1. **GENERAL INFORMATION**
2. **OBJECTIVE**

This document describes the requirements for the collection, processing, and shipment of serum biospecimen samples from research sites to a central storage site. Adherence to this protocol will ensure that all samples are stable and accounted for during the collection and transport process.

1. **SCOPE**

This protocol applies to all biospecimen samples collected during the course of the study as well as all staff responsible for collecting, processing, and recording such samples.

1. **RESPONSIBILITIES**
2. All personnel involved in specimen handling will be trained and certified on the United States and International laws governing the handling and transport of blood-borne, bio-hazardous materials through their site’s recognized local agency(s).
3. The Principal Investigator (PI) at each research site is responsible to ensure the proper handling of study samples.
4. Each research site is responsible for organizing and documenting sample shipments to the central processing and storage facility.
5. The central processing and storage facility is responsible for receiving and documenting sample shipments, storing samples from all centers, and transferring samples to other laboratories as requested.
6. **ATTACHMENTS**
   1. Appendix 1: Biological Sample and Shipment Notification Form (CARE Consortium example)
   2. Appendix 2: Serum Preparation Schematic
   3. Appendix 3: Summary of Shipping Information
   4. Appendix 4: Shipping Labels (Example)
   5. Appendix 5: Specimen Labels (Example)
7. **SUPPLIES**
8. **SITE REQUIRED EQUIPMENT**

The following materials and equipment are necessary for the processing of specimens at the collection site**:**

* + - 1. Personal Protective Equipment: lab coat, nitrile/latex gloves, and safety glasses
      2. Tourniquet
      3. Alcohol prep pad
      4. Gauze pad
      5. Bandage
      6. Butterfly needles
      7. Tube holders/Vacutainer adapter if not attached to needle
      8. Microcentrifuge tube rack
      9. Sharps bin and lid
      10. Centrifuge balance tube

In order to process samples consistently across all projects and ensure the highest quality samples possible, sites must have access to the following equipment:

Centrifuge capable of ≥ 1500 rcf (1500 x g)

Centrifuge rotor capable of holding desired sized tubes

-80°C non-frost free freezer

1. **SPECIMEN COLLECTION KIT SUPPLIES**
2. Plastic biohazard bag with absorbent sheet
3. 1 BD Vacutainer 10.0 ml, plastic, serum SST tube with clot activator
4. 10 x 2.0ml cryovials with red caps for collection of serum
5. 1 x 1.0ml disposable graduated transfer pipettes for serum
6. 25 cell cryobox per patient
7. Bio-Pen for labeling the 10.0ml BD Vacutainer
8. Labels: Kit Label
9. Labels: Subject and Site ID Label
10. Labels: Collection Tubes and Cryovials Label

### PACKAGING SUPPLIES

1. **PRIMARY RECEPTACLE:** Cryovial that holds biospecimen aliquots
2. **SECONDARY RECEPTACLE**: Leak-proof, biohazard-labeled plastic bag, capable of withstanding pressures to 95kPa during air transport, containing absorbent material.
   1. **OUTER SHIPPING PACKAGE:** Insulated bio-shipment box

a. For example, a 16”x16”x16”inner styrofoam box placed inside a 17”x17”x17”outer cardboard box.

* 1. **DRY ICE**: Enough to fill styrofoam box entirely after samples are packed

a. For the example, box described above, approximately 18-20 kg (40-45 lbs) is needed

* 1. **CENTRAL FACILITY PROCESSING FORM**: Place in separate plastic bag and include in shipment
  2. **COURIER LABEL:** Individual site’s address entered in the “Sender” area and the central storage facility’s address in the recipient area
  3. **SHIPPING LABELS:** Required to communicate the contents of the package to the courier
     1. Fragile label
     2. UN 3373 (Category B)
     3. “This side up” arrow labels
     4. Class 9 (dry ice)

