# COMPARATIVE *IN VITRO* ANALYSIS OF A BALANCED ELECTROLYTE SOLUTION VERSUS AN UNBALANCED ELECTROLYTE SOLUTION, FOR PROCESSING OF RESIDUAL PUMP BLOOD USING CELL SAVER FOR PATIENTS UNDERGOING ELECTIVE CARDIAC SURGERY

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## AUTHORS DECLARATION

Dissertation submitted in fulfilment for the degree of Master of Technology, Clinical Technology: Cardiovascular Perfusion. The research represents the original work of the author, and additional sources of information were specifically acknowledged in the text. The research presented in this dissertation has not been submitted to any other tertiary institutions.

The research described in this dissertation was carried out under the supervision of Professor J K Adam at the Department of Clinical Technology, Faculty of Health Sciences, Durban University of Technology. The research was conducted in the Department of Cardiothoracic Surgery, Inkosi Albert Luthuli Central Hospital, Durban, South Africa.

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## ABSTRACT

### Introduction

A large volume of residual haemodilute blood remains in the cardiopulmonary bypass (CPB) circuit after termination of the bypass. It is common practice in many centres to process residual pump blood with an autologus cell salvage system (ACSS), thereby producing a re-suspended red blood cell (RBC) concentrate and attenuating the need for donor blood RBC concentrate. It has also become standard practice to wash donor pack red blood cells (PRBC) before adding it to neonate cardiopulmonary circuits (Swindell et al., 2007). Manufactures of ACSS recommend 0.9% sodium chloride (NaCl) as a wash solution for processing salvaged blood. Previous studies have demonstrated that washing PRBC with normal saline results in acid-base (Huber et al., 2013) and electrolyte derangements (Varghese et al., 2007). Infusion of normal saline in healthy volunteers also results in significant changes in osmolality (Williams et al., 1999). The use of normal saline as a wash solution in processing residual CPB blood requires investigation.

### Aims and Objectives

This was a prospective, quantitative *in vitro* investigation to analyze and compare the quality of residual pump blood post CPB that had been washed with either an unbalanced electrolyte solution (0.9% normal saline) or a balanced electrolyte solution (Balsol®). Both are crystalloid solutions. The primary objective of the present study was to measure and compare the pH, electrolytes, metabolites, osmolality and strong ion difference (SID) of residual pump blood to the pH, electrolytes, metabolites, osmolality and SID of processed cell saver blood, which was washed with either 0.9% normal saline or Balsol® solution. The secondary objective was to measure and compare protein levels (albumin and total protein) in residual pump blood to protein levels in processed cell saver blood, that is washed with either 0.9% normal saline or Balsol® solution. The final objective was to determine the volume, haematocrit and haemoglobin yield post cell saver processing, from the input volume of residual pump blood when washed with either 0.9% normal saline or Balsol® solution. This was the first study of this nature done in the South African population group.

**Methodology**

In this investigation in a series of forty patients (n=40) undergoing elective cardiac surgery with CPB, the first twenty patients were allocated to the NaCl control group (n=20) and the second twenty patients were allocated to the Balsol® interventional group (n=20). The extracorporeal circuit consisted of a standard integral hollow fibre membrane oxygenator and tubing that was primed with 1500-1800 millilitres of balanced crystalloid solution (Balsol®), for both the control group and the interventional group, and addition of 5000 iu heparin. The balanced crystalloid solution (Balsol®) is the approved standard CPB priming solution for all cardiac procedures at Inkosi Albert Luthuli Central Hospital.

This setup was used with the Stockert *S5* roller pump heart lung machine. The operations were performed as per protocol with standard non-pulsatile CPB and hypothermia was maintained at 28 – 32 ºC (core) and haemodilution (haematocrit 20 % to 30 %). A standard flow rate of 2.4 L/min/m² was used. Cardio protection consisted of either cold Blood Cardioplegia using the Buckberg 4:1 ratio, being four parts blood to one part cardioplegia (with the 35ml of 20 % Dextrose + 1 gram Magnesium Sulphate added per 500ml), or 20ml/kg cold St Thomas II cardioplegia (with addition of 10ml of 8.5% $NaHCO_3$ + 100mg lignocain per litre). Topical cooling was achieved with ice cold 0.9 % saline. Maintenance fluid used during CPB was Balsol® for both the control and the interventional groups. Calcium, potassium and sodium bicarbonate was administered as required during CPB to correct deficits for both groups. Weaning of CPB was performed after re-warming to a rectal temperature of at least 35 ºC for both study groups.

