

BODY FLUID EXPANDERS

US Patent Application No. 12/667,309

Inventor: Douglas Rees PhD MRSNZ

Aqix Ltd, London, UK



2008

BODY FLUID EXPANDER

Field of the Invention

5 The invention relates generally to body fluid expanders. In particular, the invention relates to physiological liquid media for use in expanding, maintaining or replacing blood or extravascular body fluid volume. It is envisaged that the invention will find use in various medical applications, including in intravenous and extravascular (e.g. peritoneal) infusion procedures.

10

Background Art

Loss of blood volume, also known as hypovolemia, can result, for example, from a number of causes including physical injury, surgery, internal haemorrhaging or burns.

15 Hypovolemia can also be induced by the intake of drugs such as diuretics and vasodilators.

A significant loss of blood volume resulting from hypovolemia can be fatal unless treated rapidly. Such blood loss leads to a drop in blood pressure and a reduction in the
20 necessary supply of blood (and with it oxygen) to essential organs and tissues. Consequently hypovolemia can result in ischemia, multiple organ failure, kidney damage, brain damage, and ultimately death.

Hypovolemia is treated by perfusing the subject with blood substitutes. These substitutes
25 act to expand blood volume, bring about rehydration and normalise blood pressure.

Examples of currently known blood substitutes or blood volume expanders include lactated Ringer's solution, Hartmann's solution, HES (hydroxylethyl starch) and isotonic saline (sodium chloride) (Chiara et al.; Crit Care Med. 2003;31(7):1915-22; Rhee et al.;
30 J.Trauma. 1998;. 44(2): 313-319; Jernigan et al.; Am Surg. 2004;70(12):1094-8; Via et al.; J.Trauma. 2001;50:1076-82).

Despite their ability to restore blood volume, the current blood volume expanders are ineffective at preventing a severe and often fatal condition known as reperfusion injury. This phenomenon is observed in subjects who have suffered from severe hypovolemia and manifests itself in the form of damage to essential organs (such as lungs, kidneys, liver, etc). The deleterious effects of reperfusion injury are usually observed between 1 to 3 days following perfusion with blood volume expanders.

There is a need to identify further solutions which are capable of compensating for the loss of blood and the loss of interstitial and extracellular fluid resulting from hypovolemia and severe burns. There is also a need to identify treatments which are effective at preventing or lowering the incidence of reperfusion injury in hypovolemic subjects.

In addition to the above, there is also a need to maintain a sufficient volume of extravascular, interstitial fluid. This fluid, which bathes and surrounds cells, is essential to maintaining the homeostasis of tissues and organs, and functions, inter alia, as a means of delivering materials to cells, removing metabolic waste, and facilitating effective intercellular communication.

Summary of the Invention

20

In one aspect, the invention provides a non-phosphate buffered body fluid expander solution, comprising calcium ions and magnesium ions at a concentration ratio of 5:1 to 1:1.

25 In another aspect, the invention provides a body fluid expander solution, comprising a non-phosphate buffer which is selected from the group consisting of TES, MOPS, BES and combinations thereof.

In yet another aspect, the invention provides a body fluid expander solution, comprising calcium ions and magnesium ions at a concentration ratio of 5:1 to 1:1, and further

30

comprising a non-phosphate buffer which is selected from the group consisting of TES, MOPS, BES and combinations thereof.

5 In one embodiment, the non-phosphate buffer is present at a concentration of from 1 to 12 mmoles/L, preferably about 5 mmoles/L

The body fluid expander solutions of the invention preferably comprise calcium ions and magnesium ions at a concentration ratio of from 4:1 to 2:1, more preferably about 3:1.

10 Preferably, the body fluid expander solutions of the invention comprise from 0.1 to 2.5 mmoles/L calcium ions and/or from 0.4 to 25 mmoles/L magnesium ions.

In one embodiment, the body fluid expander solutions comprise calcium ions at a concentration of from 1.0 to 2.5 mmoles/L, preferably from 1.1 to 1.4 mmoles/L, more preferably from 1.2 to 1.3 mmoles/L, even more preferably about 1.25 mmoles/L.

The body fluid expander solutions of the invention preferably comprise magnesium ions at a concentration of from 0.2 to 0.6 mmoles/L, more preferably from 0.3 to 0.5 mmoles/L, even more preferably about 0.45 mmoles/L magnesium ions.

20

In one embodiment, calcium and magnesium ions are present at a concentration of about 1.25 mmoles/L and about 0.45 mmoles/L respectively.

25 In one embodiment, the body fluid expander solutions of the invention are blood substitutes for expanding and/or replacing blood volume. In yet a further embodiment, the body fluid expanders are extravascular fluid substitutes, e.g., interstitial fluid substitutes.

30 In addition to the above, the invention also encompasses concentrated forms of the solutions defined herein. For example, 1 to 50X, preferably 5 to 20X concentrates are encompassed.

In another aspect, the invention provides solutions as defined herein for use as medicaments and blood volume expanders.

- 5 In a further aspect, the invention provides solutions as defined herein for use in treating hypovolemia and/or burns, and for use in preventing and/or ameliorating reperfusion injury.

- 10 In yet a further aspect, the invention provides solutions as defined herein for use in: (a) fluid replacement therapy, (b) perfusing a body cavity (e.g. the abdominal or thoracic cavity) of a subject undergoing a surgical procedure, and/or (c) intravascular or extravascular delivery of therapeutic, test and/or synergistic agents to a subject.

- 15 The invention also encompasses the use of a solution as defined herein for the manufacture of medicaments and blood volume expanders, e.g. for treating hypovolemia or for treating the loss of extracellular and interstitial fluid in subjects suffering with burns.

- 20 The invention also provides the use of a solution as defined herein for the manufacture of a medicament for (a) treating the loss of interstitial fluid in a subject suffering with burns, (b) treating respiratory and/or metabolic acidosis in a subject, (c) perfusion of the abdominal cavity during peritoneal dialysis of a subject with acute renal failure or an acute toxicity condition, or (c) preventing and/or ameliorating reperfusion injury.

- 25 In yet a further aspect, the invention encompasses uses of solutions of the invention for delivering a therapeutic, test and/or synergistic agent to a subject, for example a biological agent, such as at least one stem cell.

- 30 In preferred embodiments, the delivery is effected by administration via an intravascular, intraperitoneal, intradermal, oral, intramuscular or topical route. Optionally, the delivery is effected by administration to the lymphatic system of a subject.

In yet a further aspect, the invention encompasses methods of treating hypovolemia and/or burns, and methods of preventing and/or ameliorating reperfusion injury, these methods comprising administering to a subject in need thereof an effective amount of a solution as defined herein.

In preferred embodiments the hypovolemia results from dehydration and/or burns and/or bleeding. In another embodiment, the hypovolemia is drug induced.

10 In a further aspect, the invention provides a method of maintaining physiological homeostasis of a tissue and/or organ in situ during a surgical procedure carried out on a subject, the method comprising perfusing said tissue and/or organ with a solution as defined herein.

15 In one embodiment of the above method, the solution is maintained at a temperature of between 4 and 20 °C such that said tissue and/or organ, when perfused with said solution, is maintained in a state of hypothermia.

In yet a further embodiment of the above method, the surgical procedure is carried out to retrieve said tissue and/or organ from a donor subject, for subsequent transplantation to a recipient subject.

In yet another aspect, the invention encompasses methods for delivering a therapeutic, test and/or synergistic agent to a subject using the solutions of the invention. In a preferred embodiment, the agent is a biological agent, such as at least one stem cell.

In preferred embodiments, the delivery is effected by administration via an intravascular, intraperitoneal, intradermal, oral, intramuscular or topical route. Optionally, the delivery is effected by administration to the lymphatic system of a subject.

30

In another aspect, the invention provides the use of a solution as defined herein for (a) dialysis of the peritoneal cavity in a subject suffering from acute renal failure or an acute toxicity condition; and/or (b) irrigation of abdominal and/or thoracic organs in a subject undergoing a surgical procedure.

5

In yet another aspect, the invention encompasses a substantially phosphate-free buffered solution for use as a medicament.

Description of Figures

10

Figure 1 Assessment of the survival of rats over time as a function of the resuscitation solution used. Rats were subjected to serial withdrawals of blood. The rats were separated into three experimental treatment groupings as follows: (1) replacement of blood with physiological saline; (2) replacement of blood with AQIX[®]RS-I solution; and (3) no replacement of blood losses. Survival of rats in each of the various groupings was monitored and the data set out in Figure 1.

15

20

Figure 2 Comparison of the total volume of blood withdrawn for each of the study arms defined with reference to Figure 1 above. Blood was withdrawn from rats as described in the Example 4 below.

25

Figure 3 Assessment of the respiratory rates of rats over time in the various study arms defined with reference to Figure 1.

30

Figure 4 Assessment of pig blood profiles over various time periods of perfusion with AQIX[®]RS-I. Pigs were infused with 1.0L of AQIX[®]RS-I solution every day for three days and blood samples were taken at various time points. Blood samples were tested, inter alia, for complete blood counts, serum chemistry determination, electrolytes, glucose, lactate, osmolality, serum enzymes (aspartate amino transferase, alanine aminotransferase),

total creatine kinase [CK] + LDH, coagulation times and fibrinogen levels. Also, blood was measured for IL-6 and TNF-alpha.

Figure 5 Comparison of the ADP:ATP ratios in CS and WS group pig kidneys before and after 6 hours of normothermic perfusion.

Detailed description of invention

10 The terms “body fluid expander” or “body fluid expander solution” or “body fluid substitute” as used herein mean a physiological liquid solution which is intended for use in replacing or expanding body fluid, and/or maintaining a sufficient volume of body fluid. The body fluids for which the solutions of the invention are intended to expand, replace or maintain include intravascular fluids (e.g. blood components such as plasma), or alternatively, extravascular fluids (e.g. interstitial fluid).

15

The terms “body fluid expander” and “body fluid expander solutions” also encompass physiological liquid media which are intended for use as a vehicle for the delivery of therapeutic, test and/or synergistic agents into the body of a subject in need of said agents, or in circumstances where the agents are to be tested, for example on non-human subjects.

20

The term “subject” or “subjects” encompasses, where appropriate, human subjects and non-human animal subjects, for example rodents, pigs, monkeys, dogs and the like.

25 Terms such as “non-phosphate buffer” and “substantially phosphate free” are intended to encompass fluid embodiments which substantially lack inorganic phosphate ions.

30 The terms “blood volume expander” or “blood volume expander solution” or “blood substitute” as used herein mean a physiological liquid solution which is intended for use in replacing, expanding or maintaining blood volume. These solutions therefore find use in substituting for a loss of blood volume resulting from hypovolemia.

The term “hypovolemia” as used herein means a state of decreased blood volume, or more specifically, a state of decreased blood plasma volume. Common causes of hypovolemia include dehydration, burns, bleeding (e.g. haemorrhaging) or the intake of certain drugs such as diuretics and vasodilators.

The term “reperfusion injury” as used herein means the injury caused to essential organs of the body following hypovolemia and subsequent perfusion with conventional blood volume expanders.

The present invention emerges from a realisation of the inventor that conventional blood volume expanders are detrimental to the maintenance of cell, tissue and organ survival and viability, and can hence contribute to the development of reperfusion injury.

In part, the present invention stems from an appreciation that the composition of blood volume expanders (and physiological media in general) should be based on a specific knowledge of the activity coefficients of ionic species within the interstitial gel phase surrounding each cell. These activity co-efficients have been calculated and the solutions of the present invention have been designed in accordance with these calculations.

The solutions of the invention make use of non-phosphate buffers, based on the realization that excess phosphate ions can be deleterious to the viability and functional integrity of perfused cells, tissues and organs. Phosphate ions inhibit glycolysis, oxidative phosphorylation (Berman & Sanders; *Circul.Res.*, 1955;3, 559-563) , creatine kinase activity (Hall & DeLuca; *Adv.Exp.Med.Biol.* 1986; 194, 71-82) and the enzymes involved in oxygen free radical scavenging (De Frietas & Valentine; *Biochemistry* 1984;23:2079). These enzymes are important in inducing apoptotic changes at the cellular level, ultimately leading to the necrosis (i.e. death) of damaged or abnormal cells, tissues and organ systems.

30

Furthermore, the use of non-phosphate buffered solutions avoids the inefficiency of conventional phosphate buffered solutions in terms of instability above pH 7.2. In particular, inorganic phosphate ions in conventional solutions are, over time, precipitated in the form of calcium phosphate (Pedersen, MD Thesis, University of Aarhus, Denmark. Publ. S A Moller Christensen A/S. 1973; 41-51). This problem is accentuated by variations in the temperature range at which the solutions are used and therefore are of limited clinical utility.

Described herein are body fluid expander solutions which, by virtue of their composition, are able to compensate either for loss of blood in hypovolemic subjects, or loss of interstitial and extracellular fluid associated with severe burns. The body fluid expander solutions of the invention provide physiological liquid media which mimic the ionic, substrate and biophysical environment of interstitial fluid (see, for example, Table II below). As such, it is envisaged that these solutions will find use as universal perfusion and preservation media. The concentrations of ion species acknowledge the activity coefficients of each ionic species within the interstitial gel phase around mammalian cells. This is in contrast to many conventional media which base their concentrations on total serum concentrations.

