Step-by-Step Instructions for the Cryopreservation of Patient X

**STEP ONE:** Instruct the hospital NOT to remove any of IV lines, and especially not the central IV line. I suggest that you might want to ask the mortician to ask this of the hospital personnel as well as the next of kin. If you can find out who the person is who prepares the body after death you can probably pay them to see to it that this is done. If you have medications, administer them as soon as possible as long as the patient is not in rigor (mortis).

**STEP TWO:** Organize and bring the necessary supplies with you:

a) IV Medication administration supplies:

![Supplies for IV Administration of Medications](image)

b) Safety and personal protection supplies:
c) Waterproofing supplies:
d) Patient cooling supplies:

- **Tarp**: 2.44 meters x 3.04 meters
- **Duct Tape**
- **Nylon Cord**
- **Bungee Fasteners x 6**
Patient Cooling Supplies

Head Ice Positioner  Body Bag (Heavy Duty)

e) Patient temperature monitoring supplies:
STEP THREE: Identify the patient's central venous line. If the patient is not in rigor (mortis) give the medications per the protocol. If you do not have some medications there is nothing that can be done about it at this point; do what you can.

You will give the medications through the patient's central venous line. This is what a central venous line looks like in place in the patient:
It may be a multiple lumen catheter so that several different things (blood + drugs) can be given at the same time:

The tip (outlet) of the catheter sits in the right atrium of the heart:
STEP FOUR: If indicated, administer the medications per steps a) through e) below. To give medications via the central venous line you can use the INJECTION PORT on the IV line. To do this you simply insert the needle of the syringe containing the medication to be given into the rubber centre on the port and inject the medication into the line as follows:

a) If there is an IV bag connected to the line (as above) then you will need to CLAMP OFF the line going to the bag so that the medication does not go up into the bag rather than into the patient as shown below:
b) Insert the needle of the syringe into the injection port:

IV line from the IV bag to the patient

Needle attached to syringe containing medication

Injection port on IV line

Catheter to the patient

c) Give the medication into the central venous catheter:
d) After giving the medications open the IV line to allow ~200 ml of solution to flow into the patient. This is done to ensure that the medications are flushed from the catheter into the patient’s circulatory system:

e) Do chest compressions with NO VENTILATION for 5 minutes:
f) Clamp the central venous line.

2) Prepare and organize the things you will to bring with you:

a) IV Medication administration supplies:
STEP FOUR: Cover the removal cot or gurney in a plastic tarpaulin 3.44 x 2.04 meters. Leave the sides of the tarp draping down around the sides of the cot.

STEP FIVE: Place the body bag on top of the tarp and unzip it so that is ready to receive the patient.

STEP SIX: Remove the patient from the gurney or tray in the morgue cooler and position her inside the open body bag.

STEP SEVEN: Place a modest amount of ice in the head ice positioner; just enough so that there will be ice UNDER the patient’s head: 4-10 cm thickness of ice at most.

STEP EIGHT: Place the patient’s head inside the head ice positioner and fill the head ice positioner with ice BUT LEAVE THE PATIENT’S MOUTH AND NOSE EXPOSED SO YOU CAN PLACE THE PROBE.

STEP NINE: Insert temperature probe deep into throat via mouth. NOTE: the patient may well be in rigor mortis (stiffening of muscles) and you may be unable to open her mouth. If the mortician cannot get the patient’s mouth open enough to get a probe in, the next best thing is to thread the probe through the nose as far as it will go. You can use either nare, left or right. It may take some effort to place the probe.

STEP TEN: Once the temperature probe is in place, secure it to the patient’s face (cheek) with sutures using a Z-shaped anchoring pattern like this:
**STEP ELEVEN:** Cover the patient’s face with a piece of aluminium foil to prevent melting ice water from entering the mouth and nose.

**STEP TWELVE:** Cover over the patient’s head in ice and fill up the head ice positioner.

**STEP THIRTEEN:** Measure and record patient's temperature. If you are using an indoor/outdoor thermometer, it only updates every couple of minutes (look at the instructions) so it may take a minute or two to get a true reading.

**STEP FOURTEEN:** Pack ice around the patient’s body in the body bag.

**STEP FIFTEEN:** Zip up the body bag as far as it will close the head ice positioner will probably stop it from closing completely.

**STEP SIXTEEN:** Bring the sides of the tarp up and over the body bag. The tarp will have metal grommets to allow it to be laced up or secured. Carefully fold the tarp over and around the patient so water will not leak out. Use nylon cord or bungee cords to hold the tarp securely in place.

