Alcor Life Extension Foundation Human Cryopreservation Protocol

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This protocol is an ideal that Alcor seeks to achieve, but that in many cases will not be possible. Obstacles preventing ideal procedures include insufficient notice of impending legal death, location of death, logistics and deployment problems, and financial constraints which are explained in more detail in the policy on Comprehensive Member Standby (CMS) on the Alcor website.

Objectives

The objective of cryonics is to stabilize critically ill patients after cardiac arrest, at cryogenic temperatures, in anticipation of future resuscitation. At Alcor, cryonics is viewed as a form of experimental critical care medicine, with members in biostasis considered patients. Because human cryopreservation is not available as an elective medical procedure, cryonics procedures can only be initiated after the pronouncement of legal death. The procedures to achieve this objective have been developed by Alcor over many years in consultation with external experts in cerebral resuscitation and tissue and organ cryopreservation.

Alcor offers whole body cryopreservation and neuro preservation. In both options the preservation of the brain as the anatomical basis of the person has the highest priority. During the initial stages of cryonics procedures the ideal objective of the Alcor protocol is to secure viability of the brain by *contemporary* biological criteria. This means that Alcor's initial stabilization procedures should not be harmful in themselves and that the reversal of these protocols should be possible in principle.

During the subsequent phase, which involves cryoprotectant perfusion and cooldown below 0 degrees Celsius to cryogenic temperatures, this objective is no longer attainable as a result of cryoprotectant toxicity and structural injury associated with thermal stress, and is replaced by the more modest objective of good ultrastructural preservation.

Non-Ideal Cases

The procedures described in this document are what is attempted under ideal logistical and biological conditions. The circumstances under which legal death occurs can be highly variable, and in many cases some or all these procedures except for cooling may be impossible. Unless members making cryopreservation arrangements express other written preferences, it is a general principle of cryonics that cryopreservation should proceed after legal death even under poor biological conditions when standard protocol procedures cannot be performed. This is done to preserve as much remaining biological information as possible because in most cases it is theoretically impossible to determine whether all brain information encoding memory and personal identity has been truly lost.

Summary of Cryonics Procedures

Alcor's cryonics protocol ideally consists of four distinct elements: (1) deployment and standby, (2) stabilization, (3) cryoprotectant perfusion, (4) cryogenic cooldown.

(1) Deployment and standby. If Alcor is notified of a pending case or emergency a standby team is deployed to the location of the patient to ensure rapid intervention after pronouncement of legal death.
(2) Stabilization. After pronouncement of legal death rapid cooling is initiated, circulation is restored, the lungs may be ventilated, and medications are administered to protect against blood clotting and keep the brain viable. In remote stabilization cases where transport to Alcor's operating room may take up to 24 hours, the blood is ideally replaced with an organ preservation solution to enhance cooling, prevent blood clotting, and protect against cold ischemia.
(3) Cryoprotectant perfusion. After arrival of the patient at the Alcor facility, the patient's blood (or organ preservation solution) is replaced with a vitrification solution. Circulation of this solution through blood vessels at cold temperatures partially replaces water inside cells with chemicals that reduce or prevent ice crystallization during further cooldown to cryogenic temperatures.
(4) Cryogenic cooldown. After cryoprotectant perfusion the patient is gradually cooled to the temperature of liquid nitrogen for long term care. In the future, as appropriately reliable equipment becomes available, cooling may terminate and long-term maintenance may occur slightly below the glass transition temperature, to minimize structural damage.

Deployment and Standby

Alcor maintains a local emergency vehicle equipped with standby and stabilization equipment and at least one complete set of kits for remote deployment, and also has access to similar cryonics emergency vehicles maintained by Suspended Animation, Inc., in Southern California and Florida. Alcor also makes an effort to maintain basic or complete kits in regional areas with a high number of cryonics members. The organization determines allocation of standby resources through periodic review of the demographics and regional distribution of its members. To minimize the chance of late or last-minute deployment Alcor encourages members to inform the organization about their health situation and uses a color-coded member tracking system that guides deployment preparations and decisions.

Alcor materials are available for family, medical caregivers and third parties about its procedures to ensure an orderly and timely transition between pronouncement of legal death and the start of cryonics procedures. Alcor will also request medical data about the terminal patient to assist in determining the time and scope of deployment. Although Alcor does not participate in pre-mortem treatment of the patient, Alcor may discuss with family and caregivers the medical management of the terminal patient. Alcor may also seek permission for placement of non-invasive monitoring devices.

Alcor maintains a Deployment Committee which normally includes its chief executive, Medical Response Director, and the Chief Medical Advisor. The committee is charged with assessing and defining Alcor's Comprehensive Member Standby policy, establishing standby deployment guidelines, and making real-time deployment decisions in emergency situations.