In US Patent 6,946,241 (which is incorporated herein by reference), the present inventor describes non-phosphate buffered liquid cell culture media. It has now been appreciated that effective blood volume expander solutions and body fluid expander solutions in general can be based on the composition of these media. It is envisaged that the solutions of the present invention will not only compensate for the loss of blood associated with hypovolemia but will also reduce or prevent the onset of reperfusion injury. It is also envisaged that the solutions defined herein will have application in the in situ maintenance of organs and tissues, which become exposed during various surgical procedures.

The body fluid expander solutions of the present invention are preferably free of serum and/or serum components. The solutions are hence free of animal derived serum proteins

and other contaminants, such that there are no undefined, extraneous serum proteins present. The absence of serum has the advantage that the solutions are “chemically” better defined than conventional serum based solutions. Furthermore, the absence of serum and serum derived components avoids concerns over the possible transmission of infectious diseases (e.g. HIV and CJD) associated with the use of this material in vivo.

For the reasons given above, the body fluid expander solutions of the present invention (and particularly those solutions intended for human use) are also preferably free of foreign or animal derived antigens, pyrogens, proteins and the like.

10

The body fluid expanders of the present invention adopt a natural physiological buffering system. Preferably this buffering system takes the form of $\text{NaHCO}_3/\text{pCO}_2$ in combination with the zwitterionic Good’s buffer, BES (N, N-bis [2-Hydroxyethyl]-2-amino-ethanesulphonic acid (Good et al.; Biochemistry 1966;5:467-477), incorporated herein by reference), which acts by virtue of its ideal pKa over a temperature range of 10 to 37°C, to provide a stable pH, an essential requisite for cellular preservation. BES has been shown to be non-toxic to cultured mammalian cells in long term studies and exhibits negligible binding of calcium or magnesium ions, so removing the potential hazard of precipitation of divalent ions which occurs when using conventional bicarbonate/phosphate or double phosphate buffer solutions. Indeed, 10X concentrates of solutions according to the invention have been experimentally shown to have a shelf life (stored at 3 to 8°C) in excess of 14 months. As alternatives to the use of BES, it is also possible to use morpholinopropane sulphonic acid (MOPS) or N-tris-(hydroxymethyl) methyl-2-amino ethane-sulphonic acid (TES). Furthermore, a combination one or more of TES, BES and MOPS could be used.

Optionally, the non-phosphate buffer (i.e. TES, MOPS or BES, or combinations thereof) is present at a concentration of from 1 to 12 mmoles/L, preferably from 3 to 7 mmoles/L, more preferably from 4 to 6 mmoles/L, even more preferably about 5 mmoles/L. Optionally, bicarbonate ions are present at a concentration of from 21 to 35 mmoles/L, preferably from 23 to 26 mmoles/L, more preferably about 25 mmoles/L.

In preferred embodiments of the invention, the non-phosphate buffer systems used allow the maintenance of a pH of from 7.05 to 7.5 over a temperature range of from 4°C to 38°C. More preferably, the pH of the solutions of the invention can be adjusted to give values ranging from 7.13 to 7.5 ± 0.5 over a temperature range of 10 to 38°C. Preferably, the solutions of the invention have a pH of about 7.46 at a temperature of about 37.4°C. It is preferred that the above pH values are maintained in vivo following administration of the solutions to a subject in need thereof.

As discussed herein, the solutions of the invention may be used for both intra- and extravascular infusion procedures. The solutions may also be used to perfuse isolated animal and human organs under normothermic conditions. When the solutions are used to perfuse isolated organs, it is preferable that the solutions are aerated with carbogen (95% oxygen/5% carbon dioxide).

The body fluid expander solutions of the present invention may also comprise in any combination, one or more, two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, eleven or more, or all of the following components: from 100 to 150 (preferably about 135) mmoles/L sodium ions, from 2.5 to 6.2 (preferably about 5) mmoles/L potassium ions, from 0.1 to 2.5 (preferably about 1.25) mmoles/L calcium ions, from 0.4 to 25.0 (preferably about 0.45) mmoles/L magnesium ions, from 96 to 126 (preferably about 118) mmoles/L chloride ions, 2 to 11 mmoles/L (preferably about 10) glucose (preferably D-glucose), from 50 to 150 (preferably about 110) μ moles/L glycerol, from 7 to 15 (preferably about 10) μ moles/L choline, from 5 to 400 (preferably about 300) μ moles/L glutamate (preferably L-glutamate), from 5 to 200 (preferably about 20) μ moles/L aspartate (preferably L-aspartate), from 100 to 2000 (preferably about 400) μ moles/L glutamine (preferably L-glutamine), from 1 to 120 (preferably about 40) nmoles/L thiamine pyrophosphate, from 40 to 70 (preferably about 50) μ moles/L D- or DL or L-carnitine (preferably L-carnitine), and from 5 to 200 (preferably about 28) m I.U./L porcine or human insulin (preferably human insulin).

Chloride ions, when present, are preferably provided as sodium, potassium, calcium and magnesium salts. Preferably, when present, choline is provided as a chloride salt.

5 Solutions encompassed by the present invention comprise a number of substrates which retain metabolic homeostasis of tissues and organs. Glucose and glycerol have been shown to be satisfactory in meeting the energy demands of isolated tissues and organs, even when they are not the preferred substrate for the organ in question (e.g. the heart) by the inclusion of physiological levels of insulin. Apart from their ability to be
10 metabolized, glycerol and glucose also have free radical scavenging and membrane stabilizing properties, which have been shown to be extremely important in maintaining the physiological viability of tissues and organs.

As described above, aspartate and glutamate can also be included in the solutions
15 according to the invention, to enhance oxidative metabolism by replenishing TCA cycle intermediates, thereby maintaining high energy phosphate levels even during ischemic insult. Similarly, glutamate is involved in maintaining intracellular oxidation-reduction potentials. It is envisaged that by optimizing the aspartate-malate and glycerol phosphate shuttles, cells will maintain an optimal NAD/NADH balance and thereby sustain adenine
20 nucleotide levels.

Thiamine plays an important role in the oxidation of α -keto acids (by the action of thiamine cocarboxylase) and prevents the accumulation of pyruvate and pyruvate aldehyde, thereby minimizing cell toxicity.

25 In the tricarboxylic acid cycle, thiamine pyrophosphate (TPP), which is included in preferred solutions of the invention, is a co-factor in the metabolism of α -ketoglutaric acid to form succinyl-coenzyme A, by oxidative decarboxylation, or to form glutamate, by reductive amination. In essence, TPP is involved in numerous interrelated
30 biochemical pathways, especially those of the Pentose Phosphate and Glycolytic pathways. The thiamine may be employed as thiamine pyrophosphate, thiamine

diphosphate or thiamine diamide. Preferably the solutions of the invention comprise thiamine as thiamine pyrophosphate chloride.

Further, the inclusion of thiamine pyrophosphate (PPi) in the formulation of RS-I would
5 also appear to be of necessity to peritoneal dialysis patients in order to prevent phosphate ion depletion and calcification as it is readily dialyzable across hemodialysis membranes (MW - 175 Dalton) and has been previously administered either intravenously or via the hemodialysis or peritoneal dialysis solution to replenish plasma phosphate and/or pyrophosphate levels.

10

The vitaminoid carnitine has been reported to have multiple effects in improving cardiac function other than by simply optimizing oxidative metabolism, such as, by promoting the utilization of alternative substrates and may additionally improve coronary blood flow. L-carnitine is preferred to the D- or DL isomers because it causes no inhibition of
15 acetyl co-enzyme A/free fatty acid metabolism. Preferably the vitaminoid component comprises 50 μ moles/L of [-]- β -hydroxy- γ -trimethylamino-butyrate hydrochloride (L-carnitine). In this invention, the preferred inclusion of the L-isomer of carnitine is intended to optimise the transport of long chain fatty acids from the cytosol into the mitochondrial matrix to the site of β -oxidation and thereby to buffer the
20 intramitochondrial acetyl CoA/CoA ratio by stimulating the synthesis of acetyl carnitine from carnitine acetyl transferase. This reduction in the ratio of acetyl CoA/CoA will result in an efflux of acetyl carnitine from the mitochondria with an associated stimulation of pyruvate dehydrogenase and reversal of fatty acid inhibition of glucose oxidation. Ultimately the optimization of free fatty acid utilization as an energy source is
25 essential for all types of cells but this must be done with preservation of carbohydrate (glucose) utilization by optimized functioning of the enzymes involved in glycolysis, eg. hexokinase, glucokinase, phosphofructokinase.

As described above, solutions of the invention may also comprise insulin. The use of
30 human recombinant insulin (e.g. expressed in *E.coli* or *S. cerevisiae*) not only precludes the risk of antigenic or viral contamination in recipient cells/tissues/organs, as may be the

case with insulin derived from other mammalian or animal sources, but also leads to a better fit being achieved of insulin molecules to human insulin receptor structure, ie. Receptor specificity will be optimized to retain the many associated functions of insulin in cellular processes.

5

Essentially, the biological effects of insulin do not simply relate to its ability to regulate carbohydrate metabolism and facilitated transport of circulating glucose into cells but also in its ability to bring about (i) the enhancement of intracellular glucokinase activity and amino-acid incorporation into protein, (ii) stimulation of DNA translation into
10 proteins, (iii) increased lipid synthesis and (iv) stimulation of sodium, potassium and inorganic phosphate transport across cell membranes.

In preferred embodiments of the invention, normal, human serum levels of insulin have been utilized. In contrast, conventional known perfusate formulations have, when
15 incorporating insulin, made use of unnatural levels of this hormone (e.g. 10 to 50 x 10⁶ mIU/L; about a million times more concentrated than the insulin found in solutions of the invention). The reason for this relates to the fact that only a small amount of the insulin exists as single molecules in such concentrations. The rest of the insulin exists in the form of large aggregates, which are ineffective at stimulating insulin receptors and are
20 hence biologically inactive. The solutions of the invention achieve normal, human serum levels of insulin by acidifying the insulin during formulation to prevent aggregate formation, so allowing individual active, molecular species of insulin to exist in solution at pH 7.3 ± 0.2.

25 The concentrations of ionic species in solution according to the invention acknowledge the activity coefficients of each ionic species and not simply their total serum concentrations. For example, serum binding of calcium and magnesium ions must be distinguished from the actual free, ionized levels of these ions. Magnesium ions are important in a number of critical cellular reactions and their extracellular presence is
30 reported to stimulate mitochondrial respiratory activity and modulate the effects of rapid

calcium influx and potassium efflux. Equally, an adequate concentration of calcium ions is important to maintain the free levels of this ion present in the circulation.

5 The ionic conductivity of the solutions of the invention is preferably comparable to that of human serum, namely $12.0 \pm 0.3 \text{ mS cm}^{-1}$ and as such maintains the ionized status of the cell membrane and activities of enzymic moieties.

10 Thus in accordance with the above, the solutions of the present invention are isosmotic to human serum (ca. 290 mOsmoles/L) and do not appear to necessitate the inclusion of plasma expanders, as demonstrated by the fact that only minor changes (ca. 8%) in hydration occur during long term (ie. 4 to 52 hr) hypothermic perfusion of the isolated rat heart and visceral nerve-muscle preparations. This may be explained by the fact that the cell membrane lies in continuity with a 99% gel interstitial phase so providing natural colloidal buffering to excess Donnan ionic equilibrium exchange across the cell
15 membrane. The majority of the osmotic pressure is provided by sodium ions and their accompanying anions, and only a small component (ca. 0.5%) can be attributed to plasma proteins.

20 The practicality of including oncotic agents is further compromised by their affinity for calcium and magnesium ions, necessitating prior dialysis in fresh solution so as not to disturb cationic composition. The labile nature of polypeptide expanders also makes them impractical through their predisposition to mechanical denaturation. Unfortunately, while these colloidal expanders are essentially non-toxic, their use is contraindicated in terms of, for example, (1) raised viscosity increases the thickness of the 'unstirred' layer
25 around cells so hindering diffusion of metabolites, (2) alteration of the surface membrane bioelectric potential so disrupting cellular metabolism and receptor activities, (3) antigenicity of proteinaceous expanders, (4) agglutination and haemolysis of RBC's and (5) blockage of microvasculature and ischemia.

30 It is envisaged that the solutions of the invention will find general use as base compositions to which additional components can be added, depending upon the specific

medical purpose. For instance, it is envisaged that the solutions of the invention could be supplemented with, for example, red blood cells (RBCs), plasma and/or platelets for the generation of artificial blood. Such blood components may be either natural or artificial. Thus, additional chemicals can be added to the base composition as and where required.

5 It is therefore intended that the solutions of the invention will find broad applicability as base compositions where a medical utility requires the expansion, replacement, maintenance and/or supplementation etc of a body fluid with a non-phosphate buffer solution.

