15: Sample Collection and Analysis

The use of fluid samples is not confined to assessment of the critically ill patient but can also be used after pronouncement, during stabilization and cryoprotective perfusion, to evaluate the effects and efficacy of cryonics procedures. The rationale for drawing and analysis of blood samples is that, for a patient who receives prompt and effective stabilization, the measured blood gas, chemistry, and electrolyte values should resemble those of a living patient.

There are a number of caveats that need to be made before further introducing the topic:

1. Because cryonics is not available as an elective medical procedure, homeostasis is typically compromised during the dying process prior to the start of stabilization procedures. As a consequence, the aim in cryonics stabilization procedures is not necessarily to maintain the blood sample values that were measured at the start of cryonics procedures, but to improve them as best as practical.

2. Administration of stabilization medications can itself alter electrolyte concentration. For example, the administration of the neuroprotectant magnesium can raise serum magnesium levels.

3. Analysis of samples is not confined to the drawing of blood samples. During remote blood substitution, samples can also be drawn from the circulating asanguineous fluid, and analysis of samples is also an option during cryoprotectant perfusion. Samples can also be drawn from the solutions themselves (prior to use) as a quality control.

4. During cryoprotective perfusion, osmolality and/or the refractive index are measured as an indicator of cryoprotectant equilibration.

This topic will not be discussed here but in the cryoprotective perfusion section of the manual.

Despite persuasive theoretical and practical arguments in favor of collecting fluid samples, there have only been a handful of cases where a concerted effort was made to collect and analyze fluid samples from the very beginning of stabilization procedures. The most important reason why cryonics organizations have routinely omitted fluid sampling and analysis is logistical. When personnel and resources compete for priority, it will often be at the expense of patient monitoring. When cryonics stabilization cases are run with insufficient people, there is little time and/or motivation to draw blood samples from the patient. In addition, drawing blood samples requires medical skills which are not likely to be encountered during local volunteer-driven cases. Unlike temperature measurements, the values obtained during blood and fluid sample analysis cannot be interpreted without basic knowledge of fluid and electrolyte balance. Prior to commercial availability of hand-held devices such as the I-STAT, on-site analysis of blood gases was not possible and samples were collected to be sent to outside labs for analysis.

Reports of Blood Gas Analysis in Cryonics Case Reports

Below is a list and short characterization of all published cryonics cases as of 2011 in which blood and perfusate samples were collected and analyzed after pronouncement of legal death:

1, 2, 3. A-1056, A-1057, and unidentified patient (Alcor, November/ December 1983): fluid samples during whole-body to neuro conversion of three patients.

4. A-1068 (Alcor, 1985): blood/perfusate samples.

5. A-1133 (Alcor, 1987): blood/perfusate samples.

6. A-1169 (Alcor, 1989): blood/perfusate samples.

7. A-1242 (Alcor, 1990): blood/perfusate samples.

8. A-1049 (Alcor, 1990): blood/perfusate samples.

9. A-1268 (Alcor, 1990): blood/perfusate samples.

10. A-1260 (Alcor, 1992): blood-perfusate samples.

11. A-1871 (Cryocare, 1995): blood-perfusate samples.

12. A-1110 (Alcor, 1997): perfusate samples

13. SA/CI-95 (Suspended Animation/Cryonics Institute, 2009): blood samples.

The last comprehensive transport and cryopreservation blood-sample analysis was done during the 1995 Cryocare James Galagher case (A-1871). Aside from one case of cryoprotectant effluent sample analysis in 1997, there was not a single documented case of transport blood gas analysis between 1995 and 2009.

During the mid-2000s, Suspended Animation staff member Aschwin de Wolf expressed renewed interest in the procedure, and both Suspended Animation and Alcor acquired the hand-held I-STAT blood gas analyzer. The first documented case of transport blood gas analysis since 1995 (albeit quite limited in nature) was reported in the 2009 Suspended Animation Curtis Henderson stabilization case report.

