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# A COMPARISON OF THE EFFECTS OF PACKED RED BLOOD CELL TRANSFUSION AND OXYGLOBIN® IN CANINE BABESIOSIS

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# A COMPARISON OF THE EFFECTS OF PACKED RED BLOOD CELL TRANSFUSION AND OXYGLOBIN® IN CANINE BABESIOSIS

By

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A dissertation submitted to the Department of Companion Animal Clinical Studies of the Faculty of Veterinary Science of the University of Pretoria in partial fulfilment of the requirements for the degree of Master of Veterinary Medicine (Small Animal Medicine).

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# 1 FOREWORD

Blood transfusion forms a mainstay of the treatment of a variety of illnesses, and is lifesaving. Nonetheless, it is not without its risks and drawbacks. Blood transfusion is a cornerstone in the treatment of canine babesiosis. The development of blood alternatives has received attention in recent times. Blood alternatives offer much of what natural blood does but without many of the associated drawbacks. These include disease transmission, transfusion reactions, poor *in vitro* and *in vivo* shelf-life and special storage and administration requirements. One product, Oxyglobin®, is the first commercially available, veterinary-licensed, haemoglobin-based oxygen carrying solution (HBOCS). Although licenced for use in canine babesiosis, this colloidal "Oxygen Bridge" has never been evaluated against the gold standard of therapy, isovolumic packed red blood cell transfusion (pRBCT).

This investigation was conducted to evaluate important aspects the equivalence of these two treatments in a field situation of naturally-infected dogs. Given the cost of HBOCS, they are unlikely to be commonly used by the practicing veterinarian in the treatment of canine babesiosis. Nonetheless, similarities in efficacy would bolster the case for and further research into blood substitutes of this and other classes, and may open the way to evaluation of HBOCS for falciparum malarial anaemia, a disease similar in many respects to canine babesiosis.

I declare that no part of this dissertation towards the degree MMedVet (Med), Department of Companion Animal Clinical Studies, at the Faculty of Veterinary Science, University of Pretoria, was submitted towards any other degree and is my own original work.

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*Love is so short, forgetting is so long*

*Pablo Neruda*

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# 5 GLOSSARY OF TERMS AND ABBREVIATIONS

**Table 1.** Glossary of terms

| | |
|---|---|
| **(t)Hb** | (Total) haemoglobin |
| **2,3-DPG** | 2,3-bisphosphoglycerate |
| **AG** | Anion Gap |
| **ALP** | Alkaline phosphatise |
| **ALT** | Alanine aminotransferase (formerly SGOT) |
| **ANCOVA** | Analysis of covariance |
| **AST** | Aspartate transferase |
| **$A_{TOT}$** | Total concentration of non-volatile weak acids, albumin & phosphate |
| **B** | B-group = receiving pRBCT |
| **BSE** | Bovine spongiform encephalopathy |
| **CFH** | Cell-free haemoglobin |
| **$CVpO_2$** | Central venous partial pressure of oxygen (oxygen extraction) |
| **Cx** | Extractable oxygen tension |
| **DBP** | Diastolic blood pressure |
| **df** | Degrees of freedom |
| **$DO_2$** | Oxygen delivery |
| **ET-1** | Endothelin-1 |
| ***F*** | F-test statistic |
| **FDA** | Food and drug administration (US regulatory body) |
| **FWA** | Free water abnormalities |
| **HbCO** | Carboxyhaemoglobin |
| **HBOCS** | Haemoglobin-based oxygen carrying solution |
| **$HCO_3^-$** | Bicarbonate |
| **Ht** | Haematocrit |
| **IL-(1,6,8)** | Interleukin (1/6/8) |
| **iNOS** | Inducible nitric oxide synthase |
| **ISA** | In-saline agglutination |
| **MAP** | Mean arterial pressure |
| **MCHC** | Mean corpuscular haemoglobin concentration |
| **metHb** | Methaemoglobin |
| **MPS** | Mononuclear phagocytic system |
| **NO** | Nitric oxide |
| **O** | O-group – receiving Oxyglobin® |
| **$O_2$cap** | Oxygen carrying capacity |
| **ODC** | Oxygen dissociation curve |
| **OSA** | Oxygen status algorithm |
| **OVAH** | Onderstepoort Veterinary Academic Hospital |
| ***P*** | P value |
| **$P_{a/v}(O_2, CO_2)$** | Arterial/venous partial pressure of oxygen/carbon dioxide (in mmHg) |
| **PCV** | Packed cell volume |
| **pRBCT** | Packed red blood cell transfusion |
| **Qx** | Cardiac compensation factor |
| **RNI** | Reactive nitrogen intermediates |
| **$S_aO_2$** | Oxygen saturation % of haemoglobin |
| **SBP** | Systolic blood pressure |
| **SD** | Standard deviation |
| **SID** | Strong ion difference |
| **SIG** | Strong ion gap |
| **SIRS** | Systemic inflammatory response syndrome |
| ***t*** | t-value |
| **$TCO_2$art** | Arterial total carbon dioxide content |
| **TNFα** | Tumour necrosis factor – alpha |
| **$VO_2$** | Oxygen ventilation |
| **WBT** | Whole blood transfusion |

# 6 GENERAL INTRODUCTION

Anaemia caused by haemoprotozoan infections such as *Babesia rossi*[1] in the dog and *Plasmodium falciparum* in man are an important cause of morbidity and mortality in tropical and subtropical parts of Africa and elsewhere in the world. A key component of the disease process is severe anaemia and concomitant tissue hypoxia; hypotension; and malaise. Blood transfusion addresses many of these by improving blood oxygen carrying capacity and delivery to the tissues, providing colloid and perfusive support and buffering acidosis. The advance of novel transfusion technologies promises to deliver many of the benefits of blood transfusion, with fewer associated problems. Use of haemoglobin-based oxygen-carrying solutions (HBOCS) in the treatment of haemoprotozoan-associated anaemia is limited by the lack of clinical trials. Oxyglobin® (HB-301, Biopure Corporation, Cambridge, MA, USA) is the first and only licenced veterinary HBOCS. A similar product for use in humans (Hemopure (HB-201)) is also available. It has been claimed that its corpuscle-free, polymerised haemoglobin structure may provide equal or superior benefits for anaemic patients in comparison to isovolumic packed red blood cell transfusions (pRBCT).

[1] Formerly referred to as *Babesia canis* subsp. *rossi*

# 7 LITERATURE REVIEW

## *7.1 Canine babesiosis*

The most common and virulent agent of canine babesiosis in South Africa is the haemoprotozoan *Babesia rossi*. (1; 2; 3) The parasite is transmitted by the bite of the brown dog tick, *Haemaphysalis leachi*. Transmission requires at least 48 hours of attachment, after which infective merozoites are passed from the tick's saliva into the bloodstream of the host. They are then internalised by the erythrocyte after which they shed their piroplasmic membrane. (4) Anaemia is caused by a combination of phagocytic, oxidative, immune- and autoimmune-mediated and parasitic-lytic processes. Death ultimately ensues from the consequences of tissue hypoxia, hypoperfusion, systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS). (5; 6) Canine babesiosis is broadly categorised as uncomplicated (pyrexia, anaemia) or complicated (of which there are many variants). (7; 8) The babesiosis caused by the dominant Southern African species, *Babesia rossi*, (9) is particularly virulent and thus serves as an excellent model for study of all the dog babesias, and possibly even human falciparum malaria. (6; 10; 11; 12; 13) This variant of canine babesiosis is typified by high mortality due to severe anemia, peracute shock-like syndromes and a host of complicated forms (cardiac, pancreatic and renal pathology, icterus, hypoglycemia, cerebral babesiosis amongst others). (14; 15; 16; 10; 12; 11; 17; 18; 19) The primary pathophysiological and most treatable process is a severe anemia. (20)

### 7.1.1 Symptomatology

Uncomplicated babesiosis in the canine presents with signs relating to acute haemolysis, including fever, anorexia, depression, tachypnoea, hyperpnoea, pallor of the mucosae, splenomegaly and a waterhammer pulse. Normal packed cell volume (PCV) reference ranges of 37 – 55 % are standard for dogs; babesiosis patients may have PCVs as low as 5%. (7; 8; 6) Simple and complex acid-base and blood gas disturbances reported by Leisewitz and others (21; 22; 23; 24; 20) relate to the severity of the anaemia, hypoperfusion, hypotension, organ dysfunction and account

for the depression, negative myocardial effects (25; 11) and compensatory attempts of the respiratory and hepatorenal metabolic mechanisms.

### 7.1.2 Pathogenesis of the anaemia of babesiosis

Canine babesiosis patients suffer from variable degrees of anaemia caused by a combination of extravascular and intravascular haemolysis, and possibly other factors as well. (26) The haemolysis caused by *B.rossi* is intravascular as well as extravascular. (7) The extravascular component occurs primarily in the spleen (hence the marked splenomegaly of this disease). Intravascular haemolysis results from a combination of direct parasitic damage, induction of serum haemolytic factors, (27; 28; 29; 30; 31) increased phagocytosis by the MPS, (32; 33) damage by the secondary immune system after formation of antierythrocyte membrane antibodies, (28; 29) intra-erythrocytic accumulation of cyclic nucleotides (34; 35) osmotic fragility of the erythrocytes (36) and oxidative stress. (37) In addition to this, soluble protozoal proteases present in the patient's bloodstream can activate the kallikrein axis and induce production of fibrinogen-like proteins, which increase erythrocyte "stickiness". This may lead to margination and sludging of parasitized and fragmented erythrocytes, exacerbating the acute anaemia. (38; 13)

HBOCS have demonstrated their safety and efficacy in various experimental and clinical models of acute anaemia, (39; 40; 41) chronic verminosis (fleas and hookworm), (42) toxicity (zinc or rodenticide toxicity), (43) and septic or non-infectious maldistributive or hypotensive states. (44; 45; 46; 47) In both babesiosis and malaria, the mechanism by which anaemia and hypoxaemia is induced is distinct from these other conditions. A combination of different processes contributes to the cellular hypoxia and hyperlactataemia. In malaria, these include microcirculatory obstruction by sludging of parasitized erythrocytes; reduced hepatic circulation and thus diminished lactate clearance; increased levels of anaerobic glycolysis by infected erythrocytes; and a thiamine deficiency. (48) Although there are some differences in the pathophysiology of babesiosis and malaria, there are many more parallels in the hypoxic results of anaemia. (19; 49; 12; 38; 8; 7) But what of the processes that lead to the anaemia?

In this, at least, there are some comparisons, and a few differences. The malarial pigment is not a feature of babesiosis. (19; 12; 8) Anaemia results, as stated earlier, from a combination of direct parasitic damage, immune-mediated erythrolysis and immune-directed erythrophagocytosis by the MPS.[2]

Lastly, although the role of endogenous hypotension-induced nitric oxide release is controversial in canine babesiosis, (50; 51) and the role of HBOCS as NO scavengers is poorly understood, (39; 47) in theory this may be one of the advantages of Oxyglobin® in the face of hypotensive shock. An infusion of such a potent NO-scavenger would therefore be expected to bring about a greater elevation and/or more rapid normalisation of hypotension than an isovolumic infusion of another fluid (blood, for example).

### 7.1.3 Acid-base and blood gas disturbances of babesial anaemia

Tissue hypoxia is a consequence of decreased red blood cell mass due to anaemia; altered haemoglobin dissociation characteristics (52; 53); altered perfusion due to disseminated intravascular coagulation or sludging (54; 55); shock and myocardial suppression (5; 25; 56; 57; 20; 16); methaemoglobinaemia (58) and possibly also endogenous production of carbon monoxide. (52; 59)

Leisewitz et al (22) explored the effects of blood transfusion on oxygen status using the oxygen status algorithm (OSA) developed by Siggaard-Andersen and others. (60; 61; 62) They showed that blood transfusion had positive beneficial effects on oxygen content and carriage, and described the changes in the ODC (oxygen dissociation curve) caused by canine babesiosis and transfusion therapy. Acidosis was demonstrated in that study not by a decreased pH (acidaemia), but rather by a base deficit (base deficit). Normoxaemia (due to an appropriate and sufficient ventilatory

[2] References (31; 29; 30; 117; 99; 180; 34; 35; 19; 4);also (36; 93; 28; 27; 33; 32; 37; 55; 12; 13), and (7; 8; 6; 49; 107)

response) was noted in the clinically affected dogs. In that study, it was noted that some patients with babesiosis presented with a mixed acid-base disturbance, but that use of the Anion Gap (AG) was necessary to understand and contextualise this. Although this was not done in the 1996 study, it was reported on in subsequent South African babesiosis studies. (22)

Cellular lactate generation and the subsequent hyperlactataemia contribute to a metabolic acidosis and an elevated AG. (63; 24; 64) In the past, it was thought that metabolic lactic acidosis was the dominant acid base change. It has become apparent that this view (based largely on the Henderson-Haselbach equation) was overly simplistic. (24) Using the Stewart approach to the analysis of acid-base the complexities of these derangements in dogs with severe babesiosis have become apparent.

Current evidence suggests that it is *highly unlikely* that dogs with complicated babesiosis have simple acid-base imbalances. Analysis of acid-base balance using the Stewart approach helps to make the multifactorial pathophysiology of these abnormalities clearer as it considers the effects of electrolytes, water balance and protein concentrations in addition to the more commonly considered variables (such as $CO_2$, $HCO^-_3$ and lactate). (65; 66; 24; 67; 68) It is also important to realise that "global" or singular measures of acid-base or blood gas status drawn from only one point in time or one source (arterial or venous) may oversimplify the situation and may miss incipient crises or ignore the dynamic effects of treatment and disease on the parameters being measured. (69; 70; 71; 72)

The traditional approach to acid-base evaluation focuses on the relationship between pH, $pCO_2$, and $[HCO_3]$ as described by the Henderson Hasselbalch equation. (65; 66; 24) This approach is limited by several factors:

(1) It does not give a complete assessment of the pathophysiologic changes in the non-respiratory (metabolic) component;

(2) it may lead to the assumption that changes in electrolytes and changes in acid-base status are unrelated; and

(3) it does not recognize changes in $[H^+]$ due to changes in plasma protein, phosphorus and urate concentrations. Stewart suggested the so-called strong ion difference (SID) approach, a quantitative method for analyzing non-respiratory acid-base imbalances. (65; 66; 73; 67)

The Stewart (SID) approach to acid-base medicine has been applied in both human and veterinary medicine. (74; 75; 67) This quantitative approach makes a clear distinction between ***independent*** variables ($pCO_2$, SID, and total concentration of non-volatile weak acids, albumin and phosphorus $[A_{TOT}]$) and ***dependent*** variables, $[H^+]$ and $[HCO_3]$. The concentrations of $HCO_3$ and $H^+$ are *dependent* on the concentrations of primary or independent variables (i.e. the dependent variables can only change when one or more of the independent variables changes). Although the concentration of weak acids is affected by acid-base changes, the body's control mechanisms are directed at manipulation of the strong ion concentrations (via renal function) and $pCO_2$ (via respiration). Changes in protein concentrations however must be considered to fully evaluate acid-base abnormalities. SID is affected by four parameters:

(1) free water (as reflected by the sodium concentration);

(2) corrected chloride ion concentration;

(3) protein (specifically albumin) concentration; and

(4) Unmeasured strong anions (lactate, ketones, phosphates and sulphates, amongst others).

Due to the relatively high concentrations of sodium ($Na^+$) and chloride ($Cl^-$) in serum, these are the major ions affecting the SID. Simplified protocols and formulae for the evaluation of non-respiratory acid-base disorders based on Stewart's SID system have been published in the veterinary literature. (74; 24; 75; 67; 65; 66)

In the Stewart system, a metabolic acidosis can be due to an abnormal $HCO_3^-$ (base deficit, or negative base excess). De Morais and Constable (76) classify SID causes of acidosis resulting from a decrease in SID caused by a decrease in strong cations or an increase in strong anions. The mechanisms include hyponatraemia (increased free water), hyperchloridaemia or increased unmeasured strong anions (organic acidosis).

In the setting of babesial anaemia, any of these situations is possible, but the latter is certainly likely, due to an increase in lactate in particular. (63) The resolution of hyperlactataemia and the lactic acidotic component of the metabolic acidosis is likely an important part of the management of the pathophysiology of the disease. (21) Other researchers have found conflicting results when sodium/free water abnormalities and chloride are concerned. (24; 17) The latter two studies, in particular, only evaluated *initial* values for their electrolytes, and not their changes in response to transfusion therapy.

#### 7.1.3.1 TISSUE OXYGEN EXTRACTION

Apart from acid-base disturbances, the derangements in lactate metabolism and evidence of tissue and organ hypoxia arising from SIRS (5) imply altered tissue extraction of oxygen from the circulation. The best manner of assessing tissue oxygen extraction is evaluation of central venous oxygen [$CVpO_2$]. (77; 78) One study suggested that peripheral venous samples were as accurate as central ones, and as arterial samples, in assessing blood gas and acid-base status. (79) Another experimental study suggested that, during cardiopulmonary resuscitation in humans, mixed venous blood was superior to arterial samples, and better reflected tissue oxygen extraction from the circulation. (80) The mixed venous oxygen tension is an indicator of the end-capillary $pO_2$. The greater the tissues' ability to extract oxygen, the lower the $CVpO_2$, as this also represents the diffusion gradient from the capillary to the mitochondria in the tissues. (81) It is a central tenet of the therapy of both babesiosis and malaria that tissue oxygen be improved, and hypoxic tissues may not be able to extract oxygen from the circulation. Therefore, molecules with a right-shifted p50 (such as Oxyglobin® or haemoglobin under the influence of the Bohr effect and 2,3-BPG) which offload $O_2$ easier would aid cellular metabolism more.

### 7.1.4 Blood pressure and canine babesiosis

Jacobson and others (50) demonstrated that 2/10 evaluated patients with severe uncomplicated babesial anaemia suffered from hypotension. They hypothesised that inflammatory mechanisms were responsible for the hypotension through their influences on vascular tone (resulting in vasodilatation), increased vascular

permeability, dehydration and myocardial depression. The usefulness of indirect oscillometric blood pressure measurement was demonstrated in that study, and the authors recommended that *"blood pressure measurement … can be used as a measure of effectiveness of fluid therapy…"* and that since even mildly affected dogs had a *"…tendency towards hypotension…"*, blood pressure measure would identify patients in need of goal-directed treatment and observation. Hypotension is hypothesised to favour the interaction between the infected erythrocytes and endothelium, facilitating the localisation of the infection and activating the coagulation cascade. (82)

NO was hypothesised to play a role in the hypotensive component of clinical babesiosis, but Jacobson and coworkers seemed to have disproved this. (50; 51) While hypotension is common, NO-mediated hypotension does not appear to be the sole aetiological agent based on these results. Inflammatory cytokines may also play a role in the induction of a maldistributive state typical of other septicaemias. (83; 6; 19) The classic "waterhammer pulse" of anaemia is one sign of altered haemodynamics. The combination of low diastolic and high systolic pressure creates a pulse differential which, combined with a tachycardia, creates the classic sensation of a bounding pulse. Ultimately, although mean arterial pressure (MAP) may be maintained, maldistribution and tissue hypoperfusion may occur during the diastolic phase of cardiac function, as in most septic states. (6; 83; 84)

In addition to its role in haemodynamic status, NO and its reactive nitrogen intermediates (RNI) may play an important role in the clearance of malarial parasites (and possibly babesial parasites?). (51) The hypothesised mechanism is different to that involved in their microbicidal effects on *Trypanosoma*, *Leishmania* and *Toxoplasma*, in that it was once thought that haemoglobin is so effective a "sink" for NO that none would escape its scavenging effects to have anti-malarial effects. (85) In fact, haemoglobin's ablity to scavenge NO is dependent on environmental oxygen saturation and may be less than complete, particularly in venous blood where, incidentally, infected erythrocytes tend to sequestrate. This may allow infected erythrocytes to come into closer apposition to the macrophages and astrocytes that secrete NO, and serve a positive clearing function for the host through the cidal effects of RNIs on the intracellular parasites. (85)

### 7.1.5 Similarities with falciparum malaria

*Plasmodium falciparum* causes the most serious of the forms of malaria, and accounts for nearly 3 million annual childhood deaths per annum in Africa alone. (86) It is estimated that there were between 300 and 660 million episodes of clinical falciparum malaria in 2002 alone. Unfortunately, over two thirds of transfusion services in developing nations cannot execute proper service and material provision. (87) Although some differences exist between canine babesiosis and childhood malaria, certain authors have drawn startling comparisons between these haemoprotozoan anaemias and their hosts:

- Both are vector-borne; (88; 13)
- Most patients are juveniles; (15; 14)
- Their body weight ranges are similar; (5; 12; 89; 90)
- Mature patients tend to suffer a disproportionately higher complication rate; (90; 89; 91; 15; 5; 92)
- Certain dog breeds (e.g. fighting breeds) may be predisposed to worse disease, as is the case with people without certain haemoglobinopathies; (12; 14; 92)
- Blood transfusion is particularly useful (and in many cases life saving) in managing the disease; (90; 6; 93; 22; 56; 91)
- Disturbances of acid-base balance, sometimes extreme, are noted. (6; 94; 22; 24; 12; 89)
- Lactic acidosis is a common feature, presumably due to either poor tissue perfusion or increase lactate generation by cells using anaerobic metabolism or due to increased oxygen demand. (89; 95; 96; 97; 98; 63; 64)

There are some differences, however. Some include:

- Malarial infections demonstrate a circadian rhythm to their pyrexia, based on the dominance of the asexual stage; (98)
- Anophelid vectors, not acarids as is the case for babesias;
- The parasitic pigment, haemozoin, is a feature of falciparum malaria and not babesiosis; (19; 13) and
- Capillary sequestrating of parasitized cells is a characteristic of malaria and not canine babesiosis. (19; 13; 85)

None of these are features of canine babesiosis.

By restricting this trial to studying the generalised state of hypoxemia and acidaemia in these diseases, the researchers expected to gain an understanding of how best to treat the specific manifestation of anaemia and, by extension, draw theoretical conclusions about treatment responses of other complicated forms of babesiosis and malaria. In both diseases, disturbances of acid-base balance, sometimes extreme, are noted, and play a major role in patient deterioration and mortality. For example, English and others (89) showed the relationship between mortality, deep breathing (as a compensatory mechanism) and metabolic acidosis. White (92) also states, *"Lactic acidosis is an important cause of death."* This concurs broadly with the findings of babesiosis researchers, such as Taylor and others (59) who demonstrated the deleterious effect on the oxygen dissociation curve of canine haemoglobin, in babesiosis-induced acidosis and hypoxia. More recent studies than that by Button (64) demonstrated the complexity of the acid-base and blood-gas relationship babesiosis (for example, Leisewitz and others (21; 23; 24)). The latter three were the most thorough investigations of blood gas and acid-base perturbations in normal and parasitaemic dogs.

In children, the usual clinical presentation of falciparum malaria is not unlike that of dogs with babesiosis (lethargy, depression, respiratory distress or Kussmaul respiration, and coma). (96) The cause of the profound respiratory distress is thought to be primarily because of metabolic acidosis. (96) The aetiology of this acidosis remains unknown, although the proposed mechanism is increased production and impaired metabolism of organic acids (lactate and ketoacids) exacerbated by pyrexia, severe anaemia, hypovolaemia, parasite metabolic byproducts, decreased hepatic function and altered rheological properties of the erythrocytes. (48; 98) The key factor in hypoxia appears to be impaired oxygen delivery due to a critical reduction in haemoglobin, and hypovolaemia. (95; 97) Dordorp and others (99) also demonstrated that there is a significant association between acidosis and the rigidity of the non-parasitised erythrocytes. An interesting parallel can be drawn here with the various mechanisms of erythrocytic damage induced by babesiosis, mentioned earlier.

### *7.2 Blood therapy*

Treatment with blood can take two forms: whole blood transfusion (WBT) or packed red blood cell transfusions (pRBCT). (91) Whole blood is preferred for coagulopathic, dehydrated, hypovolaemic patients. In the South African veterinary context, the use of pRBCT is preferred because it: (20; 56; 22; 7)

- Allows for the collection of fresh (frozen) plasma for treatment of other conditions;
- Platelet transfusion is rarely required even in the face of profound thrombocytopaenia of babesiosis; (100)
- Transfusion volumes of 20 ml/kg provide fluid and erythrocyte-haemoglobin benefits without the higher volume load of WBT. The latter may provoke lung oedema in these patients; (101)
- The chief benefits of blood transfusion derive from oxygen carriage and acid buffering, components of therapy not provided in any great measure by plasma. (20; 102; 94; 103; 21; 22)
- Blood therapy decreases mortality in both canine babesiosis (104; 56; 20; 21; 22) and human falciparum malaria. (90; 94; 98)

Blood therapy may however serve as a transmitter of infectious diseases from the donor to the recipient (veterinary or medical), including:

- Babesiosis; (8; 7; 105)
- Leishmaniasis; (106)
- Hepatitis Virus B and C; (98)
- Hepatitis A and G, parvovirus B19, HTLV-I and -II viruses; (107)
- Human immunodeficiency virus; (107; 98) and
- TT Virus in human patients; (98)

Furthermore, blood transfusion may provoke a variety of transfusion reactions, including acute and delayed haemolytic and nonhaemolytic, febrile or other reactions. (107) Cross-matching, often unavailable or impractical in veterinary or rural African contexts, is required to eliminate some of these consequences, but is far from a guarantee of safety, especially with repeated transfusion. (108) Obonyo and co-workers commented that "...*transfusion with screened blood provided a survival advantage... in contrast, transfusion with unscreened blood decreased survival...*" (109)

Blood has a poor shelf-life (30 days at 4°C), and extended storage (past 7 days) decreases 2,3-DPG levels, resulting in a left-shifted oxygen dissociation curve (ODC) and poor peripheral oxygen offloading. (52; 59) Furthermore, the accumulation of methaemoglobin (110) and potentially endotoxin-like erythrocyte membrane fragments and free cytosolic stroma may harm the patient or hamper recovery. (111) These products of cellular aging and storage can induce production of a number of cytokines such as TNFα, IL-1, IL-6 and IL-8, thereby worsening a patient's status despite or even because of transfusion. (112) In addition, blood transfusion has cost factors associated with it, as well as being a putative cause of immunosuppression. (113; 107; 114) Even cost-saving measures such as reclaiming blood from sluices in orthopaedic theatres has been attempted and found to be just as expensive as standard transfusions. (107)

Since some cases of malaria are characterised by sequestration of RBC within capillaries, essentially leading to microcirculatory "damming", transfused RBC may not improve oxygenation of tissue beds supplied by such capillaries, and may even worsen the syndromes seen and thus the hypoxia and acid-base disturbances. (13; 55; 19; 99; 10) It has been demonstrated using mathematical models as well as with *in vitro* and *in vivo* experiments that erythrocytes aggregate under conditions of reduced arteriolar pressure (such as in hypotensive shock caused by babesiosis and malaria). (115) This drop in arteriolar pressure results in an increase in venous resistance and the exaggeration of the parabolic profiles of intravascular blood velocities consequential to increases in blood viscosity and increased shear rates. (115) The intrinsic internal resistance of the red blood cell (endogenous or transfused) to oxygen diffusion is also not negligible. In a mathematical model, Clark and others (116) demonstrated that the internal resistance of the erythrocyte to oxygen diffusion only determines the *minimum possible* unloading time. This minimum time is an important intrinsic property of the red cell, but it does limit the *absolute efficiency* of oxygen delivery in a situation of dire and exigent need. Given a situation such as exists in malaria and presumably in babesiosis as well, where RBC have decreased survival times, leading to: (117)

- altered deformability; (99)

- decreased survival (including transfused erythrocytes); (117)
- auto-oxidation of Hb to metHb in infected erythrocytes; (110) and
- reduced affinity of Hb for 2,3-DPG and molecular changes in the haemoglobin molecule resulting to lower 2,3-DPG and temperature sensitivities and higher $O_2$ affinity. (110)

In such situations, the use of haemoglobin-based oxygen carrying solutions (HBOCS) may provide the solution to these problems.

Lastly, blood may become contaminated by bacteria, resulting in sepsis, and necessitating vigilant handling and quality controls. There are also concerns that it does not increase either global or regional oxygen utilisation in anaemic septic patients, that it hampers right ventricular ejection (102) and is highly dependent on the vagaries of supply. Unfortunately, more than two thirds of developing nations do not have adequate transfusion services, one of the major limitations of pRBCT. (98)

### 7.2.1 Indications for blood therapy[3]

- Anaemia of most causes;
- Tissue hypoxia;
- Methaemoglobinaemia;
- Haemoglobinopathies;
- Surgery.

### 7.2.2 Effects on acid-base and oxygenation status

In a series of articles over 40 years, various South African researchers have described the beneficial effects of blood transfusion on acid-base and blood oxygenation status in canine babesiosis patients. Although there are supporting articles which have led to the core supporting publications (e.g. references (20; 49; 91; 21; 59)), the nucleus of this discussion is the work of Le Roux, (56) Malherbe, (104) and Button, (20; 64) which

[3] References: (120; 135; 39; 178; 113; 45; 158; 46; 47; 40); also (43; 179; 123; 164; 122; 182; 121; 181); and (41; 42; 96; 123; 164)

culminated in the studies by Leisewitz and others. (21; 22) The mixed acid-base descriptions of canine babesiosis by Leisewitz et al (24) are an important supporting work, but did not deal with blood transfusion *per se*, only the baseline status of this class of patient.

Malherbe's 1976 study gave the first real indication of the complexity of the acid-base disturbances of babesiosis, and the effects of treatment thereof, but had no control group or randomisation, samples taken were jugular (venous) blood and drawn into sodium heparin (thereby influencing Stewart-based physiological measurements)[4] and treatments included sodium bicarbonate. (104) This study also demonstrated the time-dependent elevations in stored RBC transfusion acidity, which the researchers attempted to negate by use of sodium bicarbonate. Button's study rectified many of these problems by using femoral arterial samples, employing the Siggaard-Anderson normogram and measuring electrolytes and lactate. (64; 60; 61; 62) However, Button only evaluated *baseline samples*, as did Leisewitz et al, (24) although he did attempt to classify patients and prognosticate based on presenting acid-base and blood gas abnormalities, a trial extended further by Nel and coworkers using lactate. (63; 118)

Leisewitz and coworkers' 1996 study is the most helpful for comparisons and predictions. (22) They used good techniques to evaluate a small sample of patients (6 dogs) over 72 hours. Parameters evaluated were similar to those used in this study, and described the undeniably positive effects of whole blood transfusion on increasing oxyhaemoglobin, oxygen content and haematocrit, decreasing carboxyhaemoglobin and acidosis, and improving the characteristics of the oxygen normogram (extractable oxygen tension, Cx and cardiac compensation factor, Qx). (21; 60; 62)

The 2001 paper by Leisewitz et al described a good many parameters in great detail, drawing not so much on the OSA of Siggaard-Andersen but more on Stewart's equations. (24) They demonstrated widely varying but severe acid-base disturbances characterised by a metabolic acidosis (base deficit) by not acidaemia (pH normal); hypocarbia; hyperlactataemic anion gap; hyperchloraemic, dilutional acidosis; and

[4]In fairness, Malherbe's study predated Stewart's work by nearly 7 years!

more. Although, as stated elsewhere, that study evaluated only abnormalities at *presentation*, it forms part of the foundation for understanding the response to transfusion (in conjunction with Leisewitz's earlier papers). (21; 22) A study on a narrower spectrum of patients with severe babesial anaemia using blood and a comparative treatment (in this case a haemoglobin-based oxygen carrying solution) would serve not only to bring these important pieces of research together, but would also serve the purpose of comparing blood to another similar treatment to put its efficacy into context, *and* as a pilot for childhood malarial transfusion studies.

## *7.3 Haemoglobin-based oxygen carrying solutions*

### 7.3.1 Basic properties

Oxyglobin® (HBOC-301, Biopure Corporation, USA) is a dark purple, sterile, polyionic colloid produced by addition of glutaraldehyde-polymerised stroma-free bovine haemoglobin in a modified lactated Ringer's solution. The final product contains 13 g/dL of haemoglobin with a P50 of 35mmHg. Oxyglobin's® ODC (oxygen dissociation curve) was once thought to be right-shifted relative to normal canine haemoglobin. Meyer (47) states a P50 of 26 mmHg for blood, whereas Cambier and others described a P50 of 30.0 $\pm$ 1.3 mmHg for dogs, somewhat closer to that of Oxyglobin®. (119) In either event, this difference may have important implications for its functional characteristics in hypoxic and maldistributive states. (120; 121; 122; 123; 113; 39)

Polymerisation of the bovine haemoglobin tetramer forms long, variable-length chains of polyhaemoglobin with molecular weights in the 65 – 130 kiloDalton range. This is important in creating the delayed exretion/metabolism characteristics of this molecule when compared to normal free haemoglobin, which is rapidly excreted via the kidneys and plays little role in oxygen transport to the peripheral tissues. (39; 113; 123; 122; 121; 120) The lack of erythrocyte membrane and RBC (red blood cell) stroma means antigenicity is reduced and thus crossmatching is unnecessary. (47)

One of the early stumbling blocks to the success of early HBOCS was the absolute dependence of the human haemoglobin on the 2,3-DPG molecule. The use of bovine haemoglobin, which utilises the chloride molecule instead of 2,3-DPG, and

polymerisation at the amino acid residues normally responsible for 2,3-DPG binding, have overcome this hindrance. (124) Polymerisation also alters the surface charge of the haemoglobin molecule, making extravasation and glomerular filtration slower. The latter is responsible, in part, for the the lack of nephrotoxicity of the HBOCS. (125)

Haemoglobin solutions will distribute throughout plasma, transporting, binding and releasing oxygen, even in vascular spaces where blood flow is suboptimal as a result of vasomotor imbalances, ischaemia, thromboembolic events or poor vascularity. Within the blood vessel, various theoretical and *in vitro* models have demonstrated that an exclusion zone exists at the periphery of the blood/plasma "column", a so-called "near wall excess phenomenon". (115; 126; 127; 47; 128; 129) Simulations have demonstrated that solutions of 30 to 100% haemoglobin can transport oxygen with similar or better efficacy than erythrocyte suspensions, because the smaller haemoglobin molecules can exist within this exclusion zone. (130; 47) King *et al* (131) also demonstrated that early fluid support of critically injured swine was far more successful in achieving resuscitation endpoints than a traditional lactated Ringer's infusion (which could not achieve said endpoints in the model used). In contrast, Driessen and others (132) found that Hb-200 did *not* improve oxygen delivery more than hetastarch, although they postulated that it might facilitate oxygen transport to tissues by diffusion. An earlier study by Sprung et al using 4% or 8% haemoglobin pyridoxalated-hemoglobin-polyoxyethylene conjugate or 8% stroma-free demonstrated little durable or significant improvements in oxygen delivery, extraction or survival over WBT, (133) while McNeil and co-workers showed that Oxyglobin could reverse global anaerobic metabolism in a hypotensive model before it even resolved the hypotension. (134) Thus, theoretical advantages of HBOCS may be just that, and there is some controversy over their true clinical benefits!

#### *7.3.1.1.1 Composition of Oxyglobin® (HB-200)*

(Tshepo Pharmaceuticals) (135):

Polymerised haemoglobin of bovine origin 13 g/dL

Modified Ringer's Lactate Solution containing:

- Water 100 g/dL
- NaCl 13 mmol/L
- KCl 4 mmol/L
- $CaCl_2.2H_2O$ 1.4 mmol/L
- NaOH 13 mmol/L (Driessen *et al* state 10 mmol/L (136))
- Sodium lactate 27 mmol/L
- N-acetyl-L-cysteine 200 mg/dL

When fully saturated, it binds approximately 1.36 mL of oxygen per gram of haemoglobin such that a plasma haemoglobin concentration of $\geq$0.6 g Hb/kg is associated with a greater than 20% increase in arterial oxygen content and a 10 to 15% enhancement of pulmonary diffusing capacity. Driessen et al also supply the following additional information: (136)

- pH 7.8
- Colloid oncotic pressure 42 torr (5.6 kPa)
  - Oncotic pressure similar to that of 5% albumin (46)
- $P_{50}$ 34 torr (4.5 kPa)
- Unpolymerised haemoglobin <5%
- Molecular weight of ~50% of haemoglobin polymers is 65 – 130 kDa (137)
- Molecular weight of ≤10% of haemoglobin polymers is >500 kDa (137)
- Free glutaraldehyde <3.5 µg/mL
- Endotoxin <0.05 EU/mL
- Osmolality similar to plasma (46)

#### 7.3.1.2 PHARMACOKINETICS

Plasma clearance is by a combination of MPS breakdown, hepatic breakdown and renal excretion of smaller polyhaemoglobin fragments (113) and follows first order pharmacokinetics. (135) Plasma concentrations of haemoglobin are proportional to dose. Normal dosage range is 10 – 30 ml/kg administered at 5 – 10 ml/kg/hour. (120; 101; 135)

#### 7.3.1.3 SIDE EFFECTS: HUMANS

Abdominal pain, asthenia, laboratory test abnormalities (↑AST, ALT, Lipase and many other colorimetric tests), pain, hypertension, jaundice, nausea, rash and haematuria. (135)

### 7.3.2 Effects on acid-base and oxygenation status

Many studies, including those sited in reviews by Standl, (114) Muir, (46) and Scott (113) cite the beneficial effects on plasmatic transport and delivery of oxygen. This is due in part to HBOCS' apparent independence from 2,3-DPG and their small molecule size (relative to the size of an erythrocyte). These benefits are thought to be due to N-terminal methionine residues in the β1 and β2 globin chain sequences which substitute for valine and histidine groups, although some have questioned whether the glutaraldehyde polymerisation process actually *robs* the polyhaemoglobin of sensitivity to the chloride ion. (137) There is *in vitro* evidence that the glutaraldehyde-induced modification of $Cl^-$-binding amino acid groups causes a complete loss of $Cl^-$ sensitivity compared to normal bovine Hb, which is an intriguing twist on the DPG versus chloride debate. (137) Other studies have demonstrated the lack of influence of chloride (and phosphate) ions on haemoglobin oxyphoric function. (119; 138)

Some trials make specific references to measured improvements in one or more of $DO_2$, $aO_2ct$, $S_aO_2$ and other parameters of oxygen carriage and delivery. (103; 139; 132; 136; 133; 140; 119) Acid-base status was not, *per se*, a focus of study in these articles. Standl and coworkers demonstrated that HBOC-201 provided superior and more rapid improvements in (skeletal muscle) tissue oxygenation and tissue oxygen extraction than a transfusion of autologous red bloods cells. (141) Another group found mixed

results (either no or some superiority of HBOCS) depending on which parameters they were measuring, and the model, (136; 132) and yet another group found *no* changes in oxygenation status with Oxyglobin® transfusion, albeit with positive effects on other parameters (with some researchers common to the previous groups!). (142; 119) A 1995 study using 8% haemoglobin or an early version of an HBOCS similarly found no enduring effects on $DO_2$, $VO_2$ or other measures of oxygen carriage. (133) In a murine malaria model, an earlier HBOCS prototype decreased lactic acidosis and anaemia after transfusion. (143)

Most authors and reviewers cited in this and preceding paragraphs seem to take it as a given (or report based on clinical measurements) that HBOCS improve some measures of oxygen transport and delivery to a mild to moderate degree. Relating clinical relevance to oxygenation status of some of these data to dogs with babesiosis or children with falciparum malarial anaemia requires a greater extrapolation of faith and data points than might be justifiable. This applies particularly to those data and conclusions from experimental trials in swine or dogs, usually involving traumatic brain injury, haemodilutive acute shock or acute haemorrhagic shock, or in people undergoing coronary bypass surgery.

### 7.3.3 Effects on blood pressure status

HBOCS are described as having potent NO-scavenging effects, as well as interacting with endothelin (ET-1) so as to increase MAP. (144; 145; 146; 147) It is debatable whether this increase in MAP may be helpful for patients already adequately compensating for hypotensive effects of disease. In regard to their scavenging, it is interesting to hypothesise on the link between an increase in NO scavenging and clearance of the parasite. It may be that increased NO scavenging, a smaller oxygen-carriage molecule able to perfuse even partially obstructed blood vessels and elevated diastolic blood pressure would be beneficial in human falciparum patients, especially those with cerebral malaria. On the other hand, these are precisely the theoretical reasons why this aspect of HBOCS, NO scavenging, might be counterproductive. One group has indicated that the ability of HBOCS to increase oxygen delivery to tissues is

limited by this very vasoactivity! (139) This was not, however, an aspect of the current study.

