Chapter 13
Collection of Blood Samples

Collection of blood samples during transport is critically important. Blood samples help to establish the patient's condition immediately prior to cardiac arrest, and provide valuable feedback on the effectiveness—or lack thereof—of cardiopulmonary support during transport. Blood samples should be drawn approximately once every 30 minutes, beginning as soon after legal death as possible (keeping in mind that the first priority is cardiopulmonary support and administration of stabilizing medications).

Laboratory Evaluation

Blood samples collected during transport are subjected to a large battery of tests. The levels of various electrolytes present in the serum or plasma (cell-free fraction of the blood) provide insight into the patient's pre-ischemic condition as well as into the effectiveness of resuscitation. Tissue enzyme levels present in the serum reflect the pre-ischemic condition, and their increase during transport can be indicative of ongoing ischemic or other injury. Bacterial cultures of blood establish a baseline for quality control of the sterile technique employed during subsequent total body washout and cryoprotective perfusion.

Blood

Different laboratory tests require different methods of blood collection (type of sample collection container used, method of sample handling, etc.), and different fractions of the blood collected. Whole blood—containing all blood elements (cells, plasma, and clotting elements)—is the sample of choice for blood gas analysis and evaluation of formed elements (cells).

Plasma is the liquid part of whole blood, which contains all the blood proteins. Serum is the liquid which remains after whole blood clots. Plasma and serum samples, which contain most of the physiologically and clinically significant substances found in blood, are used for most biochemical and physiological studies of relevance to cryonic suspension patients. They also provide useful electrolyte evaluation, enzyme analysis, glucose concentration, protein determination, and bilirubin level.

Venous, Arterial, And Capillary Blood

Blood is pumped from the heart through the arteries and into the capillaries, then into the veins, which return the blood to the heart and lungs.

Venous blood returns to the heart from the tissues through the veins. It carries waste products back to the central circulation for distribution to the lungs, kidneys, liver, and other organs, where they can be metabolized and excreted. Since venous blood represents physiologic conditions throughout the body, and can be easily obtained, it is used for most laboratory procedures.
Figure 13-1: The major components of blood.

Figure 13-2: Formation of serum.
Arterial blood leaves the heart through the arteries and is distributed to the tissues via the capillary network, to replenish tissues with oxygen and nutrients. Arterial puncture is not to be carried out during transport operations except by qualified personnel who are thoroughly trained in this technique and who have had extensive outside experience in its application. However, if an arterial line is present at the time of clinical death, it may be used to gather arterial blood samples where appropriate.

Blood in the capillaries (peripheral blood) does the real work of the circulatory system—exchanging fluids, nutrients, and wastes between blood and tissues. Capillary blood samples are most useful for evaluating hemoglobin and hematocrit as well as for evaluating total protein concentration in plasma via the indirect method of refractometry. Collection of capillary blood will not be covered in this manual.

![Capillary Fluid Exchange Diagram]

*Figure 13-4: The capillary system.*

**Quantities And Containers**

Sample collection during transport of cryonic suspension patients is carried out using a stoppered, evacuated glass tube, either with or without anticoagulant, additives, or preservatives (indicated by color-coded stoppers), depending upon the test for which the blood is being collected. The system in use at the time of this writing is the Vacutainer blood collection system, manufactured by Becton-Dickinson. This system consists of an evacuated, rubber-stoppered glass collection vial, a plastic needle/tube holder, and a special double-ended needle which mates to the holder. Blood is collected by carrying out vein puncture with the needle in the holder and then sliding the tube into the holder and puncturing its stopper with the other end of the double-pointed collection needle, which projects into the holder. Blood is drawn into the tube by the vacuum within.

Normal collection volume is 15 cc for all samples except those collected for glucose determination (gray stopper), which are 3 cc.
Select a venipuncture site, usually the antecubital fossa.

Using a circular motion, cleanse the area first with povidone-iodine solution and then alcohol.

Screw the Vacutainer needle into the sleeve (top left).

Apply a soft rubber tourniquet above the venipuncture site (top right).

Remove the needle cover. With the bevel facing up, insert the needle into the patient's vein at a 15° angle (bottom left). When a drop of blood appears just inside the needle holder, gently push the Vacutainer tube into the needle sleeve, so the blood enters the tube. Try to keep the needle still to prevent it from perforating the patient's vein.

When the tube is filled, remove the tourniquet, and pull the Vacutainer tube off the needle end. Withdraw the needle, using a dry sponge to apply direct pressure to the puncture site (bottom right). After 2 or 3 minutes, remove the sponge, and cover the site with an adhesive bandage.

Figure 13-4: Drawing venous blood samples.

Preparing For Blood Collection

Assemble the equipment required for blood collection. This will consist of a Velcro tourniquet, povidone-iodine solution swabs, Vacutainer needle holder, 20 gauge or 21 gauge multidraw Vacutainer needle, appropriate blood collection tubes (see discussion below for
tube selection), and a gauze pad and plastic tape to control post-stick bleeding.

