Biospecimen Collection, Processing and Shipment

This Standard Operating Procedure (SOP) provides instructions for PNRR study team members how to order supplies, collect and process the biospecimen samples and ship the collected biospecimen samples to Indiana University Genetic Biobank (IUGB) for long-term storage and further processing.

I. Blood Biospecimen Collection Kits

All biospecimen collection kits are assembled at Indiana University (IU) and are shipped to each site upon request. The PNRR site personnel will be responsible for informing IU of the number of required blood and saliva collection kits, shipping supplies, and the requested date of arrival, with at least 10 business days advanced notice, via the PNRR-portal at http://kits.iu.edu/pnrr

Blood Collection Kits:

Each blood collection kit contains the following items:

1. **Sample Collection and Processing Form**
   A paper copy of the Sample Collection and Processing Form pre-labeled with the kit number/barcode.

2. **Biohazard 95k Pressure Bag (95kPa)**
   Clear sealable plastic bag, marked with the red biohazardous symbol. The bag is pre-labeled with the kit number/barcode. A sheet of absorbent material is included in the bag.

   Note: *absorbent material to be inside the sealed bag is a DOT/IATA requirement.*

3. **Blood collection tubes**
   Each blood collection kit includes two (2) 10ml Ethylenediaminetetraacetic acid (EDTA) tubes with purple tops and two (2) 5ml Serum Separation Tubes (SST) with a yellow top.

4. **Sterile pipettes and mixing vial**
   Four (4) sterile 1ml disposable pipettes and one (1) sterile mixing vial.

5. **Cryovials**
   Seventeen (17) 2ml aliquot tubes (cryovials) for long-term cold storage of the serum, plasma, and buffy coat aliquots. The cyrovials are pre-labeled with the blood kit number and barcode, as well as the specimen type. Furthermore, they have colored tops for easy identification of the type of specimen collected in each of them:

Sample Type	Cryovial Top Color
serum	yellow
plasma	purple
buffy coat	clear

6. **Cryobox**
   25-cell cryobox for storage, freezing and shipping the aliquot tubes. Cryobox is pre-labeled with kit-number/barcode.

7. **Extra Labels**
    1. Five general labels with lines for the PNRR site coordinator to write in the Site ID and Individual ID. These labels should be applied to the EDTA and SST tubes prior to blood collection.
    2. One additional kit number/barcode label.
    3. Two extra specimen labels for additional plasma cryovials, in case more than 10ml of plasma is collected.
    4. One extra specimen label for additional serum cryovial, in case more than 5ml serum is collected.

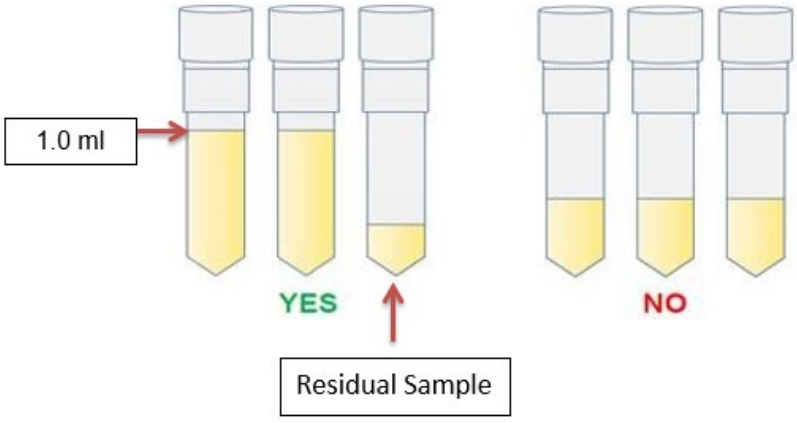
II. Blood Collection and Processing

II.a. General Remarks

- Blood should be collected from study participants at the enrollment visit. Ideally, the blood sample is collected on the same day the patient is examined by the enrolling physician. If that is not feasible, then the blood sample must be collected within three months of the enrollment visit.