Unless unforeseen circumstances (such as a last-minute remote case) do not permit full deployment, Alcor stabilization protocol ideally requires *four team members* to be present at the start of cryonics procedures. To avoid fatigue and errors, standby team members are rotated in pairs, on a 12-hour cycle, to allow for sufficient rest and sleep. The stabilization team will typically be headed by Alcor's Medical Response Director, who is a nationally certified paramedic. Additional team members may include other Alcor staff members with EMT (emergency medical technician) training, local volunteers with cryonics stabilization training, or a Standby Team of Suspended Animation, Inc, which is composed of trained staff members and consulting professional perfusionists and surgeons. If there is insufficient notice for Alcor or Suspended Animation, Inc., to reach the location of a cryonics case before legal death, emergency stabilization may be performed entirely by local volunteer team members. At Alcor's discretion, or member choice, stabilization may also be performed entirely by Suspended Animation, Inc.

Alcor offers education and training to its members and interested medical professionals in basic human cryopreservation procedures. In addition, anyone who feels motivated to participate actively in cases may seek more advanced training. A network of volunteers and trained members may be called upon to assist in remote cases or basic logistical or stabilization tasks.

Stabilization

The objective of stabilization is to maintain viability of the brain by contemporary biological criteria after legal pronouncement of death. To achieve this purpose four different procedures are ideally employed:

1. Cardiopulmonary Support. Circulation is restored to provide oxygenated blood to the brain and to enhance cooling. Depending on specific circumstances, the lungs may be ventilated.
2. Induction of Hypothermia. The temperature of the patient is lowered to just above 0 degrees Celsius to depress metabolism.
3. Administration of Medications. Drugs are administered to improve circulation, inhibit blood clotting, and to protect the brain.
4. Blood substitution. If the patient is distant from Alcor's facilities, and if it is logistically possible to do so, the blood of the patient is substituted with an organ preservation solution to enhance cooling, prevent blood clotting, and protect against cold ischemia.

Cardiopulmonary support, induction of hypothermia, and administration of medications are initiated as quickly as possible after death is pronounced. In practice, none of these procedures alone is sufficient to maintain the brain in a viable state. To ensure that these interventions are executed concurrently, a minimum number of four team members will be present at the start of stabilization. Their tasks will include data collection for subsequent review and analysis.

Stabilization procedures end when either the temperature of the patient has been lowered close to the freezing point of water or when blood washout is started to prepare for cryoprotectant perfusion. In remote cryonics cases blood substitution is an option prior to transport to the cryonics facility.

Cardiopulmonary Support

Cardiopulmonary support (CPS) is distinguished from cardiopulmonary resuscitation (CPR) because the objective of circulation and ventilation in cryonics is not resuscitation of the patient but to prevent (additional) ischemic injury.

The three objectives of cardiopulmonary support are:

1. Restore circulation of oxygenated blood to the brain
2. Circulate medications
3. Improve the rate of external cooling

After pronouncement of legal death the patient is transferred to the portable ice bath and mechanical cardiopulmonary support is started. Mechanical devices allow for consistent and aggressive chest compressions, permitting continued CPS during transport of the patient. They also prevent fatigue of standby team members and release team members to perform other important tasks. The preferred method of cardiopulmonary support is battery-powered mechanical active-compression decompression. The second preferred option is gas-powered mechanical active-compression decompression. The third option is gas-powered conventional mechanical chest compression. When mechanical devices are not available or not functional, manual compression-decompression chest compressions should be initiated through the use of the Cardiopump. Conventional (i.e., hands only) chest compressions should only be pursued when all other options are exhausted.

In line with recent CPR guidelines, Alcor emphasizes the importance of continuous and vigorous chest compressions. Continuous chest compressions induce moderate air movement in and out of the lungs, help to mitigate the risk of reperfusion injury and hyperventilation when metabolism is depressed by hypothermia.

If medical professionals are available to place a secure airway to initiate positive pressure ventilation an inspiratory impedance threshold valve (ITV) should be placed between the endotracheal tube (or King Airway) and the oxygen source to prevent ventilations during the decompression phase of chest compressions. The goal is to maximize cardiac output. The chest compression-to-ventilation ratio is 30:2 and should be reduced to 60:2 below

32 degrees Celsius. No positive pressure ventilations should be initiated after 30 minutes of normothermic circulatory arrest.

Unless surgical expertise is available to perform surgery with minimal interruption of circulation, CPS should continue until the patient has reached a core temperature of 20 degrees Celsius to prevent ischemic injury during preparation for blood substitution or cryoprotective perfusion.

Induction of Hypothermia

External cooling of the patient should be started immediately after pronouncement of legal death to depress metabolism. The patient is moved from the bed to a portable ice bath (PIB) that contains ice and cold water to facilitate cooling during transport, and increase cooling rate. The patient should be completely immersed in ice and water with a primary emphasis on the head and areas with major surface vessels such as the neck, axilla and groin. Because the total area of contact between dry cubed ice and the patient is inevitably limited, some water is essential, to maximize heat transfer. It should cover as much of the patient's skin as possible, and is circulated via a system of perforated tubing attached to a submersible pump. Water is flowed rather than sprayed over the patient, to reduce the risk of infection via airborne droplets if the patient has a contagious disease.