A Brief History of Blood Gas Analysis

The history of blood gas analysis starts with the discoveries of oxygen, carbon dioxide, the physiological mechanisms of gas exchange, followed by the identification and biochemical characterization of hemoglobin. The introduction of the pH scale goes back to S. P. L. Sorensen who sought a more elegant replacement for expressing the molar concentration of hydrogen ions. The first blood pH electrode was introduced in 1925. After publication of the first temperature correction tables in 1948, temperature-controlled blood pH equipment became commercially available in the mid-1950s.

Increased use and acceptance of blood gas analysis followed its successful use during the polio epidemic that ravaged Copenhagen, Denmark

in 1952 where the mortality rate dropped from 90% at the beginning of the epidemic to 25% at the end. On-site blood gas analysis moved from the lab to the bedside in order to identify cases of inadequate ventilation and gas exchange. The post-war period also gave rise to a new generation of blood gas analysis technologies, including the introduction of transcutaneous technologies as an alternative to the use of electrodes for the measurement of pH, oxygen, and carbon dioxide. In 1972 the pulse oximeter was introduced, making it possible to non-invasively calculate arterial oxygen saturation using a patient's finger or earlobe.

The use of bedside blood gas analysis suffered a setback when regulatory agencies dictated that only licensed technologists were able to operate blood gas analyzers. As a consequence, blood gas analysis increasingly moved back to clinical pathology laboratories. In recent decades the introduction of portable hand-held blood gas analyzers such as the I-STAT are reversing this trend as these devices do not require the same certification as that is needed to operate conventional blood gas analyzers. The use of pulse oximetry has further reduced the need for conventional (invasive) blood gas analysis.

Blood Gas Analysis in Cryonics

There are three distinct methods available for blood and perfusate gas analysis in cryonics:

1. Blood sample collection by the cryonics organization and (delayed) off-site analysis by a third party (usually a laboratory).
2. Blood sample collection by the cryonics organization and delayed off-site analysis by the cryonics organization.
3. Blood sample collection by the cryonics organization and on-site analysis by the cryonics organization.

With the exception of the 2009 Suspended Animation case listed above, all blood analysis in cryonics has been done after completion of procedures by either a third party or the cryonics organization. The recent commercial availability of hand-held blood gas analyzers such as the I-STAT has made

on-site blood gas analysis and real-time intervention a realistic and cost-effective possibility.

In some cryonics cases, specific parameters have been measured on-site while arterial blood samples were chilled for lab analysis at a later point. For example, Alcor case A-1068 documents on-site pH measurements using a portable pH meter, and in the past blood glucose kits were included in Alcor's standby kits. Other monitoring devices that can provide (real-time) information about gas exchange and ventilation efficacy, such as pulse oximeters and end tidal CO2 detectors, are covered in the general chapter about monitoring.

During stabilization procedures such as cardiopulmonary support (CPS), blood gas analysis is only possible by drawing a blood sample from the patient and analyzing it on-site or submitting it to a laboratory at a later date. In the case of blood substitution and cryoprotective perfusion, blood or perfusate analysis can be conducted by using inline blood gas analyzers such as the CDI™ Blood Parameter Monitoring System. This system uses optical fluorescence and reflectance technologies to continuously monitor blood gas parameters (up to 11 in the latest version). The target market for such devices is extracorporeal perfusion, but these technologies can also be used in cryonics procedures such as blood substitution and cryoprotective perfusion. The system uses a shunt line that allows the circulating blood (or perfusate) to come into direct contact with the sensor. One of the caveats of such systems is that it can only measure values within a specific range. This range usually is adequate for conventional perfusion procedures, but in some circumstances (such as pH and temperature) the values that are observed in cryonics may fall outside this range. As of writing, there is no documented example of using such inline devices for the measurement of physiological parameters in asanguineous solutions at ultra-profound or subzero temperatures.