1. **BIOSPECIMEN COLLECTION**

**See Appendix 2 for detailed schematic which depicts serum preparation.**

1. **GENERAL Collection INFORMATION**
2. Prepare collection kit prior to sample collection and follow Universal Precautions at all times.
3. All collection tubes and cryovials should be labeled prior to drawing subject’s blood. See Appendix 5 for examples of all labels.
   1. Label all collection tubes with two labels: Subject ID label and Collection Tube/Cryovial Labels
      1. Write Subject ID and Site ID on Subject ID label for 10ml Serum tube using Bio-pen and place label on container.
      2. Collection Tube/Cryovial Labels contain preprinted study name, sample type, specimen number, and kit number.
         1. Specimen number uniquely identifies that specimen.
         2. Kit number ties all samples collected from one subject at one visit together.
4. Label all cryovials with the Collection Tube/Cryovial labels only.
   1. Red capped cryovials for serum collection.
   2. Place label with the barcode toward the top of the cryovial.
5. Track the patient closely and obtain the blood draw as soon as possible (within 24 hours of injury for acute sample).
   1. Using a blood collection set, collect blood into the 10ml Serum tube using your institution’s recommended procedure for standard venipuncture technique. Collect blood as soon as possible after injury.

The following techniques shall be used in order to prevent possible backflow:

* + 1. Place donor’s arm in a downward position.
    2. Hold tube in a vertical position below the donor’s arm during blood collection.
    3. If no other blood tubes are being drawn, release tourniquet as soon as blood starts to flow into tube.
    4. Make sure tube additives do not touch the stopper or the end of the needle during venipuncture.
  1. 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’s vacuum is designed to draw appropriate volume of blood into the tube.
  2. Immediately after blood collection, invert tube 10 times to ensure complete mixing of whole blood and tube additives.
     1. An inversion is one complete turn of the wrist (180 degrees) and back.

1. **BIOSPECIMEN PROCESSING**

### GENERAL Processing INFORMATION

1. 30 minutes after blood collection, or when blood has completely clotted, centrifuge the balanced tubes for 15 minutes at 1500 rcf (xg) at room temperature. **It is critical that the tubes be centrifuged at the appropriate speed and temperature to ensure proper serum separation.** Refrigeration prior to centrifugation is not permitted.
2. Remove the serum, being careful not to agitate the packed blood cells at the bottom half of the collection tube, by tilting the tube and placing the pipette tip along the lower side of the wall without touching red blood cells so that serum is not contaminated by cell material.
3. Using one of the provided disposable graduated transfer pipettes, transfer serum into the pre-labeled cryovials.
4. Aliquot 0.5 ml per cryovial (total vials = 8-10 with 0.5ml each).
   1. The serum tube should yield, on average, half the initial blood volume of serum for a total of 8-10 2ml cryovial tubes per subject with 0.5 ml serum per cryovial tube.
   2. In order to ensure the central storage facility 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 0.5 ml. Over-filled tubes may burst once placed in the freezer, resulting in a loss of that sample.
   3. If there is biological material remaining that will not fill a subsequent cryovial, that remaining amount should still be included and shipped to the central storage facility.
      1. For example, if 2.7ml of sample is obtained, you should fill 5 cryovials with 0.5ml, and one additional cryovial tube with the remaining 0.2ml.
5. Be sure to place serum in the cryovials with red caps labeled with the “serum” labels. Take caution not to disturb red blood cells at the bottom half of the tube.
6. After all serum has been removed, dispose of collection tube according to your site’s guidelines for disposing of biomedical waste.
7. Place the labeled cryovials in the 25 cell cryobox **in numerical order according to the specimen barcode** and place upright on dry ice. Do not put multiple subjects’ samples in the same cryobox.
8. Transfer cryobox to -80°C freezer and store all samples upright at -80°C until shipped on dry ice.
9. Fill out all appropriate fields on the specimen log.
10. **BIOSPECIMEN PACKAGING AND SHIPPING INSTRUCTIONS**
11. **GENERAL Collection INFORMATION**

Dry ice sublimates immediately upon contact with ambient temperatures, do not initiate biospecimen packaging procedures until all the following conditions have been met:

* 1. The adequate number of subject specimens has been collected.
  2. The packaging and shipping materials are at hand and have been prepared.
  3. The courier (i.e. FedEx) has been contacted for pickup.
  4. **Dry Ice should be in** **pellet form!** Chunks/blocks of dry ice can crush specimens and break shipping packaging.