Concurrent start of aggressive cardiopulmonary support increases the cooling rate by moving warm blood from the core of the patient to the surface for heat exchange. The objective of all these procedures is to achieve the fast cooling rates that are seen in cold water immersion without sacrificing cardiopulmonary support and medication administration.

A minor degree of internal cooling during stabilization can be achieved by cooling the medications and fluids before they are administered. Mannitol should be exempted from this procedure because the solution will crystallize if it is maintained at low temperatures.

Cooling the patient should continue without interruption during transport to the funeral home or during surgical procedures. Logging the temperature of the patient is important to monitor the effects of cooling efforts and for subsequent case reporting.

Because even the fastest cooling rates cannot stay ahead of ischemic injury without circulation of oxygenated blood and administration of neuroprotective medications, induction of hypothermia cannot be a substitute for these interventions. This is particularly important during the start of stabilization procedures because energy depletion is running faster than cooling can depress metabolism.

If no ice bath is available, a heavyweight body bag can be used to surround the patient with ice without spilling and leaking.

In typical cases, the patient should not be cooled below the freezing point of water (0 degrees Celsius). The patient may only be cooled below the freezing point of water if

Alcor has made the decision that long time delays before stabilization, or expected during transport, will make cryoprotectant perfusion impossible. In such cases the patient must be held at the temperature of dry ice (- 78.5 degrees Celsius), with the understanding that this will inflict very severe brain injury as a result of freezing. If a patient is frozen, special care must be taken to avoid thawing and re-freezing, which will cause even more damage. The application of dry ice without cryoprotectant perfusion (so-called "straight freezing") should be viewed as a desperation measure which cannot be reversed.

Administration of medications

Administration of medications should be started as soon as the patient has been placed in the portable ice bath. If the patient already has a patent intravenous line in place, or if no portable ice bath is available, the administration of the first medications can start sooner. Under no circumstances should Alcor team members start or authorize the administration of medication prior to pronouncement of legal death.

Each medication falls into one of three categories:

1. Small volume medications (such as heparin and streptokinase)
2. Large volume fluids (such as hydroxyethyl starch and mannitol)
3. Fluids that require gastric administration (Maalox)

The administration of the small-volume medications and the large-volume fluids should commence at the same time. This is particularly important if the patient is severely dehydrated at the start of stabilization procedures. The simultaneous administration of the small-volume medications and the large-volume fluids can be achieved either by pushing the small medications into the line or by establishing a second IV line.

If there is no delay between pronouncement of legal death and the start of stabilization procedures the full set of medications should be administered.

Small Volume Medications

(1) Propofol (200 mg - fixed dosage)

Propofol is a *general anesthetic* and is used for two reasons. The first reason is to reduce metabolic demand, and the second reason is to prevent the theoretical possibility of recovery of awareness due to aggressive cardiopulmonary support.

(2) Sodium Citrate (10 grams for patients < 100 kg, 20 grams for patients > 100 kg)

Citrate is an *anticoagulant* that prevents the formation of blood clots that can interfere with blood circulation and cryoprotective perfusion. It is administered as a custom formulation of 20% sodium citrate in water, packaged in 50 mL sterile vials.

(3) Streptokinase (250,000 IU – fixed dosage)

Streptokinase is a *thrombolytic* used to break up existing blood clots that can interfere with blood circulation and cryoprotective perfusion.

(4) Heparin (100,000 IU – fixed dosage)

Heparin is an *anticoagulant* that prevents the formation of blood clots that can interfere with blood circulation and cryoprotective perfusion. Heparin loses effectiveness at low pH (pH < 6.7), so control of pH is important during a cryonics stabilization. This is why other *anticoagulants* are also important.

(5) Aspirin (300 mg –fixed dosage)

Aspirin is an *anti-inflammatory* and *anti-platelet* agent that is used to inhibit platelet aggregation.

Note: Aspirin is reconstituted with 5 ml THAM and injected into THAM bottle.

(6) Vasopressin (200 IU – fixed dosage – intermittent administration)

Vasopressin is a *vasopressor* that is used to increase blood pressure during cardiopulmonary support. There is no need to administer vasopressin if the patient's temperature is near or below +20 °C at time of administration as it is ineffective at cold temperatures.

(7) Epinephrine (30 mg – fixed dosage - intermittent administration)

Epinephrine is a *vasopressor* that is used to increase blood pressure during cardiopulmonary support. There is no need to administer epinephrine if the patient's temperature is near or below +20 °C at time of administration as it is ineffective at cold temperatures.