Blood Sample Collection

If a decision is made to draw blood and perfusate samples, the first step is to ensure the presence of the proper supplies and qualified personnel to draw samples. Whereas some blood gases can be measured non-invasively, a

complete panel of blood gases, chemistries, and electrolytes requires drawing a blood sample from the patient. In principle, a blood sample can be drawn by inserting a needle into a vessel and drawing a suitable amount of blood. In practice, team members may want to place an IV to allow for repeated sampling from the same location. It is strongly recommended to place a separate IV line for blood sample collection. If blood samples are drawn from the same IV that is used for medications administration the likelihood of delays, errors, and faulty samples is increased.

Blood samples do not necessarily have to be drawn from a vein. It is possible to obtain blood samples from an artery, such as the radial artery at the wrist. Since this procedure is not recommended for non-professionals, it will not be discussed in this document. A special case of collecting arterial blood gases is to draw a sample from the femoral artery. There is not a realistic option during the initial stages of stabilization, but it is a possibility when surgery is performed to cannulate the femoral artery for blood washout.

There are various methods to collect venous blood samples. Unless the blood is analyzed on-site with the I-STAT, all methods require venipuncture and a collection tube for the blood. One advantage of using the I-STAT for blood sample analysis is that only a very small sample is required – just a few drops.

The most routine method for phlebotomists is to perform venipuncture with a blood collection tube. The most popular system is BD's vacutainer. The vacutainer system consists of a plastic holder, double sided-needle, and a collection tube. One side of the needle is used to puncture the skin and the other side enters the plastic holder. After the needle is correctly inserted into the vein the rubber top of the blood collection tube is pushed into the needle in the plastic holder. The vacuum in the tube draws the blood into the blood collection tube. The vacutainer system allows for the drawing of multiple samples by changing the blood collection tubes. A major advantage of the vacutainer system is that it ensures the right amount of blood is drawn at a safe speed. There are also a number of disadvantages. Unlike conventional venipuncture, in the vacutainer procedure there is no blood flashback to confirm correct placement of the needle in the vein.

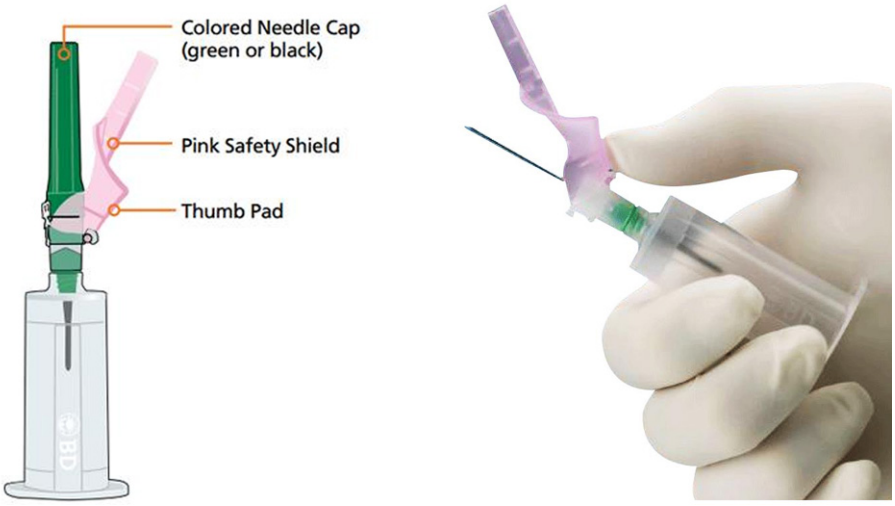

Figure 15-1. BD Vacutainer Eclipse Blood Collection Needle with Luer Adapter.