**STEP SEVENTEEN:** Transport the patient to the perfusion facility (mortuary). Have someone continue to log time/temperature readings en route. Assign someone to do this and make sure they know what they are doing. Have them demonstrate that they are capturing data.

**STEP EIGHTEEN:** Remove the patient from the gurney and pace her on the operating table leaving her inside the body bag.

**STEP NINETEEN:** Expose the neck on both sides and clear and area for surgery to expose the internal carotid arteries and internal jugular veins.

**INSTRUCTIONS FOR CRYOPROTECTIVE PERFUSION:**

1.0 Preliminary preparation

1.1 Clean all containers and utensils to be used in preparing perfusate thoroughly.

1.2 Double (two times) rinse the containers with distilled water after they have been cleaned.

1.3 If there is to be a delay between cleaning the containers and using them wrap them in plastic bags to ensure they remain clean. Be sure the containers are THOROUGHLY DRY before wrapping them in plastic, otherwise they will grow mold.

2.0 Perfusate Preparation
2.1 Prepare the following quantities and concentrations of perfusate:

a) 15% (v/v) glycerol – 10 L
b) 30% (v/v) glycerol – 10 L
c) 58% (v/v) glycerol – 20 L

2.2 Perfusates (containers) should be kept at following temperatures (if possible):

15% (v/v) glycerol – 10 L - 0°C
30% (v/v) glycerol – 10 L - 0°C
58% (v/v) glycerol – 20 L -15°C

Otherwise, store the perfusate on crushed ice taking care not to allow melting ice water to contaminate the perfusate. Keep the tops and necks of the bottles out of the ice bath.

2.3 Filter all perfusates through a 0.5 – 5μm filter BEFORE use. The perfusates should be filtered into clean containers that close tightly. This should be done as soon as possible because the patient will die within the next 48 to 72 hours and it will take a long time to prepare the perfusate. The 58%(v/v) glycerol is very slow to filter: IT CANNOT BE FILTERED AS IT IS ADMINISTERED BECAUSE IT IS TOO VISCIOUS AND TAKES TOO LONG TO FILTER.

2.4 If you do not have d-lactose, you can substitute mannitol on an equimolar basis as shown here:

<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>Molecular Weight (MW)</th>
<th>Target Molarity (mM)</th>
<th>Target Grams/Liter</th>
<th>Target Grams/20 Liters</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Mannitol</td>
<td>182.17</td>
<td>200.000000</td>
<td>36.4340</td>
<td>728.6800</td>
</tr>
<tr>
<td>2 Glucose</td>
<td>180.20</td>
<td>13.400000</td>
<td>2.4147</td>
<td>48.2936</td>
</tr>
<tr>
<td>3 TRIS Hemihydrochloride</td>
<td>139.40</td>
<td>30.000000</td>
<td>4.1820</td>
<td>83.6400</td>
</tr>
<tr>
<td>4 Potassium Chloride</td>
<td>74.56</td>
<td>10.000000</td>
<td>0.7456</td>
<td>14.9120</td>
</tr>
<tr>
<td>5 Calcium Chloride 10% (w/v) soln. (See Table 2)</td>
<td>111.00</td>
<td>1.000000</td>
<td>0.1110</td>
<td>2.2200</td>
</tr>
<tr>
<td>6 Magnesium Chloride 20% (w/v) soln. (See Table 2)</td>
<td>95.20</td>
<td>4.000000</td>
<td>0.3808</td>
<td>7.6160</td>
</tr>
<tr>
<td>7 Heparin</td>
<td>-</td>
<td>-</td>
<td>1,000 IU</td>
<td>20,000 IU</td>
</tr>
</tbody>
</table>
### TABLE 2

<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>Percentage Solution</th>
<th>Grams/mL</th>
<th>Target Grams/Liter</th>
<th>Target mL per 20 Liters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium chloride</td>
<td>10%</td>
<td>0.1000</td>
<td>0.1110</td>
<td>22.20</td>
</tr>
<tr>
<td>Magnesium chloride</td>
<td>20%</td>
<td>0.2000</td>
<td>0.3808</td>
<td>38.08</td>
</tr>
</tbody>
</table>

### 4.0 Set-Up of the Circuit for Cryoprotective Perfusion:

4.1 The circuit consists of a minimum 5-liter reservoir with a bottom outlet (10 liters is preferred). The reservoir should be filled, if possible, using a funnel with a short length of tubing attached. The end of the tubing can be positioned below the liquid surface when adding additional perfusate. This will reduce the amount of air introduced into the perfusate when additional volume is added.

