ABG Draft
Revised 9/26/96

PROTOCOL FOR OBTAINING ARTERIALIZED BLOOD GAS

1. Introduction:

Several smaller studies have demonstrated that acid-base status in postmenopausal women affects bone turnover and calcium excretion. As all people age, renal function gradually deteriorates, leading to a very mild metabolic acidosis. It appears that bone is then called upon to buffer this acid load.

While the gold standard measurement is an arterial blood gas, acid-base status can be accurately determined by obtaining an arterialized venous blood sample. Small shunts exist normally between arterioles and venules. In this procedure the participant's hand is warmed long enough for these small shunts to open. At that point there is enough mixing of arterial and venous blood locally that a venous sample very closely correlates with arterial values for pH, carbon dioxide (CO_2), and bicarbonate (HCO_3).

The objectives for this part of the examination are:

a) Obtain arterialized venous blood in a syringe for determination of pH, pCO_2, pO_2, and bicarbonate HCO_3;
b) Obtain serum for an archived sample;
c) Freeze and store archived serum at -20°C until one storage box is filled and then ship to BRI.

2. Equipment:

Gloves, disposable, non-sterile
Horizontal centrifuge
Non self-defrosting freezer, -20°C
Dry ice
Plastic disposable transfer pipettes (Fisher #13-711-5A)
Cryotube storage boxes (100 cell); 100 cell cryotube inserts
Insulated shipping boxes for sending cryotubes to BRI
Tourniquets
21 gauge butterfly needles (Vacutainer #367281)-19 gauge may be used in participants with appropriate veins-makes blood draw easier
T connector extension with heplock (Baxter #2N3326)
Alcohol wipes (Kendall Alcohol Prep #6818)
2X2 gauze pads, sterile (Kendall Gauze Sponges #1806)
Radiometer SE 1403 kit-non-vented, non-aspirated-contains 5cc waste syringe and 3cc heparinized syringe-1 per participant
10 cc syringes (B-D #301604)-1 per participant
Band-aids
Tape (3M Micropore, 1" or 1/2")
3cc syringes (B-D #309586)-2 per participant
Sodium heparin 100 USP units/ml for heparin flush-4 ml per participant
21 gauge straight, hollow needles to draw heparin into 3cc syringes-2 per participant
7cc SST tube (B-D #6517)-one per participant
2.0 cc cryotubes (Applied Scientific #AS-2305)-3 per participant V12 cohort, 2 per participant all other participants
Cup of regular ice
Microtemp 2000 T-pump
16" X 21" Seabrook reusable heating pad

ABL5 Blood Gas Machine with one red and one yellow QC control ampoule per participant
Thermometer for taking participant's temperature

3. Procedure:

A. PREPARATION

- Heating pad should be cleaned between uses with a surface cleaner and disinfectant such as Cavicide Hospital Disinfectant and Decontaminant.
- Thirty minutes before the first participant arrives, attach the reusable pad to the heating pump and plug it in. It has an internal thermostat set to 42°C and will automatically warm to this temperature. The pad will take approximately 30 minutes to warm to this temperature.
- Prepare the heparin flush syringes by drawing up 200 units (2cc of 100 USP units/ml) sodium heparin into each of the 3cc heparin flush syringes. Set the heparin flush syringes aside for use later.
- Explain procedure to participant. Suggested text: "As women get older, the amount of acid in their blood rises very slightly. This may contribute to thinning of the bones. To look at the amount of acid in your blood, we need to draw the blood in a special way. We'll put a regular blood drawing needle in a vein in your hand and then warm your hand in a heating pad for 15 minutes before drawing the blood."
- Take participant's temperature. Record temperature on "Arterialized Venous Blood Gas" data collection form.

B. VENOUS ACCESS:

- Wash hands thoroughly. Put on gloves
- Place a preprinted label showing the participant's SOF ID code on the SST red top tube and the pre-heparinized (contains dry heparin) 3cc syringe from the SE 1403 kit. It is essential to then check the ID code on the tube and the syringe to ensure that the specimen being collected belongs to the participant. This can best be done by holding the tube and syringe next to the ID number on the participant's chart and calling out the number. Then ask the participant to say her name aloud and verify it against the name on the chart.
- Place tourniquet on participant's forearm and, after cleaning skin with alcohol wipe, insert butterfly catheter into a dorsal hand vein with heplock at end. Insertion site must be at or distal to the wrist. If venipuncture is difficult, you may warm the participant's hand (using heating pad if you like) to make veins more prominent.
- Secure the butterfly in place on the participants's hand with tape and cover with tegaderm.
- Attach the 10 cc syringe to end of butterfly and draw 7 cc's of blood for an archived sample. Place it in red top SST tube for serum prep. See Part E for archiving steps.
- Leaving butterfly in place, remove the tourniquet now. The arterialized blood gas will be drawn later without a tourniquet.
- Flush IV with 200 units (2cc of 100 USP units/ml) of sodium heparin flush using one of the 3cc heparin flush syringes prepared earlier.