In principle, it is possible to secure the vacutainer needle to allow for intermittent blood samples, but during transport of a cryopatient team members may prefer to place an intravenous line. Blood samples can be drawn with a syringe, using a stopcock or directly from the line, and transferred to a blood collection tube. The disadvantage of this method is that the amount of blood and the speed at which the blood are drawn are not determined by the system. Another disadvantage, compared to the vacutainer system, is that such an "open system" can lead to spilling of blood during collection. Such events can be prevented by using a so-called "butterfly needle" which has flexible tubing attached to the small needle to connect a vacuum blood collection tube or syringe.

The small volume necessary to do on-site blood analysis with the I-STAT permits capillary blood sampling. Capillary blood collection involves the puncture of a well vascularized portion of the skin, such as the fingertips, to collect a few drops of blood from the patient. Like other venipuncture systems, capillary blood collection systems are available with safety features and anti-coagulant coating. One limitation of capillary blood sampling is that the obtained blood contains undetermined proportions of blood from

arterioles, venules, capillaries, and interstitial and intracellular fluids, which makes it harder to determine the proper reference values.

Blood collection tubes often come in different colors to reflect the various additives (or lack thereof) to the tubes. For example, for some tests whole non-coagulated blood is required whereas for other tests a gel is added to separate the serum after centrifugation. The meaning of the different colors is standardized between manufacturers. To prevent additives from one sample contaminating subsequent samples, a recommended order of draw has been established.

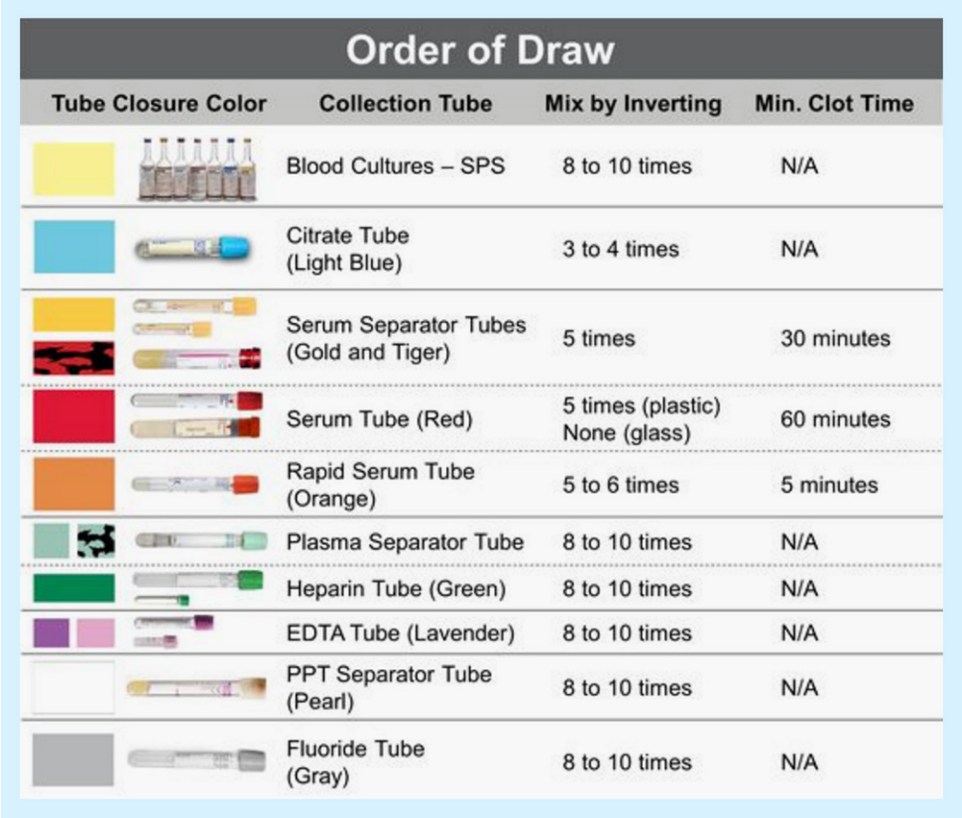

Figure 15-2. Example of Vacutainer® colors and order of draw

Perfusate Sample Collection

Collection of perfusate samples is relatively straight forward. Unlike the collection of blood samples, the drawing of perfusate samples does not require