The reservoir should be wrapped in some kind of insulating material if possible. Use of a polyurethane insulated cold beverage dispenser like this would be ideal:

![Insulated Reservoir (Beverage Cooler)](image)

But, a small blanket or a number of turns of fabric will do.

The circuit should be safe and simple:
4.2 Flow is controlled by clamping and unclamping the occluding forceps as shown.

4.3 Pressure is generated by elevating the reservoir to a height of 120-160 cm above the patient’s head (measuring from solution surface in container). A lightweight aluminium step ladder is ideal for this, as is a ceiling mounted hook and pulley or block and tackle.

4.4 If possible, a standard aneroid blood pressure gauge (sphygmomanometer) can be connected to the arterial line (line to the patient) and pressure can be read from the gauge directly.

4.5 This requires that the gauge be protected from the liquid with a transducer protector and stopcock as shown above. It is imperative that the fluid side of the transducer be completely filled with perfusate and the pressure monitoring line purged of air before this assembly is connected to the arterial line. Mr Smith had some of these protectors, in-line connectors and an aneroid gauge. If he sent them along, the diagram above shows how to set up the pressure monitoring assembly using them.

4.6 **IMPORTANT:** An accurate pressure will be obtained ONLY if the gauge-transducer assembly is at the level of the patient’s carotid arteries; an easy way
to ensure this is to position them at the level of the heart in the chest, as shown above.

5.0 Cannulation of the Patient:

5.1 Patient is placed on the operating/embalming table

5.2 If it is mandatory that ice bags be used, ensure that the head is thoroughly packed in bags (packets) of ice. Leave the neck and subclavian area exposed so that surgery for cannulation can be done.

5.3 Raise both right and left **common** carotid arteries and **internal jugular veins**. It is critically important that internal jugular veins be isolated and incised and not the external jugular veins.
5.4 Pass ties (twine or 0-silk suture) under all four vessels both proximally and distally as shown:

**CAUTION:**

Before opening the carotid arteries ligate (tie off) both the right and left distal ties. This will prevent perfusate from traveling down the vertebral arteries and escaping from the distal opening of the common carotid artery incision.

5.5 You MUST tie off the carotid arteries below the level of the cannula or you will have perfusate loss. This will occur because perfusate will flow down the vertebral arteries (retrograde) to the aortic arch and then exit through the open end of the incision in the common carotid arteries.
5.6 You must also tie off the distal internal jugular veins (going towards the body) in order to prevent blood from seeping out from the body and contaminating the venous effluent (perfusate exiting the head). If this happens it will be impossible to determine the cryoprotectant concentration or to know when the blood has been washed out of the head (brain).

CAUTION:

Before opening the internal jugular veins you must tie off (ligate) the distal ties. This will prevent blood from flowing out of the body into the venous effluent. If blood contaminates the perfusate flowing out of the head (brain) it becomes impossible to determine the glycerol concentration or to know when the blood has been washed out of the head/brain.

5.7 Use a Y-connector to connect both of the carotid artery cannulae to the line supplying the perfusate to the patient as shown in the photo below:
5.8 It is imperative that the tubing and cannula contain no air! You must thoroughly de-bubble all tubing and be certain that there is no air in the cannulae. Allow a small amount of perfusate to drip or trickle out of the cannulae as you insert them into the arteries. DO NOT ALLOW AIR TO BE PRESENT AS SHOWN IN THE PHOTO BELOW:
.5.9 Once the cannulae are in place, ligate the proximal ties around each arterial cannula tightly to secure the vessel wall to them.

5.10 After tightening the proximal ties around the arterial cannulae back-tie the cannula using the distal ligatures. This will hold the cannulae in place and prevent them from slipping out of the vessel. This is especially important when using a gravity feed set-up because there is likely to frequent tension on the arterial line (from the reservoir to the patient) as the reservoir is periodically refilled with perfusate.

This is illustrated in the diagram below:
5.11 When the cannulae are secured in place and the internal jugular vein have been incised perfusion can begin.

5.12 Remove the occluding clamps starting with those leading to each cannula.

5.13 Remove the last occluding clamp on the arterial line.

Flow should begin to the patient and within 30 to 90 seconds you should observe venous drainage from the wounds.