- Blood is collected via standard venipuncture procedure at the enrollment site. Two (2) 10ml EDTA (purple-top) and two (2) 5 ml SST (gold-top) tubes should be collected from each participant for the PNRR biorepository, and the samples should be processed and frozen within 2 hours of the blood draw.

- Serum and plasma aliquots should be filled to 1ml, with the final aliquot being a residual (less than 1ml); see diagram below. This may result in extra, un-used cryovials. The unused cryovials should be removed from the cryobox before samples are shipped to IU.



II.b. Blood Collection

1. Prior to the blood draw, each collection tube should be labeled with the Site ID and the participant's Individual ID using the blank labels provided in the blood collection kits.

2. Per standard laboratory testing procedures, serum tubes should be filled prior to EDTA tubes to prevent anticoagulant contamination.

3. Immediately after blood collection, gently invert/mix the blood collection tubes 8-10 times.

4. Record the date and time of draw on the Blood Collection and Processing Form. Also record the date and time the patient last ate. Some biomarkers are influenced by the time elapsed since the last meal, so it is important to have that information available.

   Note: *if the research participant gives you a time period when they last eat instead of an exact time, record the end time of that period. Example: if patient indicates their last meal was between 6:30 and 7:00, record 7:00 on the sample collection and processing form*

II.c. PLASMA processing (EDTA tubes)

5. The plasma collection tubes should be centrifuged as soon as possible after the blood draw (max. 30 minutes after draw) for 15 minutes at 1500 x g. If a centrifuge with refrigeration capabilities is available, the temperature should be set at 4°C.

   Note: *buffy coat layer forms best within 30 minutes of collection. It also reduced the risk of hemolysis*

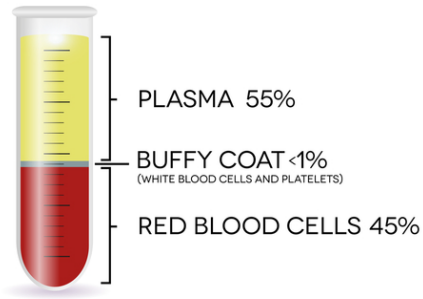
   Note: *most standard blood centrifuges allow speed settings in either Relative Centrifugal Force (RCF) or Revolutions Per Minute (RPM). If RCF is used, the speed should be set at 1500; for RPM setting the required speed depends on the radius of the centrifuge. The PNRR site personnel shall verify the required RPM-setting to reach 1500 x g (for table-top centrifuges the RPM setting is between 2000-3000, the exact number is radius dependent).*

6. While centrifuging, record the start time and the set temperature of centrifugation on the biospecimen collection and processing form. It is acceptable to start the spin of the EDTA tubes as room temperature, as refrigerated centrifuges cool down relatively fast to +4˚C.

   Note: *If a refrigerated centrifuge is not available, the EDTA tubes may be centrifuged at room temperature and the room temperature should be recorded on the Sample Collection and Processing Form.*

7. After centrifugation is complete, the EDTA tubes should be carefully removed from the centrifuge to avoid disturbing the cell layers and placed it in a test tube rack.

8. There are three distinct layers after centrifugation: the supernatant (plasma), buffy coat (which contains the white blood cells used for DNA extraction) and the red blood cells (RBCs).



NOTE: *if no buffy coat is visible at the plasma / RBC border, please check the centrifuge setting. If the centrifuge setting was lower than 1500 x g, please place the EDTA tubes back*

into the centrifuge and spin the samples for another 10 minutes at the correct centrifugation speed.

9. Verify that the blood kit number on the pre-labeled aliquot tubes matches the number on the Blood Collection and Processing Form.

10. Using a clean transfer pipette, transfer 1 ml aliquots of plasma into each pre-labeled plasma (purple-capped) cryovial. Each EDTA tube should yield, on average, 4.5 ml of blood plasma for a total of 8-10 plasma aliquots per subject. Record the number of aliquots created on the Blood Collection and Processing Form. If the final aliquot is less than 1ml (residual aliquot), record the volume of the residual aliquot and the last four digits of the barcode for that vial.