Some groups have demonstrated that it is the molecular mass and well as the dioxygenation characteristic of a particular cell-free haemoglobin (CFH) molecule that determines its NO-scavenging potency. (148) Other studies hypothesised that "overoxygenation" of the tissues is the causal drive behind the vasoconstriction. (149), and Palmer is of the opinion that both mechanisms may be responsible for the hypertensive effects, and that this can be mitigated by a couple of pharmacotherapeutic-molecular manipulations. (150) The concerns raised by the *ex vivo* and *in vivo* results of testing relate to the dangers of excessive vasoconstriction in HBOCS-transfused patients, particularly aneurysm-repair patients. This has been the main barrier to FDA approval of phase III studies thus far, and Palmer's suggestions may hold the solution to overcoming them. (150) Nonetheless, Katz and other warnings about the hypertensive potential of HBOCS seem to be related mainly to HBOCS's propensity to sharply increase MAP if administered briskly to rapidly exsaguinating patients. Sharp increases in MAP might increase uncontrolled bleeding. In addition, they might influence coagulation pathways, although this was not assessed in any good canine study. (151; 152; 153)

HBOCS transfusion leads to a rapid and sustained increase in MAP, moreso than an isovolumic lactated Ringer's solution or Hetastarch infusion (154; 155; 156) and equal to an infusion of Hetastarch. (132; 87) When compared to blood, even as little as 50% as much (as the amount of blood transfused) led to the same or a greater increase in blood pressure, although it was usually short-lived and dose-dependent and accompanied by a compensatory decrease in heart rate. (157) This was contradicted by other studies which found increases in heart rate and MAP during almost the entire course of treatment. (136; 132) Rice and coworkers remarked on the *initially* positive effect (they also followed some patients for 72 hours) and lack of adverse responses, and followed by stating that physiological haemodynamic markers such as heart rate and blood pressure could be reliably used to guide fluid therapy with HBOCS, *in haemorrhagic shock states*. (154) King and co-workers found that HBOCS such as Oxyglobin® were superior to standards of care such as lactated Ringer's, mannitol and

blood transfusion, for improving tissue perfusion *but* that parameters such as heart rate and blood pressure were *inadequate* or *misleading* endpoints for treatment due to the vasopressor effects which led to earlier normalisation of these figures than actual tissue perfusion. (131)

The putative mechanisms of the transient hypertension are threefold: (87; 147; 51)

- Inhibition of NO by direct NO uptake or iNOS (inducible NO synthase) inhibition; (46)
- Stimulation of endothelin production; (147) or
- Potentiation of the adrenergic $\alpha 1$ and $\alpha 2$ receptor responses to catecholamines. (46)

In theory, a canine babesiosis model of anaemia would be subject to one or more of these mechanisms. This haemoparasitic anaemia has been associated with insignificant increases in endogenous NO production (51; 147) and endothelin and catecholamine production are appropriate homeostatic responses to the circulatory imbalances caused by the anaemia and possible organ failure. It should be noted that Muir and Wellman listed over a dozen proposed mechanisms for HBOCS vasoconstriction, some more theoretical or better proven than others. (46)

In an experimental trial comparing HBOCS with traditional fluid and pressor/osmotherapy, King *et al* (131) concluded that Oxyglobin® was superior for the treatment of traumatic brain injury, although MAP and HR were inadequate endpoints to evaluate the progress of HBOCS resuscitation. They also stated that HBOCS infusion led to a transient state of global tissue *hypoperfusion*, presumably due to the vasopressive action of the HBOCS. In two studies by Driessen and coworkers, (136; 132) Oxyglobin® was shown to provide mixed benefits to oxygen carriage and haemodynamic status. Benefits varied on the parameter studied. Importantly, arterial oxygen content ($aO_2ct$) and total haemoglobin (tHb) were *not* significantly improved, while heart rate, systemic acid-base balance and MAP normalised. Systemic oxygen delivery did improve, but only at the maximal dose of 30 ml/kg. (132)

Mullon and others (158; 159) did not note a vasoconstrictive response to multiple HBOCS infusions in a human patient with immune mediated haemolytic anaemia,

which is corroborated in a study of patients with sickle cell anaemia, (160) and in experimental trials in sheep and human patients under going aortic surgery. (158) Thus, haemodynamic effects in canine babesiosis and falciparum malaria remain to be elucidated.

### 7.3.4 Other effects

Apart from the effects of volume overload, concerns have been raised over the relationship which may exist between HBOCS administration and the potential for increased mortality from bacterial endotoxin in animals with pre-existing septicaemia. This has been suggested but never satisfactorily demonstrated. (125; 46; 158; 159) In addition, one study found that repeated transfusions of Oxyglobin® produced no histopathological or immunopathological signs of organ damage in dogs, (125) while another by the same principal author demonstrated the innocuous nature of anti-bovine Hb glutamer antibodies in human patients who had received multiple transfusions of Hemopure. (161) Other effects include discolouration of the mucous membranes, pigmenturia, alteration of blood chemistry test results, (162) pyrexia and transient vomition. (120; 135; 163) The recent trial in 6 dogs by Keri et al is particularly useful with regards to the clinicopathological changes seen. This must be mitigated by the observation that different analysers may be used in other studies (as was the case with this study), but the findings are valuable nonetheless. Various deviations were seen over the trial period:

- **Elevations –** MCHC, $pH_{art}$, $TCO_2art$, $HCO_3^-{}_{art}$, amylase, albumin, total protein, globulin, calcium, phosphorus, total bilirubin, carboxyhaemoglobin and methaemoglobin;
- **Decreases** – PCV, RBC count, haemoglobin, creationine, cholesterol, ALT and ALP. (163)

Keri et al (163) also reported extensively on the serum and mucous membrane discolouration, although they did not specifically mention the pigmenturia aluded to by other authors mentioned previously. Lobetti and Jacobson (17) found that canine babesiosis could result in an asymptomatic pigmenturia (haematuria). This research elucidated the generally non-pathogenic nature of the pigmenturia and ascribed any

renal lesions to the concurrent hypoxemia caused by the haemoparasitic anaemia. Given the innocuous nature of this pigmenturia, the analogous pigmenturia caused by Oxyglobin® infusion (as noted by Giger and others (164)) would not be expected to be a confuser for any significant renal disease. Interestingly, Meyer (47) commented that a major advantage of Oxyglobin® lay in its structure and renal safety when compared to *"...hemoglobin dimers with their... renal toxicity"*, which seems, at least partly, to contradict Lobetti and Jacobson.

Several authors have raised concerns about the safety of HBOCS in the setting of preexisting inflammatory disease, as mentioned earlier. (158; 159; 112; 111) It was theorised by these authors that HBOCS may increase the risk of sepsis-induced complications through unknown mechanisms, although proof of this has never been conclusively demonstrated. (114) Another concern that was raised relates to the lack of the normal intraerythrocytic mechanisms protecting against the inherent proreactivity of the free haemoglobin molecule; haemoglobin is a reactive molecule that can generate oxidant species. Within the erythrocyte, various enzymatic pathways and antioxidant molecules maintain a state of constant redox equilibrium, whereas outside the erythrocyte, these mechanisms do not exist to the same level. However, the immunoreactivity of free bovine haemoglobin does not appear to be as immunoreactive as that of other species', and the glutaraldehyde polymerisation process appears to decrease immunogenicity considerably. (125) Yet another worry relates to the formation of increased amounts of methaemoglobin from the free polymerised haemoglobin molecule, which would further decrease the effectiveness of the transfusion. (157) In addition, some concern has been voiced over the transmission of bovine pathogens such as the prion agent of bovine spongiform encephalopathy. The manufacturer states that the peroxidation process renders the transfusion free of these and all other agents of disease, and only BSE-free herds of cattle form the source of the bovine haemoglobin. (120)

In a flawed, uncontrolled and retrospective article, Grundy and Barton came to the conclusion that Oxyglobin® transfusion was associated with an increased risk of mortality (165) although this was refuted by Day and others (44) and apparently also by the careful study of Hamilton and others. (125) One research study into HBOCS

infusion after traumatic brain injury not only evaluated efficacy, but also such safety aspects as neuropathology, cardiac dysfunction, coagulopathy and cytokine release, and found no evidence that HBOC-201 led to elevations in any of these safety criteria, further strengthening the case for HBOCS' safety profile. (166)

### 7.3.5 Conclusion: Project Justification

The weight of evidence is such that a trial comparing packed red blood cell transfusion (pRBCT) and Oxyglobin® for the treatment of canine babesial anaemia is justifiable on the grounds of anticipated efficacy and safety profiles of both agents. This clinical experiment would form a basis for comparison with subsequent trials in an analogous clinical trial in children suffering from falciparum malarial anaemia. The basis for comparison would be efficacy, not safety. Measures of efficacy in this setting are the effects of treatment on subjective (habitus/mentation and appetite) and objective criteria (blood gas, acid-base and haemodynamics). It was not the authors' intention to study *every* aspect of the clinical efficacy of Oxyglobin® in all permutations of babesiosis, but rather to focus primarily on the treatment of the anemia caused by babesiosis as a disease, and on transfusion as the most important supportive treatment measure. The evaluation of changes in blood gas and acid-base status is the standard means of evaluating the efficacy of transfusion therapy for anemia. This particular clinical trial was not intended to answer questions regarding safety since the safety profiles and side effects of both agents are well described, generally minor, rare or controllable.

# 8 MATERIALS AND METHODS

## *8.1 Study population*

### 8.1.1 Study setting

Onderstepoort Veterinary Academic Hospital (OVAH), Outpatients Clinic and Intensive Care Unit.

### 8.1.2 Study population

Dogs with severe *Babesia rossi* anaemia in the Onderstepoort area, permanently residing at an altitude of 1,250m above sea level, similar to those used by Leisewitz and others. (22)

### 8.1.3 Sampling frame

*Babesia rossi* positive patients (on blood smear) admitted via Outpatients for pRBCT transfusion therapy due to anaemia.

### 8.1.4 Sample size

12 dogs, 6 in each group (O for Oxyglobin®, B for pRBCT).

### 8.1.5 Study design

Prospective, longitudinal, random, positive control, experimental clinical trial.

## *8.2 Inclusion criteria*

Patients from the study population with a body mass at admission of 8 to 25 kg, PCV of 10 – 20 % (by microhaematocrit), normal blood glucose and in-saline agglutination (ISA) negative.

## *8.3 Exclusion criteria*

- Patients suspected of or with clear evidence any other serious diseases, including canine monocytic Ehrlichiosis; complicated forms of babesiosis; with a history of chronic illness or treatments;
- Fractious patients;
- Patients expiring before transfusion would be excluded from the study, but not once transfusion had been initiated.
- ISA positivity or >40% spherocytosis
    - Immune-mediated erythrolysis in O-group patients would have been determined by continued Oxyglobin® transfusion requirements, as indicated by precipitous or Oxyglobin®-resistant declines in plasma haemoglobin concentration; hypoxemia; or increases in spherocytosis by >20%; in any case, this did not occur during the trial;
- Patient ill or anorectic for 4 days or longer.

## *8.4 Patient processing*

### 8.4.1 Overview

Once patients were diagnosed with babesiosis on the basis of a blood smear, initial samples for baseline PCV, blood glucose and ISA were taken by an Outpatients clinician, sister or final-year veterinary student using a 1 ml preheparinised syringe from the accessory cephalic vein. Patients fulfilling the in- and excusion criteria were admitted, weighed and temperature taken; basal heart rate counted and then placed in left lateral recumbency. Blood pressure readings were taken (Cardell Model 9402 BP & $SpO_2$ model, Minirad International, Pennsylvania USA), and under local anaesthesia, a right jugular multilumen catheter (CS-22703-E triluminal catheter, Arrow International Inc, Pennsylvania USA) was inserted using a cut-down technique. Immediately prior to or after jugular sampling, a femoral arterial sample was taken using a pre-heparinised 1ml syringe with 27 gauge needle attached. Basal samples for full haematology (using Cell-dyne 3700, Abbott Laboratories, Ilinois USA) and a chemistry profile (using a Nexct, Alpha Wassermann, Bayer Healthcare, Leverkeusen, Germany) were processed by the laboratory as for normal medical patients. Blood gas

and acid-base analysis was performed within 20 minutes on a Blood Gas 865 (Bayer Healthcare, Leverkeusen, Germany). The distal port of the catheter was used for transfusion of either packed red blood cells or Oxyglobin® (20 ml/kg at 5 ml/kg/hour or 4 hours), and one of the other ports for sampling.

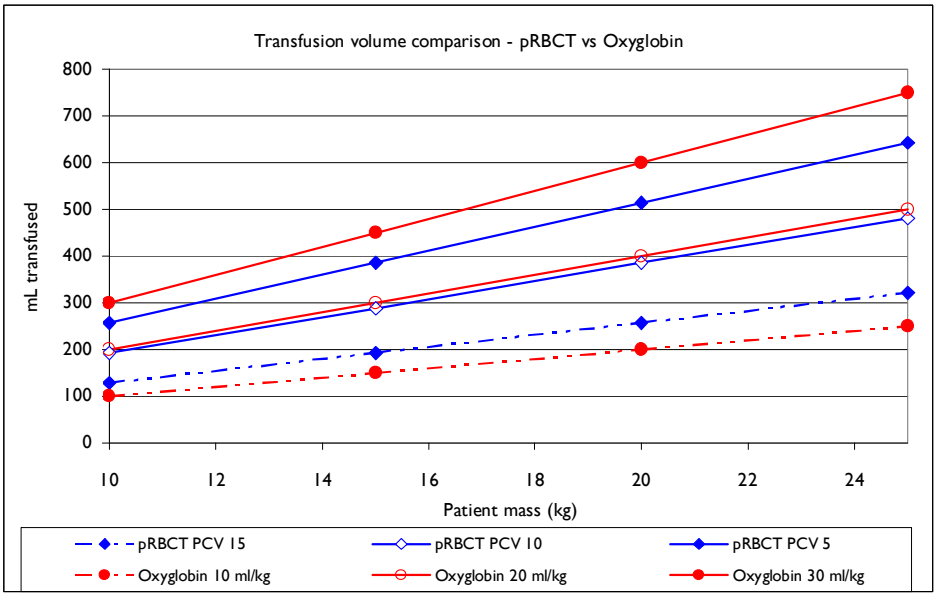
#### 8.4.1.1 TRANSFUSION VOLUME CALCULATIONS

The transfusion volume for whole blood is given by the formula: (22)

$$\frac{PCV_{required} - PCV_{patient}}{PCV_{donor}} \times 90 \times bodymass(kg)$$

Where the PCV required is usually taken as 25, and the PCV of the donor blood is 45 (WBT) or 70 (pRBCT). At this dosage, there is a divergence in the administration volumes of PRBCT and Oxyglobin® (see below). It is therefore necessary to correct for this discrepancy in PRBCT formula results and the Oxyglobin® dosage of 10 – 30 ml/kg. Since the figure (taken from the research protocol) shows the close relationship between volumes, the total volume of Oxyglobin® transfused was equal to the amount of PRBCT that would normally have been given.

| Body weight (kg) | pRBCT PCV 15 | pRBCT PCV 10 | pRBCT PCV 5 | Oxyglobin® 10ml/kg | Oxyglobin® 20ml/kg | Oxyglobin® 30ml/kg |
|---|---|---|---|---|---|---|
| **10** | 129 | 193 | 257 | 100 | 200 | 300 |
| **15** | 193 | 289 | 385 | 150 | 300 | 450 |
| **20** | 257 | 386 | 514 | 200 | 400 | 600 |
| **25** | 321 | 482 | 643 | 250 | 500 | 750 |



Thus the total dosage ranges for Oxyglobin® fell within the recommended Oxyglobin® dosage range of 10 – 30 ml/kg. Since dogs with a PCV of <10 were excluded from the trial, patients received 20 – 25 ml/kg (taken from graph & table above).

#### 8.4.1.2 POST-TRANSFUSION PROTOCOL

After 4 hours, once the transfusion was completed, a solution of lactated Ringer's solution (Sabax ringer lactate, Adcock Ingram Critical Care, Aeroton, South Africa) was substituted at maintenance rates (45 ml/kg/day) for the remainder of the study. Although this solution is lactated, and some authors state that hepatic dysfunction may dimish lactate recycling by the liver, it is a standard of care at the OVAH when supplemented with potassium at 20 mmol/L of Ringer's, and thus was used to enable comparability in later studies. (95; 97; 89; 96; 63)

Diminazene aceturate (Dizene, Virbac, Midrand South Africa), fipronil (Frontline Plus, Merial SA, Halfway House South Africa) and fenbendazole (Panacur BS, Intervet, Isando South Africa) treatment was administered only after basal sampling. Fenbendazole was administered orally q24h for three days.

### 8.4.2 Subjective evaluation & rubric

Subjective measurements of appetite and habitus were made in an unblended fashion by the researcher using a standardised chart and standardised feeding protocol. Patients' nutritional requirements over 72 hours were calculated on the basis of the standard formula kcal/day = **[70 + (30 × body weight in kg)]** and amalgamated with caloric content of the standard diet (193 kcal/can; Hill's Prescription Diet a/d, Hill's Pet Nutrition, Topeka, Kansas USA). The rubrics used for assessment are given in Table 2.

| **Table 2.** Rubrics for assessment of Habitus and Appetite | | |
|---|---|---|
| | Habitus | Appetite |
| 0 | Moribund | No interest or appetite |
| 1 | Severely depressed; only slight response to noxious stimulus | Slight interest, licks food |
| 2 | Depressed; muted but appropriate response to noxious stimulus | Eats < 50% of food offered in <10 minutes |
| 3 | Normal | Eats 50 – 100% of food offered in <10 minutes |
| 4 | Hyperexcitable | Eats all food and begs for more |

It was accepted that some animals might normally eat slower than others but given that many animals in the OVAH catchment area are subject to poor husbandry, the provision of such a palatable diet was expected to promote a vigourous appetite in animals whose appetite was restored. The nutritional planning chart designed for the study is given below. Coloured codes indicate the actual amount offered to approximate the amounts indicated off the table:

**Table 3.** Nutritional planning chart

| | | | (1/4 of a day's worth) | | | | (½ a day's worth)* | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| kg | Kcal | Cans/day | t=0 | t=1 | t=4 | t=8 | t=24 | t=48 | t=72 | TOTAL |
| 8 | 372 | 1.9 | 0.5 | 0.5 | 0.5 | 0.5 | 1.0 | 1.0 | 1.0 | **5** |
| 9 | 408 | 2.1 | 0.5 | 0.5 | 0.5 | 0.5 | 1.1 | 1.1 | 1.1 | **5** |
| 10 | 444 | 2.3 | 0.6 | 0.6 | 0.6 | 0.6 | 1.2 | 1.2 | 1.2 | **6** |
| 11 | 480 | 2.5 | 0.6 | 0.6 | 0.6 | 0.6 | 1.2 | 1.2 | 1.2 | **6** |
| 12 | 516 | 2.7 | 0.7 | 0.7 | 0.7 | 0.7 | 1.3 | 1.3 | 1.3 | **7** |
| 13 | 552 | 2.9 | 0.7 | 0.7 | 0.7 | 0.7 | 1.4 | 1.4 | 1.4 | **7** |
| 14 | 588 | 3.0 | 0.8 | 0.8 | 0.8 | 0.8 | 1.5 | 1.5 | 1.5 | **8** |
| 15 | 624 | 3.2 | 0.8 | 0.8 | 0.8 | 0.8 | 1.6 | 1.6 | 1.6 | **8** |
| 16 | 660 | 3.4 | 0.9 | 0.9 | 0.9 | 0.9 | 1.7 | 1.7 | 1.7 | **9** |
| 17 | 696 | 3.6 | 0.9 | 0.9 | 0.9 | 0.9 | 1.8 | 1.8 | 1.8 | **9** |
| 18 | 732 | 3.8 | 0.9 | 0.9 | 0.9 | 0.9 | 1.9 | 1.9 | 1.9 | **9** |
| 19 | 768 | 4.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 2.0 | 2.0 | **10** |
| 20 | 804 | 4.2 | 1.0 | 1.0 | 1.0 | 1.0 | 2.1 | 2.1 | 2.1 | **10** |
| 21 | 840 | 4.4 | 1.1 | 1.1 | 1.1 | 1.1 | 2.2 | 2.2 | 2.2 | **11** |
| 22 | 876 | 4.5 | 1.1 | 1.1 | 1.1 | 1.1 | 2.3 | 2.3 | 2.3 | **11** |
| 23 | 912 | 4.7 | 1.2 | 1.2 | 1.2 | 1.2 | 2.4 | 2.4 | 2.4 | **12** |
| 24 | 948 | 4.9 | 1.2 | 1.2 | 1.2 | 1.2 | 2.5 | 2.5 | 2.5 | **12** |
| 25 | 984 | 5.1 | 1.3 | 1.3 | 1.3 | 1.3 | 2.5 | 2.5 | 2.5 | **13** |

*only ½ was indicated as patients were fed every 12 hours during this period, and only the periods of *appetite assessment* were included in these calculations. Feedings at the other 12-hour intervals (t=36, 60) were not assessed *per se* even though feeding was performed using the same formula.

| 1/2 a can | 3/4 of a can | 1 can | 1+1/2 cans | 2 cans | 2+1/2 cans |
|---|---|---|---|---|---|

### 8.4.3 Blood pressure measurement

Blood pressure was measured first, before other interventions, in patients placed in left lateral recumbency.[5] Cuff size was estimated to be 40% of limb diameter, and measured from the left median artery just proximal to the medial left elbow. Simultaneous to the first oscillometric measurement, femoral pulse rate was measured. If the measured heart rate and actual (femoral) pulse differed by >20 bpm, the cuff was released, readjusted, and the reading discarded. This was done to ensure accurate blood pressure measurements by using the patient's own pulse as the control. Accurate heart rate measurements by the Cardell monitor were then more likely. A *post-hoc* correlation between measured heart rate and femoral pulse was performed to test the validity of this procedure.

Three measurements of systolic, diastolic and mean arterial pressure (SBP, DBP and MAP, respectively) and heart rate were taken, the cuff released and replaced, and two more readings performed. Jacobson and others (50) demonstrated the effects of natural infections with *Babesia rossi* on the blood pressure status of canine patients. Although that research used a different device for measurement than the more modern equipment used in this studies, the cutoff values described in that article were used as reference ranges:

**Table 4.** Blood pressure cutoff values from Jacobson and others **(50)**

| | Hypotension | Borderline | Normotension | Borderline | Hypertension |
|---|---|---|---|---|---|
| **DBP** | <65 | 65 – 73 | **74 – 104** | 105 – 112 | >113 |
| **MAP** | <76 | 76 – 86 | **87 – 127** | 128 – 138 | >138 |
| **SBP** | <118 | 118 – 129 | **130 – 172** | 173 – 184 | >184 |

### 8.4.4 Temperature

Rectal temperature was measured using a digital thermometer (Hartmann Thermoval Rapid, Hartmann Australasia) at each time interval.

[5] Since the jugular catheter was placed in the right side, it made sampling easier without having to roll the patient over.

### 8.4.5 Venous blood gas sampling

Venous blood sampling during the trial was performed from the central venous catheter. At admission, patients were placed in left lateral recumbency with the neck extended and gently held in place by an assistant. A 5 x 10 cm area over the right jugular furrow was clipped, aseptically prepared and surgically draped. 2 ml of Lignocaine (Bayer AG, Isando South Africa) was infiltrated into the area directly over the jugular vein. A two or three-lumen, Arrow multilmen catheter was placed via an open, aseptic technique, each port flushed with 2.5 ml of heparinised saline, and basal samples collected from the distal port. Placement in the right atrium or distal cranial vena cava was established by choosing catheter length according to patient size or a radiograph in a few cases. Using a staged technique whereby 2 ml of blood was withdrawn using a 2.5 ml syringe preflushed with balanced heparin; a similar syringe was used to withdraw a sample for placement into a serum vacutainer, and a 1 ml, heparinised syringe used for venous blood gas collection, followed by replacement of the initial 2ml of blood and then 2 ml of heparin-saline flush to clear the lumen and prevent thrombogenesis within the tip. The venous blood gas sample was plugged with a rubber stopper and placed in a polystyrene, icepack-cooled box while the arterial sample was drawn and processed in the same manner.

### 8.4.6 Arterial blood gas sampling

Arterial blood gas samples were withdrawn from alternate femoral arteries, disinfected, femoral arterial puncture using a pre-heparinised 1ml syringe with 29 gauge needle attached. After arteripuncture, pressure was placed on the site by an assistant, for at least 30 seconds, to prevent haematoma formation.

## *8.5 Sample processing*

A 2 – 3 ml EDTA Vacutainer sample (for baseline haematology) was taken from the multilumen jugular catheter after its insertion and fixation. Serum samples were allowed to clot for 3 – 4 minutes before centrifugation at 8,000 rpm for 8 minutes. Serum was decanted into plastic aliquots, labelled by patient and time (e.g. p1t24 =

patient 1, time 24 hours) and stored at -20°C in the laboratory of the section of Clinical Pathology for later analysis. Venous blood gas samples were processed within 20 minutes. Arterial blood samples were capped with a rubber stopper and processed in the same manner as venous blood gas samples.

## *8.6 Calibration of blood gas/acid-base/haemoxymetry analyser*

This was performed independently by the laboratory technicians, using standard protocols for the OVAH Clinical Pathology section.

## *8.7 Data entry*

Data was entered from printouts and written records into an MS Excel 2003 spreadsheet (Microsoft Corporation, Redmond, WA, USA).

## *8.8 Statistical analysis techniques*

Statistical analysis was performed with the assistance and advice of a biostatician using R (R Development Core Team (2005). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria). Once the validity of the core model had been assessed, further analysis using 2-tail t-tests was completed using Ms-Excel 2003 (results included in appendices).

### 8.8.1 Power analysis and limits of detection

Pre-trial patient numbers were calculated using Win Episcope 2.0 (Epidecon/Gobierno de Aragon, http://www.clive.ed.ac.uk/winepiscope). *Post-hoc* power analysis was performed to confirm that the tests used demonstrated 80% power at a P of $\leq$0.05 for all parameters used, and their limits of detection. All core statistical analyses were done in R version 2.1.1. (2005), or MS Excel 2003. Student's t tests with Welch's correction for unequal variances (R) were used to compare pH, $pCO_2$, $pO_2$, $HCO_3^-$ and MAP at each of the seven sampling times (167). Habitus and appetite were tested with Wilcoxon rank sum test. *Post hoc* power analyses were done by using the

average of all 14 means and standard deviations for each measurement (7 sampling times multiplied by two treatments). For all variables and parameters not evaluated initially in this manner, an F test was performed to test homescedascity of variance. Arrays with equal variances were compared using a 2-tail Student t-test type 2 using the Excel function **=ttest(array1,array2,2,2)** and those with unequal variances using a heteroscedascitic type 3 Student t-test **=ttest(array1,array2,2,3)**. F-values $\leq$0.05 indicated unequal variance and were the basis for distinction between the choice of tests. The comparisons between groups O and B were done for each time interval (e.g. $t_O$=0 vs $t_B$=0). If *any* F-statistic was $\leq$0.05, then a type 3 t-test was performed for all data of that parameter, across *all* times, including to compare the baseline data. Only if *all* F-statistics were >0.05 was a type 2 t-test performed to compare the treatment groups across time intervals, in an iterative fashion (i.e. $t_{O\,vs\,B} = i_0^{72}$). The comparison of terminal versus initial data used the same test type (2 or 3) as the main body of testing elected for that parameter, if *any* variance during that time had demonstrated unequal variance.

#### 8.8.1.1 POWER

The tests demonstrated that the study had 80% power to detect a difference of:

- 0.056 units in pH
- 8.26 mmHg in $pCO_2$
- 18.7 mmHg in $pO_2$
- 5.8 mmol/L in $HCO_3^-$
- 31.6 mmHg in MAP

These were considered the core parameters for evaluation, all others being secondary.

#### 8.8.1.2 ANCOVA TESTING

Additionally, to control for confounding effects that the measurement in the previous sampling time had on the present measurement, an analysis of covariance (ANCOVA) was performed for each of the parameters. This was performed by fitting a linear

model to the data (for example, pH at time t = [$b_1$ + $b_2$] × [pH at time t-1] + [$b_3$ × value]). When $b_2$ was not significant, it was deleted from the model and only the effect of treatment was retained. All models were checked for normality of errors and homoscedasticity. If these were violated, outliers were deleted or data transformed. *Post hoc* power analyses for $b_3$ were done by using the average of all seven means and standard deviations for each measurement time (combining treatments) to obtain a mean and standard deviation for time t-1. $b_1$, $b_2$ and the residual standard error was calculated as the average for each of these values for the seven sampling times.

#### 8.8.1.3 POWER

Due to the small sample size it is hard to rigorously assess the assumptions of the ANCOVA. The tests demonstrated that the study had 80% power to detect a difference of:

- 0.05 units in pH
- 6 mmHg in $pCO_2$
- 17 mmHg in $pO_2$
- 3.2 mmol/L in $HCO_3^-$
- 24 mmHg in MAP

### 8.8.2 Blood pressure validation technique

Indirect oscillometry is not as accurate as direct arterial blood pressure measurement. (50) Nonetheless, it is helpful and highly repeatable, especially if multiple measurements are taken and cuff size is appropriate. The Cardell monitor used also measured pulse rate. Simultaneous manual measurements of the femoral pulse and by the monitor were compared since there was no reason to suspect that the individual measurements at different time slots could not be used as independent data points.

### 8.8.3 Lactate measurement

Lactate measurement can be affected by a number of factors, such as sample

processing, serum spectrophotometric artefacts and sample storage. (168) Samples containing Oxyglobin® must be analysed on a particular device approved by the German Society of Clinical Pathologists but kits for this device became unavailable in South Africa early into the trial, and thus special sample collection, processing and storage for lactate assays was abandoned.

### 8.8.4 Statistical shortcomings and cautions

Patient numbers were restricted to 6 in each group so it is hard to test true efficacy and survival differences (for which a minimum of 12 patients would have been required in each group). Efficacy is best determined by differences in clinicopathological measurements and subjective criteria (which were not blinded) and some caution should be exercised due to the propensity for Type II error to occur with these limitations. Nonetheless, relatively good power was demonstrated by having a fairly homogenous "typical" population of patients with moderate disease. Herein lies another limitation of the study: true efficacy (as measured not only by clinicopathological or subjective criteria) is best evaluated by using sicker patients, but it was deemed too complex a step to take before patients such as the current 12 had been evaluated first. Recognising these limitations of the study would set the benchmarks for expanding it to larger, sicker and/or human patients.

# 9 RESULTS

## *9.1 Brief overview of results presentation*

Results are presented in a set order (Blood gas; Acid-base; Blood pressure; and Habitus & appetite), in the subcategories Baseline; t=0 to t=72, and study completion. Data is presented graphically as XY scatterplots or columns with time (hours, 0 to 72) on the X axis, pRBCT data in blue (—◆—) and Oxyglobin® data in red (—●—). Where appropriate, Y (vertical) error bars (± 1 standard deviation) are also shown, with pRBCT error bars in solid blue ( I ) and Oxyglobin® error bars overlapping them in broken red ( I ). Where appropriate, reference ranges are indicated on the Y-axis gridlines as dotted green lines (●●●●●●●●●), with the appropriate values indicated just above. In tables, **pRBCT data are in blue**, while **Oxyglobin® data are in red**. Means and standard deviation data are in **bold**. Significant differences are grouped in coloured boxes, usually yellow (if only 1 exists on a table) or more colours. Near-significant differences (P > 0.05 but mentioned in text) are indicated in pale yellow. In larger tables with multiple comparisons, matching colour boxes were compared to one another and found to have *P*<0.05.

## *9.2 Baseline samples (t=0)*

### 9.2.1 Comparison of groups at study initiation

**Table 5.** Basic patient information.

| | **Medians** | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | ***12*** |
| Group | **pRBCT** | **Oxy-globin®** | **B** | **B** | **O** | **O** | **B** | **B** | **O** | **O** | **O** | **B** | **O** | ***B*** |
| Age (months) | **9** | **18** | 12 | 6 | 24 | 7 | 8 | 48 | 36 | 12 | 24 | 10 | 12 | *8* |
| Wt (kg) | **20.45** | **15.31** | 17.2 | 21.9 | 14.5 | 8.6 | 22 | 22 | 22.5 | 11.5 | 19 | 12 | 16.1 | *19* |
| Transfusion volume (ml/kg) | **20.43** | **18.48** | 20.3 | 19.2 | 17.2 | 14.5 | 17.7 | 20.9 | 16.6 | 21.7 | 19.7 | 20.8 | 23.3 | *20.5* |
| Diminazene administered (ml) | **1.4** | **1.05** | 1.2 | 1.47 | 1 | 0.56 | 1.54 | 1.5 | 1.6 | 0.77 | 1.33 | 0.84 | 1.1 | *1.33* |
| Gender | | | FE | FE | FE | FE | FE | ME | ME | ME | FE | ME | FE | *ME* |

| | | | | |
|---|---|---|---|---|
| Males | **ME** | 3 | **MN** | 2 |
| Females | **FE** | 3 | **FN** | 4 |

M/FE = male/female (entire)

Groups were compaired with the F-test for unequal variances and all variances were found to sufficiently alike to perform type 2 testing as described in section 8.8.1. The F-statistic for volume transfused in ml/kg approached significance ($F$ = 0.0503) but neither a type 2 or 3 t-test were significant (see table in Appendix 12.1.2.2).

### 9.2.2 Overall comparison of core baseline parameters at study initiation

At the start of the two treatments there were no significant differences in any of the major parameters measured:

**Table 6.** Comparison of baseline core parameters

| Parameter | Mean O ± SD (min-max) | Mean B ± SD (min-max) | Test | t or W | df | *P* |
|---|---|---|---|---|---|---|
| pH | 7.39 ± 0.068 (7.28 – 7.46) | 7.423 ± 0.02 (7.40 – 7.45) | Welch two sample t tests | -1.06 | 5.87 | 0.33 |
| $pCO_2$ | 24.3 ± 7.8 (15.2 – 34.6) | 25.4 ± 3.3 (19.7 – 29.8) | | -0.34 | 6.76 | 0.75 |
| $pO_2$ | 92.1 ± 15.1 (75.7 – 116.0) | 85.9 ± 12.1 (74.6 – 103.8) | | 0.77 | 9.58 | 0.46 |
| $HCO_3^-$ | 14.6 ± 6.1 (12.1 – 18.0) | 15.9 ± 2.2 (6.8 23.4) | | -0.49 | 6.23 | 0.64 |
| MAP | 91.8 ± 13.1 (74 – 106.2) | 88.3 ± 21.0 (62 – 117) | | 0.34 | 8.39 | 0.74 |
| Habitus | 1.5* (1 – 2) | 1.67* (1 – 2) | Wilcoxon rank sum test | 15 | | 0.64 |
| Appetite | 0* (0 – 0) | 0* (0 – 0) | | 15 | | 0.64 |

*Median value

Thus any subsequent difference would mean that the one treatment is significantly different to the other at that time interval, and a lack of any significant difference just that.

### 9.2.3 Description of baseline parameters, t=0 hours

#### 9.2.3.1 BLOOD GAS PARAMETERS

Baseline parameters for factors describing blood gas status and influencing said parameters are summarised in table 7:

| **Table 7.** Baseline blood gas parameters | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Reference values** | **Oxyglobin® ± SD** | **pRBCT ± SD** | ***t*** | **Df** | **P** | **Test type** |
| pH (arterial) | 7.350 – 7.450 | 7.39 ± 0.068 (7.28 – 7.46) | 7.42 ± 0.02 (7.40 – 7.45) | -1.06 | 5.87 | 0.33 | 3 |
| $pCO_2$ | 35.0 – 45.0 mmHg | 24.3 ± 7.8 (15.2 – 34.6) | 25.4 ± 3.3 (19.7 – 29.8) | -0.34 | 6.76 | 0.75 | 3 |
| $pO_2$ | 75.0 – 100.0 mmHg | 92.1 ± 15.1 (75.7 – 116.0) | 85.97 ± 12.1 (74.6 – 103.8) | 0.77 | 9.58 | 0.46 | 3 |
| $CVpO_2$ | 47.9 – 56.3 mmHg [169] | 35.9 ± 9.6 (20.4 – 46.9) | 39.3 ± 12.3 (30.6 – 62.5) | | | 0.60 | 2 |
| Temperature | 37.8 – 39.3 ºC | 39.9 ± 0.9 (39.1 – 41.4) | 39.6 ± 0.6 (38.9 – 40.3) | | | 0.44 | 3 |
| tHb | g/dL | 3.6 ± 0.4 (3.1 – 4.1) | 3.9 ± 1.3 (2.3 – 5.7) | | | 0.67 | 3 |

#### *9.2.3.1.1 Assessment*

Welch 2-sample t-tests were performed on baseline blood gas (and tHb/temperature) values to determine if differences existed between treatment groups at study initiation. The mean values of the two groups demonstrated a compensatory respiratory alkalosis, normal pH and $pO_2$, with no significant differences existing between groups O and B. Patients in both groups were anaemic and pyrexic. Unequal variances were noted in all parameters barring $CVpO_2$, in which both groups showed comparable oxygen extraction (see 9.2.3.2.1.3)

### 9.2.3.2 ACID-BASE PARAMETERS

| **Table 8.** Acid-base parameters at t=0 | | | | | | |
|---|---|---|---|---|---|---|
| | **Reference values** | **Oxyglobin® ± SD (min – max)** | **pRBCT ± SD (min – max)** | ***t*** | **Df** | **P** |
| **$HCO_3^-$** | 13.5–23.9 mmol/L | 14.6 ± 6.1 (12.1 – 18.0) | 15.9 ± 2.2 (6.8 – 23.4) | 0.49 | 6.23 | 0.64 |
| **Deficit of expected compensatory response*** | | -4.4 ± 3.8 (-7.97 – 0.52) | -4.1 ± 2.0 (-7.18 – 0.86) | | | 0.89 |
| **Anion gap** | 13 - 21 mmol/L | 11.0 ± 12.1 (-0.3 – 27.0) | 8.7 ± 7.1 (-1.8 – 13.2) | | | 0.76 |
| **Sodium** | 144.2–149.8 mmol/L | 138.4 ± 6.1 (126.7 – 143.2) | 138.7 ± 3.9 (134.1 – 145.2) | | | 0.93 |
| **Chloride, Corrected** | 113.8–118.9 mmol/L | 123.4 ± 6.8 (113.9 – 129.1) | 123.7 ± 6.2 (119.7 – 132.8) | | | 0.96 |
| **Chloride gap** | -2.5–2.5 mmol/L | -7.7 ± 6.0 (-16.3 – 2.4) | -5.4 ± 4.2 (-10.4 – -0.7) | | | 0.56 |
| **Strong ion difference** | 27.1–32.3 | 13.4 ± 8.4 (3.4 – 23.3) | 16.0 ± 10.4 (1.3 – 25.5) | | | 0.71 |
| **Free water change** | -0.4–4.0 | -0.74 ± 0.5 (-1.6 – -0.41) | -0.56 ± 0.4 (-1.0 – -0.11) | | | 0.63 |
| *no reference range applicable | | | | | | |

#### *9.2.3.2.1 Assessment*

The mean values of the two groups revealed a hypoalbuminaemic alkalosis (see later); hyperchloraemic acidosis; and a dilutional acidosis, with no significant differences between groups at the outset of the study.

##### 9.2.3.2.1.1 Acidaemia

Both groups entered the trial with an acidaemia (base deficit; Table 8). Neither ANCOVA nor type 3 t-testing demonstrated any differences at t=0.

##### 9.2.3.2.1.2 The respiratory response to acidaemia

According to Day (170) and de Morais and others, (65; 66) each decrease of 1 mmol/L in $HCO_3^-$ should be matched by a 0.7 mmHg compensatory decrease in $pCO_2$. Using the formula:

$$[(HCO3^- - 18.7) \times 0.7] - (pCO_2 - 31.5)$$

(with values derived from the means of the OVAH reference ranges), these values were calculated and compared to the expected deviations. At the outset, both groups of patients had a more vigourous response than predicted by the standard formula (Table 8).

##### 9.2.3.2.1.3 Oxygen extraction (central venous $pO_2$)

Oxygen extraction by tissues is determined by the central venous oxygen $pO_2$ (169; 78)

At the outset of the trial, all patients had increased oxygen extraction as indicated by decreased $CVpO_2$.

##### 9.2.3.2.1.4 Anion gap, sodium, chloride and SID

9.2.3.2.1.4.1 Anion gap

Anion gap (AG) never approached statistically significant levels and averages were

uniformly below reference ranges throughout the study period. In conjunction with a low chloride gap (Table 3), this is classified as a hyperchloraemic acidosis. Although lactate levels were not measured, Nel and others (64; 63) indicated that a high anion gap lactic acidosis was ubiquitous in canine babesiosis.

Caution should be exercised in interpreting data and statistical significance as data sets were incomplete due to equipment malfunction resulting in no AG values being returned for some patients at some time points. Also, variance was very high for this parameter (see Figure 16). For example, at t=0, O group patients' AG ranged from -0.3 to +27.0.

9.2.3.2.1.4.2 Sodium (Na+) trends

Patients in both groups entered the trial with moderate hyponatraemia, a characteristic abnormality of childhood falciparum malaria thought to be due to inappropriate urinary sodium loss. (98)

9.2.3.2.1.4.3 Chloride ($Cl^-$) and the Chloride Gap

Chloride values were corrected for sodium using the formula: (24; 65; 76)

$$Cl^-_{corrected} = Cl^-_{patient} \times Na^+_{mean\ normal} / Na^+_{patient}$$

As noted in the study by Leisewitz et al, (24) dogs entered this trial uniformly hyperchloraemic. Variances were not significantly different ($F > 0.05$, see Appendix).

When chloride changes were calculated using the formula: (24; 66; 76)

$$\textbf{Chloride gap} = Cl^-_{mean\ normal} - Cl^-_{corrected}$$

The values obtained indicated a hyperchloraemic acidosis for patients in both groups.

9.2.3.2.1.4.4 Strong ion difference (SID)

Patients entered the trial with a low SID (albeit with large variances, Table 8). This is typical of an acidotic state (65) and is a consequence of the hyponatraemia (dilutional acidosis) and hyperchloridaemia (hyperchloraemic acidosis) noted in these patients. Hyperlactataemia and changes in other unmeasured anions and cations (not measured in this trial but evaluated by other workers e.g. Button, (64) Nel (63) and Leisewitz et al, (24)) would contribute to the remainder of any SID.