Immediately on termination of CPB a blood sample was taken from the sampling manifold of the CPB circuit for pre wash analysis. Residual pump blood was then flushed out with one litre of Balsol® solution for both groups and collected into the Medtronic autolog cell saver reservoir to be processed. In the control study group 0.9% NaCl was used as the wash solution and in the interventional study group Balsol® solution was used as the wash solution. After processing of the salvaged blood is complete, a blood sample was taken for post wash analysis. Clinical data recorded for pre and post wash samples included: pH, $pCO_2$, $pO_2$, $[K^+]$, $[Na^+]$, $[Cl^-]$, $[Ca^{2+}]$, lactate, glucose, $[HCO_3^-]$, $TCO_2$, haematocrit, haemoglobin (GEM 4000® premier™ blood gas analyser) blood volume (Medtronic autolog) and SID (calculated as per equation). Inorganic phosphate, total magnesium, albumin, total protein (Siemens Advia 1800 blood gas analyser) and osmolality (Gonotech osmometer) were also measured.

**Results**

There was a highly significant decrease ($p < 0.05$) within the NaCl group after washing with $pCO_2$ (28.3 ± 2.9 vs. <6.0 ± 0.0), [$K^+$] (4.5 ± 0.5 vs. 1.0 ± 0.7), total magnesium (1.7 ± 0.7 vs. 0.29 ± 0), ionized calcium (1.0 ± 0.09 vs. 0.1 ± 0.03), inorganic phosphate (0.9 ± 0.4 vs. 0.09 ± 0.04) and SID (27.1 ± 2.1 vs. 18.4 ± 2.2). There was a highly significant increase ($p < 0.05$) within the NaCl group after washing with pH (7.5 ± 0.1 vs. 7.7 ± 0.1), [$Na^+$] (132.9 ± 3.2 vs. 146.3 ± 1.9), [$Cl^-$] (107.8 ± 3.1 vs. 127.4 ± 2.1) and osmolaltity (256.9 ± 38.4 vs. 296.2 ± 57.5).

There were highly significant decrease ($p < 0.05$) within the Balsol® group after washing with $pCO_2$ (30.15 ± 6.0 vs. 18.9 ± 4.9), [$Na^+$] (134.7 ± 2.2 vs. 125.6 ± 1), [$Cl^-$] (108.8 ± 2.7 vs. 100.2 ± 1.4), ionized calcium (0.9 ± 0.1 vs. 0.02 ± 0.04), inorganic phosphate (0.8 ± 0.2 vs. 0.1 ± 0.024) and osmolality (288.8 ± 20.6 vs. 272.8 ± 19.9). There were highly significant increase ($p < 0.05$) within the Balsol® group after washing with pH (7.5 ± 0.1 vs. 7.7 ± 0.1), [$K^+$] (4.2 ± 0.4 vs 4.6 ± 0.3). Total magnesium and SID were similar after washing within the Balsol® group. Albumin and total protein revealed similar significant decreases within both groups after washing.

There was a highly significant difference ($p < 0.05$) in the change between groups after washing in all the variables measured, except for pH, inorganic phosphate, lactate, glucose, albumin, total protein, haematocrit, haemoglobin, and blood volume. Total carbon dioxide and [$HCO_3^-$] were not compared because they were incalculable by blood gas analyser in the NaCl group.

**Conclusion**

This investigation concluded that the balanced electrolyte solution Balsol® used for washing residual CPB blood results in a re-suspended RBC concentrate, with an osmolality and electrolyte profile that is superior compared to washing residual CPB blood with 0.9% NaCl solution.