Sample Tube Selection

Four kinds of Vacutainer tubes will be used for sample collection during transport:

1) *Tiger Stopper*, 15 cc capacity, (red and gray swirl) are glass tubes which contain an additive to promote clotting and a silicone gel used to separate the formed elements of the blood from the serum during centrifugation. They are used for collection of blood for analysis of electrolyte concentrations and clinical chemistries.

2) *Yellow Stopper*, 15 cc capacity, are tubes containing culture media for evaluation of bacterial contamination of the patient's blood. The stopper of Yellow Stopper tubes must be thoroughly cleansed before being used. This must be done to prevent bacterial contamination of the blood and tube contents, which would interfere with evaluation of the patient's blood cultures. (See Procedure for Collection of Blood Culture Sample on page 13-8.)

3) *Gray Stopper*, 5 cc capacity, are tubes containing potassium oxalate as the anticoagulant and sodium fluoride as an antimetabolite, and are used to collect blood for glucose evaluation. The sodium fluoride acts to inhibit red cell metabolism, thus preventing the red cells from using up glucose in the sample and distorting the results of the blood glucose evaluations.

4) *Purple Stopper*, 5 cc capacity, are tubes containing EDTA as an anticoagulant, and are used to collect blood for hematocrit determinations and complete blood counts.

Initiating Blood Sample Collection

Adequacy of staffing and the logistics of each situation will determine how soon after deanimation blood samples can be drawn. Ideally, if adequate personnel are available, red, green, yellow, and gray stopper tubes should be drawn immediately after legal death is pronounced and resuscitation and administration of stabilizing medications is proceeding. In reality, limitations on availability of personnel, logistics of access to suitable vessels during resuscitation, and placement of an IV will usually dictate a considerable delay between pronouncement of legal death and collection of the first blood sample.

Where possible, blood samples should be collected at regular intervals during transport and external cooling. As a minimum, hourly samples should be obtained and, if possible, samples should be collected approximately every 30 minutes.

Label all Vacutainer tubes carefully and clearly with the patient's name, Alcor #, date and collection time, and any other relevant information, such as "start of resuscitation". Number the tubes consecutively with a Sharpie indelible felt tip marker.

Venipuncture must be performed carefully to avoid hemolysis or hemoconcentration of the sample, and to prevent hematoma formation.
Select a venipuncture site. The most common site will be the antecubital fossa of the arm opposite the one being used for IV fluid administration. If you can't feel a vein distinctly, don't attempt venipuncture. Never use the antecubital vessels of an arm in which an IV is being given. The procedure for collection of the sample is as follows:

1) Separate and peel open the package containing the collection needle. Do not break open the package by bending the package over the needle.

2) Thread the needle into the holder and remove the remaining packaging. The needle which will enter the patient's skin should remain covered with its protective plastic shield.

3) Place the Vacutainer tube in the holder and push the tube onto the needle until the top of the stopper is even with the guide line on the holder.

4) Apply the tourniquet and select the sample site.

5) Using a circular motion, disinfect the skin with a povidone-iodine solution (Betadine) swab, starting in the immediate area where the stick is to be made and smoothly working outward. Never go back over a previously cleansed area.

6) Remove the needle cover. With the bevel facing up, insert the needle into the patient's vein at a 15° angle. When the first drop of blood appears just inside the needle holder, gently push the Vacutainer tube into the needle's sleeve, so the blood enters the tube. It is important to keep the needle still to avoid perforating the patient's vein and producing a hematoma.

7) When the tube is filled, remove the tourniquet and pull the Vacutainer tube off the needle end. Withdraw the needle from the patient's vein using a dry, sterile gauze sponge to apply direct pressure to the puncture site.
9) Immediately after removal of the tube containing the sample, gently invert the tube to mix the blood with the anticoagulant and/or preservative present within. Do not shake the tube, as this disrupts blood components and denatures plasma proteins, interfering with subsequent laboratory evaluation.

Hazards And Troubleshooting: Blood Sample Collection.

If a local swelling (hematoma) occurs during venipuncture, the needle must be promptly withdrawn and the patient restuck. If a hematoma begins to develop during blood collection, collection should proceed as long as there is a free flow of blood into the tube. Secondary sticks should always be done above the previous site.

If the flow of blood into the sample tube is erratic, sluggish, or stops abruptly, and there is no hematoma formation, the position of the needle should be checked and the bevel of the needle rotated or the needle withdrawn very slightly. If these maneuvers fail to establish free flow of blood into the tube, the needle should be withdrawn and a restick attempted elsewhere.

Because the patient will be heparinized, it may be difficult or impossible to secure hemostasis at the stick site without the use of a pressure dressing. If the patient is not being actively moved, the multisample Vacutainer needle and holder may be left in the vessel and gently secured with tape so that multiple samples may be collected over time. If repeated venipuncture is necessary, bleeding may be controlled with a gauze pad held in place with a pressure dressing. Only light pressure should be used to secure hemostasis. If this fails, a piece of Gelfoam hemostatic agent (if available) may be applied to the stick site with a gauze pad and a pressure dressing applied over it.