an additional invasive step because the samples can be drawn from the arterial or venous line. This also provides an opportunity to draw an undiluted initial blood sample at the start of washout in the field or in the OR. As the blood is gradually washed out, these samples will progressively become more diluted. At this point it should be noted that drawing a sample during the later stages of blood washout or cryoprotective perfusion does not guarantee that a pure perfusate sample will be obtained because blood components can still be released into the effluent as a result of delayed opening of vessels.

One exception to the non-invasive nature of perfusate sample collection is when a sample is obtained from the burr holes of the patient to monitor cryoprotectant equilibration (see the chapter on cryoprotective perfusion).

Perfusate samples can be obtained directly from the line by using a "screw-type" syringe with a Luerlock end. The syringe is either used directly in the sample port of the line, or from a bypass line with a sample port to prevent errors such as inadvertently introducing air into the circuit. If a (relatively) pure sample of the perfusate is required as a control sample it must be taken from the arterial line at the start of perfusion or during priming. If a perfusate sample is drawn to obtain information about the patient, the sample needs to be drawn from the venous line. A safer alternative to using arterial samples to check the perfusate is to simply draw a sample from the perfusate container or during priming of the circuit.

After drawing the perfusate sample it is stored in the appropriate container or tube for later analysis or analyzed on-site to obtain direct feedback.

Blood Sample Analysis

In the past, Alcor has used conventional blood testing equipment to test blood samples, but since such relatively bulky devices confer little benefit over a handheld blood analyzer they will not be discussed here. If the samples are sent out to a third-party lab for analysis, the samples should be refrigerated after collection, and preferably be shipped at that temperature as well. If the blood samples will not be analyzed before seven7 days, the samples can be frozen to dry ice temperature.

The I-STAT is a portable blood gas analyzer made by the Abbot Corporation. For cryonics casework it has a number of distinct advantages:

1. It is light (18 oz), portable, and can be included in standby kits or travel bags.
2. It permits on-site analysis of blood and perfusate samples.
3. Only a few drops of blood are required for analysis.
4. It does not require difficult operating instructions for calibration or use.
5. A wide variety of cartridges allows for different kinds of tests.
6. The data can be uploaded to a computer or printed on-site

Figure 3. I-STAT with cartridges

As of writing, cartridges are available for the following blood measurements:

Chemistries/Electrolytes

Sodium (Na)
Potassium (K)
Chloride (Cl)
TCO2
Anion Gapa
Ionized Calcium (iCa)
Glucose (Glu)
Urea Nitrogen (BUN)
Creatinine (Crea)
Lactate

Hematology

Hematocrit (Hct)
Hemoglobin (Hgb)a

Blood Gases

pH
PCO2
PO2
TCO2a
HCO3a
Base Excess (BE)a
sO2a

Coagulation

ACT Kaolin
ACT Celite®

Cardiac Markers

PT/INR
cTnl
CK-MB
BNP

To date, there is only documented cryonics case in which the I-STAT was used in a cryonics case, and there remain a lot of unknowns. One of the current unknowns is how well the I-STAT will perform with asanguineous samples. For example, MHP-2 is a high potassium intracellular perfusate but the reportable range for the I-STAT is 2.0-9.0 mmol/L (for a complete overview of the I-STAT functionalities, cartridges and reportable ranges, see the manufacturer's website).

The I-STAT does not require much in terms of maintenance but it is important to run the most recent software and to ensure that the cartridges are stored properly and not used beyond their expiration dates.

Interpretation of Blood Samples

In an ideal cryonics case (a cryopreservation conducted at a hospital), the patient would not suffer ischemia and we would expect and aim for blood gas and chemistry values to be within the normal range until the blood of the patient is washed out with a suitable solution.