**Key words**

Cardiopulmonary bypass; Residual pump blood; Autologous cell salvage system; Unbalanced electrolyte solution, Balanced electrolyte solution.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| **ACD-A** | acid citrate dextrose solution A |
| **ACSS** | autologous cell salvage system |
| **ACT** | activated clotting time |
| **ADH** | antidiuretic hormone |
| **ATP** | adenosine triphosphate |
| **BB-HS** | bicarbonate-buffered hemofiltration solution |
| **BE** | base excess |
| **CABG** | coronary artery bypass grafting |
| **CAT** | continuous autotransfusion |
| **CPB** | cardiopulmonary bypass |
| **CS** | cell saving |
| **DAT** | discontinuous autotransfusion |
| **FFP** | fresh frozen plasma |
| **g** | grams |
| **g/dL** | grams per decilitre |
| **g/L** | grams per litre |
| **Hb** | haemoglobin |

| | |
|---|---|
| **HES** | hydroxyethyl starch |
| **$[HCO_3^-]_{HH}$** | Henderson–Hasselbalch actual bicarbonate |
| **HCT** | hematocrit |
| **ICU** | intensive care unit |
| **IU** | international units |
| **kPa** | kilopascals |
| **LDH** | lactate dehydrogenase |
| **L/min/m²** | litres per minute per meter squared |
| **m** | mass |
| **meq/L** | milliequivalents |
| **mEq/kg/hr** | milliequivalents per kilogram per hour |
| **mg/dL** | milligrams per decilitre |
| **min** | minutes |
| **ml** | millilitres |
| **$mm^3$** | millimetre cubed |
| **mOsm/kg** | milliosmoles per kilogram |
| **mmHg** | millimetres mercury |
| **ml/hr** | millilitres per hour |

| | |
|---|---|
| **mmol/hr** | millimoles per hour |
| **mmol/L** | millimoles per litre |
| **MRI** | magnetic resonance imaging |
| **$ms^{-1}$** | meters per second |
| **N** | Newtons |
| **NaCl** | sodium chloride |
| **$NaHCO_3$** | sodium bicarbonate |
| **NHLS** | national health laboratory services |
| **NS** | not significant |
| **$pCO_2$** | partial pressure of carbon dioxide |
| **PLT** | platelets |
| **$pO_2$** | partial oxygen pressure |
| **PRBC** | packed red blood cells |
| **PRC** | packed red cells |
| **r** | radius |
| **RBC** | red blood cells |
| **RL** | Ringers lactate |
| **rpm** | revolutions per minute |

| | |
|---|---|
| **SCADS** | small capillary arteriolar dilations |
| **SD** | standard deviation |
| **SID** | strong ion difference |
| **$sO_2$** | oxygen saturation |
| **TAA** | thoracic aortic aneurysm |
| **$TCO_2$** | total carbon dioxide |
| **U/L** | units per litre |
| **v** | velocity |
| **vs.** | versus |
| **WBC** | white blood cells |

## LIST OF SCIENTIFIC NOTATIONS

| | |
|---|---|
| **≈** | approximately |
| **ºC** | degrees celsius |
| **ρ** | density |
| **F (g)** | gravitational force |
| **g** | gravitational |
| **µg** | micrograms |
| **µm** | micrometers |
| **µmol.gHb$^{-1}$** | micromoles per gram haemoglobin |
| **[$Ca^{2+}$]** | calcium ion concentration |
| Δ | change |
| **[$Cl^{-}$]** | chloride ion concentration |
| **[$HCO_3^{-}$]** | bicarbonate ion concentration |
| **[$K^{+}$]** | potassium ion concentration |
| **[$Mg^{2+}$]** | magnesium ion concentration |
| **[$Na^{+}$]** | sodium ion concentration |
| **[$PO_4^{3-}$]** | phosphate ion concentration |

# CHAPTER ONE: INTRODUCTION

Cardiac surgery and extracorporeal technology have developed considerably since the mid 1950's, but the advent of cardiac surgery performed under extracorporeal circulation gave rise to an enormous demand for homologous banked blood. Although the meticulous improvement of surgical technique, extracorporeal circulation, and post operative management have contributed to a marked reduction in morbidity and mortality, the development of cardiac surgery to its present day would not have been possible without homologous blood substitution. Improvements in blood bank technology and research have dramatically improved the ability to store blood and its components. Currently PRBC can be stored for up to 42 days, however, this increased storage time is not without risk. Stored blood undergoes time dependant biochemical and morphologic changes that are collectively known as "storage lesions" (Offner, 2004), that can contribute towards patient pathology. Recent evidence reported that PRBC stored for more than two weeks was associated with increased risk of post operative complications and reduced long and short term survival (Koch et al., 2008). Evidence also suggests that the number of PRBC transfused is significantly correlated to long and short term survival (van de Watering et al., 2006 and van Straten et al., 2011). Decreasing the demand thus decreasing the supply is an effective means to reduce stock piling of homologous blood, and therefore the supply of fresher homologous blood with a shorter storage (shelf) time can be achieved. Effective blood management protocols and blood salvage techniques will help achieve these goals.