11. Using a clean transfer pipette, transfer the buffy coat from each EDTA tube into the two (2) individual cryovials (clear-capped). Record the number of buffy coat aliquots created on the Biospecimen Collection and Processing Form.

    Note: *The buffy coat is a layer of white blood cells. To ensure that the DNA yield is sufficient, the entire layer must be collected, which usually means that some RBC's are also collected, which explains why buffy coat samples usually appear red or pink. An instruction video for buffy coat collection is posted on the PNRR website.*

12. Place the filled plasma and buffy coat vials into the provided cryobox cap-side up. Remove all unused purple-top cryovials.

II.d. SERUM processing (SST tubes)

13. After the blood draw, the blood in the SST tubes must clot for at least 20-30 minutes. Centrifuge the SST tubes at any temperature for 15 minutes at 1500 x g.

    Note: *most standard blood centrifuges allow speed settings in either Relative Centrifugal Force (RCF) or Revolutions Per Minute (RPM). If RCF is used, the speed should be set at 1500; for RPM setting the required speed depends on the radius of the centrifuge. The PNRR site personnel shall verify the required RPM-setting to reach 1500 x g (for table-top centrifuges the RPM setting is between 2000-3000).*

14. While centrifuging, record the start time of centrifugation on the biospecimen collection and processing form.

    NOTE: *If only a small amount of serum is separated from the RBC layer, or if the gel is still sitting on the bottom of the blood tube, the tube was not centrifuged at the appropriate setting. Check the centrifugation speed and time. Re-centrifuge the SST tubes at the correct speed for an additional 10 minutes.*

15. Using a clean transfer pipette, carefully transfer the serum from both SST tubes into the mixing vial provided in the blood collection kit.

16. Mix the collected serum in the mixing vial by drawing the serum up into the pipette 4-6 times. Alternatively, cap the mixing vial tightly and carefully invert 4-6 times.

17. Using a clean transfer pipette, transfer 1 ml aliquots of serum into the pre-labeled serum cryovials (yellow-capped). Each serum tube should yield, on average, 2.5 ml of blood serum for a total of 4-5 serum aliquots per subject. Record the number of aliquots created on the Blood Sample Collection and Processing Form. If the final aliquot is less than 1ml (residual aliquot), record the volume of the residual aliquot and the last four digits of the barcode on the Biospecimen Collection and Processing Form.

18. Transfer all serum aliquots into the cryobox, cap-side up. Remove all unused yellow-cap cryovials from the cryobox.

19. After all blood has been aliquoted and cryovials placed in cryobox, close cryobox and seal it. Place the closed/sealed cryobox into the biohazard bag, remove the air and seal the biohazard bag for storage. Freeze the samples as soon as possible after processing is complete. Be sure to freeze the samples cap-side up. Record the time that aliquots were frozen on the Biospecimen Collection and Processing Form.

    NOTE: *If a -80°C freezer is not immediately accessible, the aliquots may be stored in a -20°C freezer or on dry ice for several hours prior to being transferred to the -80°C freezer.*

20. Discard the SST and EDTA collection tubes and processing materials according to site guidelines for disposing of biomedical waste.

III. Blood Sample Shipments (frozen)

III.a. Shipment Supplies

- Required materials to transfer specimens from the enrollment center to IU will be assembled at IU and shipped to each site. The PNRR site coordinators will be responsible for informing IU of the materials the site requires at least ten (10) business days in advance, using the PNRR-portal: http:// kits.iu.edu/pnrr

- The provided shipping materials include:
    1. Styrofoam insulated shipping boxes
    2. Pre-addressed shipping labels
    3. Dry ice shipping labels
    4. “UN3373: Biological Substance, Category B” stickers
    5. “Fragile” stickers

- Insulated shipping boxes are available in two sizes. Small shippers hold samples from one to three individuals (plus dry ice). Large shippers should be used to send samples from four to eight individuals.

    NOTE: *All PNRR blood samples must be shipped on dry ice. The enrollment centers are responsible for providing the required dry ice for the shipments.*

III.b. Shipping Instructions

- **All blood samples MUST be shipped to IU within 2 weeks of collection.**

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- Samples can be shipped to IU on Monday, Tuesday, or Wednesday. Do not ship samples on Thursday or Friday.