10 The solutions of the invention find use in methods for treating hypovolemia or the loss of interstitial and extracellular fluid brought about in subjects suffering from severe burns. In addition, the solutions of the invention are applicable in methods of preventing and/or ameliorating reperfusion injury. The solutions of the invention therefore find use as medicaments. For treating hypovolemia and for preventing/ameliorating reperfusion

15 injury, it is preferable to systemically administer the solutions of the invention by the intravenous route. However, in addition to replacing, maintaining or expanding blood volume, it is envisaged that the solutions of the inventions will also find other uses which include the following; for maintaining, preserving and irrigating tissues and organs in situ during surgical procedures, for perfusing the abdominal cavity of a subject suffering with

20 an acute renal failure or an acute toxicity condition, and for preserving donor tissues and organs in situ during surgical procedures for their removal from the donor patient. Thus, as well as being administrable through the intravascular route, the solutions of the invention may also be administered by other routes, for example, using intraperitoneal, intradermal, intramuscular, topical or oral routes. For the treatment of burns, it is

25 preferable to administer the solutions locally by topical application to the site of the burn itself. In addition however, dehydration brought about as a result of severe burns can be treated through systemic administration of the solutions. In preferred embodiments, the methods, solutions and medicaments of the invention are for use in treating mammalian subjects, e.g., humans.

30

As mentioned above, it is envisaged that one application of the solutions defined herein is for preserving donor organs in situ before, during and after surgical procedures for their removal from the donor subject. Use of the solutions of the invention in this regard could involve, for example, whole body infusion (ECMO procedure) with donor kidneys, heart, liver, etc being harvested from a donor cadaver followed by normothermic reperfusion of the isolated organ. Preliminary studies (not shown here) have indicated that AQIX[®]RS-I as defined herein (see Examples) has the ability to re-animate hypothermically preserved cadaver human organs allowing such organs to be used in preclinical drug bioassay trials, thereby addressing the problem of Adverse Drug Reactions (ADR) reported to occur using in vitro and in vivo drug assessment in animal models.

It is also envisaged that the solutions of the invention will find use as media for the effective delivery of agents to a subject in need thereof, or in circumstances where the agents are to be tested, for example on non-human subjects. For example, the solutions of the invention may find use as diluents for the delivery of pharmaceutical, test or synergistic agents or, alternatively, for the delivery of stem cells to a subject in need thereof. The delivery of stem cells could, for example be brought about by suspending the stem cells in media as defined herein, and delivering the resulting suspension directly to the tissue or organ where required. Alternatively, delivery may be effected by administration of the suspension to the lymphatic system.

It will be appreciated that the general methods of administering blood volume expanders, known in the art, can be applied when using the solutions of the invention. In particular, methods for regulating the level of administration required in a particular situation (eg. by reference to maintaining adequate blood pressure and cardiovascular function) can be applied using the information available to the skilled person, having regard to the state of the art.

It will be further appreciated that standard perfusion techniques, known in the art, for bathing and irrigating tissues and organs in situ during surgical procedures and for

dialyzing body cavities, can be applied when utilizing the solutions of the present invention.

5 The invention envisages that specific sub-components of the solutions of the invention can be used individually or in any combination.

Particular embodiments of the invention are described below by way of the following examples. The examples are provided to illustrate embodiments of the invention but are not to be considered as limiting in any way.

10

EXAMPLE 1: Formulation of AQIX[®] RS-I solution

Formulation

15

In the following, thiamine pyrophosphate (cocarboxylase), Sigma C4655 was prepared as a 0.4 mg/mL stock solution in MilliQ (endotoxin-free) purified water [or equivalent ATSM Type II; NMT 18.0 MΩ-cm at 25 °C], and stored frozen in dark glass vials. Choline chloride (Sigma C7527) was prepared as a 17.5 mg/mL stock solution in MilliQ
20 endotoxin-free purified water and stored frozen in glass vials. Human recombinant insulin (Sigma I0259/I2643) was prepared as a 0.5 I.U./mL stock solution in endotoxin-free MilliQ purified water acidified to pH 2.4 with 0.12N hydrochloric acid and stored frozen in glass vials.

25 In the following preparations, endotoxin-free MilliQ purified water [or equivalent ATSM Type II; NMT 18.0 MΩ-cm at 25 °C] was used throughout, both in the initial stirring, and in the final dilution.

30 For the preparation, a stainless steel container was filled with 8 litres of MilliQ purified water and, the following ingredients were weighed out and added while constantly stirring, in the following order: 642.96 grams of sodium chloride (CFK0484), 37.28

grams of potassium chloride (BDH10198), 18.38 grams of calcium chloride dihydrate (BDS10117), 9.14 grams of magnesium chloride hexahydrate (BDH101494) and 106.61 grams of BES free acid (Sigma B6266), 1.84 milligrams of thiamine pyrophosphate (Sigma C9655) (using 4.6 mL of the stock solution), 0.9899 grams of L-carnitine (Sigma C0238), 0.1397 grams of choline chloride (Sigma 7527) in the form of 8 ml of the stock solution, 1.013 grams of glycerol (Sigma G2025), 2.8 I.U. of human recombinant insulin (5 ml of the stock solution), 0.310 grams of L-aspartate sodium salt (Sigma A6683), 180.2 grams of anhydrous D-glucose (Sigma G7021), 5.07 grams of L-glutamate sodium salt (Sigma G5889) and 5.84 grams of L-glutamine (Sigma G5763). The whole was stirred until completely dissolved and then the final volume of 10 litres was produced by adding further MilliQ purified water.

The ingredients utilised in the formulation of the solution for clinical Phase 1 trial applications will be of UPS classification and manufactured under GMP definition.

15

The solution was filtered through a sterile filter (0.2 μm Sartobran PH) into 100 mL sterile sealed glass bottles.

This solution is a 10 times concentrate of the solution intended for use. When needed, it can be diluted with the appropriate quantity of MilliQ purified water [or equivalent ATSM Type II; NMT 18.0 M Ω -cm at 25 °C].

20

For preparation of the 1X solution, 100 ml of the above 10 times concentrate may be diluted with 900 mL of double deionised or endotoxin-free MilliQ purified water [or equivalent ATSM Type II; NMT 18.0 M Ω -cm at 25 °C] to 1 litre with the addition of 2.1 g of endotoxin-free sodium bicarbonate (Sigma S4019) and stored at 8-10 °C prior to use. Sodium bicarbonate is not added to the concentrate solutions before they are stored, since extended storage of the concentrate containing bicarbonate ions may cause precipitations of calcium carbonate.

25
30

For use as a body fluid expander solution, each litre of the solution may contain 100 mg/L of chloramphenicol (Sigma C3175) or other conventional antibiotics, antifungal agents to prevent the risk of bacterial infection.

5

The following factors should be taken into account when preparing the solution:

- 1). The method of assembly of the solutions and, specifically;
- 10 2). Use of endotoxin-free MilliQ purified water [or equivalent ATSM Type II; NMT 18.0 MΩ-cm at 25 °C] to make up all stock solutions and the 10 times concentrate bottles of manufactured solutions according to the invention;
- 15 3). The method of preparing sterile stock solutions according to the invention and concentrates do not involve autoclaving or gamma-irradiation, e.g. irradiation of the solution to achieve sterility will result in degradation of glutamine, glucose, insulin and thiamine pyrophosphate components;
- 20 4). The use of glass bottles for storage of all 10 times stock concentrate solutions;
- 5). Preparation of solubilised insulin by acidification at pH 2.4 plus storing insulin ingredients and stock solutions at – 20 °C;
- 25 6). Preparation of thiamine pyrophosphate plus TPP stock solutions stored at – 20 °C under dark conditions (see reason below);
- 7). Preparation of Choline chloride plus stock solutions stored at – 20 °C;
- 30 8). Use of magnesium chloride hexahydrate (i.e. 6H₂O). This is because if the dehydrate salt is used then it adsorbs water so the weight used to calculate the precise magnesium

ion content will be in error- this is a common reason for wrongly made up Krebs solutions in terms of correct magnesium ion and calcium ion levels.

It is envisaged that the inclusion of all preferred components as described in this example, will allow these components to work in synergy to produce an overall balanced physiological effect.

Manufacturing specifications

1. Stock solutions: Various stock concentrations of solutions according to the invention namely, 1X., 10X and 20X for long-term storage have been prepared, but the preferred stock concentrates are 10X concentrates using endotoxin-free MilliQ water [or equivalent ATSM Type II; NMT 18.0 MΩ-cm at 25 °C] and sterile filtered into sealed 100 mL bottles for storage under dark conditions at 3 to 8°C. Stock solutions are reconstituted for use as 1X concentrate solutions by the addition of 100 mL of 10X concentrates of stock solutions to 900 mL of double deionised or endotoxin-free MilliQ purified water [or equivalent ATSM Type II; NMT 18.0 MΩ-cm at 25 °C] with the addition of 2.1 g of sodium bicarbonate to give a final pH of 7.22. ± 0.04 at 20°C. Sterile stock 10X concentrations of solutions according to the invention have a pH of 4.6.± 0.2 and have been shown to be retained as such for periods of up to five years. The recommended manufactured shelf-life of 10X stock concentrates of solutions according to the invention is 14 months when stored at 3 to 8 °C under dark conditions.

2. Cocarboxylase: Stock solutions of thiamine pyrophosphate chloride (cocarboxylase) are prepared at 18.4 g/mL using endotoxin-free MilliQ purified sterile filtered into dark sealed vials to prevent the photon degradation of thiamine pyrophosphate and stored frozen prior to the assemblage of 10X stock concentrates of solutions according to the invention.

3. Insulin: Human recombinant insulin is prepared as acidified (pH 2.4) stock concentrated solutions at 0.5 m I.U./mL using endotoxin-free MilliQ purified water and

sterile filtered into sealed vials and stored frozen prior to the assemblage of stock concentrates of solutions according to the invention.

4. Choline: Stock solutions of choline chloride are prepared at 17.45 mg/mL using
5 endotoxin-free MilliQ purified water and stored frozen in sealed vials prior to the assemblage of stock concentrates of solutions according to the invention

5. Chloramphenicol is not an essential component of solutions according to the invention but is preferably added, either for storage, or after the storage vials have been opened, to
10 ensure sterility during extended exposure of the solutions to the atmosphere and to lower the risk of infection to subjects treated with the body fluid expander solutions of the invention.

EXAMPLE 2: Final composition of AQIX[®]RS-I solution

15

The following Table summarises the composition of AQIX[®]RS-I solution for use as a body fluid expander.

TABLE I

Component	Concentration
NaCl	110.00 mmoles/L
KCl	5.00 mmoles/L
CaCl ₂	1.25 mmoles/L
MgCl ₂	0.45 mmoles/L
NaHCO ₃	25.0 mmoles/L
BES	5.00 mmoles/L
D-Glucose	10.00 mmoles/L
Glycerol	0.11 mmoles/L
L-Glutamate	0.30 mmoles/L
L-Glutamine	0.40 mmoles/L

L-Aspartate	0.02 mmol/L
L-Carnitine	0.05 mmol/L
Choline Chloride	0.01 mmol/L
TPP (cocarboxylase)	40.00 nmol/L
Human recombinant insulin	28 mIU/L

EXAMPLE 3: A comparison of the chemical constituents of AQIX[®]RS-I, human serum and interstitial fluid

5

A comparison of the various levels of components present in human serum, interstitial fluid and AQIX RS-I is given below in Table II.

TABLE II

10

Component	Human serum	RS-1	Interstitial Fluid [analysed or *estimated]
Sodium ions	131 – 148 mmol/L	135 mmol/L	136 mmol/L
Potassium ions	3.4 – 5.2 mmol/L	5.0 mmol/L	4.4 mmol/L
Calcium ions	1.12 – 1.46 mmol/L	1.25 mmol/L	1.18 mmol/L
Magnesium ions	0.38 – 0.72 mmol/L	0.45 mmol/L	0.51 mmol/L
Chloride ions	101 – 111 mmol/L	119 mmol/L	117 mmol/L
Bicarbonate ions	21 – 29 mmol/L	25 mmol/L	23.9 mmol/L
Organic acid	6.4 mmol/L	5 (BES) mmol/L	7 mmol/L
Glucose	3.6 – 6.1 mmol/L	10 mmol/L	3.3 - 3.6 mmol/L
Glycerol	31 – 131 mmol/L	110 mmol/L	87 mmol/L
Glutamate	20 – 110 mmol/L	300 mmol/L	cf. serum values
Glutamine	140 – 570 mmol/L	400 mmol/L	cf. serum values
Aspartate	1 – 11 μ mol/L	20 μ mol/L	cf. serum values

Carnitine (recomb.)	35 – 85 $\mu\text{mol/L}$	50 $\mu\text{mol/L}$	cf. serum values
Choline	18- 70 $\mu\text{mol/L}$	10 $\mu\text{mol/L}$	cf. serum values
Thiamine pyrophosphate	6 – 135 nmol/L	40 nmol/L	cf. serum values
Human insulin (recomb.)	6 – 35 mIU/L	28 mIU/L	24 mIU/L
pH @ 37.4 °C	7.32 – 7.45	7.30 – 7.46	7.35 – 7.38
Albumin	0.65 mmol/L	NIL	0.19 mmol/L
Osmolality (mOsm/kg water)	264-290	265-286	*264-282
Specific Conductivity (mS cm^{-1})	11.7 – 12.3	11.9 – 12.6	* 11.8 – 12.2

EXAMPLE 4: A study to investigate the effect of intravenous infusions of AQIX[®]RS-I solution using a rat hemorrhagic model

5

Surgical Procedures in the animal Lab

The model for the establishment of a central arterial access in the rat was developed at American University of Beirut (AUB).