- Wrap hand and forearm in heating pad which has been warmed to 42°C as snugly as possible, leaving no air between hand and pad. The pad may be secured **loosely** with tape or string wrapped around the outside of the pad. **Caution should be used to ensure that the tape or string is not snug enough to alter blood flow in the participant's arm.** The end of the heplock should extend out the front of the pad past the edge of the pad which covers the fingers.

- Wait fifteen minutes.

- Leave the hand wrapped in the heating pad. Using the second 3 cc heparin flush syringe prepared earlier, inject 100 units (1 cc of 100 USP units/ml) sodium heparin into the end of the IV.

- Withdraw 3-5cc from the IV using the waste 5cc syringe from the SE 1403 kit. Discard this syringe of blood/heparin mixture-this is to ensure that all the heparin has been withdrawn from the IV line.

- Attach the pre-heparinized (contains dry heparin) 3cc syringe from the SE 1403 kit to the IV.

- Withdraw 3cc of blood. Expell any air from the syringe. Cap syringe immediately and place on ice. The minimum amount of blood needed for blood gas analysis is 1cc but 3 cc is best for optimal analysis.

- Unwrap hand and remove butterfly. Apply band-aid to venipuncture site.

- Heating pad should be cleaned between uses with a surface cleaner and disinfectant such as Cavicide Hospital Disinfectant and Decontaminant. It does not have to be unplugged or cooled before cleaning. After the last participant of the day, heating pad should be unplugged, cleaned and stored for next use.

QC Checklist for arterialized blood gas protocol

- ☐ Heating pad warmed
- ☐ Prepare heparin flush syringes
- ☐ Explain procedure to participant using correct text
- ☐ Take participant's temperature and record
- ☐ Wash hands, put on gloves
- ☐ Label SST tube and blood gas syringe
- ☐ Insert butterfly into dorsal hand vein
- ☐ Draw archive serum sample
- ☐ Heparinize IV
- ☐ Warm hand in heating pad
- ☐ Wait 15 minutes
- ☐ Draw off waste
- ☐ Draw arterialized venous blood gas
- ☐ Heating pad cleaned for next participant

C. CARE OF THE ABL5 BLOOD GAS MACHINE

I. Preparing the ABL5 for sample measurement:
(This can be done in the morning before participants arrive)

- Remove from "STANDBY"- In order to save on supplies such as calibrating solutions and gases, you will place your ABL5 into the STANDBY mode at the end of each day it is used. The procedure for placing the ABL5 into standby is described below. When in the STANDBY mode only a Cal 2 is performed every 4th hour to keep the analyzer ready for use at a short notice. When you arrive in the morning, you must remove it from the STANDBY mode.

- You should see "STANDBY" in the upper left hand corner. To exit the STANDBY mode, press the softkey under the word "Exit" in the lower right hand corner. This causes the machine to perform a CAL 1 procedure ("CAL 1" will show in the upper right corner while it is calibrating. After 2 3/4 minutes you should see a reading of the Cal 1 pH, a "READY" message (upper left), and a green light to the left of the target sign. The ABL5 is now ready for action.

II. Daily Maintenance: to be done, recorded and initialed each day on the "Maintenance Schedule for ABL5"

- Check solution levels- Because of the design of these bottles, almost all the solution will be used before it is necessary to install a new bottle. See page 7.2.5 of Operator's Manual or page 8 of Short Form Instructions for changing solution bottles.

- Check thermal paper supply:- Replace when low. Instructions on page 7.2.1 of Operator's Manual or page 15 of Short Form Instructions.

- Check pressure in gas cylinders- It is not empty until the indicator is below the green (50 psi). (see pg 7.2.6 of Operator's Manual for instructions on changing the gas tanks). They should last about 6 months.

- Examine/clean inlet: Wipe off any blood. If very dirty, see page 7.2.6 of Operator's Manual.

- Check waste level in container- If full, replace. See Page 7.2.6 of Operator's Manual or page 14 of Short Form Instructions for replacement directions.