9.2.3.2.1.4.5 Free water abnormalities (FWA)

At the outset, both groups showed a free water abnormality (FWA), in the form of a dilutional acidosis. Normal values for free water change are -0.4 to 4.0. (24) F-tests showed that variances were unequal at t=48 and thus t-tests were of the Welch-corrected (type 3) form.

### 9.2.3.2.1.5 Protein baseline measurements

**Table 9.** Mean baseline plasma protein, albumin and globulin values

| | Reference ranges | O ± SD (min – max) | *B ± SD* (min – max) |
|---|---|---|---|
| **Total serum protein (TSP)** | 53–75 (mmol/L) | 62.4 ± 7.7 (55.5 – 76.9) | 65.3 ± 16.2 (54.5 – 93.8) |
| **Albumin** | 27–35 (mmol/L) | 21.3 ± 4.1 (15.0 – 26.5) | 23.1 ± 1.9 (20.3 – 24.9) |
| **Globulin** | 20–37 (mmol/L) | 41.1 ± 7.3 (30.7 – 50.4) | 42.2 ± 17.7 (31.7 – 73.5) |
| **A/G ratio** | 0.6–1.2 | 0.54 ± 0.16 (0.32 – 0.81) | 0.61 ± 0.20 (0.28 – 0.79) |
| a $P = 0.053$; b $P = 0.018$; c $P = 0.0002$; d $P = 0.003$ | | | |

Ranges and standard deviations are given in the appendices. Patients were mildly hypoalbuminaemic and hyperglobulinaemic at presentation. One patient (patient 6, group B) had a persistently hyperglobulinaemia throughout the study but since protein

measurements were made in the post-study period, this was not detected until after trial completion. No blood smear evidence of Ehrlichiosis was found at presentation or on subsequent evaluation of the patient file 9 months after trial completion. The patient was not readmitted for treatment for Ehrlichiosis during this period.

#### 9.2.3.3 HAEMODYNAMIC PARAMETERS

**Table 10.** Baseline blood pressure parameters

| Reference values | | Oxyglobin® ± SD | pRBCT ± SD | *t* | Df | P |
|---|---|---|---|---|---|---|
| **DBP** | 74 – 104 mmHg | 65.9 ± 13.0 (48.6 – 83.4) | 63.0 ± 19.4 (41.2 – 87.0) | | | 0.77 |
| **MAP** | 87 – 127 mmHg | 91.8 ± 13.1 (74.0 – 106.2) | 88.3 ± 21.0 (62 – 117) | 0.34 | 8.39 | 0.74 |
| **SBP** | 130 – 172 mmHg | 127.6 ± 13.6 (107.8 – 146.6) | 136.9 ± 17.6 (114.4 – 165.8) | | | 0.33 |
| **Measured PR** | 90 – 120 | 145.5 ± 21.6 (125 – 181) | 131.4 ± 21.5 (106.8 – 161.8) | | | 0.28 |
| **Femoral Pulse** | | 147.5 ± 27.0 (108.8 – 182.4) | 124.2 ± 19.7 (104 – 150) | | | 0.12 |

##### *9.2.3.3.1 Assessment*

The results of a Pearson's product-moment correlation of the data were:

**t=15.0881, df = 77, P-value <2.2e$^{-16}$**

**Correlation ($r^2$) = 0.8644371**

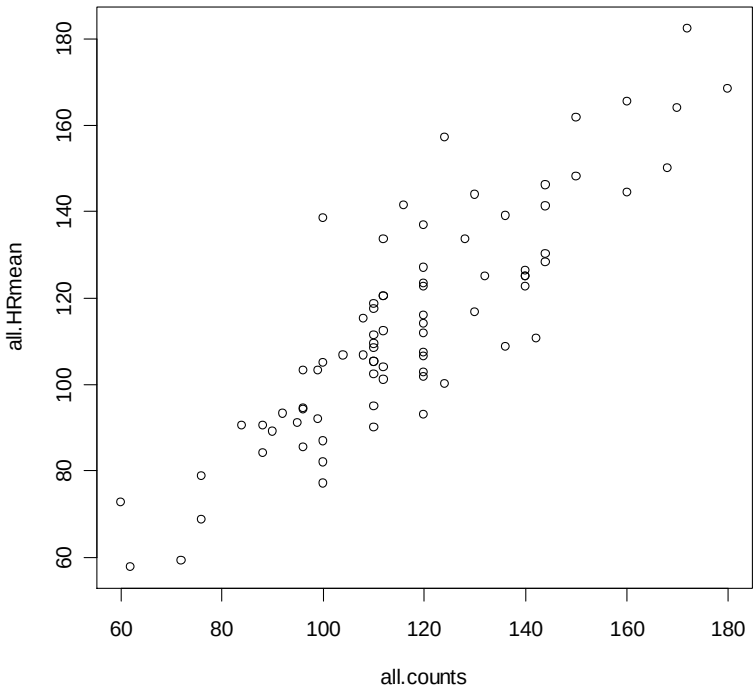



**Figure 1.** Correlation (XY scatterplot) of palpated (X, all.counts) versus machine measured (Y, all.HRmean) pulse rates.

Thus a highly significant correlation existed, demonstrating that the monitor was accurate at measuring pulse rate and thus lending some more weight to oscillometric blood pressure measurements made simultaneously by this device. It should be noted that babesiosis can cause significant cardiac arrhythmias and that this may affect oscillometric peripheral pulse measurements. (11; 25)

The mean values of the two groups demonstrated a low-normal (Oxyglobin®) or marginally low (pRBCT) mean arterial blood pressure. Nonetheless, there was no statistically significant difference (P = 0.74) between the groups. Systolic BP was in the lower end of the normal range or in the borderline zone, while DBP was in the hypotensive bracket described by Jacobson and others. (50)

Dogs were tachycardic at admission, a state consistent with anaemia and pyrexia.

#### 9.2.3.4 HABITUS & APPETITE EVALUATION

**Table 11.** Baseline habitus & appetite parameters

| | Oxyglobin® | pRBCT | W | P |
|---|---|---|---|---|
| **Habitus*** | 1.5 | 1.67 | 15 | 0.64 |
| **Appetite*** | 0 | 0 | 15 | 0.64 |

*Median, not mean values

##### *9.2.3.4.1 Assessment*

A Wilcoxon rank sum test was used to compare groups at the outset with regard to subjective criteria. Patients were all depressed, anorexic, anaemic and pyrexic upon admission (Tables 7 & 11). The two treatment groups did not differ in this regard.

### 9.2.4 Trends and differences, t=0 to t=72 hours

#### 9.2.4.1 BLOOD GAS PARAMETERS

**Table 12.** 72-hour trends: blood gas parameters. Matching colour boxes indicate that the difference noted refers between those two values e.g. $pCO_2$ t=0 vs t=72 for pRBCT

| | | Time | O* | B* | O | B | O | B | O | B | O | B | O | B | O | B |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Reference ranges | 0 | 0 | 1 | 1 | 4 | 4 | 8 | 8 | 24 | 24 | 48 | 48 | 72 | 72 |
| **pH** | 7.340–7.440 | | 7.39 ± 0.068 | 7.42 ± 0.02 | 7.398 ± 0.049 | 7.400 ± 0.014 | **7.438 ± 0.046 a** | **7.392 ± 0.025 a** | 7.436 ± 0.04 | 7.400 ± 0.025 | 7.429 ± 0.04 | 7.395 ± 0.014 | 7.420 ± 0.022 | 7.393 ± 0.042 | 7.412 ± 0.027 | 7.409 ± 0.019 |
| **$pCO_2$** | 23.1–39.9 mmHg | | 24.3 ± 7.8 | **25.4 ± 3.3 c** | 24.6 ± 7.2 | 26.1 ± 3.1 | **23.9 ± 6.3 b** | **29.2 ± 3.9 b** | 25.4 ± 7.4 | 29.0 ± 3.2 | 28.8 ± 6.3 | 29.3 ± 1.9 | 28.5 ± 4.5 | 29.1 ± 3.0 | 31.3 ± 2.6 | **29.9 ± 2.6 c** |
| **$pO_2$** | 74.2–103.8 mmHg | | 92.1 ± 15.1 | 85.9 ± 12.1 | 87.6 ± 9.7 | 89.4 ± 9.1 | 92.9 ± 13.4 | 88.3 ± 8.5 | 88.9 ± 9.0 | 82.4 ± 10.2 | 91.0 ± 15.7 | 84.2 ± 5.6 | 77.6 ± 8.5 | 88.3 ± 16.5 | 78.9 ± 4.6 | 82.5 ± 7.6 |
| **$CVpO_2$** | 47.9 – 56.3 mmHg | | 35.9 ± 9.6 | 39.3 ± 12.3 | 31.4 ± 6.7 | 36.5 ± 6.1 | 30.3 ± 7.0 | 34.6 ± 6.9 | 31.1 ± 10.3 | 35.7 ± 4.0 | 34.1 ± 6.6 | 36.8 ± 2.7 | 29.9 ± 6.9 | 34.7 ± 6.9 | 29.5 ± 4.5 | 35.6 ± 6.6 |
| **Haemo-globin** | 12-18 g/dL | | **3.6 ± 0.4 d (3.1 – 4.1)** | **3.9 ± 1.3 e (2.3 – 5.7)** | 3.1 ± 0.4 (2.7 – 3.7) | 4.0 ± 0.9 (3.1 – 5.3) | 4.8 ± 1.6 (3.5 – 7.1) | 6.7 ± 1.4 (5.3 – 9.0) | **3.9 ± 0.3 f (3.5 – 4.2)** | **7.9 ± 1.9 f (5.4 – 10.6)** | **4.1 ± 1.2 g (2.6 – 5.3)** | **8.0 ± 2.4 g (5.5 – 11.60)** | 6.1 ± 2.9 (3.2 – 10.1) | 7.0 ± 1.5 (5.5 – 9.0) | **8.0 ± 2.5 d (6.1 – 11.6)** | **9.0 ± 1.4 e (6.6 – 10.0)** |
| **Temp-erature** | 37.8 – 39.3 ºC | | **39.9 h (39.1 – 41.4)** | **39.6 i (38.9 – 40.3)** | 39.3 (37.4 – 40.9) | 39.4 (38.6 – 40.3) | 39.9 (37.8 – 41.4) | 40.1 (39.9 – 40.3) | 39.7 (37.2 – 41.2) | 39.0 (38.5 – 39.3) | 39.0 (37.8 – 40.0) | 38.3 (37.8 – 39.1) | 38.5 (38.1 – 39.5) | 38.6 (37.9 – 40.3) | **38.0 h (37.5 – 38.5)** | **38.5 i (38.7 – 38.3)** |

***O = Oxyglobin®** ***B = pRBCT**

| *P*-values: | a <0.001 | b <0.001 | c = 0.026 | d = 0.006 |
|---|---|---|---|---|
| e <0.001 | f = 0.004 | g = 0.007 | h = 0.002 | i = 0.009 |

#### *9.2.4.1.1 Assessment*

##### 9.2.4.1.1.1 pH

All patients' pH values remained within the reference range during the trial (ANCOVA), barring some exceptions at individual time points, with the only significant difference occuring at t=4 (Table 11, a). F-test statistics (see Appendices) showed unequal variances and thus a type 3 t-test was performed. Significance was *approached* at t=4 (*P*=0.064).

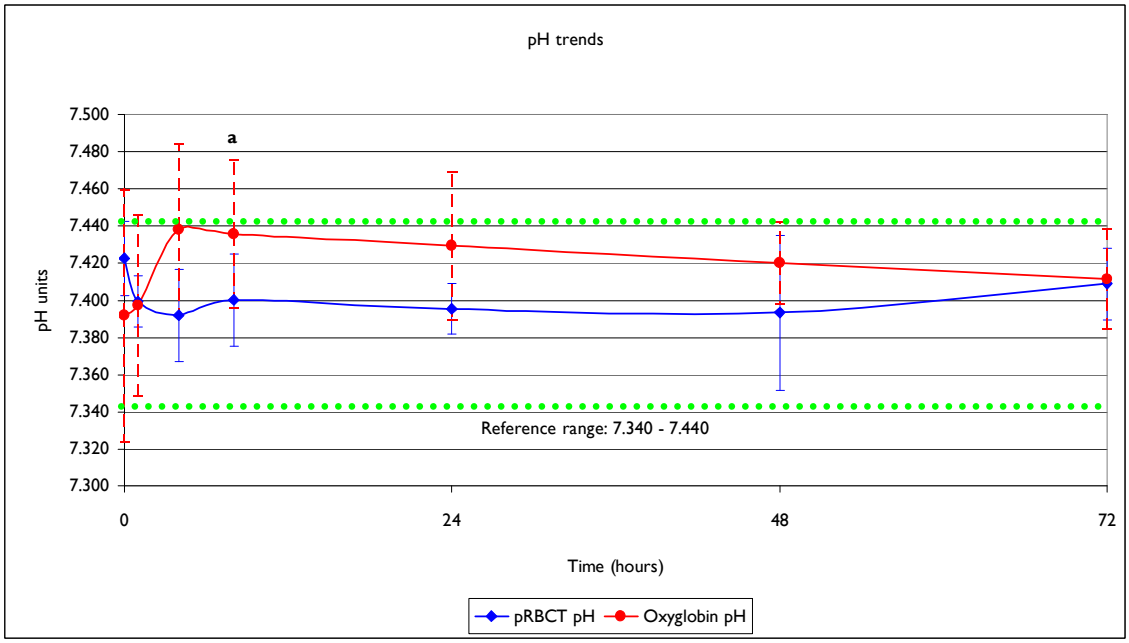



**Figure 2.** pH trends over 72 hours. a = point of near-significant difference (*P* = 0.064).

##### 9.2.4.1.1.2 $pCO_2$

$pCO_2$ was low throughout the trial, although within the OVAH reference ranges. (24) Nonetheless, B group patients experienced a significant increase in $pCO_2$ over the course of treatment. In fact, patients treated with pRBCT experienced an early and significant increase in carbon dioxide (Table 12, Figure 3).

**Table 13.** Student t-test results comparing $pCO_2$ at each time interval to baseline values, with significant differences ($P$ <0.05) or near-significant differences (0.05<$P$<0.1) highlighted in colour.

| TTESTS | | pRBCT type 2 test | F-test | Oxyglobin® type 2 test | F-test |
|---|---|---|---|---|---|
| t=0 vs | t=1 | 0.720 | 0.890 | 0.937 | 0.878 |
| | t=4 | 0.103 | 0.717 | 0.940 | 0.669 |
| | t=8 | 0.084 | 0.922 | 0.807 | 0.925 |
| | t=24 | 0.033 | 0.232 | 0.326 | 0.718 |
| | t=48 | 0.076 | 0.851 | 0.306 | 0.317 |
| | t=72 | 0.026 | 0.604 | 0.094 | 0.054 |

Table 13 can be interpreted using the example of t=0 vs t=24. At this time, there was a significant ($P$=0.033) difference between pRBCT values and the baseline, but not for Oxyglobin ($P$=0.326). Table 13 should be interpreted with caution for the reasons explained in paragraph 8.8.1.2, wherein the effects of a previous value at time t-1 may influence the value at time b. This analysis was not performed for the purposes of Table 13. Nonetheless, it highlights a trend which is subjectively observable in Figure 3. Furthermore, the F-test performed on the data showed no evidence of significantly unequal variances at t=4 and thus using the type 3 t-test (as unequal variances existed at other points in the data), significance was not achieved at this time. The ANCOVA $P$-values are reflected in table 12.

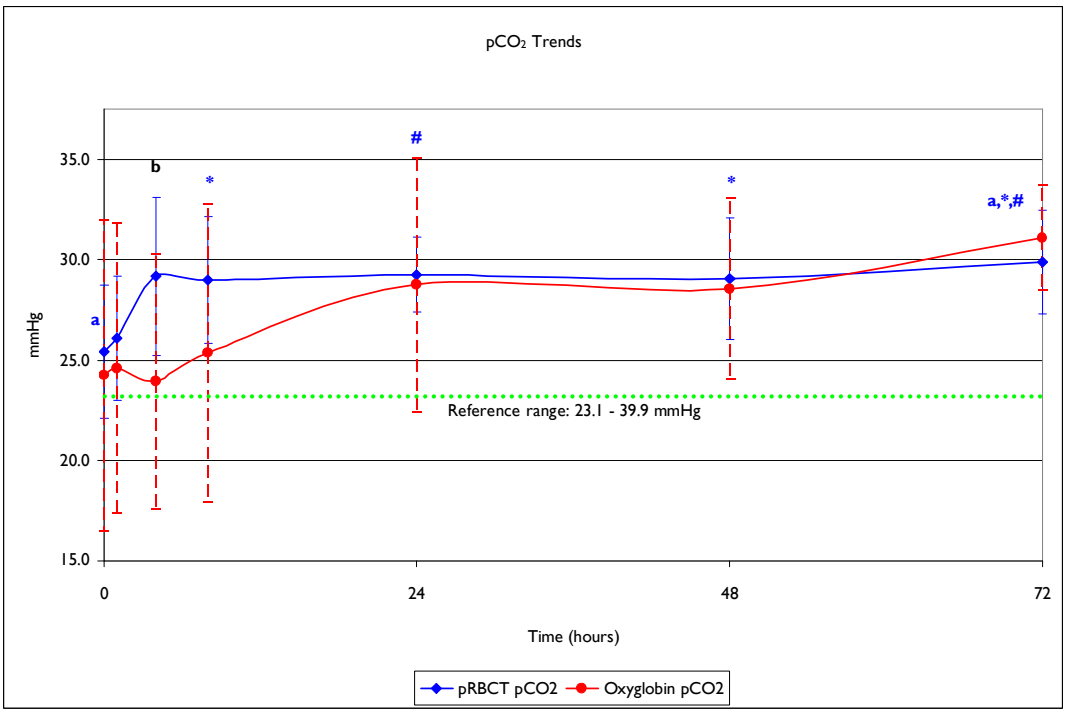



**Figure 3.** Trends in $pCO_2$ over 72 hours. Significant differences from tables 12 and 13 (a, *, #) marked on graph.

#### 9.2.4.1.1.3 $pO_2$ trends

Although individual patients may have been just within the reference limits of hypoxaemia during the trial, the overall trend was one of normoxaemia. There were no significant differences between groups, although group O patients' t=72 $pO_2$ was almost significantly lower ($P$ = 0.095) than initial means (78.9 ± 4.6 mmHg vs 92.1 ± 15.1 mmHg).

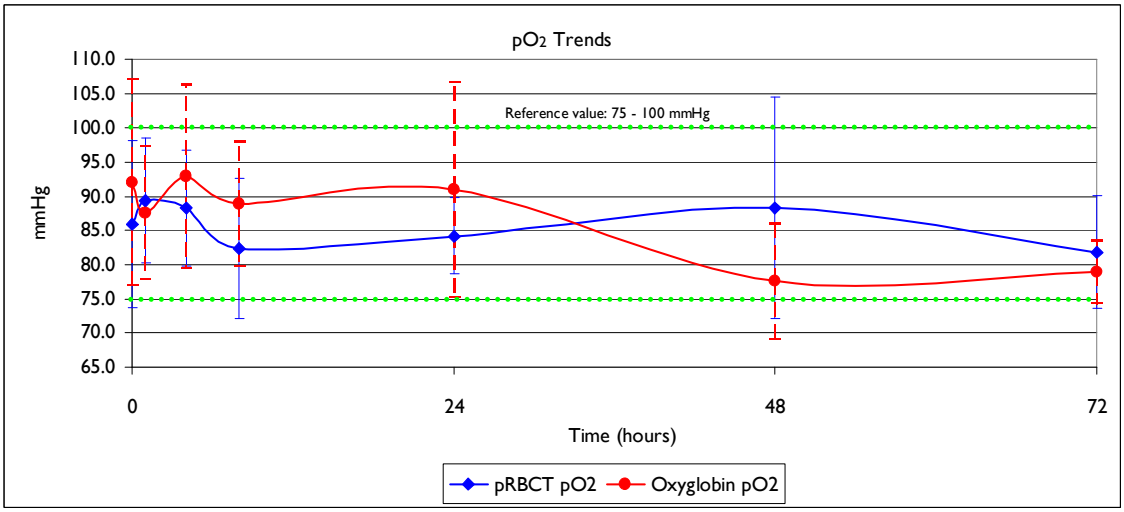



**Figure 4.** $pO_2$ trends.

#### 9.2.4.1.1.4 CVpO2

Central venous pO2 was decreased throughout the trial and did not increase. This implies increased oxygen extraction by the tissues despite either treatment, and that neither treatment significantly decreased peripheral oxygen hunger. Significance was never approached by either treatment.

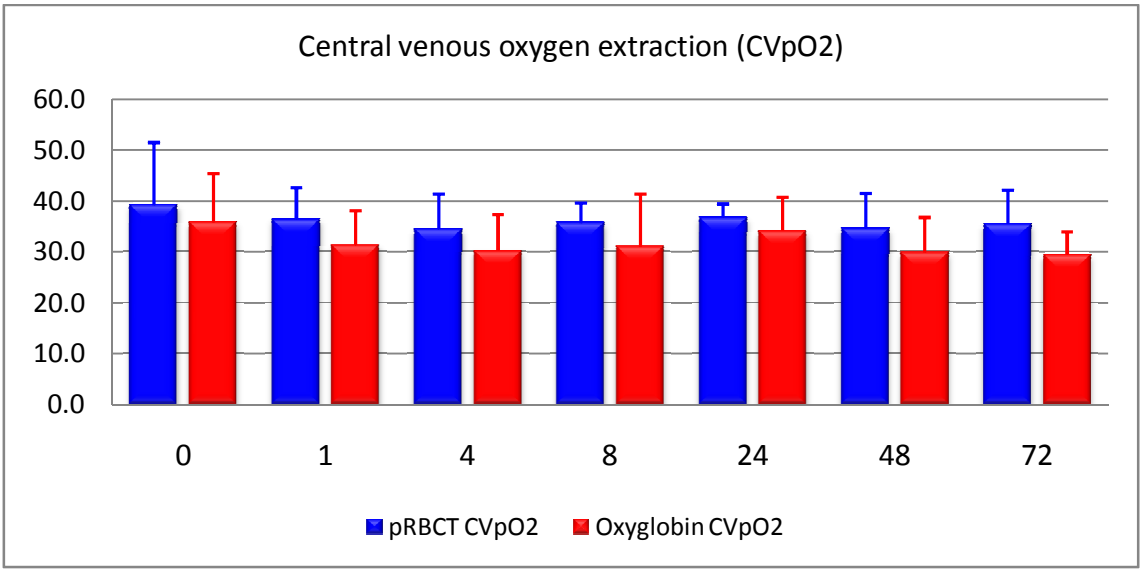



**Figure 5.** $CVpO_2$ (central venous oxygen extraction) trends over trial period.

#### 9.2.4.1.1.5 tHb

Total haemoglobin increased significantly for both groups over the course of treatment (Figure 6, a [P < 0.001] & b [P < 0.006]). pRBCT-treated patients had a significantly higher tHb than Oxyglobin®-treated patients at t=8 and t=24 (P = 0.004 and P = 0.019, respectively).

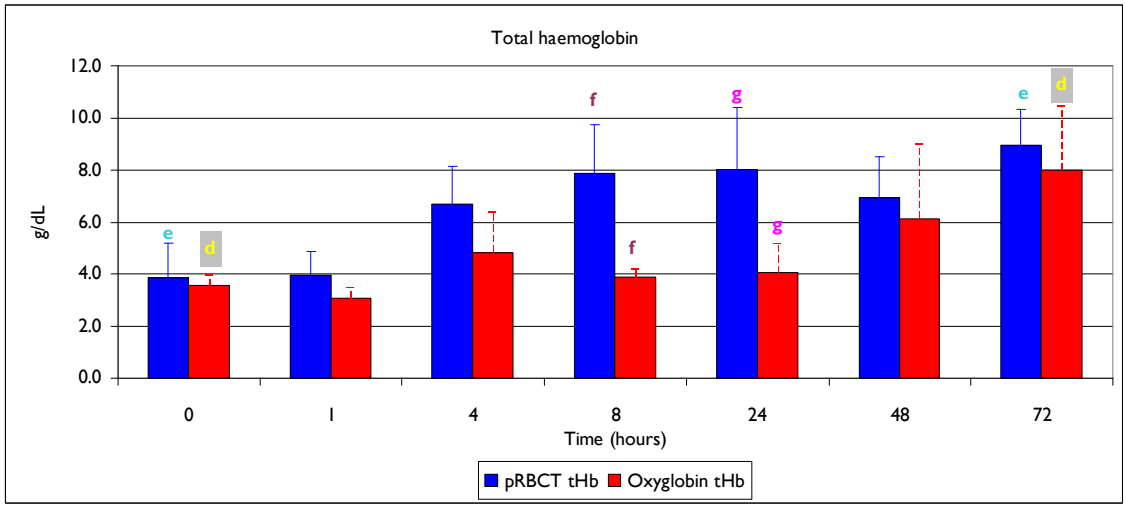



**Figure 6.** tHb trends over time. Significant differences annotated and coloured as in table 12.

Furthermore, although the trends in tHb were generally upwards, group B tHb increased to a significantly higher amount by t=1, while the Oxyglobin® group tHb never approached significance until t=72, when Oxyglobin® would have been almost completely excreted (Table 14). Since some F-test values were significant ($F < 0.05$) in group O, both groups were compared using type 3 t-tests, even though testing was within groups, not between groups (in order to maintain comparability by using similar standards).

**Table 14.** Point-versus-point 2-sample t-test *P* values comparing baseline tHb against subsequent values. Significant values in yellow* (*P* <0.05), near-significant differences in cream (0.05<*P*<0.1).

| | Group B | | Group O | |
|---|---|---|---|---|
| | T test | F test | | F test |
| t=1 vs t=0 | 0.894 | 0.453 | 0.132 | 0.852 |
| t=4 vs t=0 | 0.013 | 0.889 | 0.211 | 0.026 |
| t=8 vs t=0 | 0.006 | 0.540 | 0.219 | 0.600 |
| t=24 vs t=0 | 0.013 | 0.294 | 0.490 | 0.074 |
| t=48 vs t=0 | 0.01 | 0.794 | 0.178 | 0.003 |
| t=72 vs t=0 | 0.0004 | 0.960 | 0.038 | 0.005 |
| t-test type | F >0.05 so type 2 but see group O | | Type 3 | |

#### 9.2.4.1.1.6 Temperature

As noted beforehand, all patients began the trial pyrexic. Resolution of this pyrexia occurred rapidly (within 24 – 48 hours for most patients). Groups O and B did not differ from each other at any time during the trial, but both were significantly different to their initial values (group B *P* = 0.009, group Oxyglobin® *P* = 0.002) using a type 3 t-test for unequal variances (see Appendix).

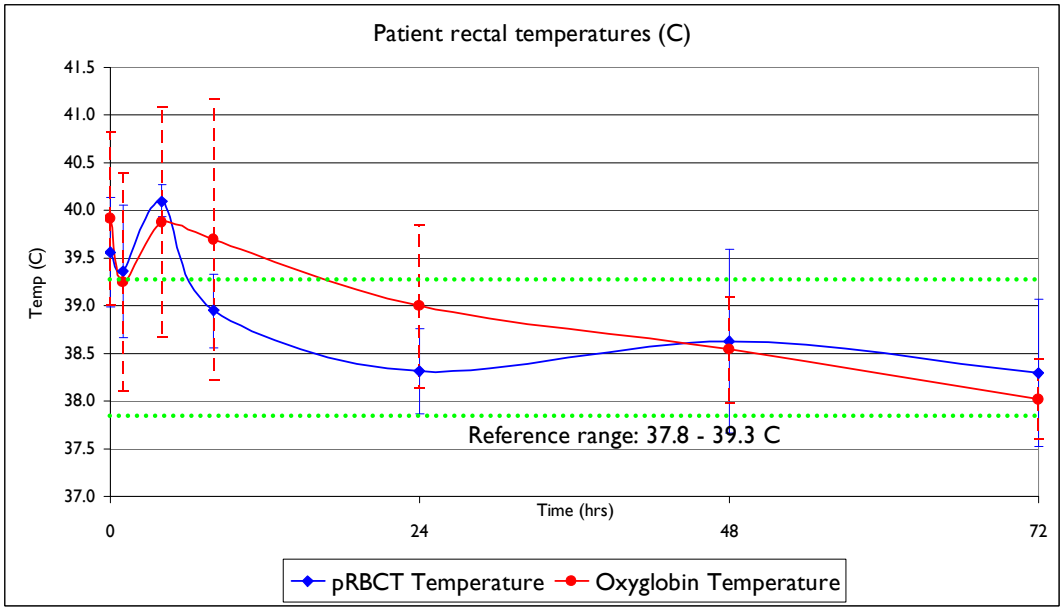



**Figure 7.** Temperature trends (in ºC) over the trial for both patient groups.

#### 9.2.4.1.1.7 Ancillary blood-gas parameters

##### 9.2.4.1.1.7.1 Arterial oxygen content ($aO_2ct$)

Although results of $aO_2ct$ are marred by small sample sizes, some interesting trends emerge. Firstly, the only significant difference exists between the t=0 and t=72 in the B group (P = 0.017). Significance was approached between groups O and B at t=24 (*P* = 0.066) and t=48 (*P* = 0.052) and due to unequal variances at t=24, type 3 t-tests were performed to take this into account (see Appendix).

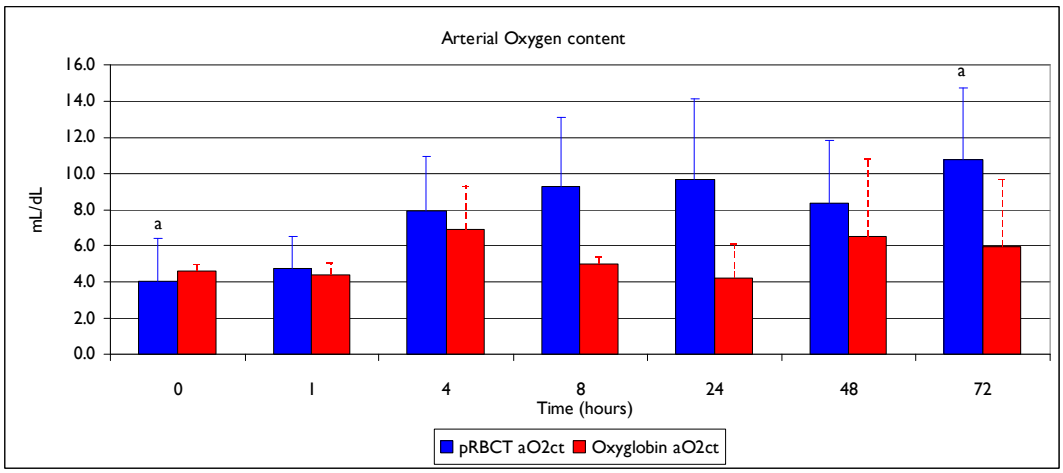



**Figure 8.** Arterial oxygen content ($aO_2ct$) trends. Significant difference "a" as noted in text.

##### 9.2.4.1.1.7.2 Methaemoglobin (metHb) levels

The reference range for methaemoglobin in dogs is <1.0% (OVAH laboratory). Sample sizes were small, marring statistical comparison, but there only appeared to be a difference at t=8 (*P* = 0.015) and t=48 (*P* = 0.003, see Appendix). This was despite wide variances (*F*-values in 12.1.8.2). One dog (patient 10, group O) had a pronounced methaemoglobinaemia (10.9%) at t=24, accounting for the large deviation (4.2% ± 4.5%) in group O at time t=24. This is far greater than the upward deviation reported by Keri et al (163), which varied from <0.5% at t=0 to >1.0% at peak of t=24.

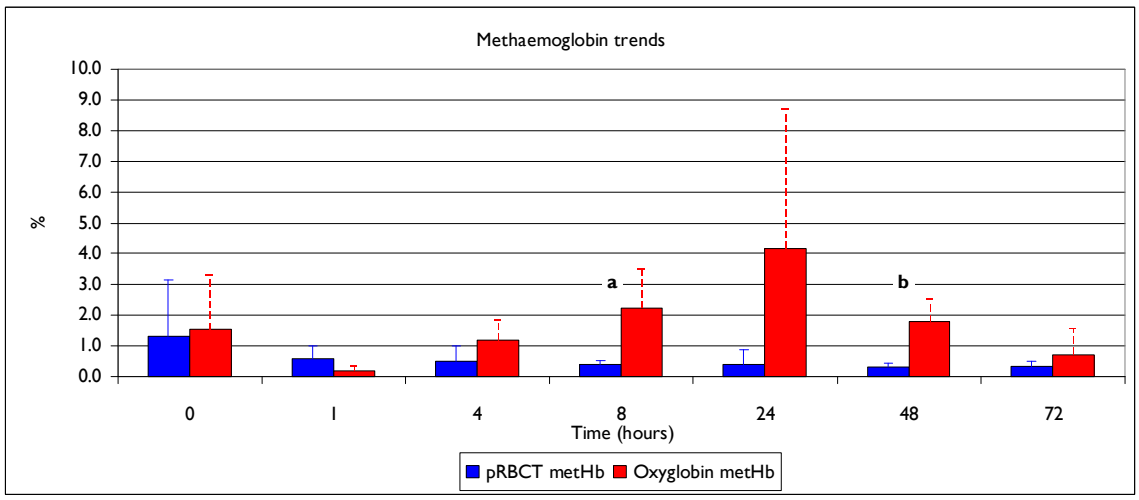



**Figure 9.** Methaemoglobin trends. Significant difference at points "a" and "b" between groups O and B.

9.2.4.1.1.7.3 Oxygen saturation ($S_aO_2$)

Oxygen saturation remained within the reference range (>92%) for both groups throughout the period, although individual measurements were below this amount (see Appendix). Significance was never achieved between groups although F-test statistics showed significant differences in variances at t=0 and 24 and almost at t=8. There was no significant difference between terminal and baseline $SaO_2$.

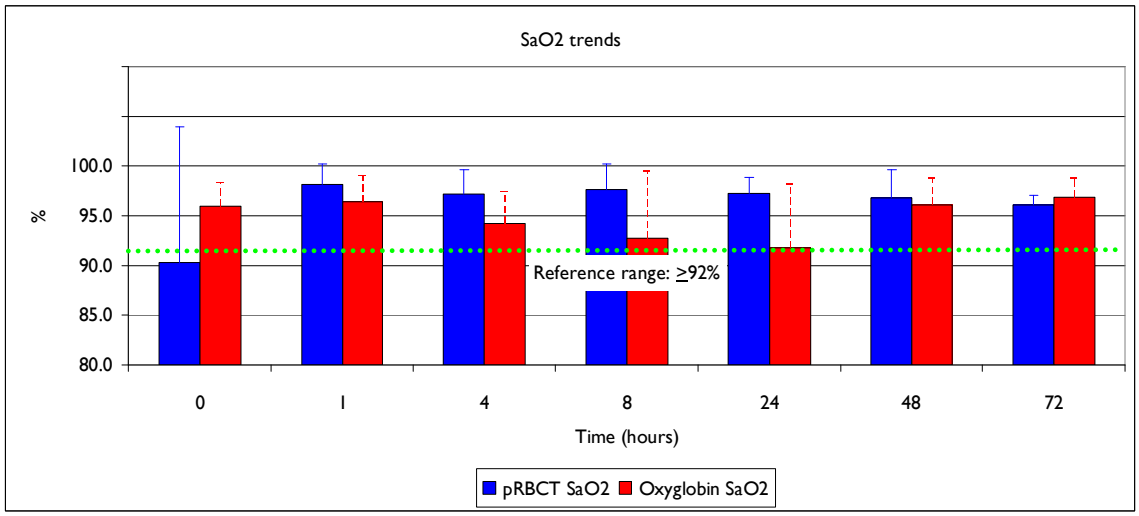



**Figure 10.** $SaO_2$ trends.

9.2.4.1.1.7.4 Carboxyhaemoglobin (HbCO) trends

The level of haemoglobin bound to CO was uniformly elevated for both groups, across the entire period. Although there was a subjective decrease in HbCO, it was not significant. O group patients had higher t=72 HbCO levels (6.9% ± 2.9% vs 3.6% ± 1.2%, *P* = 0.07, Figure 11, b) than group B (see Appendix). Group B HbCO declined to near-significant levels compared to baseline measurements (*P* = 0.094, Figure 11, a).

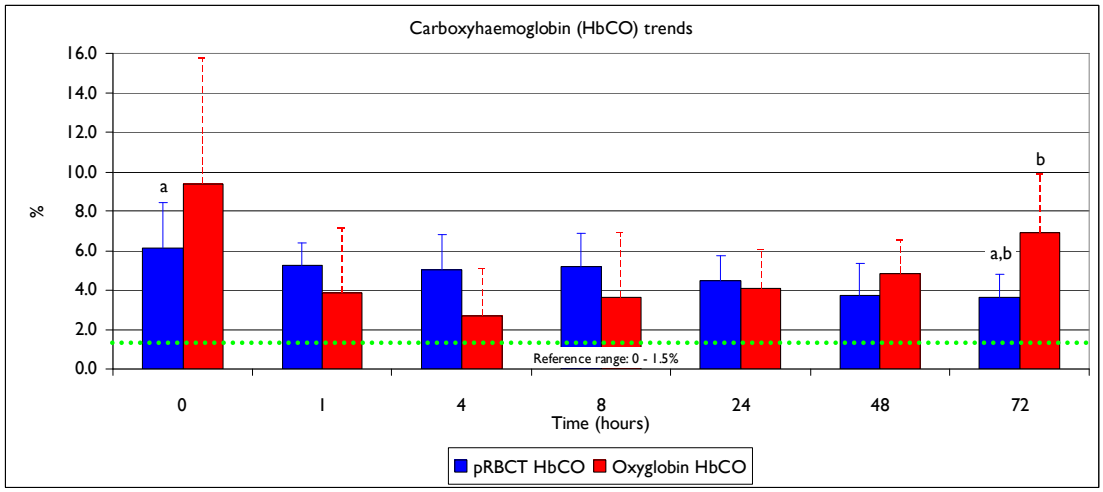



**Figure 11.** Carboxyhaemoglobin (HbCO) trends. Reference range ≤1.5% of tHb.

9.2.4.1.1.7.5 $O_2$ capacity of haemoglobin ($O_2$cap)

The reference range for $O_2$cap (16 – 24 mL/dL) was never reached by patients in either group. As with some other ancillary blood-gas parameters, sample sizes were small and thus statistical evaluation (including F-testing) was possibly unreliable. Nonetheless, a *P* of <0.05 was never broached. At t=24, pRBCT $O_2$cap was greater than that of O group patients (9.5 ± 4.2 mL/dL vs 4.4 ± 1.9 mL/dL, *P* = 0.052). In addition, values in group B in particular were greater than baseline values (Table 15, Appendix).