- The blood kits and shipping materials provided by IU are designed to comply with all DOT/IATA regulations in regard to human blood shipments. Please assemble the shipment as instructed to assure compliance.

- Notify IU of incoming sample shipments via email prior to shipment using the pnrr@iupui.edu email address. The shipment tracking number should be included in the shipment notification.

  Note: *all study personnel preparing the frozen human specimen shipments must have current DOT/IATA training. IATA training certification expires after two years, and must be renewed every 24 months.*

Packing the blood sample

1. Only one participant's blood sample should be included in each cryobox/kPa95 bag. Multiple cryoboxes may be sent in one shipping container as long as there is room for an adequate amount of dry ice (minimum 2 kg). A maximum of three samples should be shipped in one small dry-ice shipping container. If more than three samples are shipped, a large dry-ice shippers should be used.

2. Verify that biohazardous pressure bags (95kPA) are sealed, and each contains an absorbent sheet as well as a cryobox.

3. The dry-ice shipping container should first be filled with a layer of dry ice, followed by a layer of cryoboxes, another layer of dry ice and another layer of cryoboxes, topped with a thick layer of dry ice to ensure that the samples stay frozen even if the shipment takes longer than 24 hours.

4. A packing list must be placed inside of the dry-ice shipper (on top of dry ice).

5. A list that details the subject ID's and collection kits contained in the shipment should be place on top of the Styrofoam lid before the outer cardboard-box is closed.

6. Weigh the package to determine the weight of the dry ice used in the shipment in kg and add that information to the dry-ice biohazardous classification sticker.

   NOTE: *The weight of the dry ice should always be rounded up. It is better to report a higher amount of dry ice than to underestimate the amount.*

Shipment labeling and paperwork

7. Log in to the IU UPS ShipExec™ portal at: https://kits.iu.edu/UPS

8. Find the "Shipping" dropdown menu in the top left corner of the screen and click on "Shipping and Rating".

9. Once the Indiana University page loads, look for the "Study Group" dropdown menu under "Shipment Information" on the right side of the screen. Choose "PNRR" from the dropdown menu.

10. After selecting "PNRR", click on the magnifying glass icon on the left side of the screen under "Ship From".

11. An address book and filter function will appear on the screen. A list of all the PNRR site addresses should appear on the right side of the screen

    Once you have found your site address, click on the "Select" button to the left of the address.

12. Make sure your address appears in the fields under "Ship From" on the main page.

    If you accidentally selected the wrong address, click on the "Reset" button on the bottom right of the screen. After the page reloads and clears the information, select "PNRR" again from the "Study Group" menu and click on the magnifying glass icon again to search for your correct address.

13. Enter the total weight of your package in the "Weight" field on the right side of screen under the name of your study.

    Leave the "Dry Ice Weight" field empty or enter "0" if shipping an ambient sample.

14. Enter the weight of the dry ice for frozen shipments in the "Dry Ice Weight" field.

    The "Dry Ice Weight" field can never be higher than the "Weight" field.

    (Steps 15-16 can be skipped if you do not need to schedule a pickup)

15. After entering the weights, click on the blue "Pick-up Request" button.

16. When the "Create Pickup Request" box pops up, enter information into all the fields provided.
    a. Enter the "Earliest Time Ready" and "Latest Time Ready" in 24-hour format.
        i. Schedule pickup at a minimum 1 hour before the "Earliest Time Ready"
        ii. Enter times in 24-hr format (Ex: 14:00 for 2pm)
    b. Choose a name and phone number that is the best contact if the UPS driver has question related to picking up your package
    c. Entering the "Room Number" and "Floor" will help the UPS driver locate your package
        i. The "Floor" field only allows numerical characters while the "Room Number" field is free text up to 8 characters.
    d. Click "Save" when done.

17. Once you are certain that all the correct information has been entered, click the "Ship" button in the bottom right corner of the screen.

