Shipment

Destination: Dr. xxxxxxxxxxxx
 c/o Dr. xxxxxx
 Address: Building : _____ Room #: _____
 Street #: _____
 City : _____ Country: _____
 Phone: _____
 Email: _____
 Alternative contact info:
 Phone #: _____ Fax #: _____
 Mobile #: _____

Mr. Abdul Khaliq Pesnani
 Consultant, Imports & Special Assignment
 CNIC# 42000-0500070-7
 CELL# 0300-8224185
 Khaliq.pesnani@aku.edu

To Whom It May Concern:

Dear Sir,

Reference to the above subject the undersigned of The Aga Khan University Hospital, Karachi, Department of Pediatrics and Child Health, an accredited for testing purpose, have to ship Human Blood samples Quantity: xxx vials in xx Boxes. Biological Substance, Category B UN 3373 Non-infectious, Non-hazardous packed with Dry Ice to xxxxxxxx For diagnostic and scientific analysis purpose.

The samples are meant solely for lab analysis purposes and are of no commercial value.

It is further certified that the samples are non-hazardous, non-infected and do not contain animal material or its by-products.

Thanking you for your cooperation and courtesy.

With regards,

Mr. Abdul Khaliq Pesnani
Consultant, Imports & Special Assignment
CNIC# 42000-0500070-7
CELL# 0300-8224185
Khaliq.pesnani@aku.edu



The Deputy Collector of Customs

Biospecimen Collection, Processing, and Shipment Manual
Dated: 28th September 2024

Karachi AFU Airport
Karachi Pakistan

SUB: **REQUEST FOR THE EXPORT THE SHIPMENT OF PATIENT SAMPLES**
MAWB#

Dear Sir,

Reference to the above subject the undersigned of The Aga Khan University Hospital, Karachi, Department of Pediatrics and Child Health, an accredited for testing purpose, have to ship Human Blood samples Quantity: xxx vials in xx Boxes. Biological Substance, Category B UN 3373 Non-infectious, Non-hazardous packed with Dry Ice to xxxxxxxx For diagnostic and scientific analysis purpose.

This is to request you to kindly allow us to export these samples as they are temperature sensitive and are packed in dry ice and shall perish if delayed in shipping.

Thanking you for your cooperation and courtesy

With regards,

Mr. Abdul Khaliq Pesnani
Consultant, Imports & Special Assignment
CNIC# 42000-0500070-7
CELL# 0300-8224185
Khaliq.pesnani@aku.edu