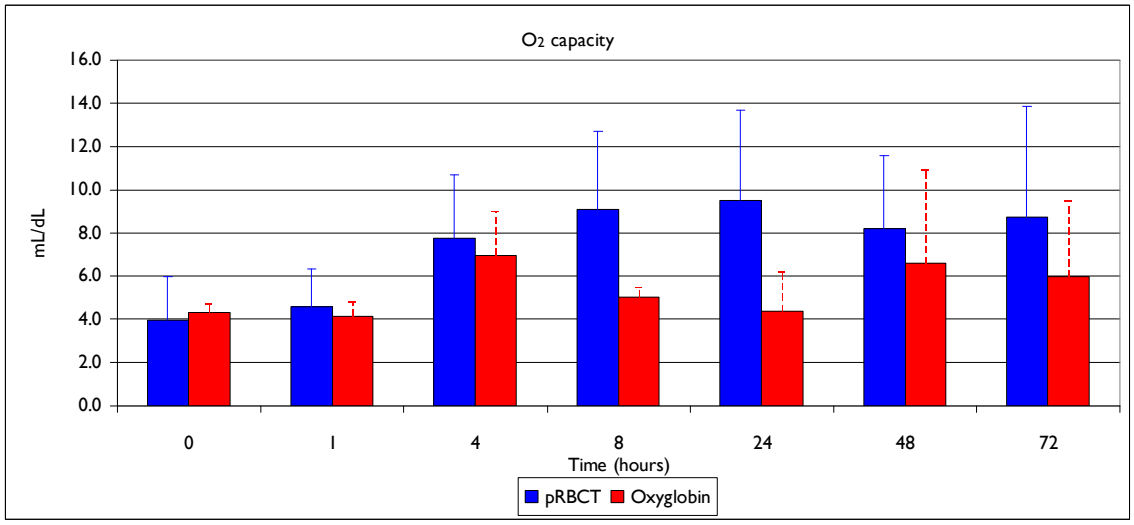



**Figure 12.** $O_2$ capacity trends.

### 9.2.4.2 ACID-BASE PARAMETERS

**Table 15.** 72-hour trends: acid-base parameters. Matching colour boxes and letter-codes indicate that the difference noted refers between those two values e.g. SID t=24 (letter g, colour green) = significant difference at this time between groups O and B

| Reference Ranges | Time= | O* 0 | B* 0 | O 1 | B 1 | O 4 | B 4 | O 8 | B 8 | O 24 | B 24 | O 48 | B 48 | O 72 | B 72 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $HCO_3^-$ | 13.5–23.9 mmol/L | 14.6 ± 6.1 | 15.9 ± 2.2 | 14.8 ± 5.5 | 15.5 ± 2.2 | 15.8 ± 5.5 | 16.8 ± 2.0 | 16.5 ± 5.4 | 17.2 ± 1.7 | 18.3 ± 3.8 | 17.2 ± 1.6 | 17.8 ± 2.3 | 17.4 ± 2.0 | 19.2 ± 2.0 | 18.3 ± 2.3 |
| Deficit of expected compensatory response** | | -4.4 ± 3.8 | **-4.1 ± 2.0 a** | -4.2 ± 3.6 | -3.1 ± 1.7 | **-5.5 ± 2.8 b** | **-1.0 ± 2.7 b** | -4.6 ± 3.8 | -1.5 ± 2.2 | -2.4 ± 4.1 | -1.2 ± 0.9 | -2.3 ± 3.0 | -1.6 ± 2.7 | -0.7 ± 1.7 | **-1.3 ± 1.1 a** |
| Anion gap | 13 - 21 mmol/L | 11.0 ± 12.1 | 8.7 ± 7.1 | 9.2 ± 10.8 | 6.6 ± 5.2 | 8.3 ± 6.6 | 7.4 ± 5.4 | 8.3 ± 4.0 | 10.3 ± 4.2 | 2.8 ± 4.5 | 8.6 ± 2.9 | 5.7 ± 4.1 | 10.7 ± 1.9 | 7.3 ± 4.7 | 11.1 ± 1.5 |
| Sodium | 144.2–149.8 mmol/L | 138.4 ± 6.1 | 138.7 ± 3.9 | 138.4 ± 5.9 | 139.9 ± 2.0 | 139.9 ± 3.9 | 140.6 ± 3.2 | 140.6 ± 2.8 | 139.3 ± 2.7 | 139.3 ± 5.2 | 143.3 ± 1.0 | 140.2 ± 3.4 | 142.2 ± 3.3 | 144.1 ± 2.7 | 142.6 ± 2.8 |
| Strong ion difference | 27.1–32.3 | 13.4 ± 8.4 | 16.0 ± 10.4 | 12.2 ± 7.7 | 13.0 ± 5.9 | 14.7 ± 4.1 | 16.2 ± 8.2 | **16.1 ± 2.2 f** | **22.2 ± 4.4 f** | **9.1 ± 6.0 g** | **20.0 ± 3.1 g** | **11.9 ± 2.8 h** | **21.4 ± 6.3 h** | 21 ± 5.6 | 24.8 ± 3.9 |
| Chloride, Corrected | 113.8–118.9 mmol/L | 123.4 ± 6.8 | 123.7 ± 6.2 | 124.5 ± 6.8 | 126.1 ± 3.9 | 124.3 ± 5.6 | 124.1 ± 4.5 | 123.8 ± 4.1 | 121.7 ± 2.9 | **129.5 ± 2.9 c** | **123.7 ± 3.4 c** | **127.4 ± 1.2 d** | **121.1 ± 4.2 d** | **123.1 ± 3.2 e** | **118.9 ± 1.8 e** |
| Chloride gap | -2.5–2.5 mmol/L | -7.7 ± 6.0 | -5.4 ± 4.2 | -7.8 ± 5.7 | -6.1 ± 1.2 | -5.8 ± 3.8 | -4.9 ± 3.1 | -5.1 ± 2.6 | -1.7 ± 2.3 | -6.5 ± 5.0 | -1.9 ± 0.7 | -5.9 ± 2.7 | -2.9 ± 3.2 | -1.6 ± 2.6 | -1.9 ± 2.3 |
| Free water change | -0.4–4.0 | -0.74 ± 0.5 | -0.56 ± 0.4 | -0.70 ± 0.5 | -0.64 ± 0.2 | -0.78 ± 0.4 | -0.58 ± 0.2 | -0.78 ± 0.4 | -0.43 ± 0.2 | -0.36 ± 0.1 | -0.45 ± 0.2 | -0.59 ± 0.1 | -0.55 ± 0.5 | -0.34 ± 0.4 | -0.51 ± 0.4 |

***O = Oxyglobin®, B = pRBCT**, a–h= significant differences ($P$<0.05); a = 0.015;b = 0.017;c = 0.039;d = 0.03;e = 0.06;f = 0.049;g = 0.018;h= 0.034. **pRBCT mean deficit discrepancy -2.0 ± 2.2 mmHg versus Oxyglobin® mean -3.6 ± 3.4

#### *9.2.4.2.1 $HCO_3^-$*

Statistical analysis by ANCOVA showed that the value of $HCO_3^-$ at t=1 had a strong effect on subsequent values for all measurements. For this reason, although Student's t-tests (with or without Welch correction/type 3) all had *P*-values >0.05, the ANCOVA tests were also performed instead, taking time t=1 into account.

Neither t-testing nor the test of choice, ANCOVA, demonstrated no difference at any time (all *P*-values >0.05).

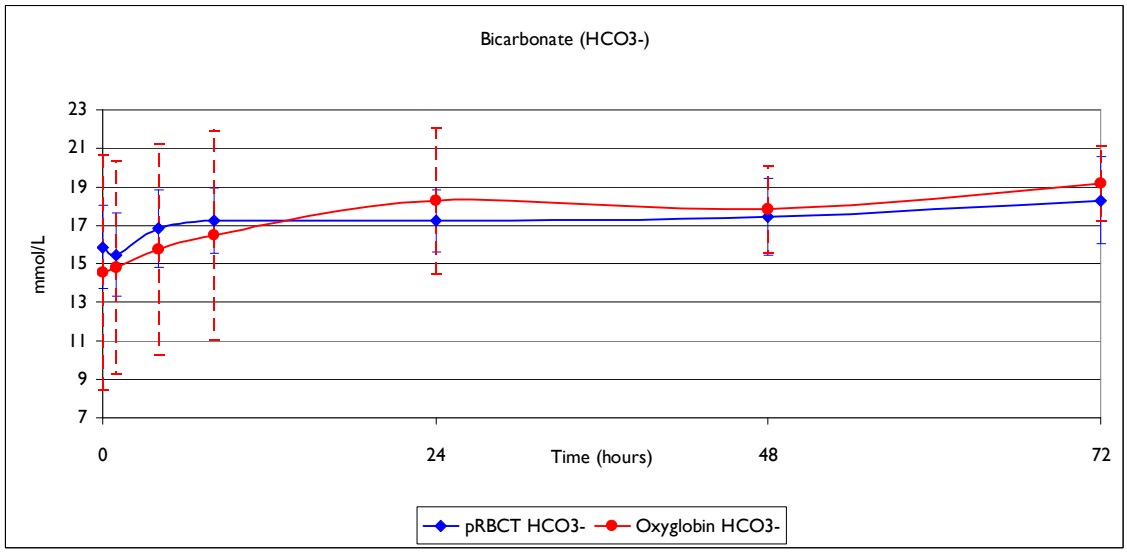



**Figure 13.** Bicarbonate trends.

#### *9.2.4.2.2 The respiratory response to acidaemia over 72 hours*

Calculation of the respiratory response – acidaemia differential showed that the pRBCT t=72 values were different to the initial values, while Oxyglobin®-treated patients' were not (pRBCT *P* = 0.02, Oxyglobin® *P* = 0.07). However, the groups only differered from each other at t=4 (pRBCT mean deficit discrepancy -0.998 mmHg [-5.74 to 2.47] versus Oxyglobin® mean -5.502 [-8.18 to -19.4, *P* = 0.017]). Variances were unequal at t=24 hence a type 3 t-test adjusted for heteroscedascity was performed as explained in section 8.8.1 of the Materials and Methods chapter. Find results of statistical analyses in the Appendices.

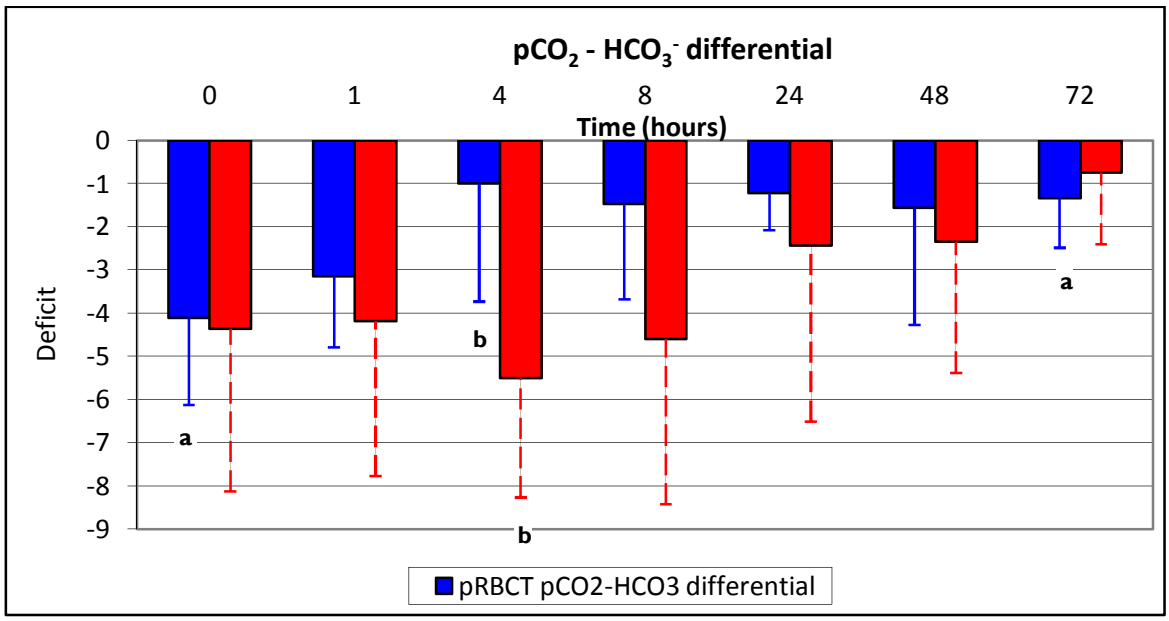



**Figure 14.** Respiratory drive differential trends. Significant differences in pRBCT groups between t=0 and t=72 ("a") and between groups B and O at t=4 ("b") annotated.

### *9.2.4.2.3 Anion Gap, Sodium & Chloride*

#### 9.2.4.2.3.1 Anion Gap trends

At t=0, patients in both groups had a mixed acid-base disturbance with a neutral pH. Normally, mixed metabolic "acidosis" (e.g. hyperlactataemic or diabetic ketoacidotic acidaemias) exhibit a normal or *increased* anion gap, and are therefore divided further into hyper- and normochloraemic anion gap acidosis. Metabolic acidosis normally results from an increase in strong anions such as chloride, lactate or ketoanions. (76) In this setting, where hyperlactataemia is a well-established aspect of canine babesiosis, (63) and where the chloride is initially normal, the normochloraemic, anion gap, metabolic acidosis of canine babesiosis is to be expected.

Data revealed that AG remained below the reference range throughout, with some patient's AG values increasing to within the range at some points. There were no significant differences between groups or within groups between baseline and terminal values. It is worth noting that younger animals may have lower anion gap figures, although figures only exist for foals and newborn puppies. (171) This may

account, in part, for the low AG values, as patient ages were often low (see Table 5).

Although no differences were noted between treatment groups, standard deviations varied widely due to outliers and low sample numbers at some intervals (Figures 13 & 14) and thus may not be sufficiently statistically rigourous to draw firm conclusions therefrom. AG being the derivative of other parameters (some measured and some calculated by the analytical device) is also an imprecise parameter for scrupulous testing as variance in root data magnifies the AG variations.

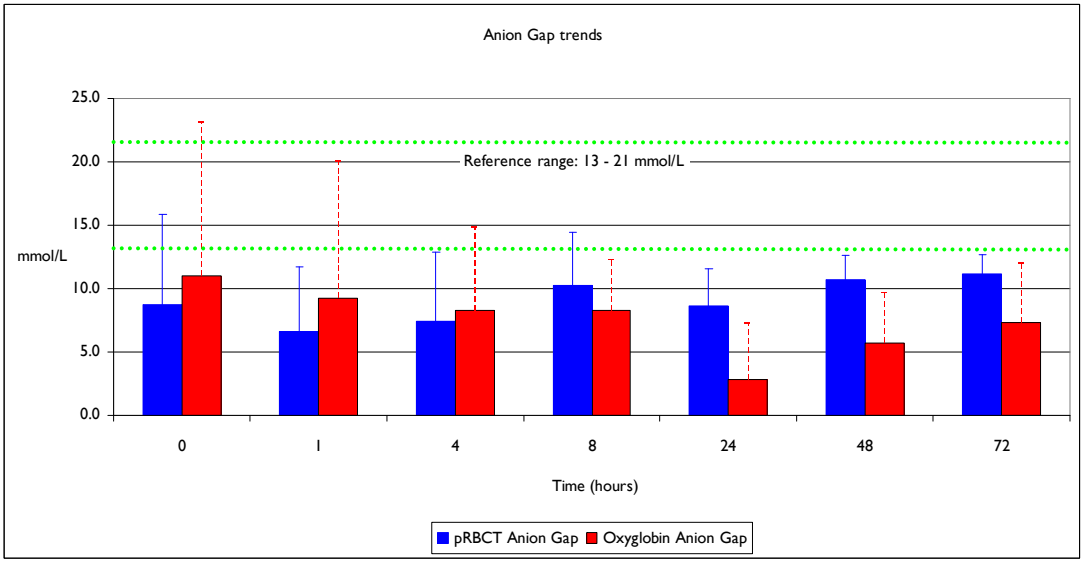



**Figure 15.** Anion Gap (AG) trends over 72 hours

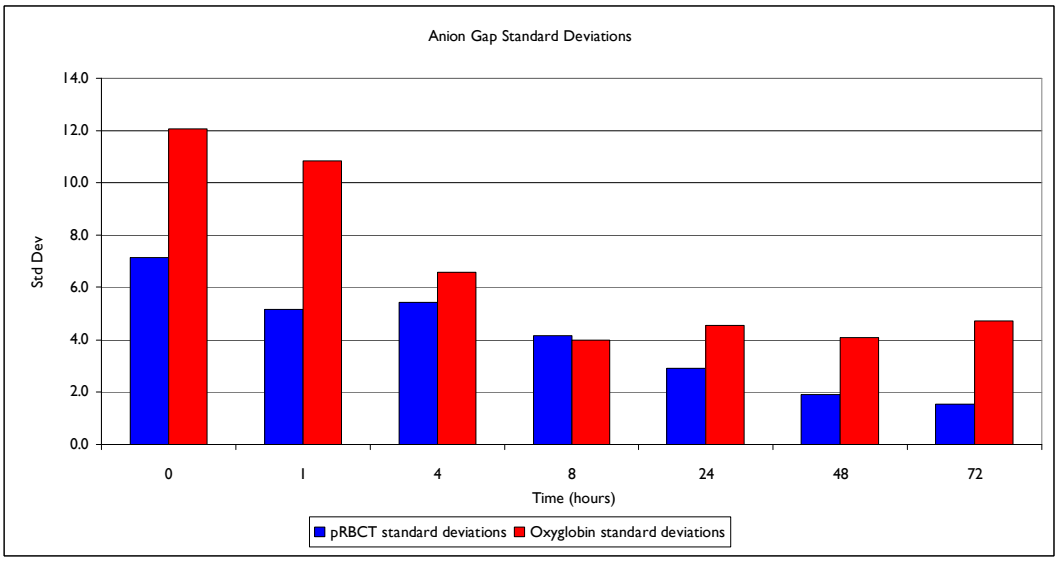



**Figure 16.** Standard deviations of Anion Gap across 72 hours

#### 9.2.4.2.3.2 Sodium shifts

Patients entered the trial uniformly hyponatraemic, leading to free water (dilutional) acidosis. This is contrary to the findings of Lobetti and Jacobson's investigation of renal involvement in canine babesiosis (17) but in agreement with the findings of Leisewitz et al. (24)

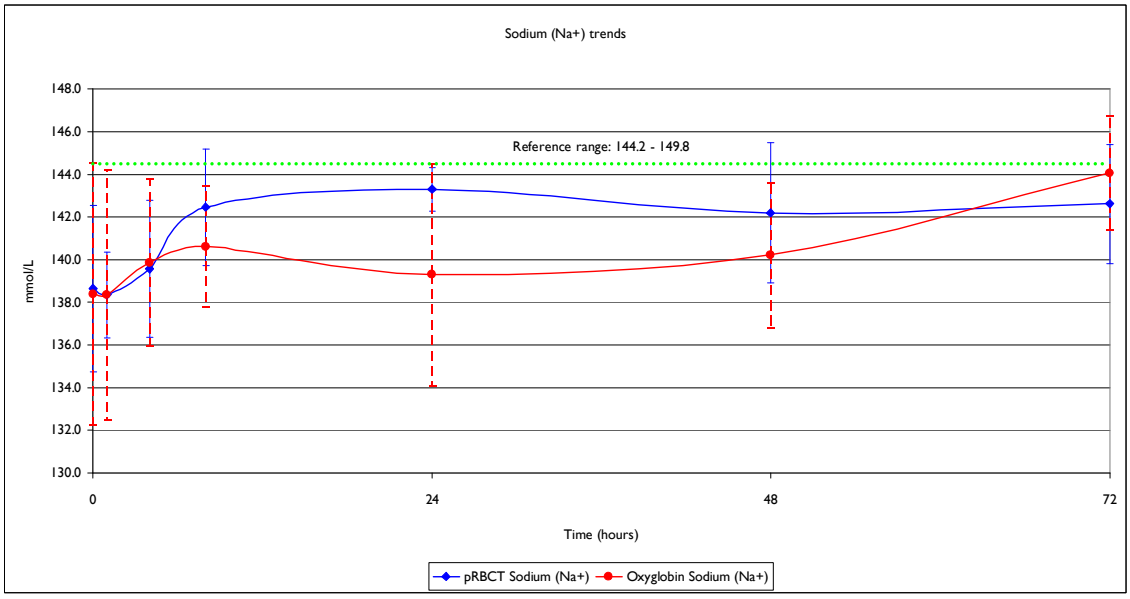



**Figure 17.** Sodium trends over 72 hours

#### 9.2.4.2.3.3 Chloride shifts

The trends over 72 hours indicated a hyperchloraemic acidosis[6] for patients in both groups. This state was not alleviated by either treatment over the entire trial (Figures 18 and 19). Patients receiving pRBCT had a more rapid return to near-normal $Cl_{corr}$ than those receiving Oxyglobin®, so that significant differences existed between groups from t=24 onwards.

[6] Adapted from de Morais & Biondo, 2006 (175):

| Lactate* | Chloride | $HCO_3^-$ | Strong anions ($Cl^- + HCO_3^-$) | AG | Type |
|---|---|---|---|---|---|
| Same | Added | ↓ | Same | Normal | Hyperchloraemic or normal AG acidosis |
| Added | Same | ↓ | ↓ | ↑ | Normochloraemic or high AG acidosis |

*or another unmeasured anion eg ketones

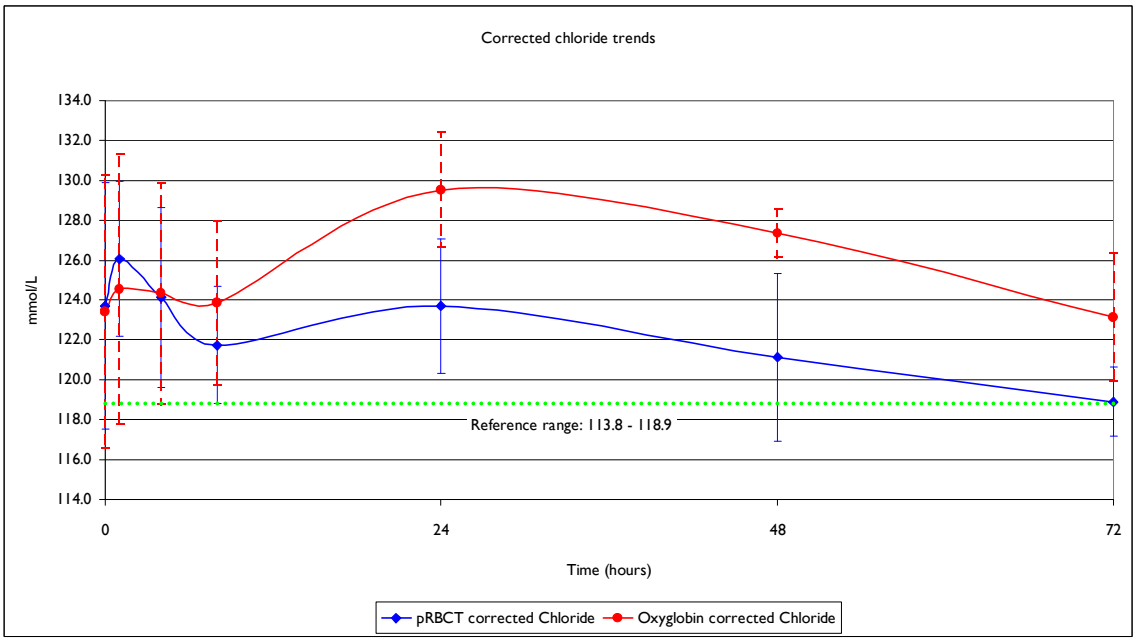



**Figure 18.** Corrected chloride trends over 72 hours.

Although chloride gap levels were not significantly different between treatment groups, pRBCT-treated patients consistently demonstrated a smaller gap and a more rapid normalisation (Figure 19, Table 15). Variances were unequal and thus a type 3 t-test was performed, but significance was never achieved although at t=8 the *P*-value was 0.078 (see Appendix).

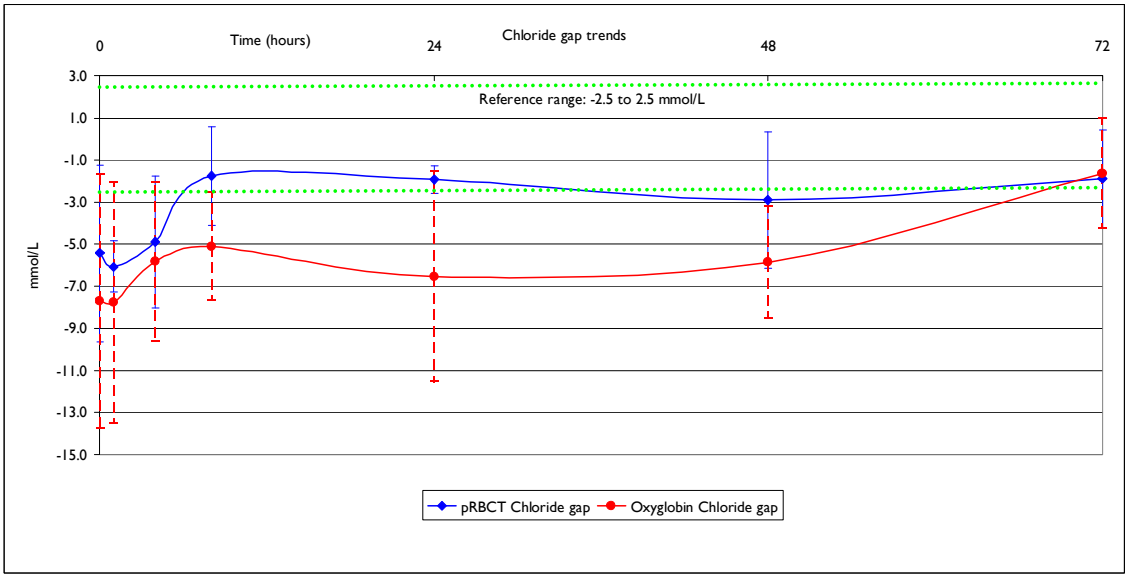



**Figure 19.** Chloride gap trends over 72 hours.

#### 9.2.4.2.3.4 Strong ion difference (SID) trends

Only two values reached the minimum of the reference range, reflective of the changes remarked upon in the paragraphs on AG, sodium, chloride and free water abnormalities. Significant differences were noted from t=8 to t=48 (see table 15). This is similar to the findings remarked on for the baseline values in (9.2.3.2.1.3.4).

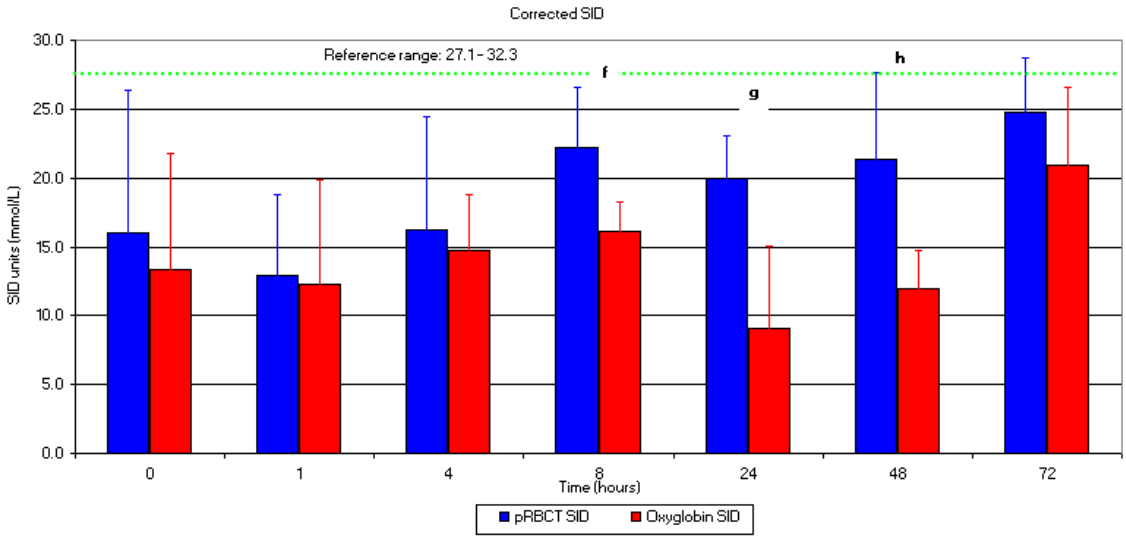



**Figure 20.** Strong Ion Difference (SID) trends. Significant differences at f ($P$ = 0.049), g ($P$ = 0.018), and h ($P$ = 0.034)

#### 9.2.4.2.3.5 Free water abnormality (FWA) trends

All patients entered the trial with a dilutional acidosis typical of either:

- Excessive natriuresis (early renal failure or renal tubular acidosis); or
- Excessive loss of sodium relative to chloride loss.

This remained the *status quo* for the entire trial, with neither group normalising completely, although both approached the lower end of normality (-0.4 units). Alhtough F-test values were significantly different at t=48, this wide difference in variation (Figure 20) did not result in a statistical difference between groups.

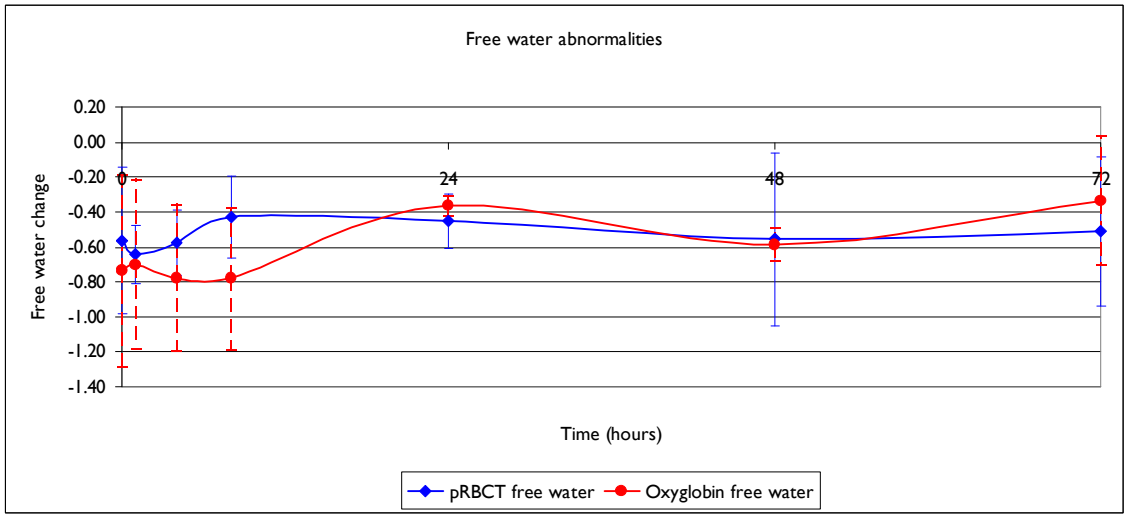



**Figure 21.** Free water abnormality trends

### 9.2.4.2.3.6 Protein trends

**Table 16.** Mean total serum protein, albumin and globulin trends. Similar-coloured boxes indicate that significant differences existed (see below for *P* values).

| | Reference ranges | 0 | 1 | 4 | 8 | 24 | 48 | 72 |
|---|---|---|---|---|---|---|---|---|
| **pRBCT TSP** | 53–75 (mmol/L) | 65.3 | 63.02 | **69.32** | **66.22** | 70 | 69.97 | 72.88 |
| **Oxyglobin® TSP** | | 62.38 | 74.38 | **93.68** | **92.43** | 79.08 | 72.36 | 68.93 |
| **pRBCT albumin** | 27–35 (mmol/L) | 23.12 | 22.98 | **23.52** | **23.13** | 22.92 | 22.82 | 23.63 |
| **Oxyglobin® albumin** | | 21.3 | 25.57 | **32.27** | **32.18** | 27.48 | 23.78 | 23.05 |
| **pRBCT globulin** | 20–37 (mmol/L) | 42.18 | 40.03 | 43.97 | 44.74 | 45.4 | 48.42 | 50.18 |
| **Oxyglobin® globulin** | | 41.08 | 48.82 | 61.42 | 60.25 | 51.6 | 48.58 | 45.88 |
| **pRBCT A/G ratio** | 0.6–1.2 | 0.613 | 0.619 | 0.583 | 0.594 | 0.553 | 0.531 | 0.542 |
| **Oxyglobin® A/G ratio** | | 0.538 | 0.535 | 0.53 | 0.533 | 0.542 | 0.512 | 0.515 |

a *P* = 0.053; b *P* = 0.018; c *P* = 0.0002; d *P* = 0.003

Patients in both groups demonstrated an elevation in total serum proteins, chiefly due to hyperalbuminaemia, from t=4 onwards. However, this was significant only group O from t=4 to t=8. (Table 16). The initial hypoalbuminaemia is consistent with the findings of Maegraith (172) and Lobetti and others. (173) Protein electrophoresis and α1-glycoprotein determinations were not performed.

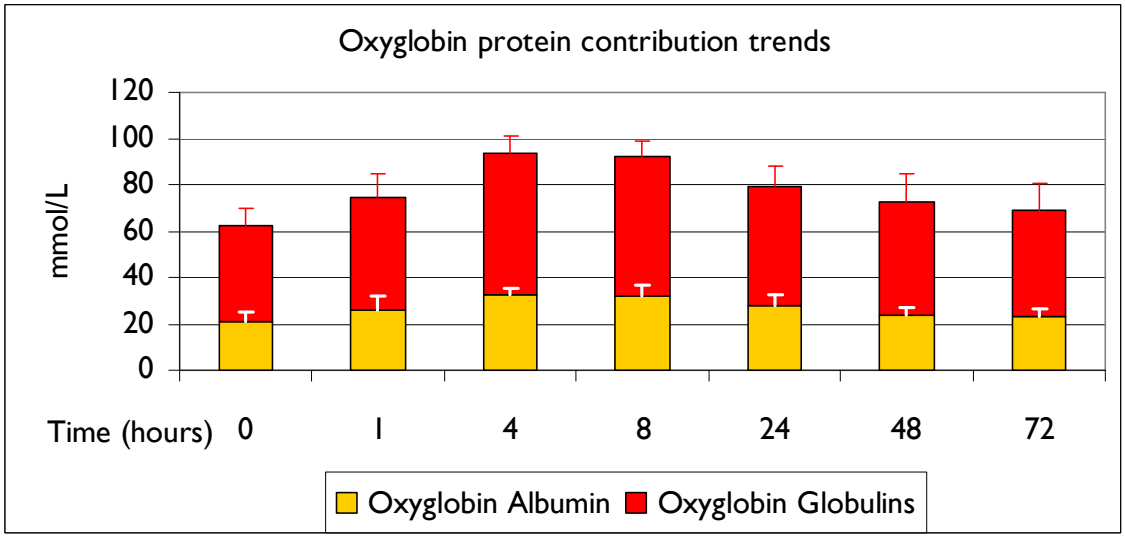



**Figure 22.** Oxyglobin® group protein (albumin + globulin) trends with standard deviation error bars.

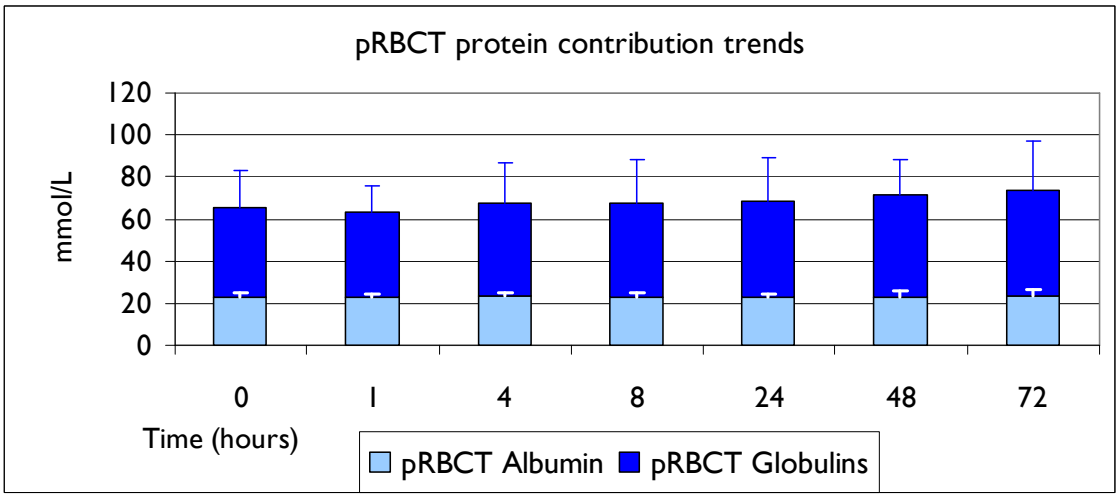



**Figure 23.** pRBCT group protein (albumin + globulin) trends with standard deviation error bars.

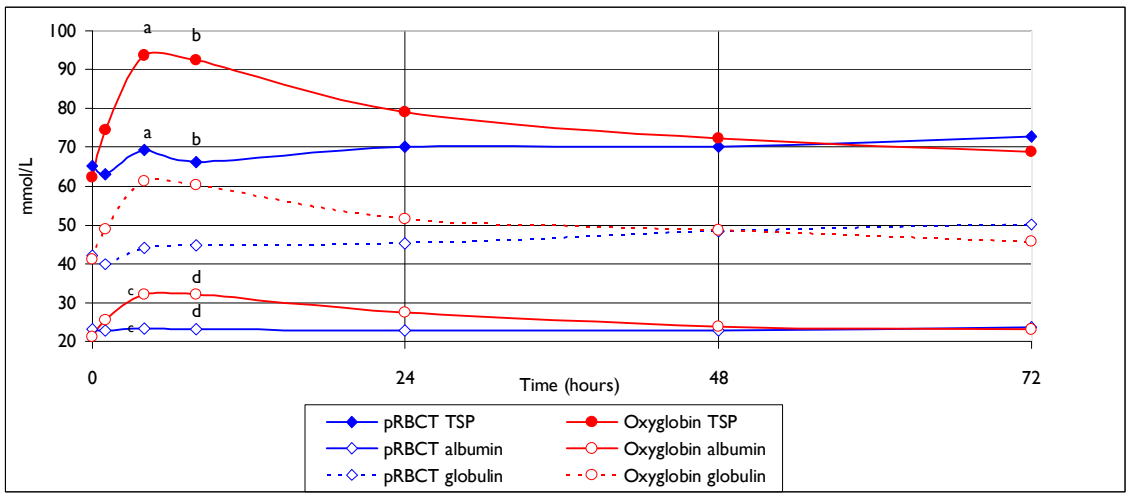



**Figure 24.** Overall protein trends. Singificant differences noted in Table 16 annotated with analogous letters (a – d).

### 9.2.4.3 HAEMODYNAMIC PARAMETERS

**Table 17.** 72-hour trends: haemodynamic. Similar coloured boxes indicate that a significant difference existed between these data points

| Reference values | O* | B* | O | B | O | B | O | B | O | B | O | B | O | B |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time t = | 0 | | 1 | | 4 | | 8 | | 24 | | 48 | | 72 | |
| SBP | 127.6 ± 13.6 | 136.9 ± 17.6 | 139.0 ± 14.3 | 132.2 ± 21.5 | 145.9 ± 16.5 | 136.5 ± 21.2 | 148.7 ± 14.8 | 128.8 ± 18.0 | 133.7 ± 15.7 | 125.3 ± 20.2 | **135.8 ± 12.1** | **116.7 ± 11.5** | 133.1 ± 14.0 | 119.5 ± 22.3 |
| MAP | 91.8 ± 13.1 | 88.3 ± 21.0 | 102.8 ± 20.7 | 91.8 ± 17.6 | 111.6 ± 17.0 | 97.3 ± 21.5 | 108.3 ± 20.3 | 94.9 ± 17.6 | 100.6 ± 17.8 | 91.8 ± 21.2 | **104.1 ± 8.3** | **87.3 ± 11.6** | 94.7 ± 13.8 | 85.9 ± 22.5 |
| DBP | 65.9 ± 13.0 | 63.0 ± 19.4 | 78.6 ± 8.7 | 70.8 ± 15.3 | 87.0 ± 22.6 | 76.5 ± 23.1 | 83.6 ± 17.1 | 72.6 ± 18.6 | 74.4 ± 74.4 | 69.4 ± 23.0 | 80.3 ± 13.1 | 69.9 ± 13.9 | 77.1 ± 15.1 | 71.2 ± 21.2 |
| Measured pulse | 145.5 ± 21.6 | 131.4 ± 21.5 | 128.8 ± 25.9 | 113.3 ± 15.7 | **129.8 ± 24.9** | **98.6 ± 11.5** | **133.0 ± 23.0** | **94.8 ± 19.0** | 115.1 ± 8.9 | 94.1 ± 25.4 | 114.0 ± 9.5 | 93.8 ± 23.8 | **115.5 ± 17.8** | **89.5 ± 7.3** |
| Femoral pulse | 147.5 ± 27.0 | 124.2 ± 19.7 | **135.8 ± 20.6** | **106.4 ± 8.1** | 124.5 ± 25.4 | 101.5 ± 16.2 | **134.8 ± 23.3** | **100.2 ± 14.5** | 118.4 ± 4.9 | 92.9 ± 30.6 | 113.4 ± 9.5 | 94.8 ± 19.6 | 115.0 ± 16.6 | 94.6 ± 17.3 |
| ***O = Oxyglobin®** | | | | | | | | ***B = pRBCT** | | | | | | |
| Significant differences: data in matching colour and text bold | | | | | | | | | | | | | | |
| *P* = 0.02 | | | *P* = 0.019 | | *P* = 0.01 | | *P* = 0.009 | | *P* = 0.009 | | *P* = 0.01 | | | |

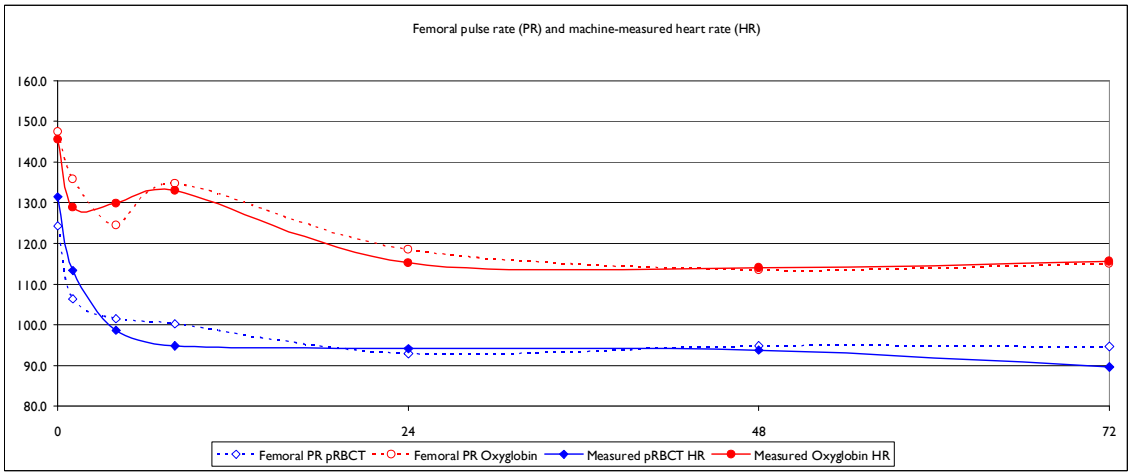



**Figure 25.** Counted femoral pulse rate (PR) and machine-measured pulse rate (HR) trends.

### *9.2.4.3.1 Assessment*

#### 9.2.4.3.1.1 Diastolic Blood Pressure (DBP)

Patients entered the trial in a state of diastolic hypotension and returned to a normotensive or near-normotensive state by t=4, maintaining said status until trial completion (see Table 4).