(8) SMT (S-methyl-isothiourea) (400 mg – fixed dosage)

SMT is a *neuroprotectant* (iNOS inhibitor) that is used to protect the brain from ischemic injury. SMT also raises blood pressure.

(9) Niacinamide (Vitamin B3) (500 mg - fixed dosage)

Niacinamide is a *neuroprotectant* (PARP inhibitor) that is used to protect the brain from ischemic injury.

(10) Kynurenine sulfate (1.5 gram – fixed dosage)

Kynurenine sulfate is a *neuroprotectant* (excitotoxicity inhbitor) that is used to protect the brain from ischemic injury.

(11) Ketorolac (7.5 to 15 mg - dosage by patient weight)

Ketorolac is a non-steroidal anti-inflammatory drug used to inhibit ischemia-induced inflammation.

(12) Gentamicin (80 mg – fixed dosage)

Gentamicin is an *antibiotic* that is used to protect the patient from microbial overgrowth during long transport times.

Large Volume Medications

(13) Vital-Oxy (formerly known as Oxynil) (70 ml or less –dosage by patient weight)

Vital-Oxy is a proprietary emulsion of the antioxidants melatonin, vitamin E (as *D-alpha tocopherol)*, PBN (*alpha Phenyl t-Butyl Nitrone)* and the anti-inflammatory agent carprofen.

(14) Hetastarch (250 ml – fixed dosage)

Hetastarch is a volume expander used to restore volume in dehydrated patients and increase cerebral perfusion during CPS.

(15) THAM (Tris (hydroxymethyl) aminomethane) (100 ml – fixed dosage)

THAM is a *buffer* that is used to mitigate acidosis. If aspirin is dissolved in THAM it is called THAM plus.

(16) Mannitol (500 ml of 20% solution – fixed dosage)

Mannitol is an *osmotic diuretic* agent that is used to reduce cerebral edema and increase blood volume.

Fluids That Require Gastric Administration

(17) Maalox (250 ml – fixed dosage)

Maalox is an *antacid* that is used to stabilize the pH of stomach contents to prevent erosion of the stomach wall by hydrochloric acid at low temperatures. Failure to prevent this can lead to contamination of the circulatory system with stomach contents and abdominal swelling during later perfusion.

If there is a delay of more than *one hour* after cardiac arrest, an *abbreviated* list of medications should be administered.

1. Sodium Citrate (if available) (10 grams for patients < 100 kg, 20 grams for patients > 100 kg)

2. Streptokinase (250,000 IU –fixed dosage)

3. Heparin (100,000 IU – fixed dosage)

4. Tempol (if available) (5 g – fixed dosage - dissolved in normal saline)

5. Gentamicin (80 mg – fixed dosage)

6. Mannitol (500 ml of 20% solution – fixed dosage)

7. Maalox (250 ml –fixed dosage)

Administration of these medications should be followed by at least ten minutes of chest compressions to distribute the medications, accompanied by surface cooling.

The vasopressors epinephrine and vasopressin should be administered intermittently to ensure higher cerebral bloodflow. The effects of vasopressor medications can be assessed through the use of end tidal CO2 monitoring.

Maalox is not introduced to the circulatory system but to the stomach of the patient. This requires the placement of the *double-lumen* King LTS-D Airway or a designated gastric tube. Unless placement of the King LTS-D Airway is not possible, the King LTS-D Airway is the preferred method for Maalox administration because it allows for simultaneous ventilation. Maalox should only be administered through the inserted gastric tube in the rear channel of the KING LTS-D Airway if the team leader has received confirmation that the KING LTS-D has *not* been accidentally placed in the trachea. A gastric tube should only be placed by an experienced medical professional.

If Alcor is not successful in persuading the patient's caregivers to leave an IV line in place, the preferred method of medication administration is intraosseous infusion. If intraosseous infusion is not available, or contra-indicated for the patient, an experienced team member should place a peripheral IV line. Central IV lines should only be placed by qualified medical professionals. Techniques such as pressure infusion should only be used by those with extensive experience such as paramedics.

Preparation of the medications should start at least one hour before the estimated time of circulatory arrest or on the way to the patient if (s)he has already been pronounced. Compounds that have been prepared in-house at Alcor should be filter-sterilized prior to administration. Mannitol should be checked for crystals before administration. If there

are crystals in the solution the solution should be warmed to dissolve them. If the crystals cannot be eliminated the fluid should not be introduced to the patient.

In instances where team members are uncertain about dosage, methods of administration, or other issues, they can contact Alcor's medical advisor, who should be available by phone at all times during standby, stabilization, and transport of the patient. Team members should not improvise on their own initiative.

The start of blood washout or cryoprotective perfusion should not be delayed to complete administration of medications. If administration of the remaining medications is still deemed desirable they can be added to the organ preservation solution during perfusion.