Specimens of this type are typically considered as Class 6.2 Category B UN3373 and as such must be triple packaged and compliant with IATA Packing Instructions 650. (See Appendix 3)

Triple packaging consists of a primary receptacle, a secondary receptacle and a rigid outer shipping packaging. The primary receptacles must be packed in a secondary receptacle in such a way that, under normal conditions of transport, they cannot break, be punctured or leak their contents into the secondary packaging. Secondary receptacles must be secured in outer shipping packaging with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or outer packaging.

1. **PACKAGING AND LABELING GUIDELINES**
   * + 1. The primary receptacle (cryovials) must be leak proof and must not contain more than 1 L of total fluid.
       2. The secondary receptacle (biohazard bag) must be leak proof.
       3. Absorbent material must be placed between the primary receptacle (cryovials) and the secondary receptacle (biohazard bag). The absorbent material should be of sufficient quantity to absorb the entire contents of the specimens being shipped. Examples of absorbent material are paper towels, absorbent pads, cotton balls or cellulose wadding.
       4. A shipping manifest of specimens being shipped must be included between the secondary receptacle and outer shipping packaging.
       5. The outer shipping packaging must display the following labels:
          1. Sender’s name and address
          2. Recipient’s name and address
          3. Responsible Person
          4. The words “Biological Substance, Category B”
          5. UN3373
          6. Class 9 label including UN 1845, and net weight of dry ice contained

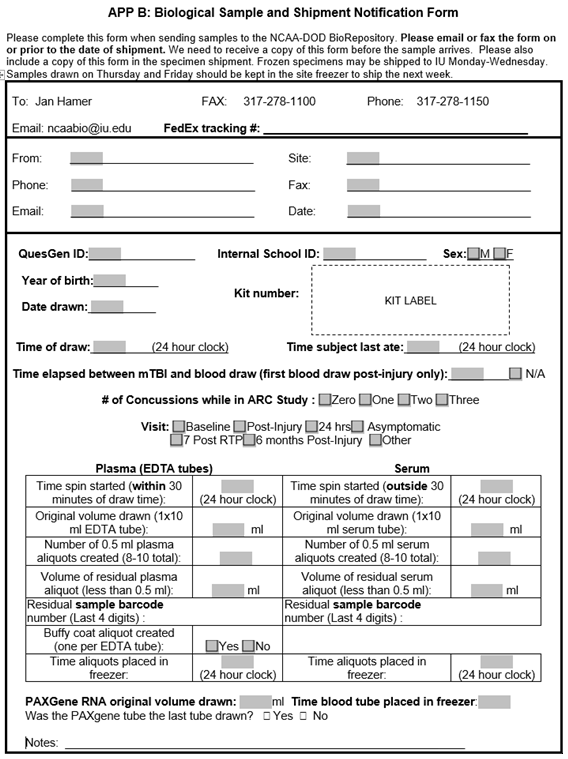
Weight of dry ice on UN1845 label must match weight recorded on courier label.

**This link describes the differences between categories A, B, and Exempt substances:** [**https://www.ups.com/content/us/en/resources/ship/hazardous/biological\_substances.html**](https://www.ups.com/content/us/en/resources/ship/hazardous/biological_substances.html)