- Perform a Cleansing program- (page 6 - Short Form Instructions)
  1. You will need the Cleaning Solution (S5332). Fill a syringe with at least 15 ul (microliters) of cleaning Solution.
  2. Press Menu softkey
  3. Press 2 on the keyboard (Maintenance). A new menu will appear.
  4. Press 2 on the keyboard (Cleansing)
  5. When prompted by the display, open the inlet to the syringe/test tube position (#1)
  6. Once you have opened it, the display will tell you to aspirate the cleaning solution (#5332) from the syringe.
  7. Push the aspirate button momentarily, and let the machine aspirate the Cleaning Solution until it beeps.
  8. Remove the syringe, wipe the inlet, and close it.
  9. The analyzer will perform a cleansing, then a rinse cycle and return to the "READY" mode after a few minutes.

- Perform a 2-Point Calibration-
  1. Hit the "Menu" softkey. Choose "1" (Calibration), then "2" (Cal 2). The machine will begin a 2-point calibration. It will take about 5 1/2 minutes to perform a 2-point calibration.
  2. When done, you can check the calibration status by hitting "Menu" softkey, then "4" for System Status, then "2" for Cal status The first page will show you the time of the last calibration. Hit "Page" softkey for the pH electrode status. There are two pH readings because it is a 2-point calibration. Hit "Page" and you will see the pCO2 electrode status, then "Page" again to see the pO2 electrode status. Things to look for are the sensitivity readings for the electrodes and the "zero" reading for the pO2 electrode.

1) Sensitivity (pH) 92-102%
2) Sensitivity (pCO_2) 85-100%
3) Sensitivity (pO_2) 5-40 mmHg
4) Zero: (pO_2) < 6 mmHg

3. You can print this by hitting the "Menu" softkey. Choose "5" (Print/Send), then "2" (Print System Status).

III. Shut down of ABL5 at the end of the day- to be done after all samples have been analyzed and any weekly or monthly maintenance has been performed and the machine has returned to a "READY" mode.

- Hit Menu softkey.
- Hit "3" on the keyboard (Utilities)
- Hit "1" on the keyboard (Standby)

QC Checklist for Preparing Blood Gas Machine

❒ Take machine off stand-by
❒ Perform daily maintenance
❒ Perform cleansing program
❒ Perform 2-point calibration
❒ Place machine into "Stand-by" mode

D. ANALYZING ARTERIALIZED BLOOD GAS SAMPLE

I. The Importance of Quality Control

We are going to use two levels of a quality control system, QUALICHECK, to evaluate the performance of the ABL5 by comparing measurements of test solutions to predetermined values. These quality control solutions are a valuable tool in detecting possible errors in blood measurement and in checking the precision and accuracy of the systems.

Each center will be testing the same lot numbers of the Yellow QUALICHECK (S2040) and the Red QUALICHECK (S2030) every time a patrticipant sample is analyzed. By looking at the recorded values of pH, pCO_2 and pO_2 values for the Yellow QC and Red QC, we will have a reference point for the participant values obtained. Only in this way will we be able to compare the values obtained at each center to those obtained at the other centers.

It is of the utmost importance that these instructions are followed to the letter each and everytime a participant sample is analyzed.

Store all ampoules of Qualichek at constant room temperature (18 to 30 degrees Centigrade).

II. Check the following items:
1. Is machine out of "Standby?"
2. Have you performed the "Daily Maintenance?"

3. Is the ABL5 in "READY" mode?
4. Green light on?
5. Do you have the following equipment ready:
   Yellow Ampoule (S2040) - unopened
   Red Ampoule (S2030) - unopened
   Participant's arterialized venous blood gas sample (in syringe from SE 1403 kit-on ice)
   Gauze pads
   "Arterialized Venous Blood Gas" Data Collection Form for the participant

III. Remember to save all the tape readouts for the yellow QC, the participant values, and the red QC. These will be an additional backup for the data collection forms on which you will record the values. Tape readouts should be stored at each clinic.