Remote blood substitution

In remote cases, blood substitution with an organ preservation solution prior to transport at hypothermic temperatures is desirable unless it is logistically impossible to do so. Remote blood substitution has the following objectives:

1. Rapid induction of ultraprofound hypothermia.
2. Prevention of clotting, red cell sludging and "no-reflow."
3. Maintaining viability of the brain during transport.

Alcor uses an Air Transportable Perfusion circuit (ATP), or, if available, the Stockert SCPC portable clinical perfusion system, to replace the blood of the patient. The organ preservation solution of choice at Alcor is MHP-2. MHP-2 is an asanguineous hyperosmolar intracellular whole body organ preservation solution.

MHP-2

Mannitol 170 mM
Adenine-HCL 0.94 mM
D-ribose 0.94 mM
Sodium bicarbonate 10 mM
Potassium chloride 28.3 mM
Calcium chloride 1 mM
Magnesium chloride 1 mM
HEPES 15 mM
Glutathione 3 mM
D-Glucose (Dextrose) 5 mM
Hydroxyethyl starch 50 g per L
Heparin 1000 I.U. per L
Insulin 40 I.U. per L

Osmolality 388-403 mOsm
pH 8.0-8.2

To facilitate rapid cooling, MHP-2 should be kept as close as possible to the freezing point of water (0 degrees Celsius). A heat exchanger built into the ATP circuit is designed to reduce the temperature to near freezing, if necessary, before the solution enters the patient. Heparin and insulin should be added to MHP-2 during extracorporeal circulation. At this point, any remaining stabilization medications (with the exception of Maalox) can be injected into the circuit as well.

Remote blood washout should only be undertaken in the absence of contra-indications for this procedure. The contra-indications for remote blood substitution range from "pre-mortem" patient pathologies to practical and logistical challenges:

Contra-indications for Remote Blood Substitution

- More than six hours since legal death occurred.

- Omitting remote blood substitution will reduce transport time significantly.
- Reaching the nearest funeral home or other location that allows blood substitution will result in excessive cardiopulmonary support times
- There are no team members with extensive experience and knowledge of cardiopulmonary bypass present on the case
- Inspection of the blood organ preservation solution (MHP2) reveals bacterial growth
- Inspection of the blood organ preservation solution composition suggests errors in perfusate composition
- The presence of systemic edema (fluid accumulation throughout the body) that may have occurred during cardiopulmonary support
- Active gastrointestinal bleeding at the time of cardiac arrest
- Prolonged splanchnic ischemia or severe abdominal swelling
- Severe pulmonary edema
- Severe cerebral edema
- Prolonged periods of warm cerebral ischemia

To facilitate a smooth transition from cardiopulmonary support to blood substitution Alcor will normally attempt to deploy a team of at least two individuals to a cooperating funeral home to set up and prime the perfusion circuit. These team members should also obtain additional ice to further cool the patient and to be used as the heat exchange medium during blood washout. In some cases (e.g., home hospice) remote blood substitution may be possible at the patient's bedside. This option should be discussed with the patient, the patient's medical surrogate, and medical caregivers in advance.

Remote blood substitution requires surgery and cannulation of the major blood vessels of the patient. The preferred procedure at Alcor is femoral-femoral cannulation. Most surgical alternatives to femoral cannulation can cause complications during cryoprotective perfusion and should only be performed by experienced surgeons in absence of the contra-indications for remote blood substitution.

Interruption of circulation should be minimized during surgery. This is particularly important if surgery is initiated when the patient's core body temperature is still close to body temperature. If extended interruptions of circulation are expected during surgery, the procedure should not be initiated until the patient's core body temperature has been lowered to 20 degrees Celsius. Cooling should never be halted during surgery; the patient should remain surrounded by ice.

As a general rule, Alcor abstains from remote blood substitution if there are no experienced clinical or research surgeons on the team (unless it is determined that a local funeral director has the required experience to do the surgery and cannulation). Alcor's staff paramedic has received the requisite surgical training, and surgeons qualified for cryonics vascular access may also be supplied by Suspended Animation, Inc., or Critical Care Research, Inc., under contract to Alcor. If there is uncertainty or debate about the presence of any of the contra-indications, Alcor shall abstain from remote blood substitution.

Remote blood substitution should only be initiated when there is either a functional ATP or a conventional perfusion circuit present. The use of embalming pumps is not permitted because such pumps do not permit adequate control and monitoring of pressure.

The purpose of the initial stage of blood substitution is to wash out the blood of the patient. When the venous effluent of the patient indicates that the blood has been washed out (as evidenced by a clear color or no further changes in color), the ATP is switched from "open circuit" (washout) mode to "closed circuit" (recirculating) mode, and MHP-2 continues to circulate through the heat exchanger until the core temperature of the patient approaches the freezing point of water. Generally speaking, the ATP is stopped when core patient temperature falls below 5 degrees Celsius, although the procedure may be aborted before this point if there is a special advantage in doing so, such as the need to coincide with available air transport schedules. The patient should be prepared for transport after closing the surgical incisions.