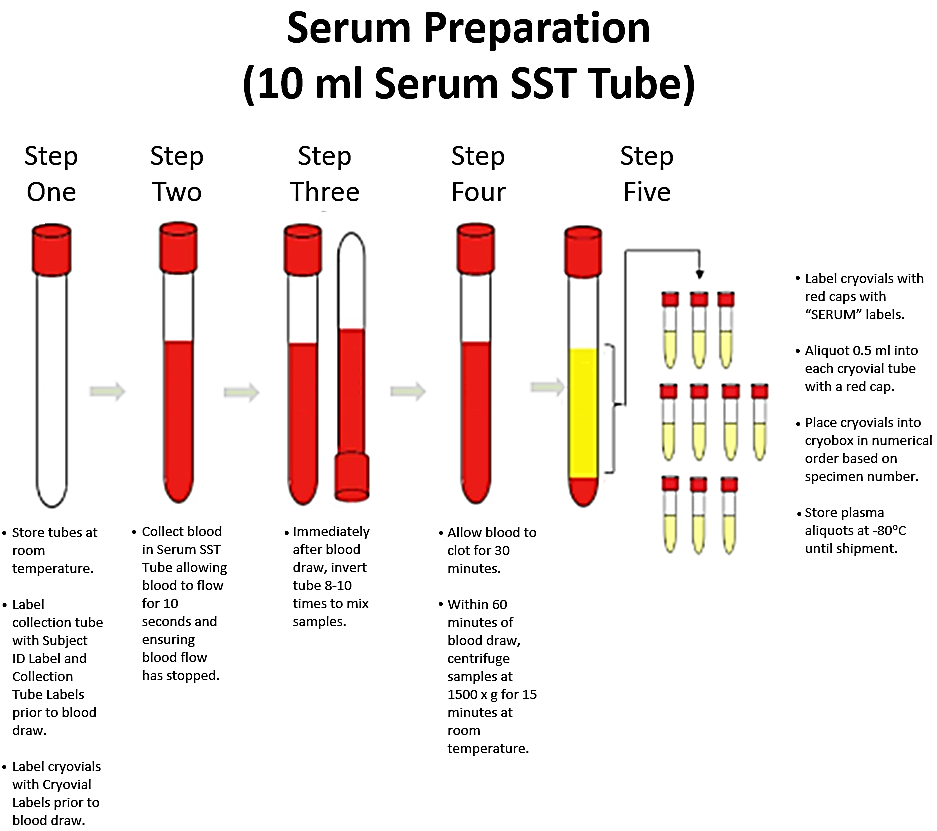
1. **PACKAGING AND SHIPPING INSTRUCTIONS**
2. Contact courier to confirm that service is available, confirm the location of the pickup, and schedule package to be picked up.
3. Notify Intended Recipient of shipment, including a copy of each Biological Sample and Shipment Form (Appendix 1).
4. Place all frozen, labeled aliquots of serum in the 25 cell cryobox.
   1. A cryobox should contain all of one subject’s aliquoted samples per collection.
   2. Cryoboxes should contain all of the specimens from the same subject, per collection.
   3. Batch shipping should be performed quarterly or when enough collections have been performed to fill a shipping box, whichever is sooner.
      1. Too many samples and not enough dry ice can compromise the samples. It is safer to send too few than to pack a shipping container too full of specimen as is could cause samples to thaw.
5. Label the lid of the cryobox with a kit number label. Place the cryobox in the secondary receptacle (remember to include the absorbent material) and ensure a tight seal on the secondary receptacle.
6. Place ~2-3 inches of dry ice in the bottom of the Styrofoam-lined shipping container.
7. Place the biohazard bag into the provided Styrofoam-lined shipping container. Cryoboxes must be placed upright so the cryovials remain upright.
8. Fill the remaining space in the Styrofoam carton with dry ice, ensuring ice surrounds and covers the cryoboxes and reaches the top of the carton.
9. The dry ice should entirely fill the inner Styrofoam box to ensure the frozen state of the specimens.
10. Replace the lid on the Styrofoam carton, place the completed Biological Sample and Shipment Notification Form (Appendix 1) in the package, and close and seal the outer cardboard shipping carton with packaging tape.
11. **Do not tape the Styrofoam box closed** as dry ice must be allowed to sublimate during shipment. Taping the lid of the Styrofoam would cause the box to be airtight, which could cause the box to explode during shipment.
12. Complete the courier return airbill with the following information:
    1. Fill in your name, address and phone number as Sender
    2. When asked if this shipment contains dangerous goods, check the boxes for “Yes, Shipper’s Declaration not required” and “Dry Ice”. Enter the number of packages (1) x the net weight of dry ice in kg (must match amount on the Dry Ice label).
13. Complete the Class 9 UN 1845 Dry Ice label (Appendix 4) with the following information:
    1. Your name and return address
    2. Net weight of dry ice in kg (must match amount on the airbill)
    3. Consignee name and address
    4. Do not cover any part of this label with other stickers, including pre-printed address labels.
14. Apply all warning labels and the completed return airbill to the outside of the package, taking care not to overlap labels.
15. Hold packaged samples in -80°C freezer until time of courier pick-up/drop-off. Do NOT package samples if you are unable to ship the same day. There is not sufficient dry ice in the above directions for storing samples multiple days outside of a freezer.
16. Specimens should be sent Priority Overnight. Shipments should be sent Monday through Wednesday to avoid shipping delays. Most couriers do not replenish dry ice if shipments are delayed or held over during the weekend.
17. Use courier tracking to ensure the delivery occurs as scheduled and is received by intended recipient.