#### 9.2.4.3.1.2 Mean Arterial Pressure (MAP)

The ANCOVA test had 80% power to detect a difference of only 24.0 mmHg, due to the wide variations in readings. This difference is considered too wide to be clinically useful, as a difference of no more than 15 – 20 mmHg was sought prior to sampling. Student's t tests with Welch correction demonstrated that patients in the Oxyglobin® group had MAP significantly higher than pRBCT patients at t=48 (t=3.132, df = 7.454, *P*-value = 0.015). A Student's t-test with Welch correction performed on the differences between time slots showed that all *P*-values were greater than 0.05 (i.e., not statistically significant) thus no changes in MAP from time segment to time segment were significant. Lastly, an ANCOVA taking time t=1 into account showed that although the difference was marginally significant at t=48, this test was more accurately performed with a t-test.

#### 9.2.4.3.1.3 Systolic Blood Pressure (SBP)

Using the criteria in Table 4, (50) patients were systolically normotensive or borderline hypotensive throughout the trial. No differences existed between groups or from t=0 versus t=72 for either group.

#### 9.2.4.3.1.4 Heart rate measured by machine (measured HR)

Final (t=72) machine measured pulse rate differed significantly from baseline machine-measured pulse rate (HR) for both group O (t=72 HR 115.5 ± 17.8 vs t=0 HR 145.5 ± 21.6, *P* = 0.035) and group B (t=72 HR 89.5 ± 23.8 vs t=0 HR 131.4 ± 21.5, *P* = 0.001). Groups differed significantly from one another at t=1, t=4 and t=72 with patients in the Oxyglobin® group consistently having higher pulse rates (Table 17 & Figure 25).

#### 9.2.4.3.1.5 Peripheral pulse measured

Although there was close correlation between measured pulse rate and palpated (counted) femoral pulse (see 8.8.2), statistical differences still existed. Femoral PR differed between groups at points (see Table 17).

### 9.2.4.4 HABITUS & APPETITE PARAMETERS

**Table 18.** Habitus & Appetite (subjective criteria) trends. Median values.

| **Reference values** | **O*** | **B*** | **O** | **B** | **O** | **B** | **O** | **B** | **O** | **B** | **O** | **B** | **O** | **B** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Time t =** | **0** | **0** | **1** | **1** | **4** | **4** | **8** | **8** | **24** | **24** | **48** | **48** | **72** | **72** |
| Habitus | 1.5 | 2 | 1.5 | 2 | 2 | 3 | **2** | **3** | **2** | **3** | **3** | **4** | 3 | 4 |
| Appetite | 0 | 0 | 0 | 0 | 0.5 | 3.5 | 0.5 | 0 | **0** | **3.5** | 3 | 3.5 | 3 | 3.5 |

***O = Oxyglobin®** | ***B = pRBCT**

Significant differences: data in bold & coloured to indicate which other data point is being compared.

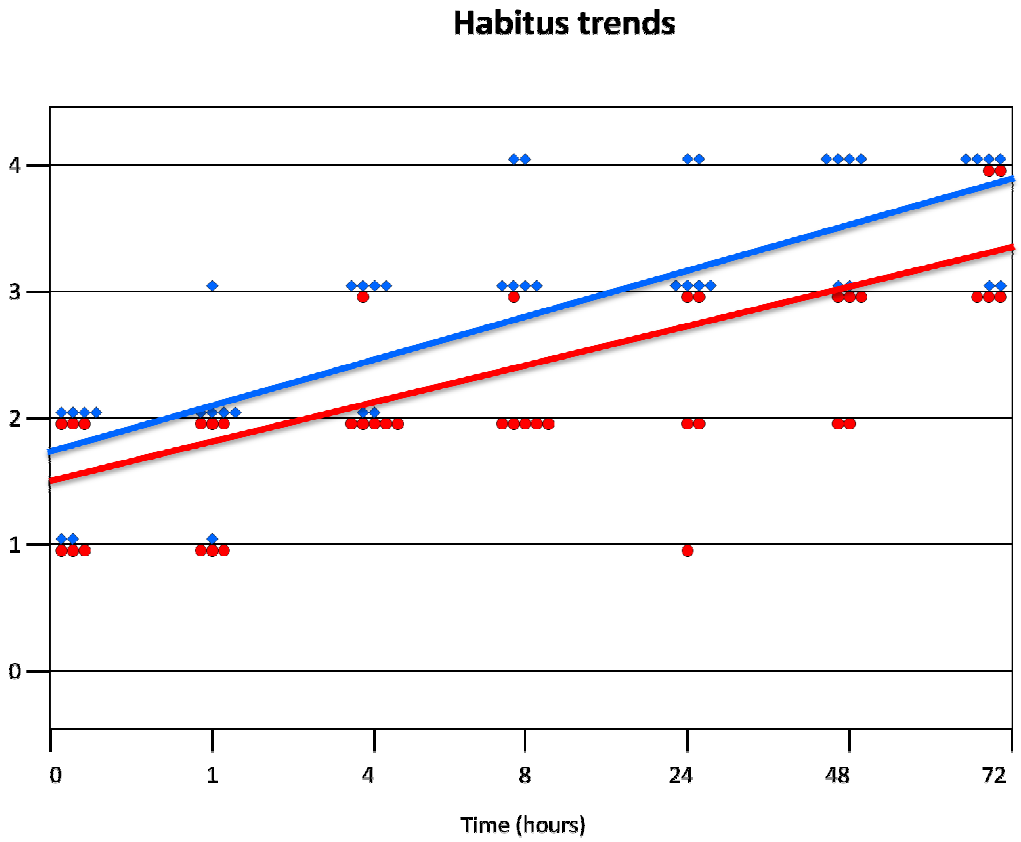



**Figure 26.** Habitus trends as scatterplot with trend lines for Oxyglobin (red) and pRBCT (blue)

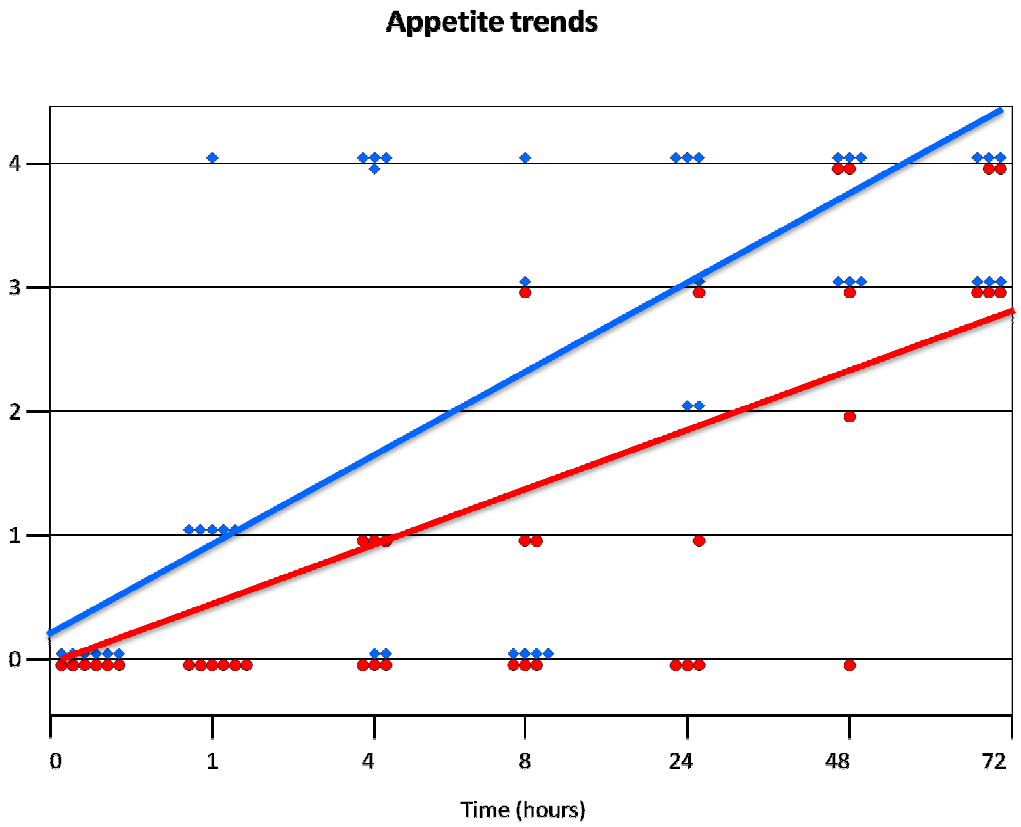



**Figure 27.** Appetite trends as scatterplot with trend lines for Oxyglobin (red) and pRBCT (blue)

#### *9.2.4.4.1 Assessment*

##### 9.2.4.4.1.1 Habitus

Patients showed a uniform and steady improvement in habitus over the study period in both groups (Table 28, Figure 28). Habitus improved somewhat better for patients transfused with pRBCT than for patients treated with Oxyglobin®. Wilcoxon rank sum tests performed for the difference at each time (non-parametric data) showed that the habitus for pRBCT patients was significantly higher at t=8 (W=2, *P*-value = 0.007), t=24 (W=4, *P*-value = 0.036) and t=48 (W=3, *P*-value = 0.04). A Wilcoxon rank sum test on the differences between time slots demonstrated that only one time difference (between t=4 and t=8) was significantly higher in pRBCT (W = 6, *P*-value = 0.02). This explains why for the next three time slots, habitus for patients treated with pRBCT was higher than Oxyglobin®-treated patients' habitus.

##### 9.2.4.4.1.2 Appetite

The pattern of improvement for appetite was similar to that of habitus, although differences between treatments were less pronounced. Overall, patients in the B group (pRBCT transfusion) performed somewhat better than those in the O group (Figures 27 & 28).

**Figure 28.** Patients at t=48; in group O (left) and group B (right). Differences in appetite can easily be appreciated.

Wilcoxon rank sum tests for the difference at each time showed that pRBCT values were significantly higher at t=24 (W=2.5, *P*-value = 0.025) than for Oxyglobin® values.

A Wilcoxon rank sum test on the differences between time slots was not significant (All *P*-values >0.05).

## *9.3 Side Effects of Treatment*

### 9.3.1 Deaths

One dog (patient 11) in group O went into acute respiratory distress and died within minutes between t=8 and t=24. A full post mortem found pathology (gross and microscopic) consistent with babesiosis, but no specific signs of morbidity related to treatment were discovered. Death was ascribed to babesiosis.

### 9.3.2 Iridis Rubeosis

Two patients in group O developed red (not injected) irises and anterior chamber pigmentation. Consultation with a veterinary ophthalmology resident was sought in both instances, and iridis rubeosis was diagnosed. This is apparently incidental and in one patient resolved within the trial period; the other patient was number 11, and died before trial completion (see 9.3.1). No further testing or evaluation (other than indirect ophthalmoscopy) was performed.

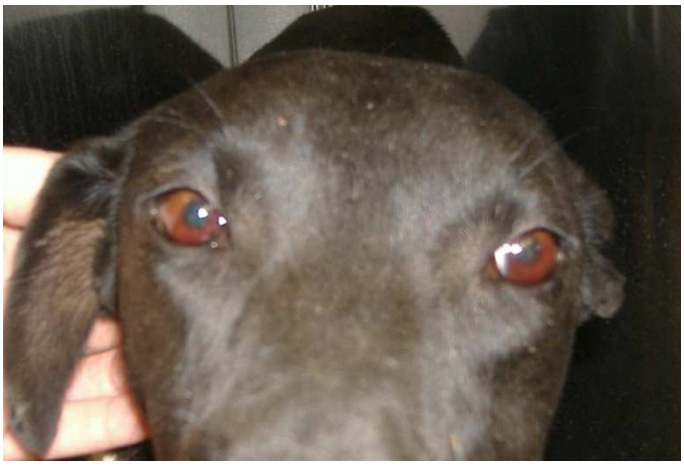


**Figure 29.** Iridis rubeosis - deposition of reddish pigment in the iris of Oxyglobin®-treated dog. More noticeable in the right eye (left side of picture) as a distinct, inferonasal reddish band involving 50% of the iris, while the normal iris has a more hazel colouration.

**Figure 30.** Iridis rubeosis. The left eye of this Malamute (patient 11) has distinct reddish tinge, especially noticeable in a patient with an amelanotic iris. The right eye seems unaffected.

### 9.3.3 Dermal pigmentation

Three patients in group O developed subcutaneous inguinal maculae of a reddish hue. Lesions disappeared by trial completion. They were non-painful and no consequences were ascribed to their presence (Figure 31).

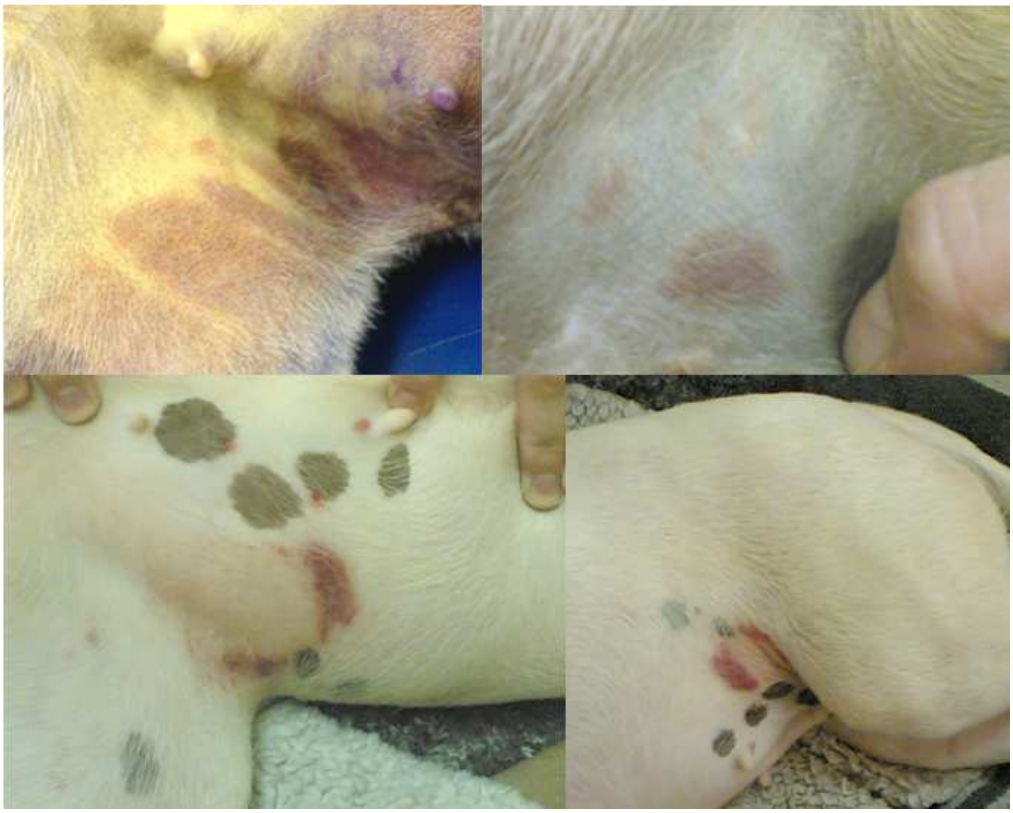


**Figure 31.** Subcutaneous pigmentation in 3 dogs receiving Oxyglobin®. Most remarkable was the consistent inguinal location of this pigmentation, and its rapid and complete resolution by t=72 hours. Patient in top left panel developed marked icterus within 8 hours of admission, a common occurrence in canine babesiosis cases.

## 10 GENERAL DISCUSSION OF RESULTS

At the outset, both patient groups were highly comparable with regard to demographics and also the grade and nature of their medical illness. Patient inclusion criteria assisted in the selection of a strictly anaemia-only group without other combinations of complications. This aided comparison and the power of a small study.

The search for blood substitutes is a holy grail of human and veterinary medicine. Blood requirements far outstrip supply, and difficulties of infection transmission, storage, transfusion reactions and logistics (such as cost) drive the search for alternatives to blood. Anemia cause by hemoprotozoan infections such as babesiosis in dogs is an example of a disease that causes significant mortality and morbidity where a blood substitute would be useful in treatment. Although the manufacturer of Oxyglobin® advertises its licence for various anaemic and maldistributive diseases including canine babesiosis, its beneficial effects in respect of this condition, and its efficacy relative to that of the established standard, blood, has never been critically evaluated in a prospective, randomized trial in naturally infected dogs. It has also never been specifically evaluated for the treatment of the *B. rossi* form of babesiosis.

The babesiosis caused by the dominant Southern African species, *Babesia rossi*, (9) is particularly virulent (10; 6) and thus serves as an excellent model for study of all the dog babesias, and possibly even human falciparum malaria. (19; 12; 13; 10) This variant of canine babesiosis is typified by high mortality due to severe anemia, peracute shock-like syndromes and a host of complicated forms (cardiac and renal pathology, icterus, hypoglycemia, cerebral babesiosis amongst others). (15; 14; 6; 16; 12; 13; 11; 17; 18) The primary pathophysiological and most treatable process is a severe anemia. Better understanding of the treatment responses of the more complex forms of babesiosis and falciparum malaria will hopefully flow from the observations made in this study.

All dogs in this study presented with moderate anemia; a mixed acid-base abnormality (respiratory alkalosis; hypoalbuminemic alkalosis; hyperchloremic acidosis; dilutional acidosis); pyrexia; anorexia; tachycardia; hemodynamic changes; and depression. No patients were markedly hypoxaemic at any time in the study barring the one patient which died, and which developed fulminant hypoxaemia and died. It is worthwhile noting that, barring the small sample numbers, $aO_2ct$ did not increase significantly for Oxyglobin®-treated patients, while it did improve in the pRBCT-treated group. This echoes findings in three other studies. (139; 136; 132)

Although Oxyglobin® is a solution of (polymerized) haemoglobin, it is interesting to note that total haemoglobin (tHb) did not increase significantly in group O patients. This is in agreement with the findings of some authors (136; 132; 142) but seems counterintuitive. By contrast, tHb in group B had already improved significantly by t=1. This may imply that the haemoregenerative process is suppressed by the normal oxygen tension created by Oxyglobin®, and only rebounds once the Oxyglobin® is excreted. Alternately, the colloid effect of the HBOC molecule can influence total haemoglobin measurements and thus it may have been an artefactual decrease. (163)

Siggaard-Andersen's method of assessing acid base balance (60) would have described these dogs with a metabolic acidosis with compensatory respiratory alkalosis "with many hidden water and electrolyte disorders", and not ascribed the alterations as being *due to* the changes in said agents. In the face of a normal pH, this is evidently a gross oversimplification. A Stewart-based (strong ion difference) approach, however, draws attention to the law of maintenance of electroneutrality and the *influences* of strong ions and free water on the acid-base status. (67)

The Stewart-based approach expands the Henderson-Hasselbachian approach of a "metabolic acidosis balanced by compensatory respiratory alkalosis" (66; 174; 118) by including the influences of electrolytes, albumin and free water. (67; 24) All dogs in this study had mixed acid-base disorders, although none as severe as those in the study by Leisewitz and coworkers, probably because that study evaluated a broader range of dogs. (24) In the current trial, all patients had a normal pH on presentation,

but the respiratory compensatory drive was apparently mildly excessive relative to the degree of base deficit, although not to the extent described in the more severe dogs in Leisewitz et al's 2001 study. Since overcompensation is not physiologically rational, this respiratory alkalosis indicates a mixed disturbance due to a respiratory alkalosis, but may also represent influences of the hypoalbuminemia on central respiratory receptors, hypotension, pulmonary afferent vagal influences, and peripheral oxygen sensor drives. (174; 24; 118) As long ago as 1976, Malherbe and others stated that *"[t]he 'actual' pH of the blood [had] been found to be of less importance than the extent of base deficit"*. (104)

The analyzer employed (Blood Gas 865, Bayer Healthcare, Leverkeusen, Germany) provided a *calculated* anion gap (AG). In order for the Stewart approach to be holistically used, the Strong Ion Difference (SID) or Strong Ion Gap (SIG) must be known. Although AG and SID/SIG are not synonymous, one study demonstrated an excellent correlation ($r$ = 0.91 to 0.99) between the "corrected" AG and SIG. (68) Calculation of the corrected AG (and thus its substitution for SIG in interpreting the data using the Stewart approach) require measurement of urate, lactate and phosphate, which are not part of a standard veterinary acid-base study. This detracts only slightly from the conclusions drawn in this study regarding acid-base status. In addition, albumin contributes significantly to SID/SIG so the elevations seen in the Oxyglobin-treated patients were probably of little significance to Stewart-based acid-base interpretation. (118; 173)

Chloride is an important anion along with bicarbonate ($HCO_3^-$) in acid-base metabolism. The chloride shift (exchange of $Cl^-$ for $HCO_3^-$ as the latter diffuses out of the erythrocyte to maintain electroneutrality) and superimposition of suspected renal tubular acidosis in babesiosis (Leisewitz, *pers comm.*, 2003) and the chloride avidity of Oxyglobin® make for an interesting and complex interaction. One can speculate that this is due to the chloride avidity of the Oxyglobin® molecule, such that it acts as a "chloride trap" or "sink", altering measurements and maintaining a wider chloride gap differential than pRBCT. This remains a tantalising speculation, and its importance is uncertain. pRBCT normalised the respiratory compensatory drive by completion of

the infusion better than did Oxyglobin®, as demonstrated by the $\Delta[pCO_2\text{-}HCO_3^-]$ (9.2.4.2.2). It also demonstrated a better overall normalisation of this drive, although by trial termination values were not significantly different between groups. This may be due to superior $HCO_3^-$ provision by the transfused erythrocytes from their intracellular stores, or by differences in proton buffering between Oxyglobin® and pRBCT. Notwithstanding this, there were no differences in bicarbonate at any time, but a significant disparity in pH at t=4. This may be interpreted in light of the moderate grade of acidaemia/acidosis of these patients, but discrepancies in buffering at peak transfusion concentrations at t=4, between the two treatments.

The dilutional acidosis results in *corrected* chloride being elevated. By using the *principles* of the law of mass action in isolation of Stewart's principles, chloride increases might create a greater inward chloride shift into the erythrocytes, thereby increasing plasma bicarbonate. More bicarbonate (and therefore $CO_2$) could be exhaled by the lungs, and thus more buffering and resolution of intracellular acidosis might occur. *However*, Oxyglobin®, being cell-free, would not be expected to experience or partake in the classical "chloride shift"; alternatively, its inherent chloride avidity might *trap* chloride as mentioned earlier, thereby creating a similar effect.

In this trial, since there were differences in corrected chloride from t=24 onwards, one might conclude there to be a significant dissimilarity in the manner of chloride handling between the two groups. This could be due to competing or interfering $Cl^-$ /$Hb^-$ handling by the kidneys, already hypoxic (17) and potentially underperfused; a respiratory acidosis (175) although this did not occur in these 12 patients; or an artefactual hypernatraemia, which was also not the case here. (175)

Seeing as previous workers have described the ubiquity of hyperlactaemia in babesiosis and malaria, (104; 24; 63; 118; 89) the outcome of this hyperchloraemic acidosis is probably really an indication of a superimposition of two processes. These

would be, namely a normochloraemic (hyperlactataemic[7]) and hyperchloraemic acidoses with the *net* results seen in these 12 patients. This is probably more likely than a "black and white" distinction between one or the other process occurring, but without the lactate measurements hoped for, it remains a speculation for this particular study. Even so, sicker patients might have a greater elevation in lactate or more profoundly altered renal acid handling, with different anion gap changes and a different clinicopathological picture. This was not seen in this trial (urine AG and fractional excretion ratios were not studied), and leaves us with teasing questions about the role of chloride in the functioning of Oxyglobin®. To further complicate matters, Buehler et al (137) reported that polymerised haemoglobin lost its oxyphoric sensitivity to the chloride ion.

Of the plasma proteins, only albumin is considered an organic anionic acid. (174; 173) Infusion of Oxyglobin® led to significant increases in albumin during the early trial period, and artefactual albumin elevations may be due to high plasma Oxyglobin® concentrations influencing measurements. This is possible since it was only at the time of peak serum Oxyglobin® concentration (t=4) that albumin differed between groups. Since albumin is a negative acute phase protein, (173) it would be reasonable to expect a hypoalbuminemia at the outset for all dogs. It is worth noting that the only other study of canine acute phase proteins (ceruloplasmin, CRP, haptoglobin) in babesiosis found a significant increase in the former two. (176) In addition, although Keri et al (163) also reported an upward albumin deviation in Oxyglobin®-infused patients, Callas and others found this only occurred at HBOC-201 serum levels below 0.7 g/dL. (177) Although the dogs evaluated by Leisewitz and coworkers generally fell into a more serious clinical subset of babesiosis, there was some overlap with dogs here. Both studies noted a high prevalence of hypoalbuminemia at admission (70% in Leisewitz et al, 100% of 11 dogs in this study). (24) Similarly, Lobetti et al reported a similar distribution of initial albumin for severe uncomplicated babesiosis patients (24.15 ± 3.95 mmol/L vs this study t=0 albumin of 22.1 ± 3.3 mmol/L) in their study.

[7] See footnote p72, which refers to paragraph 9.2.4.2.3.3 (Chloride shifts)

(173) Using the Stewart terminology, all dogs admitted in all three studies had a hypoalbuminemic alkalosis.

Although globulins do not contribute to acid-base balance according to the Stewart approach, (169; 66) 63% of dogs (7/11) in this study also presented with hyperglobulinemia, compared with 45% in the study by Leisewitz (24) but only 2/10 of the severe complicated and 4/40 of all patients (including healthy ones) in the study by Lobetti et al. (173; 24; 67) The differences were small and probably unimportant, but reasons remain obscure. The subjective (but statistically insignificant) hyperglobulinaemia in group O from t=4 onwards is interesting, but likely due to spectrophotometric artifacts caused by the presence of the agent in serum. (120; 163)

The perturbations in albumin are also the most likely cause of the lack of a high anion gap despite the likelihood of hyperlactatemia. (24) Nel and others have demonstrated that the lactic acidosis of babesiosis resulted in a high anion gap acidosis, due to poor perfusion and oxygenation of tissues. (63) The anaerobic conditions created by the anemia result in elevated lactate levels. (63) Although this trial did not measure lactate concentrations *per se*, it is reasonable to expect little deviation from previous results for hyperlactatemia in this condition. The hyperlactatemia in combination with albumin deviations might account, in part, for any anion gap abnormalities expected, although none were noted. (24) Although endpoint lactates could not be determined, resolution of protein, acid-base and subjective criteria, as well as the results of Nel and others (63) would seem to indicate normalization of lactate due to improved tissue oxygenation and perfusion, in both groups. (149; 104; 118)

All patients in the trial were pyrexic, a prevalent state in both babesiosis and falciparum malaria. It should be noted that the antibabesial product used (Dizene) contains a nonsteroidal agent (dipyrone) which might contribute to resolution of the pyrexia.

In many respects the two treatment groups were equivalent or at least very similar at the outset and completion of the trial. Differences *were* noted throughout the trial

for different parameters and at different times, but (barring the subjective parameters, dermal and ocular changes in particular) were mostly of little discernable clinical impact. The resolution of acidosis is a direct consequence of improved circulating volume; improved tissue oxygen delivery; improved hemodynamics; and removal of the inciting agent (the *Babesia* parasite). Both treatments achieve this in a similar manner and period, although there may have been different pharmacodynamics.

The contrasts between Oxyglobin®- and pRBCT-treated dogs were most marked only when the subjective criteria were compared. Dogs in the B group had a near-normal appetite within 24 hours of admission, whereas those treated with Oxyglobin® needed 72 hours to reach the same level of improvement. A lesser effect was seen with regard to habitus. The possible reasons for this remain speculative. The difference argues strongly for positive effects of blood transfusion (other than simple volume and oxygen-carrying replacement). The eventual recovery and equivalence of Oxyglobin®-treated dogs may be a result of the appropriate, endogenous, vigourous regenerative responses which would be present in all dogs with babesial anemia. It may however also be postulated that the late improvement in the Oxyglobin®-treated dogs was due to the completion of its excretion, and that some effect or component of the Oxyglobin® actually *suppressed* appetite and habitus despite clinicopathological and blood pressure improvements.

A sinus tachycardia is an appropriate, homeostatic, physiological response to severe anemia. Babesiosis is well-described cause of the systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS). (5) Inflammatory cytokines, (83) inducible nitric oxide (51) and hypoxemia may all result in a maldistributive state, if it were not for the appropriate physiological responses. The classic “waterhammer pulse” of anemia was clearly demonstrated by low diastolic and high systolic pressures (a “bounding” heart) while all dogs maintained a normal MAP. The lack of a clear difference between the two agents’ effects on MAP barring one divergence at t=48 is noteworthy. This is despite the potent nitric oxide (NO)-scavenging and inactivating properties of the Oxyglobin® polymers, and their possible activity as endothelin-1 antagonists. (146; 145; 144) NO is a potent vasodilator

produced both constitutively and inductively by the vascular endothelium (144), and one would expect a NO scavenger to elevate blood pressure more than a less effective scavenger or placebo. By contrast, the findings of this study would imply that either these properties are less potent in this setting than others have proposed, or that isovolumic blood and Oxyglobin® transfusions possess equivalence in this regard. Although the results showed that Oxyglobin® did produce elevations in blood pressure (particularly in the first eight hours), significance was not achieved during this period due to wide variations in response between dogs causing overlapping data distributions. It is the authors' inference that although both treatments help to maintain and normalize blood pressure, this benefit is similar and cannot be dissected out from the beneficial effects of the parasiticidal treatment, volume of transfusion, or some other effect. In fact, Jacobson and others showed (51) that nitric oxide may not be as important in canine babesiosis as once speculated.

This particular study cohort was defined in order to limit the costs of the study (large dogs) and any danger to dogs arising from multiple sampling times (small dogs). Exclusion of all complicated forms of babesiosis allowed the cohort to be as homogenous as possible with respect to presenting abnormalities and demographics; it also allowed the study to retain a high degree of power despite a small sample size. It was not the author's intention to study every aspect of the clinical efficacy of these remedies in all permutations of babesiosis, but rather to focus primarily on the treatment of the anemia caused by babesiosis as a disease, and on transfusion as the most important supportive treatment measure. The evaluation of changes in blood gas and acid-base status is the standard means of evaluating the efficacy of transfusion therapy for anemia. (22; 24) In this respect, it seems reasonable to utilize Oxyglobin® for the treatment of canine babesiosis, and to expect comparable blood gas, acid-base and blood pressure improvement results, with only transient differences in subjective criteria of assessment.

# 11 CONCLUSIONS

The use of HBOCS such as Oxyglobin® represents a viable and safe alternative to packed red blood cell transfusion in haemoprotozoal anaemias such as those caused by *established Babesia rossi* and by extension, probably also *Plasmodium falciparum*. This prospective, randomized clinical trial demonstrated and described a variety of effects of the HBOCS on an animal disease model or a common human disease. Oxyglobin® established the typical clinical and clinicopathological response patterns in patients when compared with a positive control. Mortality was not a studied end-point in this trial, and further clinical experiments with larger numbers, sicker patients and negative controls receiving only lactated Ringer's solution would be a desirable next step in determining survival differences. Nonetheless, this trial demonstrated sufficient equivalence of efficacy of pRBCT and Oxyglobin®, and by extension, this should also be true for Hemopure® in falciparum malarial anaemia (with some subjective and objective minor differences between pRBCT and Oxyglobin®).

# 12 APPENDICES

## *12.1 Data arrays*

### 12.1.1 General remarks

#### 12.1.1.1 PATIENT IDENTIFICATION

Patients are identified in tables by their patient number (1 – 12) and/or group (O or B or pRBCT vs Oxy). **pRBCT** data are in **blue** and **Oxyglobin®** in **red**.

#### 12.1.1.2 STATISTICAL EVALUATION

For specific techniques, see the section dealing with statistical techniques. In tables, means, minimum/maximum figures for ranges and standard deviation are listed in tables, where appropriate. Tables are divided into pRBCT data above Oxyglobin® data, and with t-test values (to 3 significant digits) in the bottom row of the column for which they are applicable. P-values comparing t=0 and t=72 values for a particular parameter are given to the right of the last value for each of the two treatment groups. Statistically significant differences are in highlighted boxes. In some instances, P-values >0.05 which approach significance are highlighted in a lighter shade. T-tests are two-tailed in all instances. All data is presented in the same order as it is discussed in the results section. Only summary data and table headers are in bold.

### 12.1.2 Patient Data

#### 12.1.2.1 PATIENT IDENTIFICATION

| Patient group/ number | Date | t=0 | Patient Name | Owner Name | Patient Number | Owner Number |
|---|---|---|---|---|---|---|
| pRBCT 1 | 10/2/2005 | 15h30 | Lady | Billingham | 179393 | 102142 |
| pRBCT 2 | 25/3/2005 | 20h30 | Wendy | Mogotsi | 178174 | 122548 |
| Oxy 3 | 26/3/2005 | 13h00 | Zoe | Kapp | 180551 | 115362 |
| Oxy 4 | 12/4/2005 | 10h30 | Snoopy | Ndhlovu | 116207 | 130938 |
| pRBCT 5 | 13/4/2005 | 19h30 | Jesse | Fairbanks | 180016 | 134254 |
| pRBCT 6 | 4/10/2005 | 21h30 | Tenboys | Mabena | 185629 | 136285 |
| Oxy 7 | 4/10/2005 | 22h00 | Puncho | Mabena | 185628 | 136285 |
| Oxy 8 | 23/10/2005 | 20h30 | Blacky | Shabangu | 186151 | 126827 |
| Oxy 9 | 12/12/2005 | 10h45 | Pelet | Richardson | 187474 | 100457 |
| pRBCT 10 | 14/12/2005 | 16h30 | Baile | Masilo | 187562 | 136984 |
| Oxy 11 | 29/1/2006 | 10h00 | Shingles | Kruger | 188850 | 125164 |
| pRBCT 12 | 16/3/2006 | 12h30 | Roxy | Letasholo | 190275 | 136554 |

#### 12.1.2.2 PATIENT DEMOGRAPHICS

| | Mean values | |
|---|---|---|
| **Group** | **pRBCT** | **Oxyglobin®** |
| Age (months) | 9 | 18 |
| Wt (kg) | 20.45 | 15.31 |
| Transfusion volume (ml/kg) | 20.43 | 18.48 |
| Diminazene administered (ml) | 1.4 | 1.05 |
| Males (entire) | 3 | 2 |
| Females (entire) | 3 | 4 |
| No significant differences between groups (*P*>0.05) | | |

All values derived from the following table:

| Patient | Pat No | Weight (kg) | Sex | Age(m) | pRBCT/Oxy ml | ml/kg | Diminazene mL administered |
|---|---|---|---|---|---|---|---|
| pRBCT 1 | 179393 | 17.2 | FE | 12 | 350 | 20.3 | 1.2 |
| pRBCT 2 | 178174 | 21.9 | FE | 6 | 420 | 19.2 | 1.47 |
| pRBCT 5 | 180016 | 22 | FE | 8 | 390 | 17.7 | 1.54 |
| pRBCT 6 | 185629 | 22 | ME | 48 | 460 | 20.9 | 1.5 |
| pRBCT 10 | 187562 | 12 | ME | 10 | 250 | 20.8 | 0.84 |
| pRBCT 12 | 190275 | 19 | ME | 8 | 390 | 20.5 | 1.33 |
| **Mean** | | **19.0** | | **15.3** | **376.7** | **19.9** | **1.3** |
| **Min** | | **12.0** | | **6.0** | **250.0** | **17.7** | **0.8** |
| **Max** | | **22.0** | | **48.0** | **460.0** | **20.9** | **1.5** |
| **StDev** | | **4.0** | | **16.1** | **72.0** | **1.2** | **0.3** |
| Oxy 3 | 180551 | 14.52 | FE | 24 | 250 | 17.2 | 1 |
| Oxy 4 | 116207 | 8.6 | FE | 7 | 125 | 14.5 | 0.56 |
| Oxy 7 | 185628 | 22.54 | ME | 36 | 375 | 16.6 | 1.6 |
| Oxy 8 | 186151 | 11.5 | ME | 12 | 250 | 21.7 | 0.77 |
| Oxy 9 | 187474 | 19 | FE | 24 | 375 | 19.7 | 1.33 |
| Oxy 11 | 188850 | 16.1 | FE | 12 | 375 | 23.2 | 1.1 |
| **Mean** | | **15.4** | | **19.2** | **291.7** | **18.9** | **1.1** |
| **Min** | | **8.6** | | **7.0** | **125.0** | **14.5** | **0.6** |
| **Max** | | **22.5** | | **36.0** | **375.0** | **23.3** | **1.6** |
| **StDev** | | **5.0** | | **10.8** | **102.1** | **3.3** | **0.4** |
| **F-test** | | **0.614** | | **0.397** | **0.462** | **0.0503** | **0.458** |
| **Type 2 t-test** | | **0.194** | | **0.639** | **0.127** | **0.480** | **0.206** |
| **Type 3 t-test** | | | | | | **0.489** | |

### 12.1.3 Haematology, Protein & Temperature Data

TSP on this table measured by refractometer.

| Patient | Time | Temp | Ht | TSP | tHb g/dL |
|---|---|---|---|---|---|
| 1 | 0 | 39.7 | 12 | | 2.3 |
| 1 | 1 | 38.7 | | | 3.9 |
| 1 | 4 | 40.1 | 20 | 48 | 5.9 |
| 1 | 8 | 39.3 | | | 7.3 |
| 1 | 24 | 38.4 | | | 6.3 |
| 1 | 48 | 37.9 | | | 5.5 |
| 1 | 72 | 37.3 | | | 6.6 |
| 2 | 0 | 40.3 | 11 | 68 | 2.8 |
| 2 | 1 | 40.3 | 8.8 | | 3.1 |
| 2 | 4 | 40.3 | | | 7.1 |
| 2 | 8 | 39.3 | 32 | | 10.6 |
| 2 | 24 | 38 | | | 11.6 |
| 2 | 48 | 39.3 | 16 | | 5.6 |
| 2 | 72 | 39.2 | 28 | | 10 |
| 3 | 0 | 39.2 | 12 | 70 | 3.1 |
| 3 | 1 | 39.3 | 8.2 | | 3.1 |
| 3 | 4 | 39.6 | 16 | | 7.1 |
| 3 | 8 | 40.6 | 7 | | 3.9 |
| 3 | 24 | 39.6 | 10 | | 4.6 |
| 3 | 48 | 38.5 | 18 | | 6.1 |
| 3 | 72 | 37.5 | | | 11.6 |
| 4 | 0 | 39.7 | 14 | 60 | 3.5 |
| 4 | 1 | 39.5 | | | 2.7 |
| 4 | 4 | 40.5 | 13 | | 4.4 |
| 4 | 8 | 39.1 | 10 | | 3.5 |
| 4 | 24 | 38.7 | 11 | | 3.7 |
| 4 | 48 | 38.1 | 15 | | 5.1 |
| 4 | 72 | 38.4 | | | 6.1 |
| 5 | 0 | 40.1 | 17 | 65 | 4.2 |
| 5 | 1 | 39.7 | 13 | | 4.3 |
| 5 | 4 | 40.3 | | | 5.3 |
| 5 | 8 | 38.5 | 24 | | 8.2 |
| 5 | 24 | 38.4 | 23 | | 7.9 |
| 5 | 48 | 38 | | | 9 |
| 5 | 72 | 38.7 | | | 9 |
| 6 | 0 | 38.9 | 14 | 95 | |
| 6 | 1 | 38.6 | | | |
| 6 | 4 | 39.9 | 23 | | |
| 6 | 8 | 38.6 | 19 | 95 | |
| 6 | 24 | 37.8 | | | |
| 6 | 48 | 38.1 | | | |
| 6 | 72 | 37.4 | 24 | | |
| 7 | 0 | 39.5 | 15 | 65 | |
| 7 | 1 | 39.6 | | | |
| 7 | 4 | 40.3 | 25 | | |
| 7 | 8 | 40.7 | 19 | 44 | |
| 7 | 24 | 40 | | | |
| 7 | 48 | 38.3 | 14 | 66 | |
| 7 | 72 | 37.9 | 22 | | |
| 8 | 0 | 39.1 | 13 | 70 | 3.9 |
| 8 | 1 | 37.4 | | | 2.81 |
| 8 | 4 | 37.8 | | | 3.5 |
| 8 | 8 | 37.2 | | | 4 |
| 8 | 24 | 37.8 | | | 5.3 |
| 8 | 48 | 38.3 | | | 10.1 |
| 8 | 72 | 37.8 | | | 7.71 |
| 9 | 0 | 40.6 | 10 | 64 | 3.3 |
| 9 | 1 | 38.8 | 11 | | 3.7 |
| 9 | 4 | 39.7 | | | 4.3 |
| 9 | 8 | 39.4 | | | 4.2 |
| 9 | 24 | 38.9 | | | 2.6 |
| 9 | 48 | 39.5 | | | 3.2 |
| 9 | 72 | 38.5 | 19 | | 6.5 |
| 10 | 0 | 39.4 | 13 | 54 | 4.3 |
| 10 | 1 | 39.9 | | | 3.2 |
| 10 | 4 | 40 | 18 | | 6.1 |
| 10 | 8 | 38.7 | 16 | | 5.4 |
| 10 | 24 | 39.1 | 16 | | 5.5 |
| 10 | 48 | 38.2 | 19 | | 6.6 |
| 10 | 72 | 38.5 | 27 | | 9.3 |
| 11 | 0 | 41.4 | 12 | 60 | 4.1 |
| 11 | 1 | 40.9 | | | |
| 11 | 4 | 41.4 | | | |
| 11 | 8 | 41.2 | | | |
| 11 | 24 | | | | |
| 11 | 48 | | | | |
| 11 | 72 | | | | |
| 12 | 0 | 39 | 17 | 60 | 5.7 |
| 12 | 1 | 39 | 16 | | 5.3 |
| 12 | 4 | 40 | 26 | | 9 |
| 12 | 8 | 39.3 | 23 | | 7.8 |
| 12 | 24 | 38.2 | 26 | | 8.8 |
| 12 | 48 | 40.3 | 24 | | 8.1 |
| 12 | 72 | 38.7 | 29 | | 9.9 |