Normal Blood Gas and Chemistry Values

		Arterial	Venous
pH	A measure of acidity or alkalinity	7.35 – 7.45	7.31 – 7.41
pO_2	Partial pressure of oxygen	75 – 100 mmHg	30 – 40 mmHg
pCO_2	Partial pressure of carbon dioxide	35 – 45 mmHg	41 – 51 mmHg
TCO_2	Total concentration of carbon dioxide	24 – 30	25 – 33
HCO_3^-	Bicarbonate	22 – 26 mEq/L	22 – 29 mEq/L
O_2 Sat	Saturation of oxygen	95 – 100% (sea level; room air)	60 – 85%
BE	Base Excess	-2 to +2 mmol/L	0 to 4mmol/L
Lactate		4.5 - 14.4 mg/dL	4.5 – 19.8 mg/dL
NA	Sodium	135 - 145 mEq/L	
K	Potassium	3.5 – 5.0 mEq/L	
Cl	Chloride	95 – 105 mEq/L	
Ca	Calcium (ionized)	2.2 – 2.5 mEq/L	
Mg	Magnesium	1.6 – 2.6 mEq/L	
PO_4	Phosphorus	2.5 – 4.5 mg/dL	
BUN	Blood Urea Nitrogen	5 – 25 mg/dL	
Creatinine		0.5 – 1.5 mg/dL	
Hgb	Hemoglobin	Male: 13 – 18 g/dL; Female: 12 – 16 g/dL	
Hct	Hematocrit	Male: 42 – 52 g/dL; Female: 37 – 47 g/dL	
Anion Gap		10 – 12 mEq/L	
Osmolality		280 – 300 mOsm/kg	
Albumin		3.5 – 5 g/dL	
Total Protein		6.0 – 8.0 g/dL	

In more realistic scenarios we expect the patient to experience circulatory arrest, (cerebral) ischemia, and hypo-perfusion during CPS. In many cases the blood and chemistry values will be outside of the normal range prior to circulatory arrest because conventional medical treatment of the patient has been halted. This means that blood and chemistry values obtained immediately at the start of stabilization are not necessarily a healthy range, but a baseline to which the effects of stabilization procedures can be compared.

As discussed in the introduction, a value outside of the normal range does not necessarily mean that the patient would be better off if his values would fall inside the normal range. Some of the body's responses to circulatory arrest or hypo-perfusion may produce abnormal values but could actually be cerebroprotective, such as a drop of pH during cerebral ischemia. Additionally, administration of medications can alter blood chemistries without such an event being indicative of a deteriorating (or improving) situation. For example, the administration non-electrolyte fluids can depress electrolyte levels in the blood.

Our understanding of what values would be "ideal" during cryonics stabilization cases is almost non-existent and, keeping these caveats in mind, our best interpretation of the results to date is to simply determine to what degree our measured values compare to normal values.

Blood and Perfusate Analysis in Cryonics Cases

In this section we attempt a general overview of the documented cryonics cases in which blood and/or perfusate analysis was conducted. One complicating factor in evaluating these cases is that cases where blood samples were analyzed also tend to be generally "good" cases. After all, sample collection and analysis is not a core stabilization procedure and is usually possible only in cases where timely intervention with sufficient personnel and equipment is available. As a consequence, we have a rather poor understanding of what blood and perfusate samples look like in non-ideal and poor cases. We can compare results with normal values but it remains difficult to tell how these values compare to what would have been the case without standby and stabilization interventions.

Below, we present notes of *selected* cryonics cases where blood and perfusate analysis was conducted.

Alcor A-1056, A-1057, and unidentified patient

For all three patients fluid samples were obtained from the body of the patients after neuro conversion. The report specifies cryoprotectant osmolalities for all three patients in fluids obtained from different parts of the body. The author suggests that the low and variable distribution of cryoprotectant can be attributed to low volumes of the cryoprotectant and ischemia-induced perfusion impairment. In one patient light microscopy shows evidence of red blood cell agglutination.