18. If no red error messages pop up at the top of your screen after clicking on "Ship", then two PDF files should have downloaded: "Shipment Receipt" & "UPS Package Label"
    a. Shipment Receipt will list a "Pickup No." that references your specific package if there is ever an issue with UPS picking up your package for scheduled pickups

19. Print out the UPS air waybill label that downloads to your computer, fold it in half, place it inside the clear UPS sleeve.

20. Peel the backing off the clear sleeve, and affix the clear sleeve to the top of the outside cardboard of the dry ice shipping container.

21. Fill out the dry ice label by providing both shipper and recipient information (see below) and the amount of dry ice used to keep the blood samples frozen. Affix the dry ice label to the front of the outside cardboard box of the dry ice shipping container.

    Recipient Information:
    PNRR at IUGB
    351 West 10th Street, TK-217
    Indianapolis, IN 46202
    Phone: 317-278-6158

    NOTE: *The courier may refuse to accept the shipment if the dry ice weight is not recorded correctly. Ensure that the same amount of dry ice is recorded on both the airway bill and the dry ice label.*

22. Affix the "UN 3373: Biological Substance, Category B" and "Fragile" stickers to the front of the outside cardboard of the dry ice shipper, next to the dry ice label.

23. Place your package in the spot designated in your pickup request, or wherever your daily UPS pickups occur.

    NOTE: If you need to reprint your air waybill or void your shipment, click on "History" at the top of the main screen.
    a. If your shipment does not automatically pop up, enter the date of shipment and then click "Search".
    b. To reprint your air waybill, click on the printer icon to the far left under "Action"
    c. To void your shipment, click on the "X" icon to the far left under "Action"

IV. Saliva Collection

If it is not possible to collect 10ml or more blood in the two provided EDTA vacutainers from a research participant, or PNRR site personnel receives notification of an insufficient DNA yield after the DNA was extracted from the buffy coats shipped to IU, personnel at the enrollment center should attempt to collect a saliva specimen from the research participant for DNA.

Saliva collection kits can be ordered via the PNRR-portal at http://kits.iu.edu/pnrr.

Each saliva collection kit includes the following items:
1. Saliva collection kit with instructions
2. PNRR Biological Sample Form
3. Biohazard pressure bag (95kPa)
4. Pre-labeled and postage-paid shipping envelope
5. UN 3343 exempt sticker

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IV.a Saliva Collection Instructions

Note: *each saliva collection kit contains an insert with detailed instructions including diagrams how to collect a viable saliva sample.*

For saliva collections at the enrollment center:

1. The patient cannot eat, drink, smoke or chew gum for 30 minutes before the saliva collection.

2. Using a blank Subject/Site ID label from the IU-provided kit, label the saliva collection tube with the subject's ID.

3. Ask the patient to spit into funnel until the amount of liquid saliva (not bubbles) reaches the fill line. It takes approximately 5 minutes to collect a sufficient amount of saliva.

4. Close the funnel lid by firmly pushing the lid downwards until the liquid inside the lid is released into the tube.

5. Hold the tube upright. Remove the funnel and screw the small blue cap on the tube. Ensure cap is secured tightly.

6. Invert sample several times to mix saliva with stabilization liquid.

7. Label the saliva sample with the subject ID.

8. Place the tube in the bio-specimen bag, peel off the blue liner and seal the bag by pressing down on the glue line.

Shipment instructions (from enrollment center):

9. Complete the "PNRR Biological Sample Form" and place form in shipment envelope together with the saliva sample itself.

10. Mail envelope to PNRR@IUGB within 48 hours after collection using the provided postage-paid padded envelopes. Verify the address and ensure that an "exempt human specimen" sticker is on the envelope.

    PNRR @ IUGB
    351 W 10th Street, TK342
    Indianapolis, IN 46202
    Phone: 317-278-6158

Mailing saliva collections kits to research participants:

1. Using a blank Subject/Site ID label from the IU-provided kit, label the saliva collection tube with the subject's ID.
2. Fill out the PNRR Biological Sample Form (contact information should be for the PNRR site personnel sending the saliva collection kit to the patient).
3. Mail the saliva collection kit to patient, in combination with a letter summarizing the request and instructions. A letter template is provided on the PNRR website.