### 12.1.4 Albumin, Globulin and Total Serum Protein

| | Reference ranges | 0 | 1 | 4 | 8 | 24 | 48 | 72 |
|---|---|---|---|---|---|---|---|---|
| pRBCT TSP | 53–75 (mmol/L) | 65.3 | 63.02 | 69.32 a | 66.22 b | 70 | 69.97 | 72.88 |
| Oxyglobin® TSP | | 62.38 | 74.38 | 93.68 a | 92.43 b | 79.08 | 72.36 | 68.93 |
| pRBCT albumin | 27–35 (mmol/L) | 23.12 | 22.98 | 23.52 c | 23.13 d | 22.92 | 22.82 | 23.63 |
| Oxyglobin® albumin | | 21.3 | 25.57 | 32.27 c | 32.18 d | 27.48 | 23.78 | 23.05 |
| pRBCT globulin | 20–37 (mmol/L) | 42.18 | 40.03 | 43.97 | 44.74 | 45.4 | 48.42 | 50.18 |
| Oxyglobin® globulin | | 41.08 | 48.82 | 61.42 | 60.25 | 51.6 | 48.58 | 45.88 |
| pRBCT A/G ratio | 0.6–1.2 | 0.613 | 0.619 | 0.583 | 0.594 | 0.553 | 0.531 | 0.542 |
| Oxyglobin® A/G ratio | | 0.538 | 0.535 | 0.53 | 0.533 | 0.542 | 0.512 | 0.515 |
| a $P = 0.053$; b $P = 0.018$; c $P = 0.0002$; d $P = 0.003$ | | | | | | | | |

#### 12.1.4.1 P-VALUES

| | 0 | 1 | 4 | 8 | 24 | 48 | 72 |
|---|---|---|---|---|---|---|---|
| **pRBCT TSP** | 65.3 | 63.02 | 69.32 | 66.22 | 70 | 69.97 | 72.88 |
| **Oxyglobin® TSP** | 62.38 | 74.38 | 93.683 | 92.43 | 79.08 | 72.36 | 68.93 |
| **P** | ***0.702*** | ***0.159*** | ***0.026*** | ***0.014*** | ***0.441*** | ***0.803*** | ***0.750*** |
| **pRBCT albumin** | 23.12 | 22.98 | 23.52 | 23.13 | 22.92 | 22.82 | 23.63 |
| **Oxyglobin® albumin** | 21.3 | 25.57 | 32.267 | 32.18 | 27.48 | 23.78 | 23.05 |
| **P** | ***0.386*** | ***0.346*** | ***0.0002*** | ***0.001*** | ***0.079*** | ***0.641*** | ***0.776*** |
| **pRBCT globulin** | 42.18 | 40.03 | 43.967 | 44.74 | 45.4 | 48.42 | 50.18 |
| **Oxyglobin® globulin** | 41.08 | 48.82 | 61.417 | 60.25 | 51.6 | 48.58 | 45.88 |
| **P** | ***0.89*** | ***0.23*** | ***0.067*** | ***0.12*** | ***0.55*** | ***0.99*** | ***0.75*** |
| **pRBCT A/G ratio** | 0.613 | 0.619 | 0.583 | 0.594 | 0.553 | 0.531 | 0.542 |
| **Oxyglobin® A/G ratio** | 0.538 | 0.535 | 0.5303 | 0.533 | 0.542 | 0.512 | 0.515 |
| **P** | ***0.5*** | ***0.36*** | ***0.482*** | ***0.46*** | ***0.91*** | ***0.84*** | ***0.79*** |

### 12.1.4.2 PROTEIN RESULTS

| | | Times | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **TSP** | | **0** | **1** | **4** | **8** | **24** | **48** | **72** |
| **pRBCT** | 1 | | 57.8 | | 55.8 | | 56.3 | 66.6 |
| | 2 | 59.3 | 63 | 59.7 | 57.8 | 57.6 | 64.3 | 51.4 |
| | 5 | 62.3 | 60.5 | 59.6 | 64.1 | 62.1 | 69.1 | 69.4 |
| | 6 | 93.8 | 86.2 | 105.7 | 104.1 | 109.2 | 102.3 | 111.6 |
| | 10 | 56.6 | 56.1 | 62.3 | 58.4 | 60.6 | 61.8 | 66.5 |
| | 12 | 54.5 | 54.5 | 59.3 | 57.1 | 60.5 | 66 | 71.8 |
| **Oxyglobin®** | 3 | 58.4 | 62.3 | 93.3 | 91.4 | 88.2 | 70.6 | 75.3 |
| | 4 | 61.6 | 64.6 | 89.4 | 73.6 | 68.9 | 77.4 | |
| | 7 | 55.5 | 69.7 | 79.4 | 91.6 | 65.8 | 56.3 | 49.4 |
| | 8 | 63.6 | 90.1 | 95.9 | 103.1 | 93.7 | 93.2 | 85.3 |
| | 9 | 58.3 | 65.4 | 97.2 | 93 | 78.8 | 64.3 | 65.7 |
| | 11 | 76.9 | 94.2 | 106.9 | 101.9 | | | |
| **Albumin** | | | | | | | | |
| **pRBCT** | 1 | | 23.5 | | 21 | | 19.3 | 22 |
| | 2 | 22.7 | 23.5 | 22.1 | 21.4 | 21.6 | 23.1 | 21.9 |
| | 5 | 24.9 | 24 | 23.4 | 25.9 | 24.4 | 26.6 | 26.4 |
| | 6 | 20.3 | 20 | 22.1 | 22.3 | 21.7 | 19.4 | 21.2 |
| | 10 | 24.9 | 24.6 | 26.3 | 25.3 | 25.2 | 25.8 | 27.4 |
| | 12 | 22.8 | 22.3 | 23.7 | 22.9 | 21.7 | 22.7 | 22.9 |
| **Oxyglobin®** | 3 | 21.3 | 24.8 | 33.5 | 32.4 | 30.7 | 24.5 | 24.9 |
| | 4 | 15 | 15.1 | 27.7 | 23.2 | 19.4 | 19.3 | |
| | 7 | 24.8 | 27.8 | 31.1 | 34.2 | 27.2 | 22.7 | 19.1 |
| | 8 | 19.2 | 28.6 | 32.4 | 35.1 | 31 | 29.1 | 27.3 |
| | 9 | 21 | 23.6 | 32.5 | 33.3 | 29.1 | 23.3 | 20.9 |
| | 11 | 26.5 | 33.5 | 36.4 | 34.9 | | | |
| **Globulin** | | | | | | | | |
| **pRBCT** | 1 | | 34.3 | 34.8 | | 37 | 44.6 | |
| | 2 | 36.6 | 39.5 | 37.6 | 36.4 | 36 | 41.2 | 29.5 |
| | 5 | 37.4 | 36.5 | 36.2 | 38.2 | 37.7 | 42.5 | 43 |
| | 6 | 73.5 | 66.2 | 83.6 | 81.8 | 87.5 | 82.9 | 90.4 |
| | 10 | 31.7 | 31.5 | 36 | 33.1 | 35.4 | 36 | 39.1 |
| | 12 | 31.7 | 32.2 | 35.6 | 34.2 | 38.8 | 43.3 | 48.9 |
| **Oxyglobin®** | 3 | 37.1 | 37.5 | 59.8 | 59 | 57.5 | 46.1 | 50.4 |
| | 4 | 46.6 | 49.5 | 61.7 | 50.4 | 49.5 | 58.1 | |
| | 7 | 30.7 | 41.9 | 48.3 | 57.4 | 38.6 | 33.6 | 30.3 |
| | 8 | 44.4 | 61.5 | 63.5 | 68 | 62.7 | 64.1 | 58 |
| | 9 | 37.3 | 41.8 | 64.7 | 59.7 | 49.7 | 41 | 44.8 |
| | 11 | 50.4 | 60.7 | 70.5 | 67 | | | |
| **A/G ratio** | | | | | | | | |
| **pRBCT** | 1 | | 0.685 | 0.603 | | | 0.522 | 0.493 |
| | 2 | 0.62 | 0.595 | 0.588 | 0.588 | 0.6 | 0.561 | 0.742 |
| | 5 | 0.666 | 0.658 | 0.646 | 0.678 | 0.647 | 0.626 | 0.614 |
| | 6 | 0.276 | 0.302 | 0.264 | 0.273 | 0.248 | 0.234 | 0.235 |
| | 10 | 0.785 | 0.781 | 0.731 | 0.764 | 0.712 | 0.717 | 0.701 |
| | 12 | 0.719 | 0.693 | 0.666 | 0.67 | 0.559 | 0.524 | 0.468 |
| **Oxyglobin®** | 3 | 0.574 | 0.661 | 0.56 | 0.549 | 0.534 | 0.531 | 0.494 |
| | 4 | 0.322 | 0.305 | 0.449 | 0.46 | 0.392 | 0.332 | |
| | 7 | 0.808 | 0.663 | 0.644 | 0.596 | 0.705 | 0.676 | 0.63 |
| | 8 | 0.432 | 0.465 | 0.51 | 0.516 | 0.494 | 0.454 | 0.471 |
| | 9 | 0.563 | 0.565 | 0.502 | 0.558 | 0.586 | 0.568 | 0.467 |
| | 11 | 0.526 | 0.552 | 0.516 | 0.521 | | | |

## 12.1.5 Blood gas & Basic information

| Pat | t= | $FiO_2$ | tHb | $pH_a$ | $pH_v$ | $pCO_2a$ | $pCO_2v$ | $pO_2a$ | $pO_2v$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 21 | 2.3 | 7.416 | 6.955 | 19.7 | 57.7 | 98.8 | 62.5 |
| 1 | 1 | 21 | 3.9 | 7.41 | 7.354 | 22.5 | 27.2 | 105.5 | 41 |
| 1 | 4 | 21 | 5.9 | 7.424 | 7.383 | 22.4 | 29 | 99.9 | 31.4 |
| 1 | 8 | 21 | 7.3 | 7.439 | 7.393 | 24.6 | 30.6 | 87.1 | 39.4 |
| 1 | 24 | 21 | 6.3 | 7.388 | 7.417 | 26.9 | 28.9 | 80.9 | 39.5 |
| 1 | 48 | 21 | 5.5 | 7.42 | 7.376 | 30 | 34.6 | 89.6 | 40 |
| 1 | 72 | 21 | 6.6 | 7.425 | 7.39 | 30.2 | 34.2 | 73.7 | 27.8 |
| 2 | 0 | 21 | 2.8 | 7.41 | 7.379 | 24.5 | 26.4 | 74.6 | 33.6 |
| 2 | 1 | 21 | 3.1 | 7.379 | 7.362 | 22.7 | 26.6 | 84.4 | 32.3 |
| 2 | 4 | 21 | 7.1 | 7.375 | 7.385 | 26.8 | 26.7 | 79.6 | 26.1 |
| 2 | 8 | 21 | 10.6 | 7.391 | 7.361 | 25.6 | 29.9 | 86.7 | 34.8 |
| 2 | 24 | 21 | 11.6 | 7.394 | 7.374 | 29.1 | 25.4 | 82 | 40.1 |
| 2 | 48 | 21 | 5.6 | 7.323 | 7.371 | 33.2 | 37.4 | 74.5 | 28.9 |
| 2 | 72 | 12 | 10 | 7.375 | 7.35 | 25 | 32.4 | 91.6 | 28.6 |
| 3 | 0 | 21 | 3.1 | 7.444 | 7.425 | 22.4 | 24.7 | 75.7 | 31.1 |
| 3 | 1 | 21 | 3.1 | 7.41 | 7.377 | 23.5 | 25.1 | 80.5 | 26.7 |
| 3 | 4 | 21 | 7.1 | 7.451 | 7.419 | 19.8 | 22.8 | 85.7 | 18.6 |
| 3 | 8 | 21 | 3.9 | 7.437 | 7.409 | 19.2 | 22.4 | 90.7 | 22.3 |
| 3 | 24 | 21 | 4.6 | 7.382 | 7.358 | 28.1 | 32.2 | 76.5 | 28.7 |
| 3 | 48 | 21 | 6.1 | 7.409 | 7.386 | 30.8 | 33.6 | 66.5 | 23.8 |
| 3 | 72 | 21 | 11.6 | 7.374 | 7.361 | 30.3 | 35.2 | 74.7 | 26.9 |
| 4 | 0 | 21 | 3.5 | 7.441 | 7.401 | 26 | 30.4 | 101.9 | 33.4 |
| 4 | 1 | 21 | 2.7 | 7.455 | 7.396 | 24 | 31.2 | 97.9 | 30 |
| 4 | 4 | 21 | 4.4 | 7.479 | 7.39 | 24.5 | 28 | 114.6 | 35.8 |
| 4 | 8 | 21 | 3.5 | 7.438 | 7.371 | 25.4 | 27 | 97.5 | 39 |
| 4 | 24 | 21 | 3.7 | 7.424 | 7.386 | 28.8 | 30.4 | 109.5 | 40.1 |
| 4 | 48 | 21 | 5.1 | 7.452 | 7.391 | 26.7 | 35 | 90.2 | 32.9 |
| 4 | 72 | 21 | 6.1 | 7.394 | 7.286 | 31.4 | 30.2 | 80.8 | 30.8 |
| 5 | 0 | 21 | 4.2 | 7.4 | 7.369 | 29.8 | 37.8 | 103.8 | 43.5 |
| 5 | 1 | 21 | 4.3 | 7.385 | 7.345 | 26 | 37.6 | 94.3 | 36.6 |
| 5 | 4 | 21 | 5.3 | 7.357 | 7.369 | 33.2 | 36.9 | 93.9 | 43.1 |
| 5 | 8 | 21 | 8.2 | 7.375 | 7.339 | 31.7 | 36.4 | 92.1 | 38.8 |
| 5 | 24 | 21 | 7.9 | 7.404 | 7.369 | 29.8 | 38.3 | 91.6 | 35.1 |
| 5 | 48 | 21 | 9 | 7.432 | 7.376 | 28.5 | 37.1 | 119.4 | 36 |
| 5 | 72 | 21 | 9 | 7.406 | 7.376 | 29.7 | 36.3 | 89.9 | 44.8 |
| 6 | 0 | 21 |  | 7.453 | 7.454 | 26.7 | 27 | 78.6 | 30.6 |
| 6 | 1 | 21 |  | 7.411 | 7.389 | 30.6 | 33.7 | 85.5 | 26.4 |
| 6 | 4 | 21 |  | 7.408 | 7.423 | 31.3 | 31.7 | 88 | 28.4 |
| 6 | 8 | 21 |  | 7.418 | 7.39 | 30.6 | 32.5 | 66 | 30.7 |
| 6 | 24 | 21 |  | 7.406 | 7.36 | 29.3 | 35.9 | 86.5 | 33 |
| 6 | 48 | 21 |  | 7.392 | 7.386 | 26.2 | 30.2 | 80.6 | 23.7 |
| 6 | 72 | 21 |  | 7.426 | 7.382 | 30.6 | 38.4 | 85.9 | 34.8 |
| 7 | 0 | 21 |  | 7.458 | 7.425 | 34.6 | 39.7 | 80.5 | 42.3 |
| 7 | 1 | 21 |  | 7.451 | 7.416 | 35.6 | 40.8 | 84.3 | 43.2 |
| 7 | 4 | 21 |  | 7.496 | 7.46 | 33.9 | 39.2 | 77.6 | 35.9 |
| 7 | 8 | 21 |  | 7.475 | 7.45 | 34.8 | 36.4 | 82.5 | 48.3 |
| 7 | 24 | 21 |  | 7.477 | 7.432 | 30.6 | 34.9 | 88.1 | 42.5 |
| 7 | 48 | 21 |  | 7.413 | 7.34 | 30.4 | 38.3 | 77.4 | 37 |
| 7 | 72 | 21 |  | 7.421 | 7.384 | 34.1 | 38.1 | 84.5 | 36.6 |
| 8 | 0 | 21 | 3.9 | 7.37 | 7.386 | 30.9 | 29.6 | 94.2 | 46.9 |
| 8 | 1 | 21 | 2.81 | 7.371 | 7.339 | 29.6 | 32.4 | 78.8 | 26.1 |
| 8 | 4 | 21 | 3.5 | 7.407 | 7.364 | 28.3 | 35.9 | 83.5 | 24.9 |
| 8 | 8 | 21 | 4 | 7.412 | 7.384 | 33.8 | 36.7 | 74 | 25.6 |
| 8 | 24 | 21 | 5.3 | 7.402 | 7.377 | 37 | 40.5 | 75.9 | 30.1 |
| 8 | 48 | 21 | 10.1 | 7.396 | 7.366 | 33.2 | 42.9 | 78.4 | 34.5 |
| 8 | 72 | 21 | 7.71 | 7.432 | 7.39 | 32.5 | 40.5 | 80.9 | 27.9 |
| 9 | 0 | 21 | 3.3 | 7.284 | 7.201 | 15.2 | 20.3 | 116 | 41 |
| 9 | 1 | 21 | 3.7 | 7.356 | 7.283 | 15.1 | 19.4 | 101.7 | 35.2 |
| 9 | 4 | 21 | 4.3 | 7.42 | 7.401 | 20.5 | 22.5 | 97.7 | 34.2 |
| 9 | 8 | 21 | 4.2 | 7.479 | 7.436 | 21.4 | 24.3 | 92.3 | 27 |
| 9 | 24 | 21 | 2.6 | 7.462 | 7.43 | 19.3 | 21.7 | 105 | 29.3 |
| 9 | 48 | 21 | 3.2 | 7.432 | 7.375 | 21.6 | 25.2 | 75.6 | 21.5 |
| 9 | 72 | 21 | 6.5 | 7.437 | 7.357 | 27.2 | 31.4 | 73.7 | 25.2 |
| 10 | 0 | 21 | 4.3 | 7.441 | 7.379 | 25.8 | 30.5 | 78.5 | 33.6 |
| 10 | 1 | 21 | 3.2 | 7.402 | 7.339 | 27.4 | 30.7 | 86.6 | 42.6 |
| 10 | 4 | 21 | 6.1 | 7.383 | 7.362 | 30.3 | 31 | 90.9 | 38.8 |
| 10 | 8 | 21 | 5.4 | 7.377 | 7.371 | 29.5 | 33.5 | 88.9 | 31.5 |
| 10 | 24 | 21 | 5.5 | 7.372 | 7.357 | 28 | 33.8 | 88.1 | 36.7 |
| 10 | 48 | 21 | 6.6 | 7.369 | 7.334 | 25.2 | 34.8 | 87.3 | 40.6 |
| 10 | 72 | 21 | 9.3 | 7.403 | 7.371 | 31.2 | 33.3 | 75.6 | 40.5 |
| 11 | 0 | 21 | 4.1 | 7.354 | 7.28 | 16.4 | 21.1 | 84.2 | 20.4 |
| 11 | 1 | 21 |  | 7.342 | 7.292 | 19.8 | 22.2 | 82.5 | 27.2 |
| 11 | 4 | 21 |  | 7.375 | 7.283 | 16.6 | 22.8 | 98.2 | 32.4 |
| 11 | 8 | 21 |  | 7.373 | 7.315 | 17.5 | 20.4 | 96.3 | 24.3 |
| 11 | 24 |  |  |  |  |  |  |  |  |
| 11 | 48 |  |  |  |  |  |  |  |  |
| 11 | 72 |  |  |  |  |  |  |  |  |
| 12 | 0 | 21 | 5.7 | 7.416 | 7.366 | 26 | 30.9 | 81.5 | 31.7 |
| 12 | 1 | 21 | 5.3 | 7.41 | 7.338 | 27.4 | 36.9 | 80.2 | 39.9 |
| 12 | 4 | 21 | 9 | 7.406 | 7.37 | 31.1 | 33.5 | 77.7 | 39.5 |
| 12 | 8 | 21 | 7.8 | 7.402 | 7.366 | 32 | 34.3 | 73.5 | 39.1 |
| 12 | 24 | 21 | 8.8 | 7.407 | 7.366 | 32.4 | 34.8 | 76.1 | 36.3 |
| 12 | 48 | 21 | 8.1 | 7.424 | 7.399 | 31.2 | 32.8 | 78.5 | 38.8 |
| 12 | 72 | 21 | 9.9 | 7.418 | 7.367 | 32.6 | 42.4 | 74.4 | 36.8 |

## 12.1.6 Blood gas statistical analyses

### 12.1.6.1 pH DATA

| | | 0 | 1 | 4 | 8 | 24 | 48 | 72 | |
|---|---|---|---|---|---|---|---|---|---|
| pRBCT pH | 1 | 7.416 | 7.41 | 7.424 | 7.439 | 7.388 | 7.42 | 7.425 | |
| | 2 | 7.41 | 7.379 | 7.375 | 7.391 | 7.394 | 7.323 | 7.375 | |
| | 5 | 7.4 | 7.385 | 7.357 | 7.375 | 7.404 | 7.432 | 7.406 | |
| | 6 | 7.453 | 7.411 | 7.408 | 7.418 | 7.406 | 7.392 | 7.426 | |
| | 10 | 7.441 | 7.402 | 7.383 | 7.377 | 7.372 | 7.369 | 7.403 | **t0vs72 type 3** |
| | 12 | 7.416 | 7.41 | 7.406 | 7.402 | 7.407 | 7.424 | 7.418 | **0.218** |
| | **Mean** | **7.423** | **7.400** | **7.392** | **7.400** | **7.395** | **7.393** | **7.409** | |
| | **St Dev** | **0.020** | **0.014** | **0.025** | **0.025** | **0.014** | **0.042** | **0.019** | |
| | Min | 7.4 | 7.379 | 7.357 | 7.375 | 7.372 | 7.323 | 7.375 | |
| | Max | 7.453 | 7.411 | 7.424 | 7.439 | 7.407 | 7.432 | 7.426 | |
| **Oxyglobin® pH** | 3 | 7.444 | 7.41 | 7.451 | 7.437 | *7.382* | *7.409* | *7.374* | |
| | *4* | *7.441* | *7.455* | *7.479* | *7.438* | *7.424* | *7.452* | 7.394 | |
| | *7* | *7.458* | *7.451* | *7.496* | *7.475* | *7.477* | 7.413 | 7.421 | |
| | 8 | 7.37 | 7.371 | 7.407 | 7.412 | 7.402 | 7.396 | 7.432 | |
| | 9 | 7.284 | 7.356 | 7.42 | 7.479 | 7.462 | 7.432 | 7.437 | **t0vs72 type 3** |
| | 11 | 7.354 | 7.342 | 7.375 | 7.373 | | | | **0.448** |
| | **St Dev** | **0.068** | **0.049** | **0.046** | **0.040** | **0.040** | **0.022** | **0.027** | |
| | **Mean** | **7.392** | **7.398** | **7.438** | **7.436** | **7.429** | **7.420** | **7.412** | |
| | Min | 7.284 | 7.342 | 7.375 | 7.373 | 7.382 | 7.396 | 7.374 | |
| | Max | 7.458 | 7.455 | 7.496 | 7.479 | 7.477 | 7.452 | 7.437 | |
| | **F-test** | **0.018** | **0.017** | **0.204** | **0.324** | **0.037** | **0.235** | **0.476** | |
| | ttest | **0.328** | **0.926** | **0.064** | **0.101** | **0.129** | **0.206** | **0.852** | |
| | **type** | 3 | 3 | 3 | 3 | 3 | 3 | 3 | |

### 12.1.6.2 $pO_2$ DATA

| pRBCT | 0 | 1 | 4 | 8 | 24 | 48 | 72 | |
|---|---|---|---|---|---|---|---|---|
| 1 | 98.8 | 105.5 | 99.9 | 87.1 | 80.9 | 89.6 | 73.7 | |
| 2 | 74.6 | 84.4 | 79.6 | 86.7 | 82 | 74.5 | 91.6 | |
| 5 | 103.8 | 94.3 | 93.9 | 92.1 | 91.6 | 119.4 | 89.9 | |
| 6 | 78.6 | 85.5 | 88 | 66 | 86.5 | 80.6 | 85.9 | |
| 10 | 78.5 | 86.6 | 90.9 | 88.9 | 88.1 | 87.3 | 75.6 | t0vs72type3 |
| 12 | 81.5 | 80.2 | 77.7 | 73.5 | 76.1 | 78.5 | 74.4 | 0.510 |
| mean | 86.0 | 89.4 | 88.3 | 82.4 | 84.2 | 88.3 | 81.9 | |
| SD | 12.2 | 9.1 | 8.5 | 10.2 | 5.6 | 16.2 | 8.2 | |
| Min | 74.6 | 80.2 | 77.7 | 66 | 76.1 | 74.5 | 73.7 | |
| Max | 103.8 | 105.5 | 99.9 | 92.1 | 91.6 | 119.4 | 91.6 | |
| Oxyglobin® | 0 | 1 | 4 | 8 | 24 | 48 | 72 | |
| 3 | 75.7 | 80.5 | 85.7 | 90.7 | 76.5 | 66.5 | 74.7 | |
| 4 | 101.9 | 97.9 | 114.6 | 97.5 | 109.5 | 90.2 | 80.8 | |
| 7 | 80.5 | 84.3 | 77.6 | 82.5 | 88.1 | 77.4 | 84.5 | |
| 8 | 94.2 | 78.8 | 83.5 | 74 | 75.9 | 78.4 | 80.9 | |
| 9 | 116 | 101.7 | 97.7 | 92.3 | 105 | 75.6 | 73.7 | t0vs72type3 |
| 11 | 84.2 | 82.5 | 98.2 | 96.3 | | | | 0.088 |
| mean | 92.1 | 87.6 | 92.9 | 88.9 | 91.0 | 77.6 | 78.9 | |
| SD | 15.1 | 9.7 | 13.4 | 9.0 | 15.7 | 8.5 | 4.6 | |
| Min | 75.7 | 78.8 | 77.6 | 74 | 75.9 | 66.5 | 73.7 | |
| Max | 116 | 101.7 | 114.6 | 97.5 | 109.5 | 90.2 | 84.5 | |
| F test | 0.650 | 0.897 | 0.341 | 0.786 | 0.044 | 0.232 | 0.280 | |
| T-test | 0.458 | 0.747 | 0.501 | 0.271 | 0.400 | 0.200 | 0.477 | |
| Type | 3 | 3 | 3 | 3 | 3 | 3 | 3 | |

### 12.1.6.3 $pCO_2$ DATA

| pRBCT | 0 | 1 | 4 | 8 | 24 | 48 | 72 | |
|---|---|---|---|---|---|---|---|---|
| 1 | 19.7 | 22.5 | 22.4 | 24.6 | 26.9 | 30 | 30.2 | |
| 2 | 24.5 | 22.7 | 26.8 | 25.6 | 29.1 | 33.2 | 25 | |
| 5 | 29.8 | 26 | 33.2 | 31.7 | 29.8 | 28.5 | 29.7 | |
| 6 | 26.7 | 30.6 | 31.3 | 30.6 | 29.3 | 26.2 | 30.6 | |
| 10 | 25.8 | 27.4 | 30.3 | 29.5 | 28 | 25.2 | 31.2 | t0vs72 type 3 |
| 12 | 26 | 27.4 | 31.1 | 32 | 32.4 | 31.2 | 32.6 | 0.028 |
| Mean | 25.4 | 26.1 | 29.2 | 29.0 | 29.3 | 29.1 | 29.9 | |
| SD | 3.3 | 3.1 | 3.9 | 3.2 | 1.9 | 3.0 | 2.6 | |
| Min | 19.7 | 22.5 | 22.4 | 24.6 | 26.9 | 25.2 | 25 | |
| max | 29.8 | 30.6 | 33.2 | 32 | 32.4 | 33.2 | 32.6 | |
| Oxyglobin® | 0 | 1 | 4 | 8 | 24 | 48 | 72 | |
| 3 | 22.4 | 23.5 | 19.8 | 19.2 | 28.1 | 30.8 | 30.3 | |
| 4 | 26 | 24 | 24.5 | 25.4 | 28.8 | 26.7 | 31.4 | |
| 7 | 34.6 | 35.6 | 33.9 | 34.8 | 30.6 | 30.4 | 34.1 | |
| 8 | 30.9 | 29.6 | 28.3 | 33.8 | 37 | 33.2 | 32.5 | |
| 9 | 15.2 | 15.1 | 20.5 | 21.4 | 19.3 | 21.6 | 27.2 | t0vs72 type 3 |
| 11 | 16.4 | 19.8 | 16.6 | 17.5 | | | | 0.087 |
| mean | 24.3 | 24.6 | 23.9 | 25.4 | 28.8 | 28.5 | 31.1 | |
| SD | 7.8 | 7.2 | 6.3 | 7.4 | 6.3 | 4.5 | 2.6 | |
| Min | 15.2 | 15.1 | 16.6 | 17.5 | 19.3 | 21.6 | 27.2 | |
| max | 34.6 | 35.6 | 33.9 | 34.8 | 37 | 33.2 | 34.1 | |
| Ftest | 0.085 | 0.087 | 0.316 | 0.084 | 0.019 | 0.404 | 0.971 | |
| Type 3 ttest | 0.745 | 0.655 | 0.122 | 0.306 | 0.875 | 0.836 | 0.459 | |

### 12.1.6.4 TEMPERATURE DATA

| Time | | 0 | 1 | 4 | 8 | 24 | 48 | 72 | |
|---|---|---|---|---|---|---|---|---|---|
| pRBCT | 1 | 39.7 | 38.7 | 40.1 | 39.3 | 38.4 | 37.9 | 37.3 | |
| | 2 | 40.3 | 40.3 | 40.3 | 39.3 | 38 | 39.3 | 39.2 | |
| | 5 | 40.1 | 39.7 | 40.3 | 38.5 | 38.4 | 38 | 38.7 | |
| | 6 | 38.9 | 38.6 | 39.9 | 38.6 | 37.8 | 38.1 | 37.4 | |
| | 10 | 39.4 | 39.9 | 40 | 38.7 | 39.1 | 38.2 | 38.5 | |
| | 12 | 39 | 39 | 40 | 39.3 | 38.2 | 40.3 | 38.7 | T0vs72 |
| | average | 39.6 | 39.4 | 40.1 | 39.0 | 38.3 | 38.6 | 38.3 | 0.04 |
| | st dev | 0.572 | 0.698 | 0.167 | 0.389 | 0.449 | 0.963 | 0.772 | |
| | min | 38.9 | 38.6 | 39.9 | 38.5 | 37.8 | 37.9 | 37.3 | |
| | max | 40.3 | 40.3 | 40.3 | 39.3 | 39.1 | 40.3 | 39.2 | |
| Oxyglobin® | 3 | 39.2 | 39.3 | 39.6 | 40.6 | 39.6 | 38.5 | 37.5 | |
| | 4 | 39.7 | 39.5 | 40.5 | 39.1 | 38.7 | 38.1 | 38.4 | |
| | 7 | 39.5 | 39.6 | 40.3 | 40.7 | 40 | 38.3 | 37.9 | |
| | 8 | 39.1 | 37.4 | 37.8 | 37.2 | 37.8 | 38.3 | 37.8 | |
| | 9 | 40.6 | 38.8 | 39.7 | 39.4 | 38.9 | 39.5 | 38.5 | |
| | 11 | 41.4 | 40.9 | 41.4 | 41.2 | | | | T0vs72 |
| | average | 39.9 | 39.3 | 39.9 | 39.7 | 39.0 | 38.5 | 38.0 | 0.002 |
| | st dev | 0.902 | 1.143 | 1.209 | 1.467 | 0.851 | 0.555 | 0.421 | |
| | min | 39.1 | 37.4 | 37.8 | 37.2 | 37.8 | 38.1 | 37.5 | |
| | max | 41.4 | 40.9 | 41.4 | 41.2 | 40.0 | 39.5 | 38.5 | |
| | F-value | 0.339 | 0.302 | 0.001 | 0.011 | 0.193 | 0.308 | 0.263 | |
| P value | t-test, Type 3 | 0.444 | 0.836 | 0.681 | 0.274 | 0.159 | 0.846 | 0.468 | |

### 12.1.6.5 THB DATA

| | time | 0 | 1 | 4 | 8 | 24 | 48 | 72 | |
|---|---|---|---|---|---|---|---|---|---|
| pRBCT | 1 | 2.3 | 3.9 | 5.9 | 7.3 | 6.3 | 5.5 | 6.6 | |
| | 2 | 2.8 | 3.1 | 7.1 | 10.6 | 11.6 | 5.6 | 10 | |
| | 5 | 4.2 | 4.3 | 5.3 | 8.2 | 7.9 | 9 | 9 | |
| | 6 | n/d | n/d | n/d | n/d | n/d | n/d | n/d | |
| | 10 | 4.3 | 3.2 | 6.1 | 5.4 | 5.5 | 6.6 | 9.3 | 0.0004 |
| | 12 | 5.7 | 5.3 | 9 | 7.8 | 8.8 | 8.1 | 9.9 | |
| | mean | 3.9 | 4.0 | 6.7 | 7.9 | 8.0 | 7.0 | 9.0 | |
| | st dev | 1.3 | 0.9 | 1.4 | 1.9 | 2.4 | 1.5 | 1.4 | |
| | min | 2.3 | 3.1 | 5.3 | 5.4 | 5.5 | 5.5 | 6.6 | |
| | max | 5.7 | 5.3 | 9 | 10.6 | 11.6 | 9 | 10 | |
| Oxyglobin® | 3 | 3.1 | 3.1 | 7.1 | 3.9 | 4.6 | 6.1 | 11.6 | |
| | 4 | 3.5 | 2.7 | 4.4 | 3.5 | 3.7 | 5.1 | 6.1 | |
| | 7 | n/d | n/d | n/d | n/d | n/d | n/d | n/d | |
| | 8 | 3.9 | 2.81 | 3.5 | 4 | 5.3 | 10.1 | 7.71 | |
| | 9 | 3.3 | 3.7 | 4.3 | 4.2 | 2.6 | 3.2 | 6.5 | |
| | 11 | 4.1 | n/d | n/d | n/d | | | | 0.006 |
| | mean | 3.6 | 3.1 | 4.8 | 3.9 | 4.1 | 6.1 | 8.0 | |
| | st dev | 0.4 | 0.4 | 1.6 | 0.3 | 1.2 | 2.9 | 2.5 | |
| | min | 3.1 | 2.7 | 3.5 | 3.5 | 2.6 | 3.2 | 6.1 | |
| | max | 4.1 | 3.7 | 7.1 | 4.2 | 5.3 | 10.1 | 11.6 | |
| *P*-value | ttest | 0.669 | 0.119 | 0.108 | 0.004 | 0.019 | 0.595 | 0.477 | |

## 12.1.7 Ancillary blood gas and acid-base data

### 12.1.7.1 Total haemoglobin (tHb)

| | time | 0 | 1 | 4 | 8 | 24 | 48 | 72 | |
|---|---|---|---|---|---|---|---|---|---|
| Prbct | 1 | 2.3 | 3.9 | 5.9 | 7.3 | 6.3 | 5.5 | 6.6 | |
| | 2 | 2.8 | 3.1 | 7.1 | 10.6 | 11.6 | 5.6 | 10 | |
| | 5 | 4.2 | 4.3 | 5.3 | 8.2 | 7.9 | 9 | 9 | |
| | 6 | n/d | n/d | n/d | n/d | n/d | n/d | n/d | Ttest 0vs72 |
| | 10 | 4.3 | 3.2 | 6.1 | 5.4 | 5.5 | 6.6 | 9.3 | **0.0004** |
| | 12 | 5.7 | 5.3 | 9 | 7.8 | 8.8 | 8.1 | 9.9 | |
| | **mean** | **3.9** | **4.0** | **6.7** | **7.9** | **8.0** | **7.0** | **9.0** | |
| | **st dev** | **1.3** | **0.9** | **1.4** | **1.9** | **2.4** | **1.5** | **1.4** | |
| | **min** | **2.3** | **3.1** | **5.3** | **5.4** | **5.5** | **5.5** | **6.6** | |
| | **max** | **5.7** | **5.3** | **9** | **10.6** | **11.6** | **9** | **10** | |
| Oxyglobin® | 3 | 3.1 | 3.1 | 7.1 | 3.9 | 4.6 | 6.1 | 11.6 | |
| | 4 | 3.5 | 2.7 | 4.4 | 3.5 | 3.7 | 5.1 | 6.1 | |
| | 7 | n/d | n/d | n/d | n/d | n/d | n/d | n/d | |
| | 8 | 3.9 | 2.81 | 3.5 | 4 | 5.3 | 10.1 | 7.71 | |
| | 9 | 3.3 | 3.7 | 4.3 | 4.2 | 2.6 | 3.2 | 6.5 | Ttest 0vs72 |
| | 11 | 4.1 | n/d | n/d | n/d | | | | **0.006** |
| | **mean** | **3.6** | **3.1** | **4.8** | **3.9** | **4.1** | **6.1** | **8.0** | |
| | **st dev** | **0.4** | **0.4** | **1.6** | **0.3** | **1.2** | **2.9** | **2.5** | |
| | **min** | **3.1** | **2.7** | **3.5** | **3.5** | **2.6** | **3.2** | **6.1** | |
| | **max** | **4.1** | **3.7** | **7.1** | **4.2** | **5.3** | **10.1** | **11.6** | |
| | **ttest** | **0.669** | **0.119** | **0.108** | **0.004** | **0.019** | **0.595** | **0.477** | |

### 12.1.7.2 Arterial Oxygen Content

| | Time = | 0 | 1 | 4 | 8 | 24 | 48 | 72 | |
|---|---|---|---|---|---|---|---|---|---|
| pRBCT | 1 | 0.8 | 2.3 | 3.4 | 4.2 | 3.6 | 3.2 | 3.8 | |
| | 2 | 3.8 | 4.3 | 9.4 | 14.3 | 15.6 | 7.3 | 13.6 | |
| | 5 | 5.7 | 5.8 | 7 | 10.7 | 10.4 | 12.2 | 11.8 | |
| | 6 | | | | | | | | |
| | 10 | 5.9 | 4.4 | 8.1 | 7.2 | 7.3 | 8.7 | 12 | **ttest 0vs72** |
| | 12 | | 7 | 11.6 | 10 | 11.4 | 10.5 | 12.7 | **0.02** |
| | **mean** | **4.1** | **4.8** | **7.9** | **9.3** | **9.7** | **8.4** | **10.8** | |
| | **stdev** | **2.4** | **1.8** | **3.0** | **3.8** | **4.5** | **3.4** | **4.0** | |
| | min | 0.8 | 2.3 | 3.4 | 4.2 | 3.6 | 3.2 | 3.8 | |
| | max | 5.9 | 7 | 11.6 | 14.3 | 15.6 | 12.2 | 13.6 | |
| Oxyglobin® | 3 | 4.3 | 4.4 | 9.7 | 5.4 | 2.2 | 2.7 | 1.7 | |
| | 4 | 4.9 | 3.8 | 5.8 | 4.6 | 4.9 | 6.7 | 7.8 | |
| | 7 | | | | | | | | |
| | 8 | | | | | 6.5 | 12.5 | | |
| | 9 | | 5.1 | 5.2 | 5.1 | 3.2 | 4.2 | 8.4 | **ttest 0vs72** |
| | 11 | | | | | | | | **0.66** |
| | **mean** | **4.6** | **4.4** | **6.9** | **5.0** | **4.2** | **6.5** | **6.0** | |
| | **stdev** | **0.4** | **0.7** | **2.4** | **0.4** | **1.9** | **4.3** | **3.7** | |
| | min | 4.3 | 3.8 | 5.2 | 4.6 | 2.2 | 2.7 | 1.7 | |
| | max | 4.9 | 5.1 | 9.7 | 5.4 | 6.5 | 12.5 | 8.4 | |
| | ttest | 0.773 | 0.774 | 0.649 | 0.111 | 0.060 | 0.494 | 0.140 | |