Use of an IV catheter for blood sample collection

(NOTE: Due to the significant risk of bacterial contamination of the sample, blood cultures may not be drawn in this fashion. See page 13-8 for instructions.)

After the administration of medications is complete, the IV site may be slowly flushed with 50 to 75 cc of normal saline and may thereafter be used to draw blood samples. The procedure for use of the IV for blood sampling is as follows:

1) Flush the solution administration set and catheter with 50 to 75 cc of normal saline by hanging a 150 or 250 cc bag of normal saline. Set the flow rate of normal saline at a rate just sufficient to maintain some fluid flow through the catheter to keep it from clotting off ("to keep open" (TKO) rate).

2) Place a gauze pad under the connection between the administration set and the hub of the catheter.

3) Prior to drawing a sample, occlude the line with a hemostat or Orange Clamp just above the luer fitting and remove the administration set from the catheter. Cover the male luer tip of the administration set with a hypodermic needle to protect its sterility.

4) Allow a few drops of saline/blood to drip from the catheter hub (apply a tourniquet above the stick site if necessary), then connect a syringe to the catheter, taking care not to pull or torque the catheter.
5) Slowly and gently withdraw 3 cc of blood, then remove the syringe and discard it and its contents. Using a fresh syringe, withdraw the required volume of blood.

6) Reconnect the administration set, remove the Orange Clamp, and begin TKO infusion of saline.

7) Attach an 18 gauge needle to the syringe and slowly fill the sample tubes with blood. Never rapidly force blood into sample tubes and never use a needle smaller than 21 gauge to fill the tubes, or hemolysis and damage to the sample will occur.

8) Label and refrigerate the sample as instructed on Page 13-9.

In most instances, blood collected after the administration of transport medications will fail to clot, even in the tiger stopper tubes, because of the heparinization of the patient prior to blood collection.

**Procedure For Collection Of Blood Culture Sample**

Blood cultures are performed to establish the bacteriologic status of the patient's blood prior to transport and perfusion. Such cultures are important not only in establishing a baseline for quality control during subsequent perfusion, but also in establishing the patient's condition prior to clinical death and helping determine to what extent, if any, bacterial invasion or overgrowth occurs during and after transport of cryonic suspension patients.

Since antibiotics interfere with microbial growth, the blood culture preferably should be drawn prior to the administration of antibiotics during transport. If necessary, antibiotics may be withheld until a culture can be drawn.

Extreme care must be used in preparing the area for venipuncture, as the skin affords a fertile field for bacterial growth. The procedure for drawing a blood culture is as follows:
1) The blood culture sample must be drawn with a conventional syringe and needle, since the sterility of the Vacutainer piercing needle within the nonsterile holder cannot be assured.

2) Prepare the skin at the puncture site by cleansing a large area with povidone-iodine swabs using a circular, outward working motion, beginning in the center (where the stick is anticipated) and working outward. Never go back over an area which has been cleansed with the same swab.

3) Wipe the povidone-iodine from the area using several alcohol swabs in the same fashion as the povidone-iodine swabs were used.

4) Prepare the Vacutainer top by swabbing with povidone-iodine (use friction) and wiping with alcohol.

5) Perform venipuncture using syringe and needle (a 3 - 5 cc syringe and a 21 gauge, 1½ in. needle are acceptable) and fill the yellow stopper tube with blood.

6) Label the tube.

**Handling Of All Samples**

Immediately after collection, each sample should be labeled in the following manner:

1) An adhesive label should be attached with the patient's name, Alcor #, the date the sample was collected, the time it was collected, the location at which it was collected (i.e., antecubital fossa, etc.), and any relevant notes, such as "start of resuscitation" or "conclusion of medication administration," etc.

2) The adhesive label should be secured to the tube with transparent plastic tape. Cover the writing on the label with the tape to guard against water smearing if the samples are refrigerated in ice.

3) Number the tube in at least two places with an indelible Sharpie marker. Enter the tube number, date, time, and location from which the sample was collected--plus any other relevant information--on the Transport Data Collection Sheet. (The tube number is very important; if the tube labels are damaged by water, the tube identity and other relevant information is there.)

All blood samples should be promptly cooled after collection and labeling, either by conventional refrigeration or packing in ice. If the patient is to be shipped after cooling and stabilization, the blood samples may be placed in the container with the patient, as long as they are properly labeled and protected from the effects of water by placement in a liquid-tight container (such as a Zip-Loc bag). Avoid placing samples on the bottom of the patient shipping container, since water may leak into the packaging and obliterate the labeling. Where possible, attach the bag containing the samples to one of the patient's arms, using adhesive or plastic tape and taking care to avoid constricting the limb.