IV. Running the Yellow QC Ampoule

- One yellow ampoule will be analyzed in duplicate before each participant sample.
- Remember, you have 30 seconds after the inlet is opened to introduce the QC solution or the blood to the inlet needle and begin aspiration.
- Hold the ampoule between your thumb and index finger with thumb and index finger on the ends of ampoule and shake vigorously for at least 15 seconds. Do not hold the ampoule by the body of the ampoule as this will change the temperature of the control solution, and the results produced.
- Tap the top of the ampoule until all the solution collects at the bottom of the ampoule. Be sure no solution remains in the neck of the ampoule.
- The following steps will be done on each yellow ampoule and then repeated (except for opening the ampoule) on the same yellow ampoule before analyzing the participant's blood gas.
- Open the inlet one stop (for syringe).
- Hit the "QC" softkey. The display will say "aspirate sample QC."
- Open the ampoule by holding it in gauze and cracking open at the neck.
- Immerse the inlet into the control solution so that it nearly reaches the bottom of the ampoule.
- Supporting the ampoule in the gauze pad, press the Aspirate button (target) momentarily. Hold the ampoule in the inlet until a **beep** is heard and the yellow LED lights.
- Remove the ampoule, wipe the inlet with the gauze, and **close** the inlet.
- Enter the information on the QC identification screen.
  1. The cursor is placed on the first data line. To move the cursor, use the ENTER key.
  2. **Op ID** - you can identify the operator- optional. Hit ENTER.
  3. **Type** - hit ENTER since you will be using QUALICHECK.
  4. **Temp** - hit ENTER since room temperature is 25°C.
  5. **Level** - since this is the **Yellow** level, hit the "Change" softkey until **YELLOW-S2040** shows; then hit ENTER.
- The results are displayed on the screen and printed out on the tape. The reading is identified as a Yellow level reading.

- **The first reading (pH, pCO2, and pO2) of the day on the Yellow control will also be your QC reading for that day to be plotted in your logbook.**
- This first reading (pH, pCO2, and pO2) will also be recorded on the first participant's data collection form as **Yellow Control #1.**
- Repeat the above steps, from opening the inlet to printing out the results, on the same (already opened) **Yellow** ampoule. Record the results (pH, pCO2, and pO2) as **Yellow Control #2.**
- If Yellow Quality control values are not in the manufacturer's given ranges, see section VII below.

V. Running the Participant Sample: **(Always wear gloves)**

- The participant sample should be in a 3cc preheparinized syringe, which has been kept in ice after the sample was drawn. The ice will slow down cell metabolism and keep the blood gas readings constant for about 20 minutes.
- You should already have expelled any air bubbles which are in the syringe. Air in the capped syringe is a potential source of error.
- The following steps will be done in sequence and then repeated twice for a total of three times on each participant sample.
- The sample in the syringe needs to be mixed well with the anticoagulant after being drawn from the participant.
- Mix the sample well for at least 15 seconds by inverting the syringe repeatedly and rolling the syringe between your hands.
- Remove the cap. Expell a few drops of blood into a piece of gauze, and withdraw the plunger slightly.
- Open the inlet. "Blood" should already be selected.
- Insert the inlet into the syringe well below the surface.
- Press the **Aspirate** button momentarily. **Wait** for the yellow LED to light and the short **beep** to sound. This tells you that the sample has been received.
- **Remove** the syringe, **wipe** with the gauze, and **close** the inlet.
- Expell any air from the syringe, re-cap the syringe, and place back on ice.
- **Enter** the participant information on the screen.
  1. The Cursor is positioned at the Op ID. Press **ENTER** to move it to the next date line.
  2. **Pt ID** - input the participant's SOF ID #, then press **ENTER.**
  3. **Type** - "Mixed Venous" is already there. Just push **ENTER.**
- You can skip the rest of the ID keys. The temperature on the machine is set to 37°C-leave it at this temperature.
- The Patient Report will appear on the screen and will be automatically printed. You should record the **pH, pCO2, pO2, and HCO3** on the Data Collection Form as Participant Reading #1.

- As soon as the "READY" sign comes back on and the green LED light is lit, repeat the above steps, from mixing the sample to printing out the report **two more times.** These will be Participant readings #2 and #3. Record them on the Data Collection Form in the appropriate spaces.