*Note: T-test values should be treated with caution due to low sample numbers.*

### 12.1.8 Ancillary blood-gas parameters: arrays and statistical results

#### 12.1.8.1 Arterial oxygen content ($AO_2CT$)

| | | 0 | 1 | 4 | 8 | 24 | 48 | 72 | |
|---|---|---|---|---|---|---|---|---|---|
| pRBCT | 1 | 0.8 | 2.3 | 3.4 | 4.2 | 3.6 | 3.2 | 3.8 | |
| | 2 | 3.8 | 4.3 | 9.4 | 14.3 | 15.6 | 7.3 | 13.6 | |
| | 5 | 5.7 | 5.8 | 7 | 10.7 | 10.4 | 12.2 | 11.8 | |
| | 6 | | | | | | | | |
| | 10 | 5.9 | 4.4 | 8.1 | 7.2 | 7.3 | 8.7 | 12 | ttest 0vs72 |
| | 12 | | 7 | 11.6 | 10 | 11.4 | 10.5 | 12.7 | 0.017 |
| | **mean** | **4.1** | **4.8** | **7.9** | **9.3** | **9.7** | **8.4** | **10.8** | |
| | **stdev** | **2.4** | **1.8** | **3.0** | **3.8** | **4.5** | **3.4** | **4.0** | |
| | **min** | 0.8 | 2.3 | 3.4 | 4.2 | 3.6 | 3.2 | 3.8 | |
| | **max** | 5.9 | 7 | 11.6 | 14.3 | 15.6 | 12.2 | 13.6 | |
| Oxyglobin® | 3 | 4.3 | 4.4 | 9.7 | 5.4 | 2.2 | 2.7 | 1.7 | |
| | 4 | 4.9 | 3.8 | 5.8 | 4.6 | 4.9 | 6.7 | 7.8 | |
| | 7 | | | | | | | | |
| | 8 | | | | | 6.5 | 12.5 | | |
| | 9 | | 5.1 | 5.2 | 5.1 | 3.2 | 4.2 | 8.4 | ttest 0vs72 |
| | 11 | | | | | | | | 0.590 |
| | **mean** | **4.6** | **4.4** | **6.9** | **5.0** | **4.2** | **6.5** | **6.0** | |
| | **stdev** | **0.4** | **0.7** | **2.4** | **0.4** | **1.9** | **4.3** | **3.7** | |
| | **min** | 4.3 | 3.8 | 5.2 | 4.6 | 2.2 | 2.7 | 1.7 | |
| | **max** | 4.9 | 5.1 | 9.7 | 5.4 | 6.5 | 12.5 | 8.4 | |
| | **ftest** | **0.262** | **0.246** | **0.856** | **0.022** | **0.187** | **0.655** | **0.968** | |
| | **ttest** | **0.680** | **0.723** | **0.631** | **0.066** | **0.052** | **0.511** | **0.149** | |

#### 12.1.8.2 Methaemoglobin data and statistical analyses

| | Patients | | | | | | | | | | | | Means | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 5 | 6 | 10 | 12 | 3 | 4 | 8 | 9 | 7 | 11 | *pRBCT* | *SD* | Oxyglobin® | SD | ftest | Ttest type 3 |
| **0** | 0.8 | 4 | 0.3 | | 0.2 | | 2.8 | 0.3 | | | | | *1.3* | *1.8* | 1.6 | 1.8 | 0.798 | 0.897 |
| **1** | 0.7 | 1.3 | 0.3 | | 0.3 | 0.3 | 0 | 0.3 | | *0.3* | | | *0.6* | *0.4* | 0.2 | 0.2 | 0.279 | 0.138 |
| **4** | 0.6 | 1.3 | 0 | | 0.3 | 0.3 | 1.8 | 0.5 | | 1.3 | | | *0.5* | 0.5 | *1.2* | 0.7 | 0.567 | 0.198 |
| **8** | 0.6 | 0.5 | 0.3 | | 0.3 | 0.3 | 2.7 | 0.8 | | 3.2 | | | *0.4* | 0.1 | *2.2* | *1.3* | 0.001 | 0.128 |
| **24** | 0.2 | 1.2 | 0.1 | | 0.3 | 0.3 | 2.9 | 1.8 | 1.1 | 10.9 | | | 0.4 | 0.4 | *4.2* | 4.5 | 0.001 | 0.197 |
| **48** | 0.3 | 0.5 | 0.3 | | 0.3 | 0.2 | 1.4 | 1.2 | 1.6 | 2.9 | | | *0.3* | 0.1 | 1.8 | 0.8 | 0.003 | 0.031 |
| **72** | 0.2 | 0.6 | 0.3 | | 0.3 | 0.3 | *1.7* | *0.2* | | 0.3 | | | 0.3 | 0.2 | 0.7 | 0.8 | 0.008 | 0.503 |
| | | | | | | | | | | | | | t0vs72 type 3 | **0.355** | | **0.631** | | |

### 12.1.9 Oxygen saturation of Hb% - data and statistical test results

Results outside reference range highlighted in pale blue. T-tests all type 3 due to unequal variances.

| | Sat% | | | | | | | | | | | | Means | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 5 | 6 | 10 | 12 | 3 | 4 | 7 | 8 | 9 | 11 | pRBCT | SD | Oxy-globin® | SD | ftest | ttest |
| 0 | 63.6 | 92.8 | 100 | 95.5 | 99.6 | 95.3 | *94.7* | *100* | *95.1* | 96.3 | *96.9* | 93.1 | **90.3** | **13.8** | **96.0** | **2.4** | 0.001 | 0.429 |
| 1 | 98.9 | 94.5 | 99.7 | 95.9 | 99.3 | 98.3 | *95* | *100* | *95.5* | 95.3 | *99.4* | 92.9 | **98.1** | **2.1** | **96.4** | **2.8** | 0.554 | 0.342 |
| 4 | 97.6 | 93.3 | 98.5 | 93.6 | 98.2 | 98.2 | *96* | *95* | *94.5* | 96.2 | *87.6* | 95.7 | **97.2** | **2.4** | **94.2** | **3.3** | 0.529 | 0.183 |
| 8 | 96.8 | 95.6 | 98.6 | 92 | 98.8 | 98.3 | *96* | *95.9* | *94.8* | 95.1 | *78.8* | 95.6 | **97.6** | **2.6** | **92.7** | **6.8** | 0.054 | 0.227 |
| 24 | 95.7 | 95.5 | 97.7 | 96.4 | 97.8 | 99.7 | *81* | *97.7* | *96.1* | 90.4 | *93.3* | | **97.3** | **1.6** | **91.7** | **6.6** | 0.008 | 0.140 |
| 48 | 97.4 | 91.8 | 100 | 95.4 | 96.4 | 98.3 | *92.2* | 96.5 | *95* | 96.5 | *100* | | **96.8** | **2.8** | **96.0** | **2.8** | 0.965 | 0.772 |
| 72 | 95.8 | 96.1 | 97.8 | 96.7 | 95.5 | 95.3 | *94.6* | *98* | *96.2* | *96* | 99.7 | | **96.1** | **0.9** | **96.9** | **2.0** | 0.127 | 0.497 |
| | | | | | | | | | ttest 0 vs 72 | | | | | **0.409** | | 0.516 | | |

### 12.1.10 Carboxyhaemoglobin (HbCO) data and statistical analyses

| HbCO | Patients | | | | | | | | | | | | Means | | | | Ftest | type 2 T-test |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 5 | 6 | 10 | 12 | 3 | 4 | 7 | 8 | 9 | 11 | pRBCT | SD | Oxy-globin | SD | | |
| 0 | 3.4 | 9 | 6.5 | | 5.5 | | 13.9 | *4.8* | | | | | *6.*1 | 2.3 | 9.4 | 6.4 | 0.139 | 0.379 |
| 1 | 3.6 | 5.1 | 6.7 | | 5.6 | 6 | 0 | 6 | | | *5.6* | | 5.*3* | *1.*2 | 3.9 | 3.4 | 0.075 | 0.368 |
| 4 | 3.4 | 3.7 | 7.3 | | 5.8 | 6.9 | 0 | 4.7 | | | *3.4* | | 5.*1* | *1.*8 | 2.7 | 2.4 | 0.547 | 0.116 |
| 8 | 3.8 | 3.8 | 6.6 | | 6.6 | 7.3 | 0 | 6.4 | | | *4.5* | | *5.*2 | 1.*7* | *3.*6 | 3.3 | 0.238 | 0.290 |
| 24 | 3.7 | 3 | 5.8 | | 5.4 | 5.7 | 1.7 | 6.3 | | 5.1 | *4.3* | | 4.5 | 1.3 | *4.1* | 1.9 | 0.437 | 0.741 |
| 48 | 3 | 2.3 | 5.41 | | 4.1 | 6.3 | 2.6 | 5.1 | | 5.1 | *6.8* | | *3.7* | 1.7 | 4.8 | 1.7 | 0.895 | 0.568 |
| 72 | 2.6 | 2.4 | 4.9 | | 4.6 | 4.5 | 3.6 | 9.1 | | | 8.1 | | 3.6 | 1.2 | 6.9 | 2.9 | 0.126 | 0.071 |
| | | | | | | | | | ttest0vs72 | | | | | 0.09 | | 0.591 | | |

### 12.1.11 Oxygen carriage capacity

| | 1 | 2 | 5 | 6 | 10 | 12 | 3 | 4 | 7 | 8 | 9 | 11 | pRBCT | SD | Oxyglobin® | SD | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **0** | 1.3 | *3.5* | 5.4 | | 5.6 | | *4.1* | *4.6* | | | | | **4.0** | *2.0* | **4.3** | 0.4 | 0.272 | 0.736 |
| **1** | 2.3 | *4.1* | 5.6 | | 4.2 | 6.9 | *4.2* | *3.5* | | | *4.8* | | **4.6** | *1.7* | **4.2** | 0.7 | 0.254 | 0.620 |
| **4** | 3.5 | *8.8* | 6.8 | | 8 | 11.6 | *9.3* | *5.8* | | | *5.7* | | **7.7** | *3.0* | **6.9** | 2.1 | 0.705 | 0.670 |
| **8** | 4.3 | *13.7* | 10.6 | | 7 | 10 | *5.2* | *4.5* | | | *5.4* | | **9.1** | *3.6* | **5.0** | 0.5 | 0.034 | 0.063 |
| **24** | 3.8 | *14.9* | 10.3 | | 7.2 | 11.2 | *2.7* | *4.7* | | 6.9 | *3.1* | | **9.5** | *4.2* | **4.4** | 1.9 | 0.226 | 0.052 |
| **48** | 3.3 | *6.7* | 11.8 | | 8.8 | 10.5 | *3* | 6.6 | | 12.7 | *4* | | **8.2** | *3.3* | **6.6** | 4.4 | 0.611 | 0.558 |
| **72** | 4 | *2.3* | 11.9 | | 12.3 | 13.1 | *1.9* | *7.7* | | | 8.3 | | **8.7** | *5.1* | **6.0** | 3.5 | 0.692 | 0.407 |
| | | | | | | | | | | | | | **ttest** | **0.111** | | **0.509** | | |

### 12.1.12 Acid-base

| Pat | t= | $HCO_3^-a$ | $HCO_3^-v$ | $BE_a$ | $BE_v$ | $AG_a$ | $AG_v$ |
|---|---|---|---|---|---|---|---|
| pRBCT 1 | 0 | 12.1 | 12 | -11.8 | -19.5 | | |
| 1 | 1 | 13.7 | 14.5 | -10.5 | -10.6 | | |
| 1 | 4 | 13.9 | 16.4 | -9.7 | -7.9 | | |
| 1 | 8 | 15.9 | 17.8 | -7.7 | -6.6 | | |
| 1 | 24 | 15.6 | 17.9 | -9 | -6.2 | | |
| 1 | 48 | 18.9 | 19.7 | -5.4 | -5 | 10.50 | |
| 1 | 72 | 19.3 | 20.2 | -5 | -4.7 | | |
| pRBCT 2 | 0 | 14.8 | 14.8 | -9.1 | -9.6 | | |
| 2 | 1 | 12.7 | 14.3 | -11.6 | -10.3 | | |
| 2 | 4 | 14.8 | 15.1 | -9.6 | -9.1 | | |
| 2 | 8 | 14.9 | 16.2 | -9.5 | -8.7 | | |
| 2 | 24 | 16.6 | 14.4 | -8.3 | -10.6 | | |
| 2 | 48 | 16.4 | 20.7 | -9.1 | -4 | | |
| 2 | 72 | 14 | 17.1 | -10.7 | -8 | | |
| Oxy 3 | 0 | 14.7 | 15.5 | -8.8 | -8.4 | | |
| 3 | 1 | 14.2 | 14.1 | -9.8 | -10.5 | | |
| 3 | 4 | 13.2 | 14.1 | -10.2 | -9.8 | | |
| 3 | 8 | 12.3 | 13.4 | -11 | -10.3 | | |
| 3 | 24 | 15.9 | 17.2 | -8.5 | -7.6 | | |
| 3 | 48 | 18.8 | 19.4 | -5.5 | -5.2 | | |
| 3 | 72 | 17.2 | 19.4 | -7.9 | -5.9 | | |
| Oxy 4 | 0 | 16.9 | 18 | -6.5 | -6 | 4.00 | 3.70 |
| 4 | 1 | 16.1 | 18.3 | -7.3 | -4.4 | 2.40 | 2.20 |
| 4 | 4 | 17.3 | 16 | -5.3 | -8.1 | 3.30 | 5.00 |
| 4 | 8 | 16.4 | 14.9 | -7 | -9.4 | 5.90 | 8.50 |
| 4 | 24 | 18.2 | 17.6 | -5.7 | -7 | 2.20 | 3.00 |
| 4 | 48 | 18 | 20.6 | -5.3 | -4 | 6.00 | 4.40 |
| 4 | 72 | 18.5 | 13.9 | -5.5 | -11.5 | 9.90 | 6.50 |
| pRBCT 5 | 0 | 17.5 | 20.7 | -6.2 | -3.7 | 13.20 | 10.30 |
| 5 | 1 | 14.8 | 19.5 | -9.1 | -5.3 | 9.40 | 10.90 |
| 5 | 4 | 17.6 | 20.1 | -6.6 | -4.2 | 9.50 | 9.80 |
| 5 | 8 | 17.9 | 18.9 | -6.2 | -6 | 13.60 | 11.60 |
| 5 | 24 | 18 | 21.3 | -5.7 | -3.3 | 8.40 | 9.80 |
| 5 | 48 | 18.4 | 21.1 | -4.7 | -3.5 | 12.80 | 12.20 |
| 5 | 72 | 17.9 | 20.5 | -5.4 | -3.9 | 10.20 | 13.90 |
| pRBCT 6 | 0 | 18 | 18.3 | -3.6 | -3.4 | -1.80 | 0.00 |
| 6 | 1 | 18.7 | 19.6 | -4 | -3.8 | -0.60 | -1.30 |
| 6 | 4 | 18.8 | 19.7 | -3.5 | -2.5 | -0.70 | 2.30 |
| 6 | 8 | 19 | 18.9 | -3.6 | -4.3 | 4.50 | 7.50 |
| 6 | 24 | 17.9 | 19.7 | -5.1 | -4.6 | 4.60 | 5.60 |
| 6 | 48 | 17.5 | 15.4 | -5.7 | -7.2 | 10.30 | 9.60 |
| 6 | 72 | 19.6 | 22.2 | -3.4 | -2.3 | 10.30 | 8.60 |
| Oxy 7 | 0 | 23.4 | 24.9 | 1.1 | 1.5 | -0.30 | 4.20 |
| 7 | 1 | 23.7 | 25 | 1.3 | 1.4 | -0.30 | 0.80 |
| 7 | 4 | 24.9 | 26.5 | 3.5 | 3.9 | 2.40 | -0.60 |
| 7 | 8 | 24.3 | 24 | 2.7 | 1.8 | 4.50 | 4.70 |
| 7 | 24 | 21.6 | 22.2 | 0.3 | -0.3 | -1.40 | 1.50 |
| 7 | 48 | 18.7 | 19.9 | -4.1 | -4.8 | 0.10 | 2.20 |
| 7 | 72 | 21.5 | 22 | -1.8 | -2.3 | 0.40 | 0.40 |
| Oxy 8 | 0 | 17.1 | 17 | -7.7 | -7.5 | | |
| 8 | 1 | 16.7 | 16.9 | -8.5 | -8.7 | | |
| 8 | 4 | 17.3 | 19.8 | -7.2 | -5.3 | | |
| 8 | 8 | 21 | 21.4 | -3.5 | -3.6 | | |
| 8 | 24 | 22.3 | 23.1 | -2.1 | -1.8 | | |
| 8 | 48 | 19.7 | 23.7 | -4.1 | -1.2 | 9.90 | 7.70 |
| 8 | 72 | 21 | 23.8 | -2 | -0.8 | 10.60 | 8.80 |
| Oxy 9 | 0 | 6.8 | 7.4 | -15.7 | -17.2 | 27.00 | 26.40 |
| 9 | 1 | 8.1 | 8.8 | -16 | -16.5 | 23.80 | 23.50 |
| 9 | 4 | 12.7 | 13.3 | -10.6 | -10.6 | 16.10 | 13.80 |
| 9 | 8 | 15.3 | 15.7 | -7.5 | -7.9 | 9.30 | 8.80 |
| 9 | 24 | 13.3 | 13.9 | -10.1 | -10 | 7.60 | 6.40 |
| 9 | 48 | 13.9 | 14.1 | -9.7 | -10.3 | 6.70 | 3.40 |
| 9 | 72 | 17.7 | 17 | -5.5 | -7.7 | 8.40 | 9.00 |
| pRBCT 10 | 0 | 16.8 | 17.2 | -6.5 | -5.6 | 10.40 | 10.40 |
| 10 | 1 | 16.2 | 15.6 | -7.7 | -8.7 | 11.10 | 13.00 |
| 10 | 4 | 17.2 | 16.7 | -6.5 | -7.4 | 10.90 | 9.40 |
| 10 | 8 | 16.7 | 18.7 | -7.5 | -5.9 | 13.00 | 9.90 |
| 10 | 24 | 15.5 | 18.1 | -8.5 | -6.3 | 10.5 | 9.2 |
| 10 | 48 | 14 | 17.9 | -10 | -7.7 | 9.10 | 7.00 |
| 10 | 72 | 18.7 | 18.6 | -4.8 | -5.6 | 12.90 | 12.90 |
| Oxy 11 | 0 | 8.5 | 9.2 | -12.4 | -13.6 | 13.40 | 15.10 |
| 11 | 1 | 10.1 | 10 | -11.6 | -12.9 | 11.00 | 12.60 |
| 11 | 4 | 9.1 | 13.5 | -11.4 | -12.9 | 11.4 | 12.3 |
| 11 | 8 | 9.6 | 13.6 | -11.2 | -12.4 | 13.50 | 14.80 |
| 11 | 24 | | | | | | |
| 11 | 48 | | | | | | |
| 11 | 72 | | | | | | |
| pRBCT 12 | 0 | 16 | 16.9 | -5.9 | -6.3 | 13.10 | 12.90 |
| 12 | 1 | 16.7 | 19 | -7 | -5.8 | 6.50 | 6.50 |
| 12 | 4 | 18.6 | 18.4 | -4.5 | -5.4 | 10.00 | 7.80 |
| 12 | 8 | 19 | 18.8 | -4.6 | -5.3 | 10.00 | 10.90 |
| 12 | 24 | 19.7 | 19.3 | -4 | -5.2 | 11.00 | 11.90 |
| 12 | 48 | 19.4 | 19.3 | -3.5 | -4.7 | 13.30 | 11.00 |
| 12 | 72 | 20.3 | 23.4 | -3.1 | -1.4 | 11.60 | 10.60 |

### 12.1.12.1 BICARBONATE T-TESTS

| | t= | 0 | 1 | 4 | 8 | 24 | 48 | 72 |
|---|---|---|---|---|---|---|---|---|
| pRBCT | 1 | 12.1 | 13.7 | 13.9 | 15.9 | 15.6 | 18.9 | 19.3 |
| | 2 | 14.8 | 12.7 | 14.8 | 14.9 | 16.6 | 16.4 | 14 |
| | 5 | 17.5 | 14.8 | 17.6 | 17.9 | 18 | 18.4 | 17.9 |
| | 6 | 18 | 18.7 | 18.8 | 19 | 17.9 | 17.5 | 19.6 |
| | 10 | 16.8 | 16.2 | 17.2 | 16.7 | 15.5 | 14 | 18.7 |
| | 12 | 16 | 16.7 | 18.6 | 19 | 19.7 | 19.4 | 20.3 |
| | mean | 15.9 | 15.5 | 16.8 | 17.2 | 17.2 | 17.4 | 18.3 |
| | st dev | 2.2 | 2.2 | 2.0 | 1.7 | 1.6 | 2.0 | 2.3 |
| | min | 12.1 | 12.7 | 13.9 | 14.9 | 15.5 | 14 | 14 |
| | max | 18 | 18.7 | 18.8 | 19 | 19.7 | 19.4 | 20.3 |
| | | | | | | t test 0vs72 | | 0.086 |
| | | | | | | | | |
| Oxyglobin® | 3 | 14.7 | 14.2 | 13.2 | 12.3 | 15.9 | 18.8 | 17.2 |
| | 4 | 16.9 | 16.1 | 17.3 | 16.4 | 18.2 | 18 | 18.5 |
| | 7 | 23.4 | 23.7 | 24.9 | 24.3 | 21.6 | 18.7 | 21.5 |
| | 8 | 17.1 | 16.7 | 17.3 | 21 | 22.3 | 19.7 | 21 |
| | 9 | 6.8 | 8.1 | 12.7 | 15.3 | 13.3 | 13.9 | 17.7 |
| | 11 | 8.5 | 10.1 | 9.1 | 9.6 | | | |
| | mean | 14.6 | 14.8 | 15.8 | 16.5 | 18.3 | 17.8 | 19.2 |
| | stdev | 6.1 | 5.5 | 5.5 | 5.4 | 3.8 | 2.3 | 2.0 |
| | min | 6.8 | 8.1 | 9.1 | 9.6 | 13.3 | 13.9 | 17.2 |
| | max | 23.4 | 23.7 | 24.9 | 24.3 | 22.3 | 19.7 | 21.5 |
| | Ftest | 0.040 | 0.063 | 0.048 | 0.022 | 0.091 | 0.763 | 0.802 |
| | ttest | 0.640 | 0.796 | 0.668 | 0.758 | 0.591 | 0.774 | 0.506 |
| | | | | | | t test 0vs72 | | 0.130 |

### 12.1.12.2 SODIUM DATA & STATISTICAL ANALYSES

Type 3 tests

| | t= | 0 | 1 | 4 | 8 | 24 | 48 | 72 |
|---|---|---|---|---|---|---|---|---|
| | | | 1 | 1 | 1 | 1 | 1 | 1 |
| **pRBCT** | **1** | 136.0 | 136.0 | 137.0 | 139.0 | 143.0 | 143.0 | 142.0 |
| | **2** | 137.0 | 138.0 | 139.0 | 140.0 | 142.0 | 140.0 | 139.0 |
| | **5** | 145.2 | 139.9 | 140.5 | 146.5 | 142.5 | 142.8 | 141.8 |
| | **6** | 134.1 | 136.9 | 135.0 | 142.4 | 144.4 | 147.9 | 147.6 |
| | **10** | 140.1 | 141.5 | 142.9 | 144.1 | 144.6 | 138.5 | 143.0 |
| | **12** | 139.5 | 137.8 | 143.0 | 142.7 | 143.3 | 140.9 | 142.3 |
| | **mean** | **138.7** | **138.4** | **139.6** | **142.5** | **143.3** | **142.2** | **142.6** |
| | **st dev** | **3.9** | **2.0** | **3.2** | **2.7** | **1.0** | **3.3** | **2.8** |
| | **min** | 134.1 | 136.0 | 135.0 | 139.0 | 142.0 | 138.5 | 139.0 |
| | **max** | 145.2 | 141.5 | 143.0 | 146.5 | 144.6 | 147.9 | 147.6 |
| | | | | | | | ttest | **0.074** |
| **Oxyglobin®** | **3** | 140.0 | 141.0 | 141.0 | 141.0 | 142.0 | 144.0 | 144.0 |
| | **4** | 140.2 | 139.1 | 141.0 | 140.6 | 142.6 | 141.2 | 141.0 |
| | **7** | 126.7 | 127.2 | 132.1 | 135.7 | 130.8 | 141.8 | 142.0 |
| | **8** | 143.0 | 142.0 | 142.0 | 143.0 | 137.9 | 135.0 | 147.4 |
| | **9** | 137.2 | 137.4 | 140.3 | 139.8 | 143.2 | 139.1 | 146.0 |
| | **11** | 143.2 | 143.4 | 142.8 | 143.6 | | | |
| | **mean** | **138.4** | **138.4** | **139.9** | **140.6** | **139.3** | **140.2** | **144.1** |
| | **st dev** | **6.1** | **5.9** | **3.9** | **2.8** | **5.2** | **3.4** | **2.7** |
| | **min** | 126.7 | 127.2 | 132.1 | 135.7 | 130.8 | 135.0 | 141.0 |
| | **max** | 143.2 | 143.4 | 142.8 | 143.6 | 143.2 | 144.0 | 147.4 |
| | **Ftest** | **0.343** | **0.035** | **0.678** | **0.945** | **0.003** | **0.913** | **0.953** |
| | **ttest** | **0.931** | **1.000** | **0.887** | **0.278** | **0.161** | **0.359** | **0.400** |
| | | | | | | | ttest | **0.079** |

### 12.1.12.3 CORRECTED CHLORIDE DATA & STATISTICAL ANALYSES

| | t= | 0 | 1 | 4 | 8 | 24 | 48 | 72 |
|---|---|---|---|---|---|---|---|---|
| **pRBCT** | **1** | | | | | | | |
| | **2** | | | | | | | |
| | **5** | 119.7 | 125.2 | 122.6 | 119.6 | 124.0 | 118.6 | 121.5 |
| | **6** | 132.8 | 131.2 | 130.9 | 126.1 | 127.4 | 122.4 | 117.7 |
| | **10** | 121.9 | 121.8 | 121.6 | 120.6 | 124.2 | 126.5 | 118.4 |
| | **12** | 120.4 | 126.1 | 121.5 | 120.7 | 119.2 | 117.1 | 118.0 |
| | mean | **123.7** | **126.1** | **124.1** | **121.7** | **123.7** | **121.1** | **118.9** |
| | std dev | **6.2** | **3.9** | **4.5** | **2.9** | **3.4** | **4.2** | **1.8** |
| | min | 119.7 | 121.8 | 121.5 | 119.6 | 119.2 | 117.1 | 117.7 |
| | max | 132.8 | 131.2 | 130.9 | 126.1 | 127.4 | 126.5 | 121.5 |
| | | | | | | | t0vs72 | **0.186** |
| | **3** | | | | | | | |
| | **4** | 129.1 | 131.2 | 129.4 | 127.7 | 130.0 | 126.1 | 122.2 |
| **Oxyglobin®** | **7** | 123.3 | 122.8 | 119.4 | 118.3 | 127.2 | 128.7 | 127.5 |
| | **8** | | | | | 133.4 | 126.5 | 119.9 |
| | **9** | 113.9 | 115.8 | 119.7 | 123.2 | 127.4 | 128.1 | 123.0 |
| | **11** | 127.4 | 128.3 | 128.8 | 126.1 | | | |
| | mean | **123.4** | **124.5** | **124.3** | **123.8** | **129.5** | **127.4** | **123.1** |
| | st dev | **6.8** | **6.8** | **5.6** | **4.1** | **2.9** | **1.2** | **3.2** |
| | min | 113.9 | 115.8 | 119.4 | 118.3 | 127.2 | 126.1 | 119.9 |
| | max | 129.1 | 131.2 | 129.4 | 127.7 | 133.4 | 128.7 | 127.5 |
| | Ftest | **0.869** | **0.388** | **0.740** | **0.601** | **0.798** | **0.071** | **0.349** |
| | ttest | **0.957** | **0.709** | **0.961** | **0.439** | **0.039** | **0.030** | **0.060** |
| | | | | | | | t0vs72 | **0.939** |

### 12.1.12.4 CHLORIDE GAP DATA & STATISTICAL ANALYSES

| | t= | 0 | 1 | 4 | 8 | 24 | 48 | 72 |
|---|---|---|---|---|---|---|---|---|
| pRBCT | 1 | | | | | | | |
| | 2 | | | | | | | |
| | 5 | -0.7 | -5.2 | -4.6 | 0.4 | -3.0 | -2.6 | -3.5 |
| | 6 | -10.8 | -8.2 | -9.9 | -3.1 | -1.4 | 1.6 | 1.3 |
| | 10 | -4.9 | -3.8 | -2.6 | -1.6 | -1.2 | -6.5 | -2.4 |
| | 12 | -5.4 | -7.1 | -2.5 | -2.7 | -2.2 | -4.1 | -3.0 |
| | **mean** | **-5.4** | **-6.1** | **-4.9** | **-1.7** | **-1.9** | **-2.9** | **-1.9** |
| | **SD** | **4.2** | **2.0** | **3.5** | **1.6** | **0.8** | **3.4** | **2.2** |
| | **min** | -10.8 | -8.2 | -9.9 | -3.1 | -3.0 | -6.5 | -3.5 |
| | **max** | -0.7 | -3.8 | -2.5 | 0.4 | -1.2 | 1.6 | 1.3 |
| | | | | | | **t0vst72 type 3** | | 0.199 |
| Oxyglobin® | 3 | | | | | | | |
| | 4 | -5.1 | -6.2 | -4.4 | -4.7 | -3.0 | -4.1 | -4.2 |
| | 7 | -16.3 | -15.8 | -11.4 | -8.3 | -13.2 | -3.7 | -3.5 |
| | 8 | | | | | -7.4 | -9.5 | 1.1 |
| | 9 | -6.9 | -6.8 | -4.7 | -5.2 | -2.4 | -6.1 | 0.0 |
| | 11 | -2.4 | -2.3 | -2.8 | -2.1 | | | |
| | **mean** | **-7.7** | **-7.8** | **-5.8** | **-5.1** | **-6.5** | **-5.9** | **-1.6** |
| | **SD** | **6.0** | **5.7** | **3.8** | **2.6** | **5.0** | **2.7** | **2.6** |
| | **min** | -16.3 | -15.8 | -11.4 | -8.3 | -13.2 | -9.5 | -4.2 |
| | **max** | -2.4 | -2.3 | -2.8 | -2.1 | -2.4 | -3.7 | 1.1 |
| | **Ftest** | **0.561** | **0.112** | **0.885** | **0.445** | **0.014** | **0.694** | **0.773** |
| | **t test** | **0.566** | **0.602** | **0.728** | **0.078** | **0.163** | **0.222** | **0.877** |
| | | | | | | **t0vst72 type 3** | | 0.608 |

### 12.1.12.5 DEFICITS OF EXPECTED RESPIRATORY COMPENSATORY RESPONSE

#### *12.1.12.5.1 Means and overall results*

| | | 0 | 1 | 4 | 8 | 24 | 48 | 72 | |
|---|---|---|---|---|---|---|---|---|---|
| pRBCT | 1 | -7.18 | -5.5 | -5.74 | -4.94 | -2.43 | -1.64 | -1.72 | |
| | 2 | -4.27 | -4.6 | -1.97 | -3.24 | -0.93 | 3.31 | -3.21 | |
| | 5 | -0.86 | -2.77 | 2.47 | 0.76 | -1.21 | -2.79 | -1.24 | |
| | 6 | -4.31 | -0.9 | -0.27 | -1.11 | -1.64 | -4.46 | -1.53 | Ttest |
| | 10 | -4.37 | -2.35 | -0.15 | -0.6 | -1.26 | -3.01 | -0.3 | 0vs72 |
| | 12 | -3.61 | -2.7 | -0.33 | 0.29 | 0.2 | -0.79 | -0.02 | 0.02 |
| | mean | **-4.1** | **-3.137** | **-0.998** | **-1.473** | **-1.212** | **-1.563** | **-1.337** | |
| | stdev | **2.0184** | **1.6537** | **2.7259** | **2.1968** | **0.8651** | **2.696** | **1.1412** | |
| | min | -7.18 | -5.5 | -5.74 | -4.94 | -2.43 | -4.46 | -3.21 | |
| | max | -0.86 | -0.9 | 2.47 | 0.76 | 0.2 | 3.31 | -0.02 | |
| Oxyglobin® | **3** | -6.3 | -4.85 | -7.85 | -7.82 | -1.44 | -0.77 | -0.15 | |
| | **4** | -4.24 | -5.68 | -6.02 | -4.49 | -2.35 | -4.31 | 0.04 | |
| | **7** | -0.19 | 0.6 | -1.94 | -0.62 | -2.93 | -1.1 | 0.64 | |
| | **8** | 0.52 | -0.5 | -2.22 | 0.69 | 2.98 | 1 | -0.61 | Ttest |
| | **9** | -7.97 | -8.98 | -6.8 | -7.72 | -8.42 | -6.54 | -3.6 | 0vs72 |
| | **11** | -7.96 | -5.68 | -8.18 | -7.63 | | | | 0.07 |
| | mean | **-4.357** | **-4.182** | **-5.502** | **-4.598** | **-2.432** | **-2.344** | **-0.736** | |
| | stdev | **3.7676** | **3.5883** | **2.7603** | **3.8241** | **4.0738** | **3.0282** | **1.6626** | |
| | min | -7.97 | -8.98 | -8.18 | -7.82 | -8.42 | -6.54 | -3.6 | |
| | max | 0.52 | 0.6 | -1.94 | 0.69 | 2.98 | 1 | 0.64 | |
| | **F test** | 0.197 | 0.114 | 0.979 | 0.249 | 0.004 | 0.788 | 0.430 | |
| | **ttests** | 0.887 | 0.538 | 0.017 | 0.121 | 0.544 | 0.666 | 0.516 | Type 3 |

#### *12.1.12.5.2 Calculation array for compensatory deficit*

| | | | |
|---|---|---|---|
| normal pCO2 | mmHg | | 31.5 |
| normal HCO3 | mmol/L | | 18.7 |
| patient pCO2 | | | x |
| patient HCO3 | | | y |
| base deficit | | | 18.7 - y |
| creates a predicted respiratory alkalosis of | | | z |
| Calculation | | | (18.7 -y)*0.7 |
| actual pCO2 differential | 31.5-x | = | |
| difference between actual and predicted | z-p | = | |

# Appendices

| patient | time | x | y | z | p | q | | | patient | time | x | y | z | p | q | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | pCO2 | HCO3 | predicted | actual diff | differential | | | | | pCO2 | HCO3 | predicted | actual diff | differential | | |
| pRBCT | | | | | | | mean | stdev | pRBCT | | | | | | | mean | stdev |
| 1 | 0 | 19.7 | 12.1 | 4.62 | 11.8 | -7.18 | | | 3 | 0 | 22.4 | 14.7 | 2.8 | 9.1 | -6.3 | | |
| 2 | 0 | 24.5 | 14.8 | 2.73 | 7 | -4.27 | | | 4 | 0 | 26 | 16.9 | 1.26 | 5.5 | -4.24 | | |
| 5 | 0 | 29.8 | 17.5 | 0.84 | 1.7 | -0.86 | | | 7 | 0 | 34.6 | 23.4 | -3.29 | -3.1 | -0.19 | | |
| 6 | 0 | 26.7 | 18 | 0.49 | 4.8 | -4.31 | | | 8 | 0 | 30.9 | 17.1 | 1.12 | 0.6 | 0.52 | | |
| 10 | 0 | 25.8 | 16.8 | 1.33 | 5.7 | -4.37 | | | 9 | 0 | 15.2 | 6.8 | 8.33 | 16.3 | -7.97 | | |
| 12 | 0 | 26 | 16 | 1.89 | 5.5 | -3.61 | -4.1 | 2.0 | 11 | 0 | 16.4 | 8.5 | 7.14 | 15.1 | -7.96 | -4.4 | 3.8 |
| 1 | 1 | 22.5 | 13.7 | 3.5 | 9 | -5.5 | | | 3 | 1 | 23.5 | 14.2 | 3.15 | 8 | -4.85 | | |
| 2 | 1 | 22.7 | 12.7 | 4.2 | 8.8 | -4.6 | | | 4 | 1 | 24 | 16.1 | 1.82 | 7.5 | -5.68 | | |
| 5 | 1 | 26 | 14.8 | 2.73 | 5.5 | -2.77 | | | 7 | 1 | 35.6 | 23.7 | -3.5 | -4.1 | 0.6 | | |
| 6 | 1 | 30.6 | 18.7 | 0 | 0.9 | -0.9 | | | 8 | 1 | 29.6 | 16.7 | 1.4 | 1.9 | -0.5 | | |
| 10 | 1 | 27.4 | 16.2 | 1.75 | 4.1 | -2.35 | | | 9 | 1 | 15.1 | 8.1 | 7.42 | 16.4 | -8.98 | | |
| 12 | 1 | 27.4 | 16.7 | 1.4 | 4.1 | -2.7 | -3.1 | 1.7 | 11 | 1 | 19.8 | 10.1 | 6.02 | 11.7 | -5.68 | -4.2 | 3.6 |
| 1 | 4 | 22.4 | 13.9 | 3.36 | 9.1 | -5.74 | | | 3 | 4 | 19.8 | 13.2 | 3.85 | 11.7 | -7.85 | | |
| 2 | 4 | 26.8 | 14.8 | 2.73 | 4.7 | -1.97 | | | 4 | 4 | 24.5 | 17.3 | 0.98 | 7 | -6.02 | | |
| 5 | 4 | 33.2 | 17.6 | 0.77 | -1.7 | 2.47 | | | 7 | 4 | 33.9 | 24.9 | -4.34 | -2.4 | -1.94 | | |
| 6 | 4 | 31.3 | 18.8 | -0.07 | 0.2 | -0.27 | | | 8 | 4 | 28.3 | 17.3 | 0.98 | 3.2 | -2.22 | | |
| 10 | 4 | 30.3 | 17.2 | 1.05 | 1.2 | -0.15 | | | 9 | 4 | 20.5 | 12.7 | 4.2 | 11 | -6.8 | | |
| 12 | 4 | 31.1 | 18.6 | 0.07 | 0.4 | -0.33 | -1.0 | 2.7 | 11 | 4 | 16.6 | 9.1 | 6.72 | 14.9 | -8.18 | -5.5 | 2.8 |
| 1 | 8 | 24.6 | 15.9 | 1.96 | 6.9 | -4.94 | | | 3 | 8 | 19.2 | 12.3 | 4.48 | 12.3 | -7.82 | | |
| 2 | 8 | 25.6 | 14.9 | 2.66 | 5.9 | -3.24 | | | 4 | 8 | 25.4 | 16.4 | 1.61 | 6.1 | -4.49 | | |
| 5 | 8 | 31.7 | 17.9 | 0.56 | -0.2 | 0.76 | | | 7 | 8 | 34.8 | 24.3 | -3.92 | -3.3 | -0.62 | | |
| 6 | 8 | 30.6 | 19 | -0.21 | 0.9 | -1.11 | | | 8 | 8 | 33.8 | 21 | -1.61 | -2.3 | 0.69 | | |
| 10 | 8 | 29.5 | 16.7 | 1.4 | 2 | -0.6 | | | 9 | 8 | 21.4 | 15.3 | 2.38 | 10.1 | -7.72 | | |
| 12 | 8 | 32 | 19 | -0.21 | -0.5 | 0.29 | -1.5 | 2.2 | 11 | 8 | 17.5 | 9.6 | 6.37 | 14 | -7.63 | -4.6 | 3.8 |
| 1 | 24 | 26.9 | 15.6 | 2.17 | 4.6 | -2.43 | | | 3 | 24 | 28.1 | 15.9 | 1.96 | 3.4 | -1.44 | | |
| 2 | 24 | 29.1 | 16.6 | 1.47 | 2.4 | -0.93 | | | 4 | 24 | 28.8 | 18.2 | 0.35 | 2.7 | -2.35 | | |
| 5 | 24 | 29.8 | 18 | 0.49 | 1.7 | -1.21 | | | 7 | 24 | 30.6 | 21.6 | -2.03 | 0.9 | -2.93 | | |
| 6 | 24 | 29.3 | 17.9 | 0.56 | 2.2 | -1.64 | | | 8 | 24 | 37 | 22.3 | -2.52 | -5.5 | 2.98 | | |
| 10 | 24 | 28 | 15.5 | 2.24 | 3.5 | -1.26 | | | 9 | 24 | 19.3 | 13.3 | 3.78 | 12.2 | -8.42 | | |
| 12 | 24 | 32.4 | 19.7 | -0.7 | -0.9 | 0.2 | -1.2 | 0.9 | 11 | 24 | | | 13.09 | 31.5 | | -2.4 | 4.1 |
| 1 | 48 | 30 | 18.9 | -0.14 | 1.5 | -1.64 | | | 3 | 48 | 30.8 | 18.8 | -0.07 | 0.7 | -0.77 | | |
| 2 | 48 | 33.2 | 16.4 | 1.61 | -1.7 | 3.31 | | | 4 | 48 | 26.7 | 18 | 0.49 | 4.8 | -4.31 | | |
| 5 | 48 | 28.5 | 18.4 | 0.21 | 3 | -2.79 | | | 7 | 48 | 30.4 | 18.7 | 0 | 1.1 | -1.1 | | |
| 6 | 48 | 26.2 | 17.5 | 0.84 | 5.3 | -4.46 | | | 8 | 48 | 33.2 | 19.7 | -0.7 | -1.7 | 1 | | |
| 10 | 48 | 25.2 | 14 | 3.29 | 6.3 | -3.01 | | | 9 | 48 | 21.6 | 13.9 | 3.36 | 9.9 | -6.54 | | |
| 12 | 48 | 31.2 | 19.4 | -0.49 | 0.3 | -0.79 | -1.6 | 2.7 | 11 | 48 | | | 13.09 | 31.5 | | -2.3 | 3.0 |
| 1 | 72 | 30.2 | 19.3 | -0.42 | 1.3 | -1.72 | | | 3 | 72 | 30.3 | 17.2 | 1.05 | 1.2 | -0.15 | | |
| 2 | 72 | 25 | 14 | 3.29 | 6.5 | -3.21 | | | 4 | 72 | 31.4 | 18.5 | 0.14 | 0.1 | 0.04 | | |
| 5 | 72 | 29.7 | 17.9 | 0.56 | 1.8 | -1.24 | | | 7 | 72 | 34.1 | 21.5 | -1.96 | -2.6 | 0.64 | | |
| 6 | 72 | 30.6 | 19.6 | -0.63 | 0.9 | -1.53 | | | 8 | 72 | 32.5 | 21 | -1.61 | -1 | -0.61 | | |
| 10 | 72 | 31.2 | 18.7 | 0 | 0.3 | -0.3 | | | 9 | 72 | 27.2 | 17.7 | 0.7 | 4.3 | -3.6 | | |
| 12 | 72 | 32.6 | 20.3 | -1.12 | -1.1 | -0.02 | -1.3 | 1.1 | 11 | 72 | | | 13.09 | 31.5 | | -0.7 | 1.7 |
| mean deficit discrepancy | | | | | | -2.0 | | | | | | | | | -3.6 | | |
| mean deficit discrepancy st dev | | | | | | 2.2 | | | | | | | | | 3.4 | | |

| | t= | 0 | 1 | 4 | 8 | 24 | 48 | 72 |
|---|---|---|---|---|---|---|---|---|
| **pRBCT** | **1** | -7.18 | -5.5 | -5.74 | -4.94 | -2.43 | -1.64 | -1.72 |
| | **2** | -4.27 | -4.6 | -1.97 | -3.24 | -0.93 | 3.31 | -3.21 |
| | **5** | -0.86 | -2.77 | 2.47 | 0.76 | -1.21 | -2.79 | -1.24 |
| | **6** | -4.31 | -0.9 | -0.27 | -1.11 | -1.64 | -4.46 | -1.53 |
| | **10** | -4.37 | -2.35 | -0.15 | -0.6 | -1.26 | -3.01 | -0.3 |
| | **12** | -3.61 | -2.7 | -0.33 | 0.29 | 0.2 | -0.79 | -0.02 |
| | **mean** | **-4.1** | **-3.137** | **-0.998** | **-1.473** | **-1.212** | **-1.563** | **-1.337** |
| | **stdev** | **2.018** | **1.654** | **2.726** | **2.197** | **0.865** | **2.696** | **1.141** |
| | **min** | -7.18 | -5.5 | -5.74 | -4.94 | -2.43 | -4.46 | -3.21 |
| | **max** | -0.86 | -0.9 | 2.47 | 0.76 | 0.2 | 3.31 | -0.02 |
| | | | | | | Ttest 0vs72 | | **0.020** |
| **Oxyglobin®** | **3** | -6.3 | -4.85 | -7.85 | -7.82 | -1.44 | -0.77 | -0.15 |
| | **4** | -4.24 | -5.68 | -6.02 | -4.49 | -2.35 | -4.31 | 0.04 |
| | **7** | -0.19 | 0.6 | -1.94 | -0.62 | -2.93 | -1.1 | 0.64 |
| | **8** | 0.52 | -0.5 | -2.22 | 0.69 | 2.98 | 1 | -0.61 |
| | **9** | -7.97 | -8.98 | -6.8 | -7.72 | -8.42 | -6.54 | -3.6 |
| | **11** | -7.96 | -5.68 | -8.18 | -7.63 | | | |
| | **mean** | **-4.357** | **-4.182** | **-5.502** | **-4.598** | **-2.432** | **-2.344** | **-0.736** |
| | **stdev** | **3.768** | **3.588** | **2.760** | **3.824** | **4.074** | **3.028** | **1.663** |
| | **min** | -7.97 | -8.98 | -8.18 | -7.82 | -8.42 | -6.54 | -3.6 |
| | **max** | 0.52 | 0.6 | -1.94 | 0.69 | 2.98 | 1 | 0.64 |
| | **F test** | **0.197** | **0.114** | **0.979** | **0.249** | **0.004** | **0.788** | **0.430** |
| | **ttests** | **0.887** | **0.538** | **0.017** | **0.121** | **0.544** | **0.666** | **0.516** |
| | | | | | | Ttest 0vs72 | | **0.071** |