10

Briefly, it consists of a lower midline laparotomy under sterile conditions after induction of full anesthesia using an IM injection of 0.1 cc Xylocaine + 0.2 cc Ketamine for every 100 gram body weight. Following this, the aorta of the rat is dissected and cannulated under direct vision using a 24 Gauge angiocath. This allows for direct access for large amounts of blood withdrawal as well as administration of solutions. As a maintenance dose, 0.2cc IM injections of Ketamine are given every 25 minutes to all rats. Animals are allowed to breathe spontaneously throughout the experiment and their respiratory rate is continuously monitored. A warmer is used to keep the rats at normal body temperature during experimentation. As a result of the lower midline laparotomy, the bowels become

15

exposed to unveil the abdominal vessels. The bowels are wrapped with wet gauze (soaked in saline) to minimize water loss. At the completion of the experiment, the rat is either sacrificed while under anesthesia or the aortic perforation at the site of the catheter is repaired using 6-0 Prolene figure of 8 sutures and closure of the laparotomy incision using 2-0 Vicryl suture in 2 layers. The rats chosen for the survival part of the study are given intramuscular antibiotics and allowed ad lib feeding post-operatively. This procedure takes 25 to 30 minutes to complete per rat.

Animals and experimental groupings

10

19 Sprague-Dawley rats of similar age (10-12 weeks) and weight (280 to 320g) were used in the experiments. The rats were randomized by random number assignment to one of 3 arms: (1) replacement of blood volume with an equal volume of physiological saline (N=5); (2) replacement of blood volume with an equal volume of AQIX[®]RS-1 solution as defined above (N=7); or (3) no replacement of blood losses (N=7). The primary end point of the study here was the death of the animal documented by cardio-respiratory arrest.

Hemorrhage and resuscitation protocols

20 The rats were subjected to serial withdrawals of blood in 2cc increments (simulating controlled hemorrhage) every 30 minutes. At the end of each withdrawal a 0.1 ml of heparinized solution (1000 units in 20 ml of normal saline) was injected into a heparin lock to avoid blood clotting. The rats were randomly allocated to one of 3 arms as defined above.

25

The data observed and collected were as follows:

- a. Number of blood withdrawals (hence volume of hemorrhage)
- b. Respiratory rate (measured every 10 minutes)
- c. Time span until death (survival time in minutes from onset of hemorrhage)
- 30 d. Visible physical changes
- e. Pathology

Statistics

Survival was defined as the time from the first withdrawal of blood to the time of documentation of cardio-pulmonary arrest. Differences were considered statistically significant when the p-value was less than 0.05 ($p < 0.05$). All values were expressed as mean \pm SD. The analyses were performed using SPSS software version 13.0 (SPSS, Chicago, Illinois).

10 *Results of study*

Summary: Rats in the ‘no’- resuscitation arm had the shortest survival times and least blood volume withdrawn. Survival time and mean volume withdrawn were significantly improved by the addition of either normal saline or AQIX[®]RS-I. AQIX[®]RS-I provided a statistically superior survival time ($p < 0.01$) and allowed for a larger volume of blood to be withdrawn compared to physiological saline ($p < 0.01$).

Survival times: The survival of rats over time as a function of the resuscitation solution used is illustrated in Figure 1. Rats in the ‘no’ resuscitation arm had the shortest survival. Survival time was significantly improved by adding either saline replacement of blood losses or AQIX[®]RS-I replacement. Both solutions showed a significant difference when compared with the survival time of non-resuscitated rats; the mean difference between AQIX[®]RS-I and ‘no’ resuscitation groups was 89 ± 13.13 minutes ($p < 0.01$), and between saline and ‘no’ resuscitation was 57 ± 15.87 minutes ($p < 0.05$). AQIX[®]RS-I was superior to saline and resulted in a statistically significant increase in the survival time. The mean difference in survival times between the AQIX[®]RS-I treated rats and saline treated rats was significant at 43.40 ± 6.90 minutes ($p < 0.01$).

Blood volume withdrawn: The total volume of blood withdrawn for each of the study arms is set out in Figure 2. Both groups of fluid resuscitated rats showed a significant difference in the volume of blood withdrawn compared to the control arm of ‘no’

resuscitation (AQIX[®]RS-I to 'no' resuscitation 5.73 ± 0.62 ml and saline to 'no' resuscitation 3.64 ± 0.70 ml, with $p < 0.01$ for both). The mean difference in volume of blood withdrawn from AQIX[®]RS-I resuscitated rats in comparison to saline resuscitated rats was found to be statistically significant ($p < 0.01$) at 2.82 ± 0.45 ml.

5

Respiratory rates: Figure 3 illustrates the respiratory rates of rats over time in the various study arms. AQIX[®]RS-I resuscitated rats survived the greatest time and showed a slower decline in the respiratory rate overall, though initially (first 100 minutes) a more rapid descent in respiratory rate was noted. The overall respiratory rate for saline resuscitated rats declined at a rate faster than that of AQIX[®]RS-I infused rats and this decline was even faster still for the rats that received no fluid infusion.

10

Conclusion

15

AQIX[®]RS-I appears to be a more effective plasma substitute than physiological saline with respect to survival time and volume blood loss in a rat model of controlled hemorrhagic shock.

EXAMPLE 5: A study to investigate the safety of using AQIX[®]RS-I as a perfusate

20

Aim of study

This study was designed as a pilot study to evaluate the safety of AQIX[®]RS-I when administered as an intravenous infusional agent in a large animal model (swine).

25

Methods

6 pigs were used in this study arm, of both genders, ranging in weight from 27 to 35 kg. The pigs were housed in the animal care facility and were kept NPO overnight on the night prior to the study. Each pig was housed in a separate cage.

30

On day 0, each pig was initially sedated using an intramuscular injection of ketamine (15 to 20 mg/kg) followed by induction of anesthesia and endometrial intubation. Anesthesia was maintained with halothane to achieve absence of response to surgical stimulation without depression of heart rate. Each pig received pre-operative antibiotic prophylaxis
5 by intramuscular injection of gentamycin 10% (1cc/10kg).

A central venous catheter was surgically placed via direct countdown, under strict aseptic conditions, into the left internal jugular vein of each pig. The central line was extensively tunneled in the subcutaneous tissues of the pig to an exit site on the lateral and dorsal
10 aspect of the pig neck and secured in place. This was done for 2 purposes; to prevent accidental catheter dislodgement and to minimize the risk of line related infections. The animals were then allowed to return to their cages to recover from the anesthetics.

On day 1, each pig was sedated using intramuscular injection of ketamine (35 mg/kg) and
15 xylazine (7 mg/kg). Baseline blood samples were drawn from the central line under aseptic technique and immediately sent to the lab for analysis. The blood profile drawn was tested for complete blood counts, serum chemistry determination: electrolytes, glucose, lactate, osmolality, serum enzymes (aspartate aminotransferase, alanine aminotransferase), total creatine kinase [CK] + LDH, coagulation times and fibrinogen
20 levels. Also, blood was taken for measurement of IL-6 and TNF-alpha, these samples were centrifuged and stored at -80 °C for later evaluation.

After the blood was drawn, each pig had 1.0 L of AQIX[®]RS-I (at room temp) infused slowly over 1-2 hours via the central line. The pig was directly monitored during the
25 infusion for any signs of ill health or unusual behavior/manifestations. The pigs were then allowed to return to their normal living quarters and were monitored by the animal house veterinarian for any signs of ill health or unusual behavior/manifestations.

The same procedure was repeated on days 2 and 3. Prior to each infusion of AQIX[®]RS-I
30 the same blood profile (described above) was drawn to evaluate for any ill effects related to the previous administration of AQIX[®]RS-I. The pigs were then monitored for 1 week

and euthanized humanely by intravenous KCl administration under sedation with ketamine. Prior to euthanasia, a final blood sample was collected

Results

5

All 6 pigs were observed for 1 week, and all showed behavioral and feeding habits that were within normal limits.

10

Blood results are set out in Figure 4. These blood results were within normal ranges of swine of this weight category with the following exceptions:

1- Several values (e.g. potassium and calcium) were out of range with respect to worldwide swine references, yet correlate well between the sample of pigs used in this study and were consistent in value.

15

2- Certain blood values (e.g. WBC and CPK) were elevated out of range yet followed an expected decline/trend over the post-operative period. All these values improved with the administration of AQIX[®]RS-I.

20

3- Lactic acid values were occasionally out of range. Statistically they were single aberrant values and do not appear to represent any trend.

No untoward side effects or toxicities were noted, as evidenced by clinical monitoring and biochemical analyses.

25

Conclusions

30

The above detailed study involving daily IV infusions of 1.0L of AQIX[®]RS-I into the pig for a period of 3 days was completed successfully. It is concluded on the basis of the results obtained that AQIX[®]RS-I possesses no apparent safety issues when administered intravenously to farm swine.

EXAMPLE 6: A study of isolated kidney function following preservation using AQIX®RS-I

5 *Methods*

Non-heart beating donor (NHBD) pig kidneys were preserved for 2 hours under ‘cold’ (0-4 °C) static (CS) conditions in either AQIX®RS-I or the commercial hypothermic preservation solutions, Soltran® or UW® or for 2 hours under ‘warm’ (31 °C) static (WS) conditions. The kidneys were subsequently reperfused with a 50:50 autologous blood/LR perfusate mixture under normothermic conditions for periods of 6 to 8 hours.

Results

Functional parameters measured in isolated pig kidney after 6 hours of normothermic perfusion with autologous blood are set out in Table III below:

TABLE III

Functional parameters after 6 hours perfusion	AQIX® RS-I 4°C storage (n=6)	AQIX® RS-I 30°C storage (n=6)	Soltran® Flush 4°C storage (n=6)	UW ® Flush 4°C storage (n=6)	P value
pH	7.37 ± 0.15	7.30 ± 0.09	7.21 ± 0.1	7.23 ± 0.12	0.1468
Bicarbonate	21.8 ± 6.83	17.6 ± 4.24	14.6 ± 3.08	15.3 ± 4.26	0.154
Base excess	-4.7 ± 9.16	-10 ± 5.9	-9.7 ± 5.90	-13.5 ± 6.4	0.248
Serum K ⁺ levels	5.83 ± 0.34	8.01 ± 1.22	7.73 ± 1.21	8.23 ± 1.27	0.003
O ₂ consumption ml/min/g	47.3 ± 12.11	28.7 ± 6.53	31 ± 6.26	33.7 ± 15.1	0.059
% weight gain	12.7 ± 9	30.3 ± 9.3	21.2 ± 7.7	29.7 ± 3.44	0.0109
Total urine output (ml)	692 ± 230	257 ± 118	536 ± 221	410 ± 153	0.0103
RBF ml/min/100g	79.3 ± 17.89	48 ± 11.28	50 ± 10.16	55.5 ± 21.9	0.0214
RVR mmHg/ml/min	0.4 ± 0.09	0.73 ± 0.26	0.52 ± 0.09	0.8 ± 0.43	0.0173

The present study revealed that AQIX[®]RS-I significantly outperformed other preservation solutions in maintaining renal function as assessed following kidney re-
 5 animation in autologous blood. Analyses of the data obtained over the reperfusion periods revealed that kidneys preserved in AQIX[®]RS-I at 4°C exhibited (1) increased oxygen consumption, (2) increased creatine clearance rates [CrCl], (3) increased renal blood flow [RBF], (4) increased urine output, (5) decreased renal vascular resistance [RVR], (6) decreased weight gain (i.e. less oedema), (7) stable blood pH and retention of
 10 acid-based balance [bicarbonate ion levels], and (8) negligible loss of intracellular K⁺. Of particular significance is the observed stability of blood pH and retention of the neutral acid-base balance (H⁺/HCO₃⁻). This observed stability indicates that in AQIX[®]RS-I preserved kidneys, the major pH buffering system, the glutamine-ammonia shuttle, had not been compromised as was observed with the other two commercially
 15 available preservation solutions (see Table III above). Of additional significance was the observation that in those kidneys preserved for 2 hours under ‘warm’ (WS) ischemic conditions in AQIX[®]RS-I, subsequent reperfusion over 6-8 hours resulted in a restoration of the ADP:ATP balance (see Figure 5).