VI. Running the Red QC Ampoule

- One red ampoule will be analyzed in duplicate after each participant sample.
- Hold the ampoule between your thumb and index finger with thumb and index finger on the ends of ampoule and shake vigorously for at least 15 seconds. Do not hold the ampoule by the body of the ampoule as this will change the temperature of the control solution, and the results produced.
- Tap the top of the ampoule until all the solution collects at the bottom of the ampoule. Be sure no solution remains in the neck of the ampoule.
- The following steps will be done in sequence on each red ampoule and then repeated (except for opening the ampoule) on the same red ampoule after analyzing the participant's blood gas.
- Open the inlet one stop (for syringe).
- Hit the "QC" softkey. The display will say "aspirate sample QC."
- Open the ampoule by holding it in the gauze and cracking open at the neck.
- Immerse the inlet into the control solution so that it nearly reaches the bottom of the ampoule.
- Supporting the ampoule in the gauze pad, press the Aspirate button (target) momentarily. Hold the ampoule in the inlet until a **beep** is heard and the yellow LED lights.
- Remove the ampoule, wipe the inlet with the gauze, and **close** the inlet.
- Enter the information on the QC identification screen.
  1. The cursor is placed on the first data line. To move the cursor, use the ENTER key.
  2. **Op ID** - you can identify the operator- optional. Hit ENTER.
  3. **Type** - hit ENTER since you will be using QUALICHECK.
  4. **Temp** - hit ENTER since 25 degrees is room temperature.
  5. **Level** - hit ENTER since **Red-S2030** is already showing.
- The results are displayed on the screen and printed out on the tape.
- This reading (pH, pCO2, and pO2) will be recorded on the participant's data collection form as **Red Control #1.**
- Repeat the above steps, from opening the inlet to printing out the results, on the same (already opened) **Red** ampoule. Record the results (pH, pCO2, and pO2) as **Red Control #2.**
- Keep reports which print out with the control and participant values on record at each clinic site.
- If Red Quality control values are not in the manufacturer's given ranges, see section VII below.

QC Checklist for Analyzing Participant Sample

- ❒ Analyze yellow control ampoule in duplicate
- ❒ Analyze participant sample in triplicate
- ❒ Analyze red control ampoule in duplicate

VII. Quality Control Ranges: what to do if your values are not in range.

- All QC readings should fall within the ranges specified with the control material. You will receive a chart listing these ranges so that you can check to see that your QC data for both the Yellow and Red levels are within range. If your values are not within range perform the following steps. For participants whose control values fall out of the manufactuer's range, record the values obtained during this section on the "Arterialized Venous Blood Gas--Supplemental" data collection form.

- If one or more componants of Yellow or Red QC readings are incorrect:

1. Repeat the reading of the QC on the previously opened yellow or red ampoule. If OK, continue with analysis of participant sample. If still incorrect, continue with instructions below.

2. Check to see that there is plenty of each solution, and the gas indicator is in the green zone. Perform a 2-point calibration (see section II above), then open a new yellow or red QC ampoule and repeat the protocol section on running that color of ampoule. If OK, continue with analysis of participant sample. If still incorrect, continue with instructions below.

3. Clean electrode holes and electrode connections. Perform a 2-point calibration (see section II above), then open a new yellow QC ampoule and repeat the protocol section on running the yellow ampoule. If OK, continue with analysis of participant sample. If still incorrect, continue with instructions below.

4. If the problem lies with the O2 value, brush the O2 electrode and remembrane (see operator's manual for instructions). Perform a 2-point calibration (see section II above), then open a new yellow QC ampoule and repeat the protocol section on running the yellow ampoule. If OK, continue with analysis of participant sample. If still incorrect, record the readings obtained on the data collection form, run the participant sample and record these readings on the data collection form. Call the Radiometer Technical Service Advisor.

5. If the problem lies with the CO2 value, remembrane the electrode (see operator's manual for instructions). Perform a 2-point calibration (see section II above), then open a new yellow QC ampoule and repeat the protocol section on running the yellow ampoule. If OK, continue with analysis of participant sample. If still incorrect, record the readings obtained on the data collection form, run the participant sample and record these readings on the data collection form. Call the Radiometer Technical Service Advisor.

6. If the problem lies with the pH, remembrane the reference electrode and clean the pH electrode with distilled water. Perform a 2-point calibration (see section II above), then open a new yellow QC ampoule and repeat the protocol section on running the yellow ampoule. If OK, continue with analysis of participant sample. If still incorrect, record the readings obtained on the data collection form, run the participant sample and record these readings on the data collection form. Call the Radiometer Technical Service Advisor.

- If there are "?" in your participant results or an Error is displayed:

  1. Check "System Status." Push Menu softkey, than push 4 (System Status), and then 1 (Records).

  2. The Records screen will tell you where the problem lies. refer to the "Key to Record Codes" (page 8.3) in your Operator's Manual, then refer to pages 8.4 to 8.9 for ways to remedy the specific problem.

E. BLOOD PROCESSING OF SST TUBE

- Allow the filled SST tube to stand at room temperature for at least 30 minutes but for no more than 60 minutes. This procedure is necessary to allow an adequate clot to form.

- After clot formation, balance the tubes of blood for centrifugation. Use a horizontal centrifuge; angle heads are not satisfactory.