Alcor A-1068

The case report for A-1068 contains an extensive discussion of blood, washout perfusate, and cryoprotectant perfusate samples. Reported cryonics transport electrolytes show values below normal for calcium and sodium, and elevated potassium, indicating hemodilution and/or inadequacy of cardiopulmonary support.

pH measurements during extracorporeal perfusion are reflected in the graph in Figure 15-4:

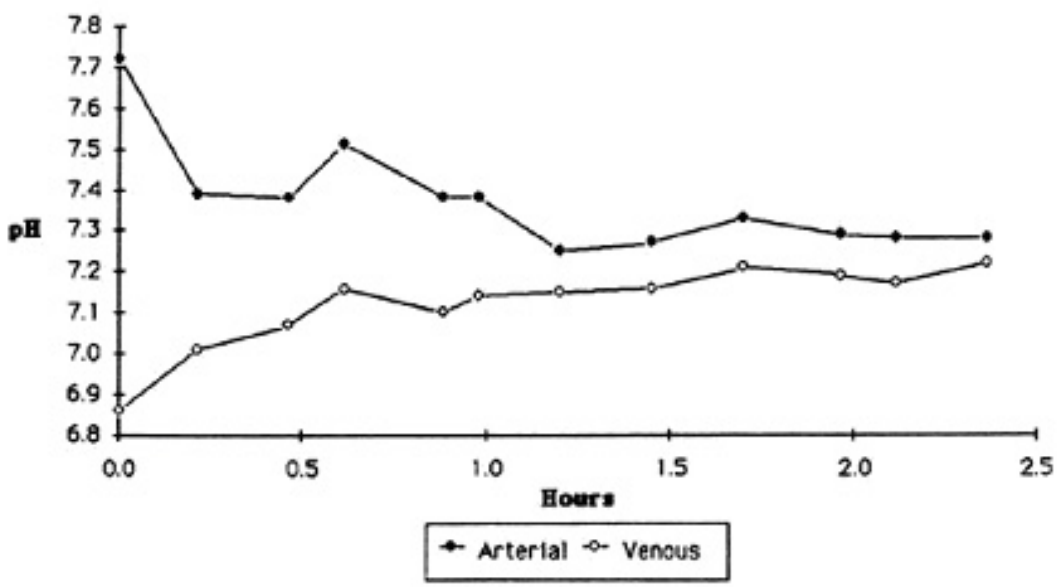

Figure 15-4. pH measurements during extracorporeal perfusion of Alcor case A-1068.

The venous drop in pH is attributed to the inadequate buffering capacity of the perfusate and the possibility of increasing HEPES is discussed. Interestingly, one of the changes from MHP to MHP-2 was to increase the concentration of HEPES from 7.2 mM to 15 mM.

There has been some debate in cryonics about the desirability of calcium in washout and cryoprotectant carrier solutions. In this case, calcium levels from both the burr hole and the recirculating cryoprotective perfusate were low but well in excess of the 50 uM/L threshold below which membrane damage is known to occur.

Alcor A-1133

This case report has an extensive appendix with graphs of blood gases, electrolytes, and enzymes data during cryoprotective perfusion. The author observes that the increase of some enzymes (SPK, LDH and SGOT) indicate progressive release from body tissues despite hemodilution.

Alcor A-1169

After 3 hours and 8 minutes of cardiopulmonary support and cooling to 23.5 degrees C, the first venous pH at the start of washout was 7.16, which may be attributed to administration of THAM during transport. After termination of blood washout, pH is 7.80, which the writer of the case report attributes to the addition of “a modest amount” of potassium phosphate to augment the HEPES buffer in the perfusate.