If practical to do so, the patient should be weighed prior and after completion of blood substitution if this capability is available at a funeral home.

Patient transport

For cases where the location of the patient is accessible more quickly, overall, by ground than by air, Alcor employs an emergency vehicle that maintains at least all the equipment that is available for remote stabilizations. Periodic inventory check-ups and test drives should ensure that the emergency vehicle is always immediately available for casework. In a typical local case the Alcor vehicle is parked close to the location of the patient. During standby the vehicle can also be used for drawing up medications and assembling equipment. The vehicle is equipped with a lift gate to transfer the portable ice bath into the vehicle. Parking should permit sufficient room for the lift gate to operate.

If the patient is located outside of the practical range of Alcor's emergency vehicle, the patient will be transported to Alcor's operating room by scheduled airline or, if appropriate financial arrangements have been made, by air ambulance. The patient is placed in a case for the shipment of "cadavers" (often a "Ziegler" case). The Ziegler case is insulated and placed in a box which is typically used for air shipment and should be available from any mortuary.

The standby team should take great care to ensure that the case does not leak water or body fluids because such events can result in the shipment being taken off the plane and held for inspection. To prevent leakage of body fluids, the patient should be placed in a body bag surrounded with ice inside the Ziegler case. To prevent leakage of water from melting ice, the ice should be placed in large (2.5 gallon) Zip Loc bags.

The quantity of ice should be sufficient to allow for at least 48 hours of transport. This quantity will vary according to the patient's weight and body temperature at the time of shipment, subject to the different ice restrictions imposed by different airlines. A chart may be provided for guidance on this topic.

If ice has been stored in a freezer, care should be taken that it has warmed to 0 degrees Celsius and is actively melting before packing with a cryonics patient. If a bag of ice has visible white frost on it, then it is too cold to use. Bags suspected of being too cold should be warmed by running water over them until all the ice inside is visibly wet and melting.

If airline regulations do not permit shipping the patient with water ice, hypothermia can be maintained by cold packs. Alternatively, Terra-Sorb hydrogel crystals can be mixed with bagged ice, using 2 teaspoons of hydrogel crystals per gallon of ice. This will convert liquid water into a gel that cannot leak. Like ice bags, cold packs and hydrogel ice bags should always be warmed enough that they don't have frost on them. Condensation of liquid water on bags or ice bags standing in room air is normal and expected.

At least one team member should be in the same airplane as the patient to intervene with airline personnel and serve as an advocate for the patient if there are unexpected delays or complications. Temperature of the patient should be logged during transport. This temperature logger should not be the same as the one that was used during stabilization, to prevent data from being lost during transport or handling.

Monitoring of Stabilization Procedures

A standby team should include one designated scribe. The main task of the scribe is to collect data and record observations during the case. At a minimum, the scribe should record and describe all the pertinent events during a case, including the following:

- Deployment and case preparation
- Medical data of the patient obtained from medical caregivers

- Time of pronouncement of legal death
- Start and completion of stabilization procedures
- Start and completion of cardiopulmonary support
- Start and completion of initial cooling
- Time of IV placement
- Time of administration of all the medications and fluids
- Intermittent temperature data
- Start and completion of surgery
- Start and completion of blood substitution
- Intermittent pressure data during blood substitution
- Any interruptions of procedures and unusual events
- Start and completion of preparation of the patient for transport

Nasal *and* rectal temperatures should be logged from the start of stabilization procedures until the completion of stabilization procedures.

End tidal CO_2 measurements should be collected during cardiopulmonary support. If available, a digital end-tidal CO_2 should be used because it provides more detailed information about the efficacy of cardiopulmonary support.

If enough personnel and expertise are available, blood samples (blood gases and electrolytes) should be collected immediately after pronouncement of legal death and at intermittent points during stabilization procedures. These samples should be sent to a lab for independent analysis.

Prior to the start of blood substitution, a sample of the organ preservation solution should be collected for in-house quality assurance purposes.

It is important to note that a scribe should go beyond merely writing down numbers. All kinds of observations are valuable, and indeed they may be crucial, at a later date, in understanding what happened during a case, and why. In addition, we strongly believe that photographs and video of procedures should be created to document a case, provided that interested parties such as relatives, medical personnel, and mortuary staff permit this. While some people have expressed concern that visual materials may be stolen or placed in public forums, we feel that they can actually protect the cryonics organization and its personnel by demonstrating that procedures were carried out conscientiously. If there is anxiety about the possible theft of records, surely the answer to this problem is to protect the records from theft, rather than to stop creating records. Alcor's signup documents clearly state that cryonics is an experimental procedure. Any experimental procedure should be documented as completely as possible, so that others can learn from it, and procedures can be improved.