#### 12.1.12.6 STRONG ION DIFFERENCE (SID) STATISTICAL DATA

All test type 2 since all *F*-values >0.05. Normal values in green boxes.

| | TIME: | 0 | 1 | 4 | 8 | 24 | 48 | 72 |
|---|---|---|---|---|---|---|---|---|
| | **1** | | | | | | | |
| **pRBCT** | **2** | | | | | | | |
| | **5** | 25.5 | 14.7 | 17.9 | 26.9 | 18.5 | 24.2 | 20.3 |
| | **6** | 1.3 | 5.7 | 4.1 | 16.3 | 17.0 | 25.5 | 29.9 |
| | **10** | 18.2 | 19.7 | 21.3 | 23.5 | 20.4 | 12.0 | 24.6 |
| | **12** | 19.1 | 11.7 | 21.5 | 22.0 | 24.1 | 23.8 | 24.3 |
| | **mean** | **16.0** | **13.0** | **16.2** | **22.2** | **20.0** | **21.4** | **24.8** |
| | **std dev** | **10.4** | **5.9** | **8.2** | **4.4** | **3.1** | **6.3** | **3.9** |
| | min | 1.3 | 5.7 | 4.1 | 16.3 | 17.0 | 12.0 | 20.3 |
| | max | 25.5 | 19.7 | 21.5 | 26.9 | 24.1 | 25.5 | 29.9 |
| | | | | | | | t0vs72 | **0.166** |
| | **3** | | | | | | | |
| **Oxyglobin®** | **4** | 11.1 | 7.9 | 11.6 | 12.9 | 12.6 | 15.1 | 18.8 |
| | **7** | 3.4 | 4.4 | 12.7 | 17.4 | 3.6 | 13.1 | 14.5 |
| | **8** | | | | | 4.5 | 8.5 | 27.5 |
| | **9** | 23.3 | 21.6 | 20.6 | 16.6 | 15.8 | 11.0 | 23.0 |
| | **11** | 15.8 | 15.1 | 14.0 | 17.5 | | | |
| | **mean** | **13.4** | **12.2** | **14.7** | **16.1** | **9.1** | **11.9** | **21.0** |
| | **st dev** | **8.4** | **7.7** | **4.1** | **2.2** | **6.0** | **2.8** | **5.6** |
| | min | 3.4 | 4.4 | 11.6 | 12.9 | 3.6 | 8.5 | 14.5 |
| | max | 23.3 | 21.6 | 20.6 | 17.5 | 15.8 | 15.1 | 27.5 |
| | **Ftest** | **0.732** | **0.670** | **0.276** | **0.273** | **0.297** | **0.221** | **0.578** |
| | **ttest** | **0.705** | **0.886** | **0.757** | **0.049** | **0.018** | **0.034** | **0.309** |
| | | | | | | | t0vs72 | **0.183** |

#### 12.1.12.7 FREE WATER ABNORMALITY STATISTICAL DATA

Values outside the reference range highlighted in light green. Significant differences in yellow. All tests type 3.

| | t= | 0 | 1 | 4 | 8 | 24 | 48 | 72 |
|---|---|---|---|---|---|---|---|---|
| **pRBCT** | **1** | | | | | | | |
| | **2** | | | | | | | |
| | **5** | -0.31 | -0.71 | -0.79 | -0.09 | -0.57 | -0.69 | -0.72 |
| | **6** | -0.11 | -0.39 | -0.34 | -0.51 | -0.30 | 0.16 | 0.12 |
| | **10** | -0.85 | -0.74 | -0.59 | -0.46 | -0.33 | -0.69 | -0.67 |
| | **12** | -0.98 | -0.73 | -0.59 | -0.64 | -0.61 | -0.99 | -0.78 |
| | **mean** | **-0.56** | **-0.64** | **-0.58** | **-0.43** | **-0.45** | **-0.55** | **-0.51** |
| | **st dev** | **0.42** | **0.17** | **0.19** | **0.24** | **0.16** | **0.49** | **0.42** |
| | **min** | -1.0 | -0.7 | -0.8 | -0.6 | -0.6 | -1.0 | -0.8 |
| | **max** | -0.11 | -0.39 | -0.34 | -0.09 | -0.30 | 0.16 | 0.12 |
| | | | | | | | ttest | **0.864** |
| **Oxyglobin®** | **3** | | | | | | | |
| | **4** | -0.51 | -0.42 | -0.47 | -0.56 | -0.38 | -0.59 | -0.77 |
| | **7** | -0.47 | -0.59 | -1.29 | -1.33 | -0.39 | -0.46 | -0.49 |
| | **8** | | | | | -0.28 | -0.69 | 0.07 |
| | **9** | -1.56 | -1.41 | -0.94 | -0.81 | -0.41 | -0.59 | -0.16 |
| | **11** | -0.41 | -0.37 | -0.40 | -0.41 | | | |
| | **mean** | **-0.74** | **-0.70** | **-0.78** | **-0.78** | **-0.36** | **-0.59** | **-0.34** |
| | **st dev** | **0.55** | **0.48** | **0.42** | **0.41** | **0.06** | **0.09** | **0.37** |
| | **min** | -1.6 | -1.4 | -1.3 | -1.3 | -0.4 | -0.7 | -0.8 |
| | **max** | -0.41 | -0.37 | -0.40 | -0.41 | -0.28 | -0.46 | 0.07 |
| | **Ftest** | **0.664** | **0.115** | **0.214** | **0.396** | **0.136** | **0.022** | **0.831** |
| | **ttest** | **0.635** | **0.840** | **0.433** | **0.198** | **0.353** | **0.909** | **0.558** |
| | | | | | | | ttest | **0.278** |

### *12.1.12.7.1 Anion Gap data and analyses*

| | | 0 | 1 | 4 | 8 | 24 | 48 | 72 |
|---|---|---|---|---|---|---|---|---|
| **pRBCT** | **1** | | | | | | | |
| | **2** | | | | | | | |
| | **5** | 13.2 | 9.4 | 9.5 | 13.6 | 8.4 | 12.8 | 10.2 |
| | **6** | -1.8 | -0.6 | -0.7 | 4.5 | 4.6 | 10.3 | 10.3 |
| | **10** | 10.4 | 11.1 | 10.9 | 13 | 10.5 | 9.1 | 12.9 |
| | **12** | 13.1 | 6.5 | 10 | 10 | 11 | | |
| | **mean** | **8.7** | **6.6** | **7.4** | **10.3** | **8.6** | **10.7** | **11.1** |
| | **stdev** | **7.1** | **5.2** | **5.4** | **4.2** | **2.9** | **1.9** | **1.5** |
| | **min** | -1.8 | -0.6 | -0.7 | 4.5 | 4.6 | 9.1 | 10.2 |
| | **max** | 13.2 | 11.1 | 10.9 | 13.6 | 11.0 | 12.8 | 12.9 |
| | | | | | | T test 0vs72 | | **0.598** |
| **Oxyglobin®** | **3** | | | | | | | |
| | **4** | 4 | 2.4 | 3.3 | 5.9 | 2.2 | 6 | 9.9 |
| | **7** | -0.3 | -0.3 | 2.4 | 4.5 | -1.4 | 0.1 | 0.4 |
| | **8** | | | | | | 9.9 | 10.6 |
| | **9** | 27 | 23.8 | 16.1 | 9.3 | 7.6 | 6.7 | 8.4 |
| | **11** | 13.4 | 11 | 11.4 | 13.5 | | | |
| | **mean** | **11.0** | **9.2** | **8.3** | **8.3** | **2.8** | **5.7** | **7.3** |
| | **stdev** | **12.1** | **10.8** | **6.6** | **4.0** | **4.5** | **4.1** | **4.7** |
| | **min** | -0.3 | -0.3 | 2.4 | 4.5 | -1.4 | 0.1 | 0.4 |
| | **max** | 27.0 | 23.8 | 16.1 | 13.5 | 7.6 | 9.9 | 10.6 |
| | **Ftest** | **0.410** | **0.254** | **0.762** | **0.953** | **0.473** | **0.362** | **0.194** |
| | **ttest** | **0.757** | **0.683** | **0.845** | **0.520** | **0.140** | **0.088** | **0.208** |
| | | | | | | T test 0vs72 | | **0.589** |

### 12.1.13 Blood pressure

| Patient | Time | Systolic | | | | | | Diastolic | | | | | | Mean arterial pressure | | | | | | Pulse | Machine measured | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | Mean | 1 | 2 | 3 | 4 | 5 | Mean | 1 | 2 | 3 | 4 | 5 | Mean | | 1 | 2 | 3 | 4 | 5 | Mean |
| 1 | 0 15h30 | 137 | 131 | 141 | 138 | 143 | 138 | 34 | 50 | 51 | 49 | 45 | 45.8 | 68 | 82 | 96 | 84 | 61 | 78.2 | 128 | 137 | 138 | 134 | 130 | 130 | 133.8 |
| 1 | 1 16h30 | 116 | 131 | 108 | 129 | 117 | 120.2 | 69 | 61 | 44 | 47 | 53 | 54.8 | 86 | 82 | 51 | 65 | 66 | 70 | 120 | 128 | 131 | 126 | 125 | 125 | 127 |
| 1 | 4 19h30 | 82 | 106 | 133 | 134 | 106 | 112.2 | 61 | 70 | 56 | 80 | 77 | 68.8 | 72 | 88 | 89 | 89 | 88 | 85.2 | 100 | 93 | 112 | 75 | 76 | 78 | 86.8 |
| 1 | 8 23h30 | 120 | 130 | 128 | 120 | 123 | 124.2 | 64 | 59 | 71 | 60 | 71 | 65 | 89 | 78 | 92 | 93 | 85 | 87.4 | 110 | 93 | 99 | 97 | 98 | 88 | 95 |
| 1 | 24 15h30 | 130 | 133 | 113 | 135 | 139 | 130 | 59 | 39 | 68 | 49 | 44 | 51.8 | 76 | 88 | 82 | 113 | 89 | 89.6 | 110 | 107 | 105 | 105 | 109 | 116 | 108.4 |
| 1 | 48 15h30 | 118 | 127 | 118 | 121 | 109 | 118.6 | 60 | 49 | 39 | 64 | 48 | 52 | 92 | 66 | 79 | 95 | 74 | 81.2 | 110 | 97 | 105 | 111 | 106 | 108 | 105.4 |
| 1 | 72 15h30 | 98 | 108 | 104 | 106 | 77 | 98.6 | 57 | 56 | 42 | 37 | 37 | 45.8 | 74 | 66 | 52 | 66 | 53 | 62.2 | 88 | 84 | 80 | 89 | 89 | 79 | 84.2 |
| 2 | 0 20h30 | 130 | 122 | 139 | 120 | 125 | 127.2 | 46 | 60 | 49 | 53 | 46 | 50.8 | 64 | 80 | 81 | 73 | 69 | 73.4 | 150 | 163 | 162 | 160 | 162 | 162 | 161.8 |
| 2 | 1 21h30 | 131 | 135 | 128 | 118 | 113 | 125 | 65 | 50 | 56 | 60 | 57 | 57.6 | 79 | 69 | 94 | 76 | 80 | 79.6 | 100 | 95 | 150 | 150 | 148 | 150 | 138.6 |
| 2 | 4 00h30 | 121 | 120 | 122 | 117 | 130 | 122 | 66 | 54 | 47 | 68 | 64 | 59.8 | 80 | 68 | 60 | 82 | 90 | 76 | 130 | 122 | 117 | 116 | 113 | 116 | 116.8 |
| 2 | 8 04h30 | 133 | 143 | 134 | 135 | 127 | 134.4 | 55 | 91 | 64 | 44 | 65 | 63.8 | 69 | 104 | 90 | 85 | 75 | 84.6 | 110 | 115 | 119 | 117 | 125 | 118 | 118.8 |
| 2 | 24 20h30 | 130 | 135 | 119 | 133 | 120 | 127.4 | 77 | 57 | 58 | 55 | 98 | 69 | 97 | 100 | 72 | 103 | 104 | 95.2 | 144 | 117 | 113 | 131 | 148 | 142 | 130.2 |
| 2 | 48 20h30 | 149 | 106 | 80 | 57 | 113 | 101 | 125 | 65 | 51 | 30 | 37 | 61.6 | 134 | 87 | 64 | 39 | 49 | 74.6 | 90 | 67 | 107 | 112 | 70 | 90 | 89.2 |
| 2 | 72 20h30 | 102 | 118 | 89 | 152 | 119 | 116 | 71 | 84 | 63 | 131 | 49 | 79.6 | 83 | 99 | 69 | 141 | 54 | 89.2 | 120 | 123 | 67 | 83 | 69 | 123 | 93 |
| 3 | 0 13h00 | 131 | 119 | 112 | 122 | 112 | 119.2 | 50 | 53 | 61 | 38 | 41 | 48.6 | 76 | 69 | 94 | 66 | 65 | 74 | 168 | 157 | 152 | 150 | 150 | 142 | 150.2 |
| 3 | 1 14h00 | 140 | 130 | 134 | 130 | 124 | 131.6 | 84 | 83 | 67 | 71 | 63 | 73.6 | 107 | 107 | 93 | 86 | 88 | 96.2 | 144 | 151 | 140 | 141 | 138 | 137 | 141.4 |
| 3 | 4 17h00 | 137 | 117 | 114 | 143 | 102 | 122.6 | 82 | 59 | 83 | 89 | 73 | 77.2 | 104 | 64 | 92 | 121 | 81 | 92.4 | 116 | 142 | 142 | 142 | 142 | 140 | 141.6 |
| 3 | 8 21h00 | 150 | 140 | 159 | 162 | 124 | 147 | 61 | 67 | 84 | 72 | 86 | 74 | 74 | 79 | 96 | 93 | 92 | 86.8 | 150 | 148 | 151 | 148 | 147 | 147 | 148.2 |
| 3 | 24 13h00 | 130 | 108 | 114 | 117 | 121 | 118 | 65 | 49 | 66 | 63 | 73 | 63.2 | 95 | 77 | 79 | 97 | 92 | 88 | 124 | 132 | 124 | 122 | 127 | 128 | 100.2 |
| 3 | 48 13h00 | 100 | 117 | 152 | 98 | 110 | 115.4 | 57 | 90 | 134 | 50 | 62 | 78.6 | 78 | 103 | 136 | 73 | 95 | 97 | 120 | 126 | 112 | 145 | 118 | 116 | 123.4 |
| 3 | 72 13h00 | 113 | 115 | 112 | 140 | 118 | 119.6 | 65 | 92 | 62 | 79 | 90 | 77.6 | 70 | 102 | 69 | 89 | 98 | 85.6 | 110 | 115 | 112 | 115 | 115 | 100 | 111.4 |
| 4 | 0 10h30 | 108 | 111 | 108 | 108 | 104 | 107.8 | 59 | 60 | 51 | 50 | 60 | 56 | 80 | 86 | 81 | 87 | 75 | 81.8 | 140 | 134 | 124 | 126 | 128 | 113 | 125 |
| 4 | 1 11h30 | 129 | 127 | 123 | 124 | 137 | 128 | 63 | 67 | 69 | 55 | 70 | 64.8 | 83 | 83 | 90 | 84 | 105 | 89 | 140 | 120 | 125 | 122 | 121 | 125 | 122.6 |
| 4 | 4 14h30 | 127 | 146 | 137 | 135 | 137 | 136.4 | 87 | 87 | 61 | 72 | 75 | 76.4 | 102 | 97 | 94 | 93 | 94 | 96 | 108 | 115 | 116 | 116 | 113 | 117 | 115.4 |
| 4 | 8 18h30 | 119 | 120 | 122 | 125 | 129 | 123 | 66 | 69 | 69 | 62 | 59 | 65 | 80 | 88 | 90 | 83 | 85 | 85.2 | 132 | 128 | 124 | 125 | 124 | 124 | 125 |
| 4 | 24 10h30 | 127 | 124 | 125 | 123 | 130 | 125.8 | 74 | 54 | 58 | 62 | 64 | 62.4 | 95 | 90 | 85 | 88 | 97 | 91 | 112 | 121 | 120 | 119 | 118 | 124 | 120.4 |
| 4 | 48 10h30 | 141 | 144 | 148 | 121 | 127 | 136.2 | 111 | 73 | 126 | 71 | 76 | 91.4 | 125 | 101 | 135 | 95 | 98 | 110.8 | 110 | 88 | 112 | 124 | 111 | 112 | 109.4 |
| 4 | 72 10h30 | 138 | 142 | 152 | 124 | 135 | 138.2 | 65 | 65 | 60 | 68 | 71 | 65.8 | 90 | 98 | 90 | 94 | 87 | 91.8 | 144 | 146 | 145 | 144 | 144 | 152 | 146.2 |
| 5 | 0 19h30 | 124 | 127 | 136 | 133 | 135 | 131 | 73 | 68 | 75 | 82 | 81 | 75.8 | 85 | 88 | 90 | 101 | 100 | 92.8 | 104 | 97 | 105 | 113 | 109 | 110 | 106.8 |
| 5 | 1 20h30 | 78 | 122 | 104 | 114 | 119 | 107.4 | 52 | 56 | 68 | 65 | 53 | 58.8 | 57 | 85 | 73 | 94 | 87 | 79.2 | 112 | 110 | 106 | 102 | 105 | 97 | 104 |
| 5 | 4 23h30 | 135 | 122 | 126 | 125 | 128 | 127.2 | 75 | 80 | 59 | 59 | 74 | 69.4 | 107 | 95 | 77 | 89 | 97 | 93 | 84 | 91 | 89 | 88 | 88 | 97 | 90.6 |
| 5 | 8 03h30 | 106 | 110 | 106 | 105 | 109 | 107.2 | 61 | 66 | 72 | 59 | 74 | 66.4 | 84 | 85 | 85 | 83 | 88 | 85 | 76 | 65 | 72 | 69 | 69 | 69 | 68.8 |
| 5 | 24 19h30 | 97 | 90 | 103 | 96 | 101 | 97.4 | 62 | 53 | 55 | 62 | 56 | 57.6 | 78 | 68 | 78 | 73 | 73 | 74 | 72 | 55 | 67 | 55 | 58 | 61 | 59.2 |
| 5 | 48 19h30 | 109 | 115 | 110 | 124 | 95 | 110.6 | 67 | 80 | 61 | 79 | 73 | 72 | 92 | 95 | 84 | 88 | 85 | 88.8 | 96 | 101 | 74 | 77 | 83 | 92 | 85.4 |
| 5 | 72 19h30 | 106 | 108 | 108 | 89 | 110 | 104.2 | 61 | 60 | 52 | 51 | 78 | 60.4 | 66 | 73 | 68 | 66 | 90 | 72.6 | 76 | 80 | 96 | 78 | 75 | 65 | 78.8 |
| 6 | 0 21h30 | 174 | 165 | 163 | 162 | 165 | 165.8 | 62 | 81 | 80 | 77 | 88 | 77.6 | 119 | 116 | 121 | 117 | 112 | 117 | 112 | 127 | 130 | 134 | 138 | 140 | 133.8 |
| 6 | 1 22h30 | 171 | 166 | 161 | 172 | 173 | 168.6 | 74 | 91 | 78 | 80 | 78 | 80.2 | 106 | 116 | 105 | 110 | 109 | 109.2 | 99 | 102 | 110 | 106 | 100 | 98 | 103.2 |
| 6 | 4 01h30 | 166 | 154 | 169 | 182 | 159 | 166 | 97 | 104 | 102 | 98 | 104 | 101 | 125 | 127 | 122 | 124 | 121 | 123.8 | 88 | 90 | 96 | 93 | 90 | 84 | 90.6 |
| 6 | 8 05h30 | 142 | 134 | 169 | 174 | 175 | 158.8 | 113 | 100 | 74 | 91 | 116 | 98.8 | 123 | 109 | 132 | 117 | 149 | 126 | 100 | 78 | 92 | 82 | 81 | 77 | 82 |
| 6 | 24 21h30 | 145 | 163 | 162 | 152 | 159 | 156.2 | 111 | 85 | 89 | 92 | 101 | 95.6 | 120 | 124 | 117 | 110 | 118 | 117.8 | 60 | 70 | 67 | 78 | 74 | 75 | 72.8 |
| 6 | 48 21h30 | 160 | 138 | 146 | 134 | 127 | 115.6 | 118 | 90 | 82 | 68 | 82 | 88 | 130 | 95 | 94 | 92 | 99 | 102 | 62 | 66 | 69 | 68 | 44 | 42 | 57.8 |
| 6 | 72 21h30 | 136 | 165 | 167 | 167 | 175 | 162 | 101 | 86 | 113 | 107 | 127 | 106.8 | 115 | 110 | 122 | 138 | 146 | 126.2 | 110 | 88 | 88 | 93 | 95 | 86 | 90 |
| 7 | 0 22h00 | 147 | 138 | 138 | 127 | 137 | 137.4 | 87 | 87 | 80 | 81 | 82 | 83.4 | 105 | 108 | 105 | 103 | 110 | 106.2 | 144 | 130 | 128 | 128 | 125 | 131 | 128.4 |
| 7 | 1 23h00 | 140 | 144 | 151 | 134 | 144 | 142.6 | 81 | 92 | 90 | 94 | 77 | 86.8 | 110 | 117 | 120 | 108 | 110 | 113 | 112 | 109 | 111 | 111 | 115 | 116 | 112.4 |
| 7 | 4 02h00 | 158 | 157 | 149 | 158 | 160 | 156.4 | 110 | 111 | 110 | 112 | 96 | 107.8 | 130 | 126 | 124 | 127 | 120 | 125.4 | 92 | 96 | 93 | 92 | 90 | 95 | 93.2 |
| 7 | 8 06h00 | 173 | 157 | 173 | 166 | 159 | 165.6 | 94 | 115 | 112 | 93 | 111 | 105 | 134 | 133 | 126 | 116 | 123 | 126.4 | 112 | 102 | 100 | 105 | 102 | 96 | 101 |
| 7 | 24 22h00 | 142 | 150 | 159 | 144 | 152 | 149.4 | 88 | 76 | 81 | 80 | 86 | 82.2 | 109 | 122 | 113 | 105 | 120 | 113.8 | 110 | 113 | 114 | 125 | 115 | 121 | 117.6 |

| | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 7 | **48** | 22h00 | 150 | 147 | 158 | 133 | 145 | 146.6 | 54 | 85 | 73 | 82 | 76 | 74 | 107 | 103 | 115 | 104 | 107 | 107.2 | **96** | 98 | 98 | 106 | 109 | 105 | 103.2 |
| 7 | **72** | 22h00 | 135 | 175 | 157 | 171 | 135 | 154.6 | 91 | 98 | 102 | 98 | 97 | 97.2 | 111 | 117 | 123 | 124 | 105 | 116 | **120** | 91 | 100 | 96 | 104 | 123 | 102.8 |
| 8 | **0** | 20h30 | 130 | 129 | 127 | 130 | 128 | 128.8 | 53 | 82 | 69 | 77 | 50 | 66.2 | 110 | 101 | 102 | 94 | 101 | 101.6 | **124** | 157 | 157 | 160 | 159 | 153 | 157.2 |
| 8 | **1** | 21h30 | 167 | 165 | 168 | 160 | 161 | 164.2 | 80 | 93 | 81 | 83 | 91 | 85.6 | 137 | 136 | 136 | 131 | 131 | 134.2 | **130** | 150 | 146 | 142 | 144 | 138 | 144 |
| 8 | **4** | 00h30 | 154 | 169 | 160 | 159 | 172 | 162.8 | 99 | 126 | 110 | 111 | 104 | 110 | 126 | 145 | 131 | 131 | 138 | 134.2 | **140** | 125 | 134 | 126 | 120 | 121 | 125.2 |
| 8 | **8** | 04h30 | 156 | 159 | 156 | 156 | 156 | 156.6 | 85 | 108 | 93 | 103 | 95 | 96.8 | 127 | 128 | 134 | 126 | 125 | 128 | **112** | 120 | 120 | 115 | 113 | 134 | 120.4 |
| 8 | **24** | 20h30 | 154 | 155 | 147 | 153 | 150 | 151.8 | 99 | 115 | 109 | 103 | 83 | 101.8 | 128 | 131 | 121 | 130 | 116 | 125.2 | **120** | 115 | 118 | 118 | 117 | 112 | 116 |
| 8 | **48** | 20h30 | 135 | 132 | 132 | 140 | 151 | 138 | 97 | 94 | 87 | 84 | 112 | 94.8 | 110 | 108 | 106 | 107 | 129 | 112 | **120** | 107 | 112 | 106 | 104 | 109 | 107.6 |
| 8 | **72** | 20h30 | 135 | 129 | 132 | 128 | 127 | 130.2 | 76 | 89 | 88 | 88 | 86 | 85.4 | 91 | 104 | 101 | 101 | 100 | 99.4 | **120** | 109 | 92 | 112 | 112 | 107 | 106.4 |
| 9 | **0** | 10h45 | 130 | 122 | 126 | 123 | 127 | 125.6 | 63 | 65 | 61 | 61 | 70 | 64 | 88 | 86 | 78 | 80 | 95 | 85.4 | **136** | 133 | 132 | 11 | 132 | 136 | 108.8 |
| 9 | **1** | 11h45 | 142 | 132 | 139 | 114 | 100 | 125.4 | 81 | 95 | 78 | 76 | 49 | 75.8 | 100 | 106 | 88 | 80 | 62 | 75 | **142** | 91 | 83 | 91 | 141 | 147 | 110.6 |
| 9 | **4** | 14h45 | 147 | 157 | 168 | 180 | 154 | 161.2 | 44 | 60 | 56 | 50 | 48 | 51.6 | 102 | 107 | 105 | 85 | 115 | 102.8 | **136** | 142 | 141 | 137 | 138 | 137 | 139 |
| 9 | **8** | 18h45 | 139 | 146 | 149 | 145 | 139 | 143.6 | 52 | 68 | 78 | 69 | 66 | 66.6 | 84 | 110 | 101 | 99 | 101 | 99 | **120** | 147 | 136 | 135 | 133 | 133 | 136.8 |
| 9 | **24** | 10h45 | 122 | 126 | 124 | 121 | 125 | 123.6 | 62 | 62 | 65 | 69 | 55 | 62.6 | 75 | 87 | 87 | 89 | 88 | 85.2 | **120** | 123 | 121 | 122 | 124 | 123 | 122.6 |
| 9 | **48** | 10h45 | 137 | 146 | 144 | 146 | 141 | 142.8 | 55 | 60 | 71 | 61 | 66 | 62.6 | 90 | 101 | 100 | 89 | 88 | 93.6 | **140** | 131 | 125 | 125 | 126 | 125 | 126.4 |
| 9 | **72** | 10h45 | 116 | 127 | 123 | 127 | 121 | 122.8 | 60 | 54 | 64 | 57 | 62 | 59.4 | 87 | 77 | 77 | 80 | 82 | 80.6 | **120** | 117 | 115 | 111 | 108 | 109 | 112 |
| 10 | **0** | 16h30 | 111 | 121 | 116 | 110 | 114 | 114.4 | 38 | 46 | 43 | 40 | 39 | 41.2 | 68 | 63 | 59 | 63 | 57 | 62 | **108** | 109 | 105 | 104 | 107 | 109 | 106.8 |
| 10 | **1** | 17h30 | 132 | 147 | 153 | 146 | 143 | 144.2 | 81 | 96 | 72 | 97 | 94 | 88 | 103 | 112 | 114 | 118 | 106 | 110.6 | **100** | 106 | 108 | 108 | 107 | 96 | 105 |
| 10 | **4** | 20h30 | 144 | 131 | 128 | 132 | 130 | 133 | 56 | 57 | 42 | 56 | 45 | 51.2 | 89 | 87 | 83 | 70 | 77 | 81.2 | **110** | 109 | 108 | 103 | 106 | 100 | 105.2 |
| 10 | **8** | 00h30 | 113 | 120 | 115 | 116 | 113 | 115.4 | 65 | 43 | 53 | 43 | 46 | 50 | 80 | 75 | 85 | 83 | 80 | 80.6 | **95** | 93 | 90 | 89 | 87 | 96 | 91 |
| 10 | **24** | 16h30 | 116 | 110 | 101 | 112 | 109 | 109.6 | 38 | 42 | 40 | 48 | 50 | 43.6 | 66 | 52 | 53 | 76 | 66 | 62.6 | **96** | 98 | 93 | 95 | 94 | 93 | 94.6 |
| 10 | **48** | 16h30 | 124 | 116 | 123 | 116 | 115 | 118.8 | 55 | 76 | 54 | 65 | 61 | 62.2 | 75 | 86 | 69 | 87 | 70 | 77.4 | **99** | 100 | 95 | 85 | 99 | 81 | 92 |
| 10 | **72** | 16h30 | 122 | 105 | 115 | 155 | 96 | 118.6 | 56 | 57 | 90 | 50 | 44 | 59.4 | 87 | 66 | 99 | 67 | 53 | 74.4 | **96** | 110 | 81 | 82 | 91 | 107 | 94.2 |
| 11 | **0** | 16h00 | 145 | 155 | 154 | 141 | 138 | 146.6 | 83 | 76 | 73 | 82 | 73 | 77.4 | 117 | 115 | 95 | 98 | 83 | 101.6 | **172** | 181 | 181 | 189 | 183 | 178 | 182.4 |
| 11 | **1** | 17h00 | 142 | 143 | 140 | 144 | 141 | 142 | 77 | 80 | 91 | 88 | 89 | 85 | 107 | 100 | 111 | 116 | 112 | 109.2 | **170** | 166 | 166 | 163 | 163 | 162 | 164 |
| 11 | **4** | 20h00 | 174 | 163 | 173 | 169 | 160 | 135.8 | 108 | 101 | 98 | 85 | 102 | 98.8 | 131 | 123 | 111 | 103 | 126 | 118.8 | **160** | 166 | 166 | 163 | 165 | 168 | 165.6 |
| 11 | **8** | 0h00 | 158 | 157 | 156 | 156 | 154 | 156.2 | 99 | 95 | 96 | 89 | 91 | 94 | 127 | 127 | 120 | 127 | 120 | 124.2 | **180** | 171 | 169 | 168 | 168 | 166 | 168.4 |
| 12 | **0** | 12h30 | 155 | 149 | 151 | 148 | 123 | 145.2 | 87 | 75 | 87 | 87 | 99 | 87 | 106 | 101 | 114 | 105 | 107 | 106.6 | **160** | 147 | 150 | 145 | 140 | 140 | 144.4 |
| 12 | **1** | 13h30 | 114 | 130 | 138 | 129 | 127 | 127.6 | 89 | 86 | 92 | 82 | 78 | 85.4 | 99 | 109 | 108 | 101 | 95 | 102.4 | **110** | 118 | 102 | 96 | 100 | 96 | 102.4 |
| 12 | **4** | 16h30 | 173 | 160 | 160 | 151 | 148 | 158.4 | 110 | 115 | 105 | 109 | 104 | 108.6 | 126 | 128 | 125 | 123 | 120 | 124.4 | **120** | 108 | 108 | 100 | 92 | 101 | 101.8 |
| 12 | **8** | 20h30 | 138 | 145 | 138 | 125 | 119 | 133 | 100 | 84 | 88 | 93 | 93 | 91.6 | 113 | 104 | 107 | 103 | 101 | 105.6 | **120** | 109 | 114 | 118 | 111 | 119 | 114.2 |
| 12 | **24** | 20h30 | 101 | 140 | 145 | 135 | 134 | 131 | 90 | 103 | 104 | 102 | 94 | 98.6 | 95 | 118 | 124 | 116 | 105 | 111.6 | **100** | 86 | 90 | 80 | 69 | 60 | 77 |
| 12 | **48** | 20h30 | 138 | 143 | 134 | 136 | 128 | 135.8 | 89 | 89 | 81 | 79 | 80 | 83.6 | 105 | 104 | 99 | 97 | 94 | 99.8 | **99** | 130 | 124 | 121 | 116 | 116 | 117 |
| 12 | **72** | 20h30 | 121 | 124 | 111 | 115 | 117 | 117.6 | 73 | 76 | 76 | 77 | 73 | 75 | 81 | 99 | 88 | 91 | 94 | 90.6 | **91** | 100 | 95 | 85 | 72 | 67 | 78 |

### 12.1.14 Habitus & appetite

| Patient | Time | | Temp | Habitus | Appetite |
|---|---|---|---|---|---|
| 1 | 0 | 15h30 | 39.7 | 1 | 0 |
| 1 | 1 | 16h30 | 38.7 | 1 | 0 |
| 1 | 4 | 19h30 | 40.1 | 2 | 3 |
| 1 | 8 | 23h30 | 39.3 | 3 | 0 |
| 1 | 24 | 15h30 | 38.4 | 3 | 3 |
| 1 | 48 | 15h30 | 37.9 | 3 | 3 |
| 1 | 72 | 15h30 | 37.3 | 3 | 3 |
| 2 | 0 | 20h30 | 40.3 | 1 | 0 |
| 2 | 1 | 21h30 | 40.3 | 2 | 0 |
| 2 | 4 | 00h30 | 40.3 | 3 | 4 |
| 2 | 8 | 04h30 | 39.3 | 3 | 0 |
| 2 | 24 | 20h30 | 38 | 3 | 4 |
| 2 | 48 | 20h30 | 39.3 | 4 | 4 |
| 2 | 72 | 20h30 | 39.2 | 4 | 4 |
| 3 | 0 | 13h00 | 39.2 | 1 | 0 |
| 3 | 1 | 14h00 | 39.3 | 1 | 0 |
| 3 | 4 | 17h00 | 39.6 | 2 | 0 |
| 3 | 8 | 21h00 | 40.6 | 2 | 1 |
| 3 | 24 | 13h00 | 39.6 | 2 | 0 |
| 3 | 48 | 13h00 | 38.5 | 3 | 2 |
| 3 | 72 | 13h00 | 37.5 | 4 | 3 |
| 4 | 0 | 10h30 | 39.7 | 2 | 0 |
| 4 | 1 | 11h30 | 39.5 | 2 | 0 |
| 4 | 4 | 14h30 | 40.5 | 2 | 1 |
| 4 | 8 | 18h30 | 39.1 | 2 | 1 |
| 4 | 24 | 10h30 | 38.7 | 3 | 1 |
| 4 | 48 | 10h30 | 38.1 | 3 | 4 |
| 4 | 72 | 10h30 | 38.4 | 4 | 4 |
| 5 | 0 | 19h30 | 40.1 | 2 | 0 |
| 5 | 1 | 20h30 | 39.7 | 2 | 0 |
| 5 | 4 | 23h30 | 40.3 | 3 | 4 |
| 5 | 8 | 03h30 | 38.5 | 4 | 0 |
| 5 | 24 | 19h30 | 38.4 | 4 | 4 |
| 5 | 48 | 19h30 | 38 | 4 | 4 |
| 5 | 72 | 19h30 | 38.7 | 4 | 4 |
| 6 | 0 | 21h30 | 38.9 | 2 | 0 |
| 6 | 1 | 22h30 | 38.6 | 2 | 0 |
| 6 | 4 | 01h30 | 39.9 | 2 | 0 |
| 6 | 8 | 05h30 | 38.6 | 3 | 3 |
| 6 | 24 | 21h30 | 37.8 | 3 | 2 |
| 6 | 48 | 21h30 | 38.1 | 3 | 3 |
| 6 | 72 | 21h30 | 37.4 | 4 | 3 |
| 7 | 0 | 22h00 | 39.5 | 2 | 0 |
| 7 | 1 | 23h00 | 39.6 | 2 | 0 |
| 7 | 4 | 02h00 | 40.3 | 3 | 0 |
| 7 | 8 | 06h00 | 40.7 | 3 | 0 |
| 7 | 24 | 22h00 | 40 | 2 | 0 |
| 7 | 48 | 22h00 | 38.3 | 2 | 0 |
| 7 | 72 | 22h00 | 37.9 | 3 | 3 |
| 8 | 0 | 20h30 | 39.1 | 1 | 0 |
| 8 | 1 | 21h30 | 37.4 | 2 | 0 |
| 8 | 4 | 00h30 | 37.8 | 2 | 1 |
| 8 | 8 | 04h30 | 37.2 | 2 | 3 |
| 8 | 24 | 20h30 | 37.8 | 3 | 3 |
| 8 | 48 | 20h30 | 38.3 | 3 | 4 |
| 8 | 72 | 20h30 | 37.8 | 3 | 4 |
| 9 | 0 | 10h45 | 40.6 | 1 | 0 |
| 9 | 1 | 11h45 | 38.8 | 1 | 0 |
| 9 | 4 | 14h45 | 39.7 | 2 | 0 |
| 9 | 8 | 18h45 | 39.4 | 2 | 0 |
| 9 | 24 | 10h45 | 38.9 | 1 | 0 |
| 9 | 48 | 10h45 | 39.5 | 2 | 3 |
| 9 | 72 | 10h45 | 38.5 | 3 | 3 |
| 10 | 0 | 16h30 | 39.4 | 2 | 0 |
| 10 | 1 | 17h30 | 39.9 | 3 | 0 |
| 10 | 4 | 20h30 | 40 | 3 | 0 |
| 10 | 8 | 00h30 | 38.7 | 3 | 0 |
| 10 | 24 | 16h30 | 39.1 | 3 | 2 |
| 10 | 48 | 16h30 | 38.2 | 4 | 3 |
| 10 | 72 | 16h30 | 38.5 | 4 | 3 |
| 11 | 0 | 16h00 | 41.4 | 2 | 0 |
| 11 | 1 | 17h00 | 40.9 | 1 | 0 |
| 11 | 4 | 20h00 | 41.4 | 2 | 1 |
| 11 | 8 | 0h00 | 41.2 | 2 | 0 |
| 12 | 0 | 12h30 | 39 | 2 | 0 |
| 12 | 1 | 13h30 | 39 | 2 | 4 |
| 12 | 4 | 16h30 | 40 | 3 | 4 |
| 12 | 8 | 20h30 | 39.3 | 4 | 4 |
| 12 | 24 | 20h30 | 38.2 | 4 | 4 |
| 12 | 48 | 20h30 | 40.3 | 4 | 4 |
| 12 | 72 | 20h30 | 38.7 | 3 | 4 |

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