Alcor A-1242

This report documents a venous pH of 6.41 at the start of washout after a prolonged period of circulatory arrest without comprehensive and ongoing stabilization procedures.

Alcor A-1049

This is one of the most comprehensive cryonics case reports ever written and contains extensive data and analysis of blood and perfusate samples. In this case, a concerted effort was made to keep the patient viable by contemporary medical criteria and the serum chemistries indicate a relatively successful effort. Up until the removal of blood, the blood looked bright red and free of agglutination and clots, indicating successful oxygenation, anti-coagulation and mitigation of red cell aggregation. pH remained slightly basic until the end of cryoprotective perfusion, indicating robust protection against ischemia and good buffering activity of the washout solution and cryoprotectant carrier solution.

This case also stands out for conducting a renal viability evaluation, which was possible because the patient was a neuro patient. The patient’s kidney was subjected to renal slice potassium / sodium ratios in a cryobiology lab and the average ratio of 3.5 corresponds to the expected value for such slices after a storage time of approximately 2.5 days.

This case report also emphasizes the importance of perfusing a dehydrated patient with elevated serum osmolality with a hyperosmolar perfusate to avoid edema and cell lysis. This is one of the reasons why MHP-2 is formulated as a hyperosmolar perfusate. The report also stresses the value of comparing transport and washout samples to a baseline blood sample to

estimate the degree of hemodilution, the recruitment of interstitial and intracellular fluid to the vascular space, and the uniformity of blood washout.

CryoCare A-1871

This case is considered one of the best stabilization cases performed in cryonics, especially in terms of rapid cooling. The pH measurements during stabilization are consistently below physiological values but the author mentions that the team deliberately aimed for a lower pH (in the range of 7.0 to 7.2) because of its protective properties during ischemia. During cryoprotective perfusion pH approximated physiological values.

Venous oxygen saturation during stabilization was above physiological values, indicating vigorous cardiopulmonary support, ventilation and rapid cooling (reducing oxygen utilization). Other indicators such as potassium and sodium were slightly outside of the normal range but not nearly near the values that would be expected in the presence of severe ischemia.

Abnormally high values were observed for glucose, which the author attributes to failure of glucose regulation. Lactate levels were also elevated and continued to increase up until cryoprotective perfusion.

Alcor A-1110

This case report presents a comprehensive series of cryoprotective perfusate sample measurements and was conducted during a period where routine blood and perfusate sampling and analysis had been all but abandoned. There is no detailed analysis of these measurements in the report. The low osmolality measurements may reflect an error in carrier solution formulation.

Suspended Animation / Cryonics Institute-95

This is the only recent case in which a transport blood sample was collected. This case is also unique for the use of the I-STAT. There is no discussion of this single blood sample and lack of a context and specific timestamp does not permit a meaningful analysis in this report.

As has been become obvious from this brief discussion of the results of blood and perfusate analysis in cryonics cases, the practice has been gradually

abandoned. In addition, there is no or little analysis in more recent case reports.

Specific Biomarkers of Cerebral Ischemia

In the forensic sciences and biomedical research it has been found that (cerebral) ischemia reduces pH, elevate lactate, reduce calcium and sodium serum levels, and elevate serum potassium levels. A more focused approach has been to look for specific biomarkers of cerebral ischemia.

To distinguish cerebral ischemia from other common insults there has been a concerted search for bloodborne biochemical markers that, individually, or in combination, can lead to a credible diagnosis of cerebral ischemia or stroke. Among those biomarkers are N-acetyl aspartate, glutamate, taurine, matrix metalloproteinase 9, brain natriuretic factor (BNP), d-dimer, and protein S100β.

To date there have not been any attempts to identify specific biomarkers, or combinations of them, to evaluate the efficacy of stabilization and cryopreservation procedures. The phenomenon of cryoprotectant toxicity may also lend itself to the identification of bloodborne compounds that are associated with cell lysis or other mechanisms of cryoprotectant toxicity.