20 The ADP:ATP ratio levels were highest in the preperfusion biopsies and indicative of the ischaemic damage sustained during the CS /WS storage periods (Figure 5). However, after 6 h perfusion, the ratio had improved in both groups showing recovery of cellular function but with no significant difference (P = 0.71) observed between the CS and WS groups of kidneys (MD Kay et al., 2006; Transplant International 20 (1), 88–92).

25

EXAMPLE 7: Assessing the capability of AQIX[®]RS-I to maintain isolated mammalian organ and tissue preparation viability over various time periods of preservation.

Methods

30

Functional viability of mammalian tissue and organ preparations was assessed following storage/perfusion of the tissues/organs for various periods in AQIX[®]RS-I solution.

Viability was assessed using a variety of functional indicators, e.g., maintenance of cell membrane potentials, neurotransmitter output, myogenicity, membrane receptor sensitivity, enzyme functions, histological changes, etc.

5 Results

Table IV below reveals the ability of AQIX[®]RS-I to maintain functional viability of various tissues and organs preparations over preservation periods of varying length (between 0.3 to 10 days).

10 TABLE IV

	<u>Species</u>	<u>Tissue/Organ Preparations</u>	<u>Max^m Days Stored</u>	<u>Preservation Conditions</u>	
				<u>in vitro °C</u>	<u>Exp. °C</u>
15	rat	jejunum	9.0	8-12	35
		jejunum	1.5	-	35
		ileum	8.0	8-12	35
		ileum	1.3	-	20-35
		colon	5.0	-	20-35
20		uterus	3.0	-	35
		uterus	10.0	8-12	35
		detrusor muscle	2.0	-	20-35
		diaphragm muscle	0.6	-	35-37
		diaphragm muscle	2.0	-	20-35
25		soleus muscle	1.1	-	20-35
		heart	0.8	-	35-37
		heart	2.1	-	20-25
		heart-lung	1.2	-	20-35
		RBC's	4.0	No haemolysis at 4°C	
30		kidney	1.0	-	20-35
		liver	0.3	-	35
		rabbit intestine (jejunum)	5.0	8-12	37
		intestine (jejunum)	2.0	-	20-37
		uterus	7.0	8-12	37
35		superior cervical ganglion	2.0	8-12	37
		ganglion	0.8	-	37
		RBC's	3.0	No haemolysis at 4°C	
	guinea pig	ileum	7.0	8-12	37
		detrusor muscle	4.0	8-12	37
40		detrusor muscle	1.0	-	20-37
		heart	0.4	-	20-37

	mouse	soleus	0.9	-	20-35
		diaphragm	1.5	-	20-35
		intercostal mepp	0.9	-	20-35
		diaphragm } discharge	1.5	-	20-35
5	pig	kidney	0.8	0-4	37
		kidney	0.8	30	37
	human	intercostal } analysis	1.3	-	37
		kidney	1.5	0-4	37

10

CLAIMS

1. A non-phosphate buffered body fluid expander solution, comprising calcium ions and magnesium ions at a concentration ratio of 5:1 to 1:1.
5
2. A body fluid expander solution, comprising a non-phosphate buffer which is selected from the group consisting of TES, MOPS, BES and combinations thereof.
- 10 3. A body fluid expander solution as claimed in claim 2, wherein said non-phosphate buffer is present at a concentration of from 1 to 12 mmoles/L, preferably about 5 mmoles/L
- 15 4. A body fluid expander solution as claimed in claim 2 or claim 3, comprising calcium and magnesium ions at a concentration ratio of from 5:1 to 1:1.
5. A body fluid expander solution as claimed in claim any one of claims 1 to 4, wherein the concentration ratio of calcium ions and magnesium ions is from 4:1 to 2:1, preferably about 3:1.
20
6. A body fluid expander solution as claimed any one of claims 1 to 5, comprising from 0.1 to 2.5 mmoles/L calcium ions and/or from 0.4 to 25 mmoles/L magnesium ions.
- 25 7. A body fluid expander solution as claimed in any one of claims 1 to 6, comprising 1.0 to 2.5 mmoles/L calcium ions.
8. A body fluid expander solution as claimed in claim 7, comprising 1.1 to 1.4 mmoles/L, preferably from 1.2 to 1.3 mmoles/L, more preferably about 1.25 mmoles/L calcium ions..
30

9. A body fluid expander solution as claimed in any one of claims 1 to 8, comprising 0.2 to 0.6 mmoles/L, preferably from 0.3 to 0.5 mmoles/L, more preferably about 0.45 mmoles/L magnesium ions.
- 5 10. A body fluid expander solution as claimed in claim 9, comprising about 1.25 mmoles/L calcium ions and about 0.45 mmoles/L magnesium ions.
11. A body fluid expander solution as claimed in any one of the preceding claims, which is free of serum and/or serum extract.
- 10 12. A body fluid expander solution as claimed in any one of the preceding claims, comprising 21 to 35 mmoles/L, preferably 25 mmoles/L bicarbonate ions.
13. A body fluid expander solution as claimed in any one of the preceding claims, comprising one or more of:
- 15 (a) from 100 to 150 mmoles/L sodium ions;
- (b) from 2.5 to 6.2 mmoles/Lmmoles/L potassium ions;
- (c) from 96 to 126 mmoles/L chloride ions;
- (d) 2 to 11 mmoles/L glucose;
- 20 (e) 50 to 150 μ moles/L glycerol;
- (f) 7 to 15 μ moles/L choline;
- (g) 5 to 400 μ moles/L glutamate;
- (h) 5 to 200 μ moles/L aspartate;
- (i) 100 to 2000 μ moles/L glutamine;
- 25 (j) 1 to 120 nmoles/L thiamine pyrophosphate;
- (k) 40 to 70 μ moles/L D- or DL or L-carnitine; and
- (l) 5 to 200 m I.U./L porcine or human insulin
14. A body fluid expander solution as claimed in claim 13, comprising one or more of:
- 30 (a) about 135 mmoles/L sodium ions;

- (b) about 5 mmoles/Lmmoles/L potassium ions;
 - (c) about 118 mmoles/L chloride ions;
 - (d) about 10 mmoles/L D-glucose;
 - (e) about 110 μ moles/L glycerol;
 - 5 (f) about 10 μ moles/L choline;
 - (g) about 300 μ moles/L L-glutamate;
 - (h) about 20 μ moles/L L-aspartate;
 - (i) about 400 μ moles/L of L-glutamine;
 - (j) about 40 nmoles/L thiamine pyrophosphate;
 - 10 (k) about 50 μ moles/L L-carnitine; and
 - (l) about 28 m I.U./L of recombinant human insulin
15. A body fluid expander solution as claimed in any one of the preceding claims, comprising an antibiotic component, e.g. chloramphenicol.
- 15
16. A body fluid expander solution as claimed in claim 15, comprising 10 to 150 mg/L, preferably about 100 mg/L chloramphenicol.
17. A body fluid expander solution as claimed in any one of the preceding claims, wherein the pH is from 7.05 to 7.5 at a temperature range of from 4 to 38°C.
- 20
18. A body fluid expander solution as claimed in any one of the preceding claims, wherein said solution is a blood substitute.
- 25
19. A body fluid expander solution as claimed in any one of claims 1 to 18 wherein said solution is an extravascular fluid substitute, e.g., a peritoneal fluid substitute.
- 30
20. A concentrated stock solution of a body fluid expander solution as claimed in any one of the preceding claims, optionally wherein the stock solution is concentrated by a factor of from 1 to 50, preferably 5 to 20.

21. A solution as claimed in any one of claims 1 to 19, for use as a medicament.
22. A solution as claimed in any one of claims 1 to 19, for use in treating
5 hypovolemia.
23. A solution as claimed in any one of claims 1 to 19, for use in treating burns.
24. A solution as claimed in any one of claims 1 to 19, for use as a blood volume
10 expander.
25. A solution as claimed in any on of claims 1 to 19, for use in preventing and/or
ameliorating reperfusion injury
- 15 26. A solution as claimed in any one of claims 1 to 19, for use as a fluid
replacement therapeutic.
27. A solution as claimed in claim 26, for use in perfusing a peritoneal cavity in a
subject undergoing surgical procedures.
20
28. A solution as claimed in any one of claims 1 to 19, for use as an intravascular
or extravascular delivery medium for in vivo delivery of therapeutic, test
and/or synergistic agents to a subject.
- 25 29. A solution as claimed in claim 28, for use in delivering therapeutic, test and/or
synergistic agents to the lymphatic system of a subject.
30. Use of a solution as claimed in any one of claims 1 to 19 for the manufacture
of a blood volume expander for treating hypovolemia.
30

31. Use as claimed in claim 30, wherein the hypovolemia results from dehydration and/or burns and/or bleeding.
32. Use as claimed in claim 30, wherein the hypovolemia is drug induced.
- 5
33. Use of a solution as claimed in any one of claims 1 to 17, for the manufacture of a medicament for treating subjects suffering with burns.
34. Use of a solution as claimed in any one of claims 1 to 19, for the manufacture of a medicament for:
- 10
- (a) treating the loss of extracellular and interstitial fluid in a subject suffering with burns;
 - (b) in situ irrigation of an abdominal or thoracic organ or tissue in a subject undergoing a surgical procedure;
 - 15
 - (c) perfusion of the abdominal cavity during peritoneal dialysis of a subject with acute renal failure or an acute toxicity condition; or
 - (d) preventing and/or ameliorating reperfusion injury.
35. Use of a solution as claimed in any one of claims 1 to 19 for:
- 20
- (a) dialysis of the peritoneal cavity in a subject suffering from acute renal failure or an acute toxicity condition; or
 - (b) irrigation of an abdominal or thoracic organ in a subject undergoing a surgical procedure.
- 25
36. Use of a solution as claimed in any one of claims 1 to 19 for delivering a therapeutic, test and/or synergistic agent to a subject.
37. Use as claimed in claim 36 wherein the agent is a biological agent, such as at least one stem cell.
- 30

38. Use as claimed in claims 36 or 37 wherein the delivery is effected by administration to the lymphatic system of a subject.
- 5 39. Use as claimed in claims 36 or 37 wherein the delivery is effected by administration via an intravascular, intraperitoneal, intradermal, oral, intramuscular or topical route.
- 10 40. A method of treating hypovolemia, comprising administering to a subject in need thereof a therapeutically effective amount of a solution as claimed in any one of claims 1 to 19.
- 15 41. A method of treating burns, comprising administering to a subject in need thereof a therapeutically effective amount of a solution as claimed in any one of claims 1 to 19.
- 20 42. A method of preventing and/or ameliorating reperfusion injury, comprising administering to a subject an effective amount of a solution as claimed in any one of claims 1 to 19.
- 25 43. A method of maintaining physiological homeostasis of a tissue and/or organ in situ during a surgical procedure carried out on a subject, wherein the method comprises perfusing said tissue and/or organ with a solution as defined in any one of claims 1 to 19.
- 30 44. A method as claimed in claim 43, wherein said solution is maintained at a temperature of between about 4 – 20 °C such that said tissue and/or organ when perfused with said solution is maintained in a state of hypothermia.
45. A method as claimed in claim 43, wherein the surgical procedure is carried out to retrieve said tissue and/or organ from a donor subject, for subsequent transplantation to a recipient subject.