**ATTACHMENTS**

Appendix 1: Biological Sample and Shipment Notification Form (CARE Consortium example)

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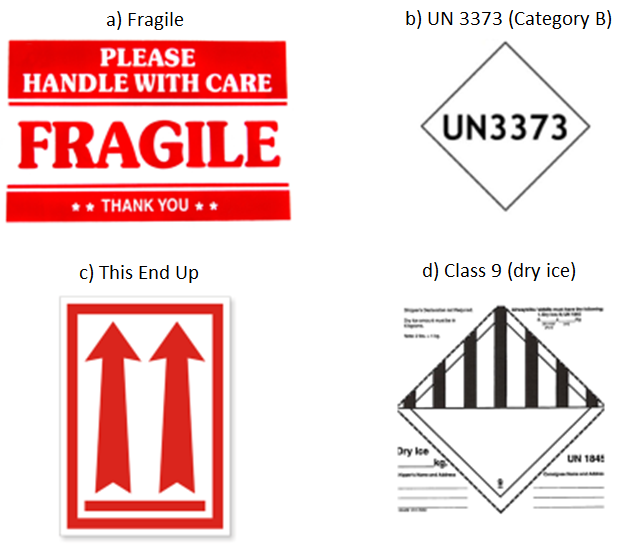
Appendix 2: Serum Preparation Schematic



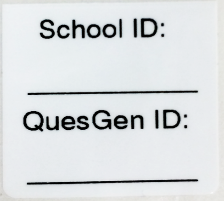
Appendix 3 SUMMARY OF SHIPPING INFORMATION

|  |  |  |
| --- | --- | --- |
| Dry Ice | Category B infectious Substance | Shipment Type |
| Dry Ice  or  Carbon Dioxide, solid | Diagnostic specimens or  Clinical specimens | Proper Shipping Name |
| UN 1845 | UN 3373 | UN Number |
| 9 | 6.2 | Hazard Class |
| III | - | Packing Group (PG) |
| 904 | 650 | Packing Instruction (PI) |
| 200 kg | 4 L or 4 kg | Max. Net qty./pkg. for Passenger Aircraft |
| 200 kg | 4 L or 4 kg | Max. Net qty./pkg/ for Cargo Aircraft |

Appendix 4 SHIPPING LABELS (EXAMPLE)

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Appendix 5 SPECIMEN LABELS (SUMMARY & EXAMPLE)

* 1. **Kit Label**
     1. Number assigned to all patient samples for one visit.
     2. Included on the Biological Sample and Shipment Notification Form, the CRF and the lid of the cryobox.
  2. **Subject ID Label**
     1. ID assigned to the subject that connects visits from one patient.
     2. ****Subject ID is typically handwritten onsite using Bio-Pen and should be included on each Biological Sample and Shipment Notification Form and CRF.
     3. In the example on the right, two Subject ID’s are provided. One unique to the site and one unique to the subject.
     4. Placed on collection tubes only and not cryovials that will be distributed to other laboratories.
  3. **Collection Tube/Cryovial Labels**
     1. Each individual Collection tube and cryovial should be assigned a unique barcode number.
     2. Each label should also include the Study Abbreviation, Sample Type, and Kit Number.