At a minimum, the team leader should be equipped with a voice recorder to document important events as they occur. Scribe notes and voice recordings are essential for constructing a correct timeline of the case. A separate scribe sheet is available for data collection during the terminal phase. All scribe sheets and voice recordings should be

surrendered to designated Alcor representatives after completing the case, and a signed, formal acknowledgment of receipt should be obtained.

Cryoprotective Perfusion

Cryoprotective perfusion is the core procedure of Alcor's human cryopreservation protocol. Without the introduction of a vitrification solution, extensive damage to the brain should be expected. To achieve good morphological preservation of the brain, the blood (or organ preservation solution) in the patient is replaced by a vitrification solution. Alcor's vitrification solution, M22, is licensed from 21st Century Medicine, Inc. It is the least toxic vitrification solution known in peer reviewed literature for its concentration, and provides strong protection against ice formation at slow cooling rates.

M22

Dimethyl sulfoxide	22.305% w/v
Formamide	12.858%
Ethylene glycol	16.837%
N-methylformamide	3%
3-methoxy-1,2-propanediol	4%
Polyvinyl pyrrolidone K12	2.8%
X-1000 ice blocker	1%
Z-1000 ice blocker	2%

A modified version of M22 is used to mitigate edema during the perfusion of whole body patients. In both whole body and neuro patients, M22 is introduced in a hypertonic carrier solution called LM5.

LM5

Glucose	90 mM
Mannitol	45 mM
Alpha-Lactose Monohydrate	45 mM
Potassium Chloride	28.2 mM
Potassium phosphate dibasic trihydrate	7.2 mM
Gluthathione reduced)	5 mM
Adenine HCl	1 mM
Sodium Bicarbonate	10 mM

The concentration of these LM5 solutes is the same in the base perfusate (starting perfusate), and the M22 solution that is added to the base perfusate, so that the concentration of these LM5 carrier solution solutes remains constant during the whole process of cryoprotectant perfusion.

Upon arrival at the Alcor facility, a median sternotomy should be performed to cannulate the great vessels of the heart (aorta and right atrium) for whole body patients. Vascular

access surgery for whole body or neuropatients at Alcor is performed by physicians or veterinary surgeons. The first step is to wash out the blood (or prior organ preservation solution) with B1 base perfusate. B1 consists of LM5 plus 1 mM calcium chloride dehydrate, plus 2 mM magnesium chloride hexahydrate, plus a proprietary additive that reduces edema.

After this step has been completed, the concentration of M22 solutes in carrier solution should be slowly ramped up in a linear fashion by progressively adding "M22 concentrate" (1.25 times normal concentration of M22 non-carrier solutes in LM5 carrier solution) to the circulating B1 base perfusate. The objective is to linearly increase the concentration of cryoprotectants in the circulating perfusate so that the arterial concentration of M22 solutes reaches 50% of target concentration in 100 minutes. The target concentration is the concentration of M22 solutes shown in the M22 composition table above, a concentration which can be created in the laboratory for refractometer calibration purposes by diluting a sample of M22 concentrate (1.25 times concentrated M22) with a 25% additional volume of B1 base perfusate.

When the arterial cryoprotectant concentration reaches 50% of target concentration, the rate of concentrate addition should be reduced to hold the arterial concentration near 50% while the venous concentration catches up. During this time, the arterial perfusion temperature should be dropped from near +3.5 degrees Celsius to -3 degrees Celsius. When the venous effluent of M22 reaches 50% of target nominal M22 concentration as measured by manual inspection of refractive index, the concentration of M22 should be rapidly increased to between 100% and 105% of target concentration. Cryoprotective perfusion is complete when the venous effluent concentration reaches 100% of target and there is little fluctuation in the refractive index of the venous effluent. In patients with extensive ischemic injury cryoprotective perfusion should be halted when no notable gains in M22 equilibration or severe cerebral edema is observed.

In neuro patients, only the head is perfused, through the carotid arteries. This procedure permits faster cooling rates, bilateral monitoring of the brain, and has been observed to produce reduced burr hole drainage and facial edema. If there is evidence that the Circle of Willis is incomplete, or damaged, the vertebral arteries should be cannulated as well. Otherwise they are clamped. During isolated head perfusion the head is secured in a cephalic enclosure and the venous return is filtered before being partly returned to the patient.

Perfusion pressure during cryoprotective perfusion should not exceed 100 mmHg for both neuro and whole body patients, measured in the arterial line. Because cryoprotectant concentration and lower temperatures both increase viscosity the pump speed needs to be reduced a number of times in the course of perfusion. Not exceeding 100 mmHg is particularly important in ischemic patients and patients with brain swelling. Perfusion pressures below 80 mmHg should be avoided.