46. A method for delivering a therapeutic, test and/or synergistic agent to a subject using a solution as claimed in any one of claims 1 to 19.
- 5 47. A method as claimed in claim 46 wherein the agent is a biological agent, such as at least one stem cell.
48. A method as claimed in any one of claims 46 and 47 wherein the delivery is effected by administration to the lymphatic system of a subject.
- 10 49. A method as claimed in any one of claims 46 and 47 wherein the delivery is effected by administration via an intravascular, intraperitoneal, intradermal, oral, intramuscular or topical route.
- 15 50. A method as claimed in any one of claims 40 to 49 or a use as claimed in any one of claims 35 to 39, wherein the subject is a human.
51. A method as claimed in any one of claims 42 to 49 or a use as claimed in any one of claims 35b to 39, wherein the subject is a non-human animal.
- 20 52. A body fluid expander solution substantially as hereinbefore described with reference to the examples.
53. A substantially phosphate-free buffered solution for use as a medicament.
- 25

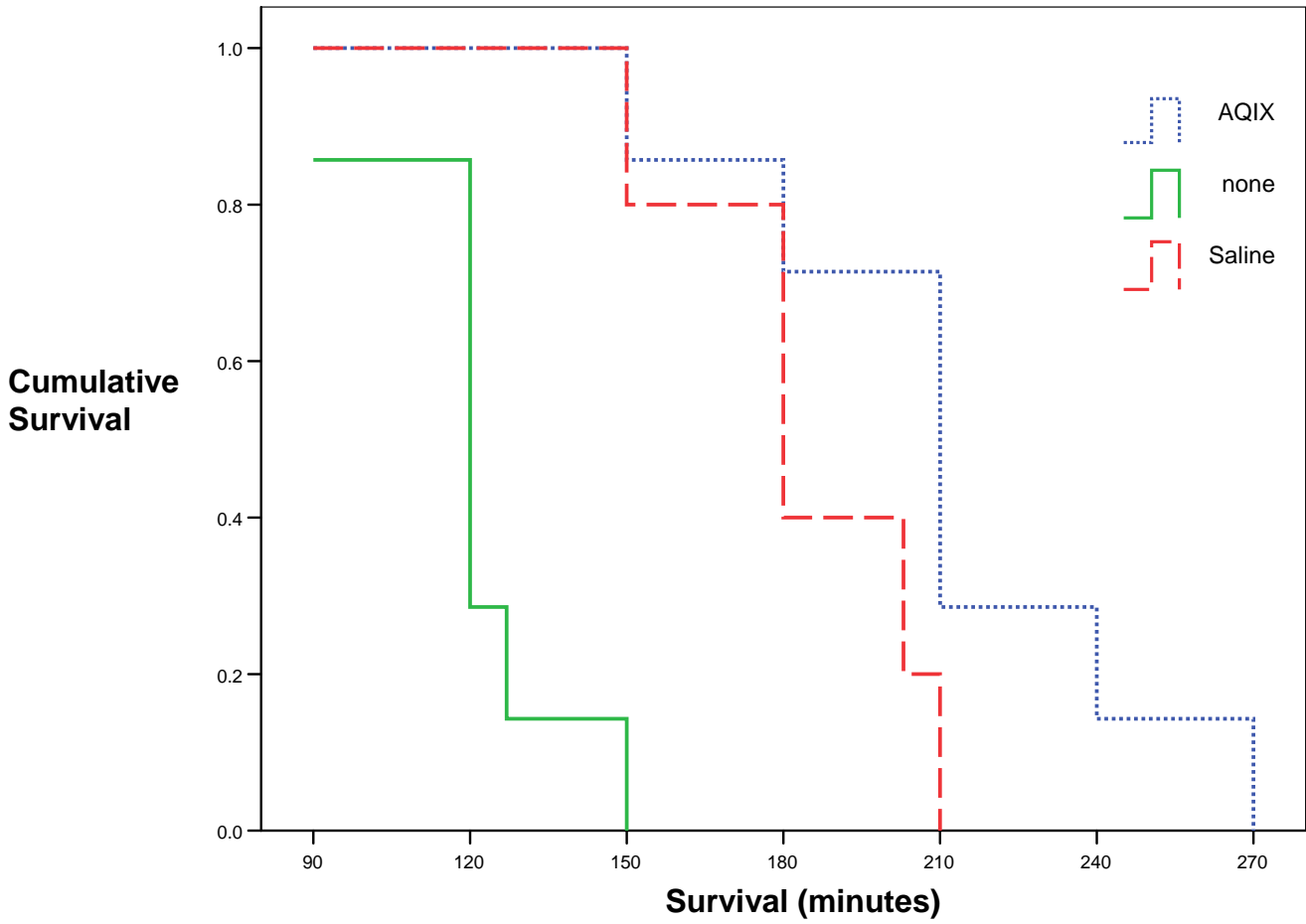
5

SHEET 1 OF 17

FIGURE 1

10

15

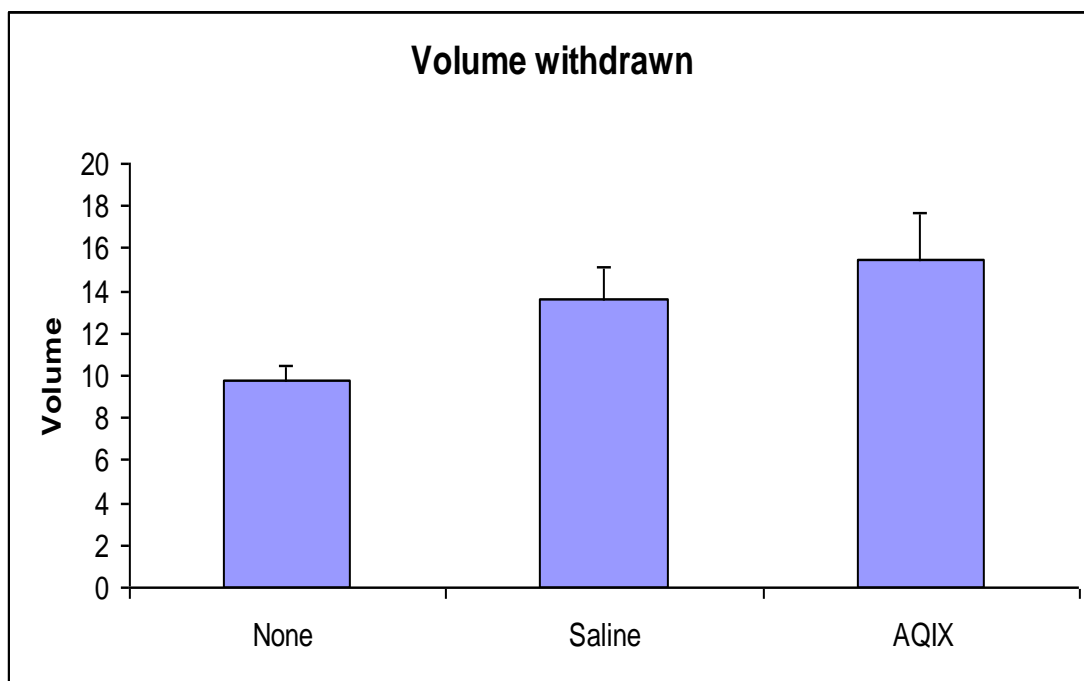


5

SHEET 2 OF 17

FIGURE 2

10



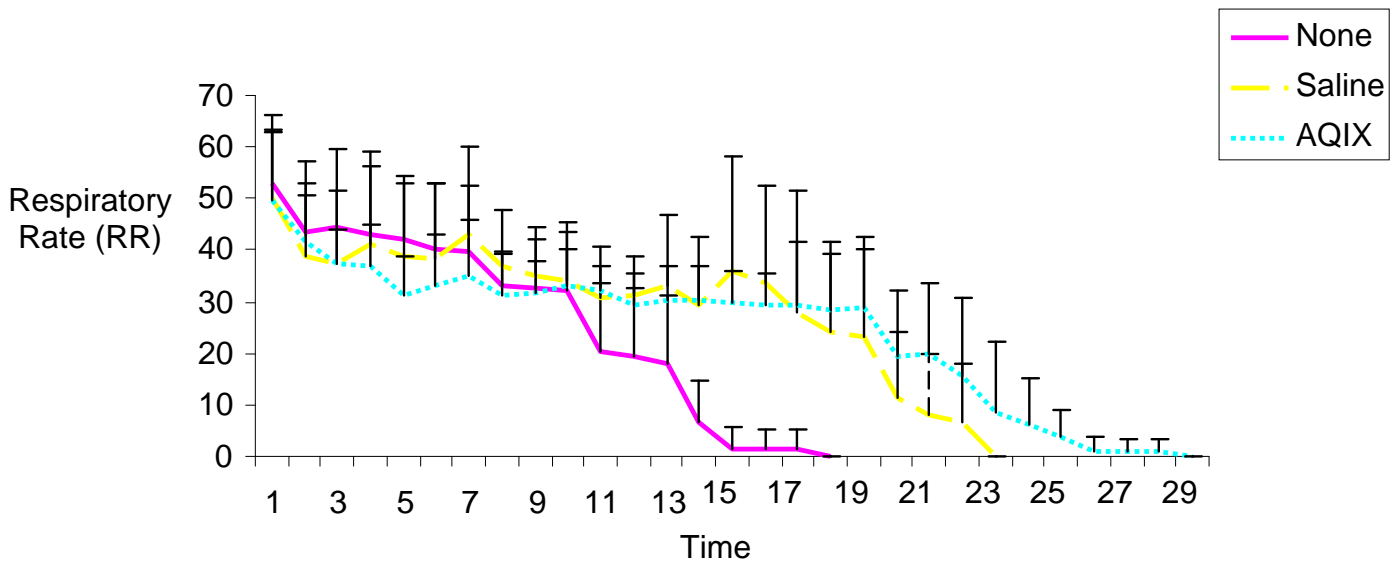
15

5

SHEET 3 OF 17

FIGURE 3

10



SHEET 4 OF 17

FIGURE 4A

Lab Data	(units)	P.PIG#1 (8/31/2006) baseline	P.PIG #1 (9/1/2006) DAY 1	P.PIG# 1 (9/2/2006) DAY 2	Reference values
CBC					
WBCs	mm ³	20400	23900	18600	7 to 20
RBCs	mil/mm ³	6.67	6.56	6.46	
Hg	g/dL	12.1	12	12	10 to 16
Hct	%	36	35	35	32 to 50
MCV	fl	54	54	54	50 to 68
MCH	pg	18	18	19	17 to 23
MCHC	g/dL	34	34	35	30 to 36
RDW	%	18	19	19	
WBCdiff					
poly	%	47	61	59	28 to 50
lymph	%	51	33	38	40 to 60
mono	%	1	6	3	2 to 10
eosino	%	1	0	0	0 to 10
Plts	mm ³	526000	522000	458000	120k to 720k
glucose					
glucose	mg/dL	77	59	97	65 to 95
BUN					
BUN	mg/dL	11	14	16	8 to 24
Creatinine					
Creatinine	mg/dL	0.8	0.9	1	1 to 3
Sodium					
Sodium	mmol/L	139	139	142	135 to 150
Potassium					
Potassium	mmol/L	3.9	3.8	3.8	4.4 to 6.7
Chloride					
Chloride	mmol/L	101	103	102	94 to 106
Carbon Dioxide					
Carbon Dioxide	mmol/L	22	26	30	
Magnesium					
Magnesium	mg/dL	2.2	2	1.9	
Calcium					
Calcium	mg/dL	9.6	9.2	9.4	10.2 to 11.9
Phosphate, Inorg.					
Phosphate, Inorg.	mg/dL	8.6	8.2	7.9	7 to 10.9
SGOT					
SGOT	IU/L	73	64	60	17 to 45
SGPT					
SGPT	IU/L	66	69	70	9 to 17
LDH					
LDH	IU/L	778	771	731	
CPK					
CPK	IU/L		2760	1887	
Osmolality					
Osmolality	mOsm/kg	286	295	295	
Lactic acid					
Lactic acid	mmol/L	5.76	1.49	1.66	
Prothrombin					
Pt	sec	10.6	10.8	10.7	
Control	sec	12	12	13	
Pt ratio					
Pt ratio		0.9	0.9	0.8	
INR					
INR		0.9	0.9	0.9	
APTT					
Pt	sec	20	19	19.5	
Control	sec	29.5	30	30.5	
Fibrinogen					
Fibrinogen	g/L	2.8	3.6	3.1	1 to 5

SHEET 5 OF 17

FIGURE 4B

5

Lab Data	(units)	P.PIG #2 (9/7/2006) baseline	P.PIG #2 (9/8/2006) DAY 1	P.PIG #2 (9/9/2006) DAY 2	P.PIG #2 (13/09/06) DAY 7
CBC					
WBCs	mm ³	24700	19200	20000	29400
RBCs	mil/mm ³	6.46	6.39	6.29	6.26
Hg	g/dL	11.2	11.1	10.9	10.7
Hct	%	32	32	32	32
MCV	fl	50	50	51	52
MCH	pg	17	17	17	17
MCHC	g/dL	35	35	34	33
RDW	%	20	21	22	28
WBCdiff					
poly	%	32	19	36	50
lymph	%	67	77	58	43
mono	%	0	4	5	6
eosino	%	1	0	0	1
Plts	mm ³	121000	586000	575000	563000
glucose	mg/dL	87	96	92	96
BUN	mg/dL	14	14	18	16
Creatinine	mg/dL	0.6	0.7	0.8	0.7
Sodium	mmol/L	139	144	149	140
Potassium	mmol/L	4.1	4.3	4.3	4
Chloride	mmol/L	99	107	110	99
Carbon Dioxide	mmol/L	32	27	30	28
Magnesium	mg/dL	2.2	2.5	2.4	2.2
Calcium	mg/dL	9.9	10.1	10.5	10.5
Phosphate,Inorg.	mg/dL	9.4	8.7	8.4	8.8
SGOT	IU/L	88	70	46	38
SGPT	IU/L	40	59	54	66
LDH	IU/L	767	795	748	623
CPK	IU/L	5234	4595	3542	743
Osmolality	mOsm/kg	287	300	312	291
Lactic acid	mmol/L	0.61	0.83	1.63	5.13
Prothrombin					
Pt	sec	11.4	12.1	11.5	11.5
Control	sec	12	12	12	12
Pt ratio		1	1	1	1
INR		1	1	1	1
APTT					
Pt	sec	17	22.5	20	19.5
Control	sec	29.5	30	30	29
Fibrinogen	g/L	3.2	3.3	2.9	2.1

