room temperature for 15 minutes at 1500 x g. **It is critical that the tubes be centrifuged at the appropriate speed to ensure proper filtering of the serum from the red blood cells.**

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 Sample Form.

15. After centrifugation is complete, the SST tubes should be carefully removed from the centrifuge and placed it in a test tube rack.

    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.*

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

17. 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.

18. Using a clean transfer pipette, transfer 1 ml aliquots of serum into the prelabeled 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 Sample 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 Blood collection and processing form.

19. Transfer all serum aliquots into the cryobox, cap-side up. Discard all unused yellow-cap cryovials.

20. Place the cryobox into the biohazard bag remove the air and seal the biohazard bag for storage/freezing. Place the samples into a -80°C freezer immediately after processing is complete. Be sure to freeze the samples capside up. Record the time that aliquots were placed into the freezer on the Blood 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.*

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

III. Sample Shipments

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 four individuals (plus dry ice). Large shippers should be used to send samples from four to eight individuals.
  NOTE: *All PNRR samples must be shipped on dry ice. The enrollment centers are responsible for providing the required dry ice for the shipments.*

IV.b. Shipping Instructions

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

- 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 Department of Transportation rules regarding the shipment of blood samples. 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.

1. Packaging the blood sample

1. Only one participant's blood sample should be included in each cryobox. Multiple cryoboxes may be sent in one shipping container as long as there is room for an adequate amount of dry ice, but please request a bulk shipper if sending more than three cryoboxes in a shipment.

2. Verify that there is an absorbent sheet in the biohazard bag with the cryobox, and ensure that the biohazardous bag is sealed.

3. The insulated shipping container should be filled first 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. Copies of the sample forms for the specimens should be included in the shipment and should be place outside the Styrofoam liner.

5. Ensure the Styrofoam lid is in place (shut) before placing tape on the outside cardboard shipping box to close it securely.

6. Weigh the package to determine the weight of the dry ice used in the shipment in kg.

   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.*

2. Shipment labeling and paperwork

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

8. Create the waybill by choosing the Study Group "PNRR", search for your address, enter total weight and dry ice weight, schedule a pickup (if needed) then click Ship.

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

10. 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.

11. 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.*

12. 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.

IV. Saliva Collection

If PNRR site personnel receives notification of an insufficient DNA yield, personnel at the enrollment center should attempt to collect a saliva specimen from the patient.

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 bag
4. Pre-labeled and postage-paid shipping envelope
5. UN 3343 exempt sticker

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

Mailing saliva collections kits to the subjects:

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.