- Centrifuge the blood for 10 minutes at room temperature at a setting known to yield a relative centrifugal force (RCF) of at least 1000 x g at the bottom of the tubes. The table below gives those combinations of centrifuge speed in revolutions per minute (rpm) and rotating radius (r) that will yield and RCF value of 1000 x g. RPM should be read from a tachometer or rev counter when the centrifuge is normally loaded. Radius (r) is measured in centimeters from the center of the rotor shaft to the bottom of the vacutainer tube when the tube is in a horizontal position.

r (cm)	12	14	16	18	20	22.5	26
rpm	2800	2600	2400	2250	2100	2000	1900

  Do not use a brake to slow down the centrifuge.

- Label each 2 cc cryotube (3 per V12 participant, 2 per all other participants) with the SOF ppt ID. Use a pen with permanent ink such as a "Sharpie." Keep the labeled cryotubes away from solvents such as alcohol or acetone as these will erase the ID code. Before transferring the serum, the red-top tube and cryotubes should be held side by side and the numbers read aloud to check that the ID code numbers match. <u>Do not set up production lines of labeled empty cryotubes.</u> The chance of error is increased by the latter procedure.

- Transfer the serum with a pipette to the labeled 2 cc cryotubes. Fill each cryotube to just above the 1 cc line that is already marked on the tube. If there is not enough serum to fill the tube to the 1 cc line, fill it as much as possible.

- If a serum sample is reddish in color, determine if it is hemolyzed or simply contaminated with red blood cells. One can tell the difference by recentrifuging the vacutainer tube. This will pellet any contaminating red cells and the serum will be clear. If the sample is hemolyzed, the red color will remain in the serum. If the participant is still in the clinic another SST tube should be obtained. Otherwise, the hemolyzed sample should be processed.

- Some caution should be used in capping the cryotubes. Screw the caps on firmly to secure them tightly, but do not apply an extreme amount of pressure. To promote rapid freezing, place the cryotubes upright in a footless metal rack that is in contact with a shelf in a -20°C freezer.

<u>QC Checklist for SST Tube Processing</u>

❒ Allow tube to sit for 30-60 minutes
❒ Balance tubes in centrifuge

- ❒ Centrifuge for 10 minutes
- ❒ Label cryotubes with "Sharpie"
- ❒ Pipette serum into cryotubes

F. TEMPORARY CRYOTUBE STORAGE AND SHIPMENT

- After samples have been frozen by placing cryotubes upright on a -20°C shelf overnight, place cryotubes in ID numerical order into a storage box using the inserts.
- Use the cryotube storage box grid for recording the position of cryotubes, by ID number, within the shipping boxes sent to BRI. (This is a back-up identification system in case the ID numbers on the tube are obliterated after prolonged storage at -70°C.) As the filled tubes are placed into the slots formed by inserts, write the ID number which is on the tube into the corresponding box on the paper grid.
- Since the box does not have a definite up or down, right or left, you will have to mark the upper right corner of the cardboard box and the insert. (The paper grid is already marked "upper right" and "upper left.") Orient the box so that the oval holes along the bottom of the box are facing toward you on one side and away from you on the other side. In a clearly visible spot in the upper right corner of the box and the insert (to the right and away from you), punch a hole in the cardboard with a single hole paper punch.
- Store samples at -20°C in the storage box until 100 cryotubes have been filled and frozen.
- When the box is ready for shipping, record on the grid form the dates over which the samples were collected, your clinic, the date the box was shipped to BRI, and the number of tubes being shipped.
- Send one copy of the form to BRI with the box and keep one copy yourself.
- When the box arrives at BRI it will be assigned a unique identifier and placed into storage. A copy of the grid with the identifier will be sent to the coordinating center.
- A box of samples should be shipped in an insulated shipping box on dry ice by a 24-hour air carrier (such as Federal Express). To ensure that the samples can be received at BRI the next day, ship Monday-Wednesday only. The insulated shipping box and carrier can be chosen by each Center. However, the shipping box must provide insulation and have inner dimensions sufficiently large to handle 1 storage box (outside dimensions 5.25 x 5.25 x 4.75 inches) and 5-10 lbs of chipped dry ice to keep the samples cold even if shipment is interrupted for a day or two. (If in doubt, err on the side of too much dry ice.)
- Serum should be shipped to:

  Don Dover
  Biomedical Research Institute
  12264 Wilkins Ave., Bay E
  Rockville, Maryland 20852
  301/881-4513
  301/770-9811 (F)