SHEET 6 OF 17

FIGURE 4C

Lab Data	(units)	P.PIG #3	P.PIG #3	P.PIG #3	P.PIG #3
		(9/14/2006) baseline	(9/15/2006) DAY1	(16/09/06) DAY 2	(20/09/06) DAY 7
CBC					
WBCs	mm ³	23100	11900	18200	26000
RBCs	mil/mm ³	6.46	6.29	6.1	6.24
Hg	g/dL	11.2	10.9	10.5	11.1
Hct	%	32	31	31	31
MCV	fl	49	50	50	50
MCH	pg	17	17	17	18
MCHC	g/dL	35	35	35	35
RDW	%	34	32	34	22
WBCdiff					
poly	%	54	28	29	59
lymph	%	45	66	67	28
mono	%	1	1	4	13
eosino	%	0	1	0	0
Plts	mm ³	455000	389000	390000	337000
glucose	mg/dL	81	96	95	78
BUN	mg/dL	11	13	11	10
Creatinine	mg/dL	0.8	0.9	0.9	0.7
Sodium	mmol/L	142	138	139	138
Potassium	mmol/L	4.1	4.1	4.5	4.5
Chloride	mmol/L	103	101	102	101
Carbon Dioxide	mmol/L	28	29	29	29
Magnesium	mg/dL	2	1.8	2.1	2.1
Calcium	mg/dL	9.7	9.7	9.7	9.6
Phosphate,Inorg.	mg/dL	7.7	7.7	8.1	7.8
SGOT	IU/L	33	36	26	22
SGPT	IU/L	26	32	35	65
LDH	IU/L	551	605	561	576
CPK	IU/L	1609	2494	1407	252
Osmolality	mOsm/kg	290	290	295	289
Lactic acid	mmol/L	0.68	0.46	1.14	0.31
Prothrombin					
Pt	sec	11.2	11.3	11.1	10.9
Control	sec	12	12	12	12
Pt ratio		0.9	0.9	0.9	0.9
INR		0.9	0.9	0.9	0.9
APTT					
Pt	sec	17	17	19.5	19
Control	sec	29.5	29.5	30	30
5 Fibrinogen	g/L	3.2	3	2.4	2.5

SHEET 7 OF 17

FIGURE 4D

Lab Data	(units)	P.PIG #4	P.PIG #4	P.PIG #4	P.PIG #4	
		(20/09/06) baseline	(21/09/06) DAY 1	(22/09/06) DAY2	(26/09/06) DAY 7	
CBC						
WBCs	mm ³	26700	15800	21300	29700	
RBCs	mil/mm ³	6.11	5.67	6.28	4.89	
Hg	g/dL	11.1	10.4	11.6	9.3	
Hct	%	33	30	34	27	
MCV	fl	54	54	54	56	
MCH	pg	18	18	19	19	
MCHC	g/dL	34	34	35	34	
RDW	%	20	20	20	25	
WBCdiff						
poly	%	69	60	48	67	
lymph	%	25	32	46	24	
mono	%	6	7	2	9	
eosino	%	0	0	4	0	
Plts	mm ³	645000	564000	586000	676000	
glucose						
glucose	mg/dL	100	65	75	70	
BUN						
BUN	mg/dL	26	10	10	15	
Creatinine						
Creatinine	mg/dL	1	0.8	0.8	0.6	
Sodium						
Sodium	mmol/L	141	139	138	142	
Potassium						
Potassium	mmol/L	4.3	4.1	4.9	4.5	
Chloride						
Chloride	mmol/L	99	100	99	98	
Carbon Dioxide						
Carbon Dioxide	mmol/L	28	34	30	34	
Magnesium						
Magnesium	mg/dL	2.3	2	2.1	2.2	
Calcium						
Calcium	mg/dL	9	9.1	9.7	10.4	
Phosphate, Inorg.						
Phosphate, Inorg.	mg/dL	8.6	6.4	7.5	9.3	
SGOT						
SGOT	IU/L	98	58	34	24	
SGPT						
SGPT	IU/L	53	55	56	54	
LDH						
LDH	IU/L	1083	977	933	586	
CPK						
CPK	IU/L	3687	2677	1346	326	
Osmolality						
Osmolality	mOsm/kg	295	284	287	292	
Lactic acid						
Lactic acid	mmol/L	3.14	1.13	0.95	1.58	
Prothrombin						
Pt	sec	9.9	10.9	10.7	10.5	
Control	sec	12	12	12	12	
Pt ratio						
Pt ratio		0.8	0.9	0.9	0.9	
INR						
INR		0.8	0.9	0.9	0.9	
APTT						
Pt	sec	22.5	20.5	18.5	17	
Control	sec	30.5	29	30	30	
5	Fibrinogen	g/L	3	3.2	2.7	2.8

SHEET 8 OF 17

FIGURE 4E

Lab Data	(units)	P. PIG #5	P. PIG #5	P. PIG #5	P. PIG #5	
		(28/09/06) baseline	(29/09/06) DAY 1	(30/09/06) DAY2	(4/10/06) DAY 7	
CBC						
WBCs	mm ³	21700	19700	20300	22700	
RBCs	mil/mm ³	6.35	5.7	5.58	6.23	
Hg	g/dL	11.1	9.9	9.8	11	
Hct	%	33	29	29	33	
MCV	fl	51	51	51	52	
MCH	pg	18	17	18	18	
MCHC	g/dL	34	35	34	34	
RDW	%	21	20	21	21	
WBCdiff						
poly	%	53	45	42	52	
lymph	%	43	49	52	41	
mono	%	3	3	3	5	
eosino	%	1	1	2	1	
Plts	mm ³	531000	456000	479000	513000	
glucose	mg/dL	171	97	84	79	
BUN	mg/dL	29	23	21	17	
Creatinine	mg/dL	1	0.8	0.7	0.9	
Sodium	mmol/L	142	144	150	142	
Potassium	mmol/L	4.6	4.7	4.6	4.6	
Chloride	mmol/L	99	104	107	98	
Carbon Dioxide	mmol/L	35	30	31	35	
Magnesium	mg/dL	2.6	2.6	2.5	2.8	
Calcium	mg/dL	9.8	10.1	10.2	10.4	
Phosphate,Inorg.	mg/dL	7.9	8.2	7.7	9.4	
SGOT	IU/L	45	34	38	23	
SGPT	IU/L	57	56	58	45	
LDH	IU/L	702	641	601	498	
CPK	IU/L	2719	1916	1606	505	
Osmolality	mOsm/kg	305	304	310	297	
Lactic acid	mmol/L	4.96	0.7	1.3	3.21	
Prothrombin						
Pt	sec	11.2	10.9	11	11.4	
Control	sec	12	12	12	12	
Pt ratio		0.9	0.9	0.9	1	
INR		0.9	0.9	0.9	1	
APTT						
Pt	sec	15.5	16.5	18	17.5	
Control	sec	30.5	29.5	30	30.5	
5	Fibrinogen	g/L	2.9	2.6	2.4	2.3

SHEET 9 OF 17

FIGURE 4F

Lab Data	(units)	P.PIG #6	P.PIG #6	P.PIG #6	P.PIG #6
		(19/10/06) baseline	(20/10/06) DAY 1	(21/10/06) DAY 2	(25/10/06) DAY 7
CBC					
WBCs	mm ³	21400	21100	20800	17000
RBCs	mil/mm ³	6.05	5.49	5.77	5.36
Hg	g/dL	11.7	10.6	11.1	10.2
Hct	%	34	31	32	30
MCV	fl	57	57	56	56
MCH	pg	19	19	19	19
MCHC	g/dL	34	34	35	34
RDW	%	16	16	16	16
WBCdiff					
poly	%	51	66	68	54
lymph	%	47	31	32	36
mono	%	2	3	0	6
eosino	%	0	0	0	1
Plts	mm ³	601000	595000	616000	711000
glucose	mg/dL	74	91	105	108
BUN	mg/dL	10	14	9	16
Creatinine	mg/dL	0.7	0.7	0.8	0.5
Sodium	mmol/L	138	139	143	145
Potassium	mmol/L	3.8	4.4	4	4.5
Chloride	mmol/L	98	99	103	100
Carbon Dioxide	mmol/L	31	32	31	40
Magnesium	mg/dL	2.2	2.1	1.7	2.2
Calcium	mg/dL	9.6	9.6	9.5	11.6
Phosphate, Inorg.	mg/dL	8.4	7.3	7.4	8.8
SGOT	IU/L	103	111	50	29
SGPT	IU/L	43	54	56	49
LDH	IU/L	867	939	738	618
CPK	IU/L	11870	11528	4305	346
Osmolality	mOsm/kg	300	301	296	309
Lactic acid	mmol/L	1.06	0.89	1.17	1.3
Prothrombin					
Pt	sec	15.5	16.2	13	11.6
Control	sec	12	12	12	12
Pt ratio		1.3	1.3	1.1	1
INR		1.3	1.4	1.1	1
APTT					
Pt	sec	21	23.5	23	23.5
Control	sec	30.5	29	29.5	29
5 Fibrinogen	g/L	3.2	2.7	2.2	1.6

SHEET 10 OF 17

FIGURE 4G

5

Lab Data	(units)	Reference values	PIG #1a (24/01/07) baseline	PIG#1 b (24/01/07) 0	PIG1#1c (24/01/07) 30 min	PIG#1d (24/01/07) 45 min
CBC						
WBCs	mm ³	7 to 20	16400	16100	8400	11100
RBCs	mil/mm ³		4.66	5.3	5.06	5.03
Hg	g/dL	10 to 16	8.5	9.7	9.6	9.5
Hct	%	32 to 50	25	28	27	27
MCV	fl	50 to 68	53	53	54	54
MCH	pg	17 to 23	18	18	19	19
MCHC	g/dL	30 to 36	34	34	35	35
RDW	%		21	20	20	20
WBCdiff						
poly	%	28 to 50	45	46	40	38
lymph	%	40 to 60	43	37	49	56
mono	%	2 to 10	5	2	3	1
eosino	%	0 to 10	6	15	8	5
Plts	mm ³	120k to 720k	476	468	394	294
glucose	mg/dL	65 to 95	42	41	46	38
BUN	mg/dL	8 to 24	11	11	14	14
Creatinine	mg/dL	1 to 3	0.6	0.6	0.9	0.9
Sodium	mmol/L	135 to 150	142	142	141	140
Potassium	mmol/L	4.4 to 6.7	4.2	3.6	6	9
Chloride	mmol/L	94 to 106	106	107	107	106
Carbon Dioxide	mmol/L		34	32	26	18
Magnesium	mg/dL		2.1	2.1	2.9	3.6
Calcium	mg/dL	10.2 to 11.9	8.9	8.6	9	9.5
Phosphate, Inorg.	mg/dL	7 to 10.9	6.5	6.5	8.5	11.9
SGOT	IU/L	17 to 45	17	20	19	26
SGPT	IU/L	9 to 17	19	22	20	21
LDH	IU/L		403	421	456	450
CPK	IU/L		313	343	392	75
Osmolality	mOsm/kg		291	291	290	288
Lactic acid	mmol/L		1.25	0.89	4.18	8.15
Prothrombin						
Pt	sec		11.5	10.9	11	11.6
Control	sec		12	12	12	12
Pt ratio			1	0.9	0.9	1
INR			1	0.9	0.9	1
APTT						
Pt	sec		22	18	39.5	51.5
Control	sec		29	29	29	29
Fibrinogen	g/L	1 to 5	3.2	3.3	3.1	3.1
pH			7.626	7.617	7.579	7.553
pCO ₂	mmHg		31.1	28.5	26.7	23.7
pO ₂	mmHg		103	141	139	131

SHEET 11 OF 17

FIGURE 4H

5

Lab Data	(units)	PIG #2a	PIG #2b	PIG#2c	PIG #2d(PIG#2d
		(27/01/07)	(27/01/07)	(27/01/07)	27/01/07)	(27/01/07)
		baseline	0min	30 min	45 min	1 hr
CBC						
WBCs	mm ³	9800	9600	10700	7300	10500
RBCs	mil/mm ³	4.26	4.22	4.78	4.71	4.67
Hg	g/dL	8.6	8.8	9.9	9.6	9.8
Hct	%	26	26	30	29	29
MCV	fl	62	61	62	62	63
MCH	pg	21	21	21	20	21
MCHC	g/dL	34	34	34	33	34
RDW	%	19	18	19	19	18
WBCdiff						
poly	%	5	19	21	43	49
lymph	%	90	73	66	48	40
mono	%	0	2	9	4	5
eosino	%	5	6	4	4	5
Plts	mm ³	458	440	371	358	353
glucose	mg/dL	47	52	92	74	67
BUN	mg/dL	10	11	14	15	18
Creatinine	mg/dL	0.6	0.5	0.7	0.8	0.8
Sodium	mmol/L	142	142	140	140	141
Potassium	mmol/L	3.7	4.2	5.2	5.7	6
Chloride	mmol/L	104	105	105	105	106
Carbon Dioxide	mmol/L	30	30	25	25	24
Magnesium	mg/dL	2.1	1.9	2.4	2.7	2.7
Calcium	mg/dL	10	9.4	9.8	9.7	9.7
Phosphate,Inorg.	mg/dL	8.8	8.9	11	11.7	12.5
SGOT	IU/L	18	17	20	23	27
SGPT	IU/L	39	36	32	32	33
LDH	IU/L	510	502	515	586	513
CPK	IU/L	277	315	361	411	369
Osmolality	mOsm/kg	293	296	300	308	302
Lactic acid	mmol/L	2.18	0.6	2.08	2.79	3.47
Prothrombin						
Pt	sec	11.3	11.1	11	10.7	10.9
Control	sec	12	12	12	12	12
Pt ratio		0.9	0.9	0.9	0.9	0.9
INR		0.9	0.9	0.9	0.9	0.9
APTT						
Pt	sec	18.5	18	17	16.5	17.5
Control	sec	30	30	30	30	30
Fibrinogen	g/L	1.3	1.2	1.1	1.1	1.1
pH		?	7.535	7.378	7.372	7.363
pCO ₂	mmHg	?	57.9	48.4	48.4	46.7
pO ₂	mmHg	?	522	472	384	439