In all cases, Alcor introduces its vitrification solution by ramping up the concentration of the cryoprotectant components linearly to avoid osmotic injury that would occur when

cells are hit with a full-strength high molar solution. M22 concentrate is gradually introduced to a mixing reservoir where the solution is continuously mixed with the previously-perfused base perfusate B1 before it is introduced to the patient. The venous effluent is partly discarded (to maintain volume of the mixing reservoir as M22 concentrate is added) and partly returned to the mixing reservoir to ensure a linear increase of the ramp and to reduce M22 volumes. In whole body patients, the circuit should also include a cardiotomy sucker to recover lost perfusate from the patient's chest cavity. A subzero heat exchanger should be used to lower the perfusate temperature below 0 degrees Celsius. At Alcor, LabView software monitors the conduct of perfusion.

Cryoprotectant Perfusion Monitoring

Scribing and monitoring continues during cryoprotective perfusion. Collection of important data is automated, but the scribe should make an effort to document the flow of the case and record data manually, including all events that may be at all pertinent. At a minimum the scribe should record the following:

- Preparation and set-up of the cryoprotective perfusion circuit
- Time of arrival of the patient
- Time of start and completion of surgery
- Start of blood / perfusate washout
- Start of cryoprotective perfusion
- Intermittent pressure readings
- Intermittent flow readings
- Intermittent perfusate and patient temperature data
- Manual refractive index measurements
- Any interruptions of procedures and unusual events
- Completion of cryoprotective perfusion.

Visual data are even more important, during cryoprotective perfusion, than during field work. Alcor maintains a video camera that monitors events from a fixed position, but its record should be supplemented by handheld camera photographs and video showing closeup details of surgical procedures, cannulation, shrinkage of the brain visible through burr holes, and other data.

The status of the brain is visually monitored through two small holes in the skull (burr holes) made using a standard neurosurgical tool (14 mm Codman perforator). This permits observation of the osmotic response of the brain. A brain with substantial ischemic injury swells, indicating disruption of the blood brain barrier, damage to endothelial cells, or compromise of water regulation of the cells.

During cryoprotective perfusion LabView software collects cryoprotectant concentration data from inline refractometers. These measurements can be consulted to look at trends but should not be used for making decisions. Protocol decisions should be guided by manual refractive index measures that are analyzed by either benchtop refractometers or handheld digital refractometers.

If practical to do so, in neuropreservation cases the cephalon should be weighed prior and after completion of cryoprotective perfusion.

Cryogenic Cooldown

After completion or termination of cryoprotective perfusion the patient will be prepared for cryogenic cooldown. A “crackphone” is placed in contact with the surface of the brain, to sonically detect subsequent fracturing events. Whole body patients should be transferred to a large insulated cooling box, and neuro patients to a small dewar. The cooldown process is software controlled. Liquid nitrogen is injected into the cooling box or dewar and vaporizes, drawing heat from the patient. A fan circulates the vapor to further enhance cooling. The temperature is dropped rapidly to approximately -110 degrees Celsius. That temperature is held at a plateau for 12 hours to allow annealing, and is then dropped more slowly over 100 hours to minimize thermal stress and fracturing. In a neuro case the final descent from around -190 degrees Celsius to -196 degrees Celsius is achieved by gradually filling the dewar with liquid nitrogen. In whole body cases the patient is transferred to a Bigfoot dewar in a precooled sleeping bag for the final descent to liquid nitrogen temperature. Because whole body transfers are done at room temperature, good logistical preparation and minimizing transfer time is of the essence.

If cryoprotective perfusion is not possible, ice formation will start below 0 degrees Celsius. As a consequence, a slow uniform cooling rate (to minimize thermal stress caused by unequal cooling) can be maintained throughout the whole temperature range.

Temperature and crackphone data are collected by the software throughout the cooling process.

Long Term Care

After cooldown to liquid nitrogen temperature (-196 degrees Celsius) the patient is maintained in a vacuum-insulated dewar until such a time in the future when resuscitation may be deemed feasible. Long-term care dewars should be equipped with level sensors and alarms. Dewar refills should follow a systematic, documented schedule.

Debriefing and Case Reports

After participation in the case, team members are required to submit scribe sheets, recordings, and other notes to Alcor. Alcor should schedule a debriefing session with all case participants and advisors as soon as is convenient after completion of the case. The objective of debriefing is to discuss strengths and weaknesses of the case in an analytical, non-confrontational manner. The debriefing session should be documented, and a transcript should be circulated among participants to check for accuracy and completeness. Usually the debriefing document should include a list with action items to be completed. A follow-up meeting should be scheduled to determine progress on these

items. These action items and their completion should also be documented in the case report.

A case report should be generated, including every pertinent detail . Alcor may decide to withhold some information to protect the privacy of the patient. Case reports should be completed within 2 months after the case and should follow a general template to allow for meaningful comparisons between cases, and meta-analysis.

After completion of the case the standby coordinator and other staff members should give priority to preparing Alcor for future cases. Equipment must be retrieved, cleaned, and refurbished, and consumable supplies must be replenished. This unglamorous routine work is obviously vital to maintaining future response capability