SHEET 12 OF 17

FIGURE 4I

5

Lab Data	(units)	PIG#3a	PIG#3b	PIG #3C	PIG#3d	PIG#3e
		(31/01/07) baseline	(31/01/07) 0min	(31/01/07) 30MIN	(31/01/07) 45min	(31/01/07) 1 hr
CBC						
WBCs	mm ³	21200	13200	13900	13600	10300
RBCs	mil/mm ³	4.73	4.14	4.18	4.12	3.97
Hg	g/dL	8.7	7.6	7.7	7.6	7.2
Hct	%	24	21	22	21	21
MCV	fl	51	51	52	52	52
MCH	pg	18	18	18	18	18
MCHC	g/dL	36	36	36	36	35
RDW	%	21	21	21	22	22
WBCdiff						
poly	%	66	61	73	75	65
lymph	%	21	27	15	17	2
mono	%	12	12	9	7	30
eosino	%	1	0	2	1	3
Plts	mm ³	300	234	225	229	205
glucose	mg/dL	90	171	150	116	80
BUN	mg/dL	11	14	15	15	16
Creatinine	mg/dL	0.5	0.6	0.9	0.8	1
Sodium	mmol/L	132	135	133	134	135
Potassium	mmol/L	3.7	5.2	5.7	6.5	6.8
Chloride	mmol/L	94	101	97	98	98
Carbon Dioxide	mmol/L	29	23	24	23	18
Magnesium	mg/dL	2.1	2.2	2.8	2.9	3.1
Calcium	mg/dL	10	8.7	9.6	9.4	9.7
Phosphate,Inorg.	mg/dL	6.6	6.8	9	10.2	11.6
SGOT	IU/L	23	18	16	26	17
SGPT	IU/L	24	14	18	19	18
LDH	IU/L	384	392	298	458	313
CPK	IU/L	493	446	482	592	545
Osmolality	mOsm/kg	273	285	280	280	281
Lactic acid	mmol/L	1.01	3.14	6.28	1.01	10.04
Prothrombin						
Pt	sec	12.1	12.2	12.1	12.2	12.2
Control	sec	12	12	12	12	12
Pt ratio		1	1	1	1	1
INR		1	1	1	1	1
APTT						
Pt	sec	18.5	19	17.5	17.5	17.5
Control	sec	31	31	31	31	31
Fibrinogen	g/L	2.4	2	2.1	2.1	2
pH		7.522	7.582	7.531	7.503	7.501
pCO ₂	mmHg	38.4	28.8	29	25.6	20.9
pO ₂	mmHg	448	397	510	490	460

SHEET 13 OF 17

FIGURE 4J

Lab Data	(units)	PIG#4a	PIG#4b	PIG#4c	PIG#4d	PIG#4e
		(03/02/07) baseline	(03/02/07) 0min	(03/02/07) 30min	(03/02/07) 45min	(03/02/07) 1 hr
CBC						
WBCs	mm ³	10800	10800	8300	6900	8200
RBCs	mil/mm ³	4.63	4.53	5.57	5.55	5.67
Hg	g/dL	8.3	8.2	10.1	10.2	10.3
Hct	%	25	24	30	30	31
MCV	fl	53	53	54	54	54
MCH	pg	18	18	18	18	18
MCHC	g/dL	34	34	34	34	34
RDW	%	19	19	20	19	19
WBCdiff						
poly	%	59	41	49	46	41
lymph	%	32	55	45	50	53
mono	%	7	0	5	1	4
eosino	%	2	4	1	3	1
Plts	mm ³	515	468	362	357	360
glucose	mg/dL	74	73	81	55	40
BUN	mg/dL	15	15	19	21	19
Creatinine	mg/dL	0.5	0.6	0.7	0.9	0.8
Sodium	mmol/L	137	137	135	138	135
Potassium	mmol/L	3.7	3.8	7.4	8.2	8.4
Chloride	mmol/L	98	98	102	104	101
Carbon Dioxide	mmol/L	30	33	22	22	17
Magnesium	mg/dL	2.2	2.2	3	3.2	3.87
Calcium	mg/dL	9.8	10.2	9.6	9.7	9.3
Phosphate, Inorg.	mg/dL	8.1	8.4	11.8	13.3	15
SGOT	IU/L	35	32	36	35	43
SGPT	IU/L	41	39	33	34	32
LDH	IU/L	573	533	497	501	544
CPK	IU/L	415	427	407	415	438
Osmolality	mOsm/kg	285	284	282	287	280
Lactic acid	mmol/L	0.63	0.72	4.74	6.54	8.06
Prothrombin						
Pt	sec	11	10.8	10.4	9.9	9.9
Control	sec	12	12	12	12	12
Pt ratio		0.9	0.9	0.9	0.8	0.8
INR		0.9	0.9	0.9	0.8	0.8
APTT						
Pt	sec	17	16.5	>180	>180	16.5
Control	sec	30.5	30.5	30.5	30.5	30.5
Fibrinogen	g/L	1.7	1.7	1.4	1.4	1.4
pH		7.396	7.386	7.538	7.615	7.511
pCO ₂	mmHg	50.8	51.1	26.9	17.9	21.9
pO ₂	mmHg	496	437	573	580	443

SHEET 14 OF 17

FIGURE 4K

Lab Data (units as indicated previously)	PIG#5a (07/02/07) baseline	PIG#5B (07/02/07) 0MIN	PIG#5C (07/02/07) 30MIN	PIG#5D (07/02/07) 45 MIN	PIG#5E (07/02/07) 1HR	PIG#5f (07/02/07) 1h30min	PIG#5g (07/02/07) 1h45min
CBC							
WBCs	11400	10500	9500	10200	11000	10000	7600
RBCs	4.11	4.06	4.03	4.17	4.31	4.19	4.36
Hg	8.6	8.4	8.3	8.6	8.8	9.1	9
Hct	24	24	23	24	25	25	26
MCV	58	58	58	58	58	60	59
MCH	21	21	21	21	20	22	21
MCHC	36	36	36	36	35	36	35
RDW	17	17	17	17	17	17	17
WBCdiff							
poly	55	44	58	58	59	61	67
lymph	0	43	35	38	32	30	26
mono	45	3	3	0	0	6	3
eosino	0	3	1	1	5	0	1
Plts	728	659	652	587	608	624	532
glucose	60	60	107	91	81	72	60
BUN	12	12	13	13	15	15	18
Creatinine	0.8	0.8	1	1.2	1.2	1.5	1.6
Sodium	141	141	139	139	141	141	154
Potassium	4	4.6	5	5.3	5.5	6.6	>8.5
Chloride	103	104	103	103	104	104	106
Carbon Dioxide	29	28	28	25	24	22	16
Magnesium	2.5	2.6	3	2.9	2.7	3	3.4
Calcium	9.8	9.8	9.3	9.4	9.6	9.3	9.3
Phosphate,Inorg.	8.2	8.2	8.4	9.7	10.2	12.7	15.5
SGOT	20	19	16	22	20	28	58
SGPT	33	31	29	31	29	28	30
LDH	484	446	411	416	410	419	493
CPK	450	442	427	450	455	473	534
Osmolality	290	290	286	288	292	293	318
Lactic acid	1.15	1.14	3.18	3.76	4.28	5.82	9.14
Prothrombin							
Pt	11.8	11.7 ?		11.6	11.4	11.2	11.3
Control	12	12 ?		12	12	12	12
Pt ratio	1	1 ?		1	1	0.9	0.9
INR	1	1 ?		1	1	0.9	0.9
APTT							
Pt	20.5	20.5 ?		20	19	18.5	19.5
Control	31.5	31.5 ?		31.5	31.5	31.5	31.5
Fibrinogen	2.1	2 ?		1.9	1.9	1.8	1.9
pH	7.61	7.654	7.633	7.573	7.624	7.61	7.62
pCO ₂	28	24.4	23.7	27.5	22.7	21.1	16.2
pO ₂	537	569	571	546	531	572	530

SHEET 15 OF 17

FIGURE 4L

Lab Data (units as indicated previously)	PIG#6a (12/02/07) baseline	PIG#6b (12/02/07) 0min	PIG#6c (12/02/07) 30min	PIG#6d (12/02/07) 45min	PIG#6e (12/02/07) 1 hr	PIG#6f (12/02/07) 1hr30'	PIG#6g (12/02/07) 1hr45'
CBC							
WBCs	13800	12600	10600	12200	12600	13000	12600
RBCs	4.96	4.68	4.33	4.43	4.51	4.72	4.71
Hg	10.4	9.9	9.3	9.6	9.7	10.1	10
Hct	30	28	26	26	27	28	28
MCV	60	59	60	59	60	60	60
MCH	21	21	21	22	21	22	21
MCHC	35	36	36	37	36	36	36
RDW	19	19	19	20	19	19	20
WBCdiff							
poly	44	40	38	33	45	44	45
lymph	44	55	54	57	48	48	51
mono	7	1	6	6	2	2	3
eosino	2	1	2	3	2	4	0
Plts	331	292	241	307	319	274	241
glucose	58	67	121	100	102	102	111
BUN	12	12	16	16	14	17	16
Creatinine	0.7	0.7	0.8	0.9	1.1	1.1	1.2
Sodium	141	140	139	140	140	140	139
Potassium	3.9	4.1	4.6	4.6	5	5.2	5.5
Chloride	103	104	105	104	104	104	103
Carbon Dioxide	26	25	23	22	23	21	22
Magnesium	2.6	2.3	2.5	2.4	2.5	2.6	2.6
Calcium	10.1	10.4	9.8	10.1	9.9	10	10
Phosphate,Inorg.	7	6.9	7	8.2	8.4	9.7	10.2
SGOT	27	29	27	26	25	26	27
SGPT	40	40	33	33	32	34	34
LDH	497	487	440	438	426	422	453
CPK	325	338	323	331	334	357	371
Osmolality	290	288	291	292	291	293	290
Lactic acid	0.93	1.75	3.77	3.84	4.46	4.4	4.96
Prothrombin							
Pt	10.3	10.4	10.4	10.3	10	9.9	10.5
Control	12	12	12	12	12	12	12
Pt ratio	0.9	0.9	0.9	0.9	0.8	0.8	0.9
INR	0.9	0.9	0.9	0.9	0.8	0.8	0.9
APTT							
Pt	17	16.5	16	15.5	>180	>180	15.5
Control	30	30.5	30.5	30.5	30.5	30.5	30.5
Fibrinogen	1.8	1.75	3.77	1.6	1.6	1.5	1.5
pH	7.546	7.576	7.568	7.57	7.565	7.541	7.55
pCO ₂	32.4	28.6	24.7	25	24.5	25.1	26
pO ₂	501	490	553	525	364	360	540

SHEET 16 OF 17

FIGURE 4M

Lab Data (units as indicated previously)	PIG#6H (12/02/07) 2hr	PIG#6i (12/02/07) 2h30'
CBC		
WBCs	12100	11600
RBCs	4.53	4.45
Hg	9.6	9.5
Hct	27	27
MCV	60	60
MCH	21	21
MCHC	36	36
RDW	20	20
WBCdiff		
poly	58	46
lymph	38	51
mono	3	2
eosino	1	0
Plts	297	270
glucose	142	168
BUN	19	17
Creatinine	1.4	1.5
Sodium	139	138
Potassium	6.1	6.7
Chloride	104	102
Carbon Dioxide	19	18
Magnesium	2.8	3.1
Calcium	10	53
Phosphate,Inorg.	10.7	10.9
SGOT	27	26
SGPT	30	34
LDH	440	432
CPK	368	365
Osmolality	293	292
Lactic acid	8.38	10.85
Prothrombin		
Pt	10.7	10.8
Control	12	12
Pt ratio	0.9	0.9
INR	0.9	0.9
APTT		
Pt	16	16.5
Control	30.5	30.5
Fibrinogen	1.5	1.5
pH	7.49	7.454
pCO ₂	25	21.4
pO ₂	463	638

FIGURE 5

5