Throughout the world health care providers strive to provide patients with optimal treatment at the lowest possible cost. The growing costs of cardiac surgery have prompted the cardiovascular surgical team to review their strategies in reducing costs in all aspects. None of these costs are more prominent than that of homologous blood. Firstly, homologous blood has a dual cost, that is it contributes to the economic costs of cardiac surgery in the hospital it is undertaken in, as well as the cost of risk of complications, such as transfusion transmitted infections and reactions to the patients to whom it is transfused (Kuppurao and Wee, 2010). Secondly, religious beliefs such as those of Jehovah's Witness have also imposed serious moral and ethical problems by patients refusing to accept any blood products. Finally, homologous blood is a scarce and precious resource that is expensive and not always available.

At the end of cardiac procedures assisted with cardiopulmonary bypass (CPB) a large volume of haemodiluted blood (1-1.5 litres) remains in the extracorporeal circuit. To reduce transfusion requirements, it is common practice that this blood can be used for autotransfusion with or without processing with a cell saver machine. Autotransfusion is any process in which the patient's own blood is salvaged and re-infused thereby attenuating the need for donor blood. Intra-operative transfusion of residual pump blood after cell saver processing is a technique in which autologous blood is collected, centrifuged and washed with 0.9% normal saline. The final product is re-suspended red blood cells with a pack cell volume equivalent to that of a unit of homologous packed red blood cells. The washing process has been also reported to ameliorate levels of micro-aggregates (Varghese et al., 2007), pro-inflammatory cytokines (Amand et al., 2002), and plasma free haemoglobin (Naumenko et al., 2008), which may result in undesirable post transfusion complications. On the other hand, re-infusion of processed cardiotomy suction blood may reduce coagulation factors resulting in a dilutional coagulopathy which increases fresh frozen plasma use (Djaiani et al., 2007). This dilutional coagulopathy may increase bleeding and result in a paradoxical increase in homologous red blood cell transfusions (Rubens et al., 2012).

Little attention has, however, been given to the manufacturers recommendation of 0.9% normal saline or sodium chloride (NaCl) as a wash solution. Evidence (Awad, Allison, and Lobo, 2008) suggests that there is nothing "normal" about 0.9% NaCl solution. 0.9% NaCl is only normal in terms of osmolality but it is not a balanced electrolyte solution and has been reported to result in hyperchloremic metabolic acidosis when large volumes are rapidly infused (Chowdhury et al., 2012). It also contributes to unwarranted electrolyte imbalances when used as a wash solution, as important ions like potassium, magnesium, calcium and phosphate that are important for cardiac function are depleted (Huber et al., 2013; Varghese et al., 2007; Halpern et al., 1996). Intra-operative cell salvage is a well established practice in many cardiac surgery units, but the quality of the salvaged and processed blood and the risks that it may pose is being questioned with the same resolve as is with banked blood.

This was a prospective, quantitative *in vitro* investigation to analyze and compare the quality of residual pump blood post CPB that had been washed with either an unbalanced electrolyte solution (0.9% normal saline) or a balanced electrolyte solution (Balsol®). Both are crystalloid solutions. The primary objective of the this study was to measure and compare the pH, electrolytes, metabolites, osmolality and strong ion difference (SID) of residual pump blood to the pH, electrolytes, metabolites, osmolality and SID of processed

cell saver blood, which was washed with either 0.9% normal saline or Balsol® solution. The secondary objective was to measure and compare protein levels (albumin and total protein) in residual pump blood to protein levels in processed cell saver blood, that is washed with either 0.9% normal saline or Balsol® solution. The final objective was to determine the volume, haematocrit and haemoglobin yield post cell saver processing, from the input volume of residual pump blood when washed with either 0.9% normal saline or Balsol® solution. This was the first study of this nature done in the South African population group.

## CHAPTER TWO: STUDY BACKGROUND AND LITERATURE REVIEW

### 2.1 INTRODUCTION

This chapter provides information on the components of an autologous cell salvage system and their function, the theory of operation of a cell saver and the different types of cell salvage devices on the market.

### 2.2 AUTOLOGOUS CELL SALVAGE SYSTEMS

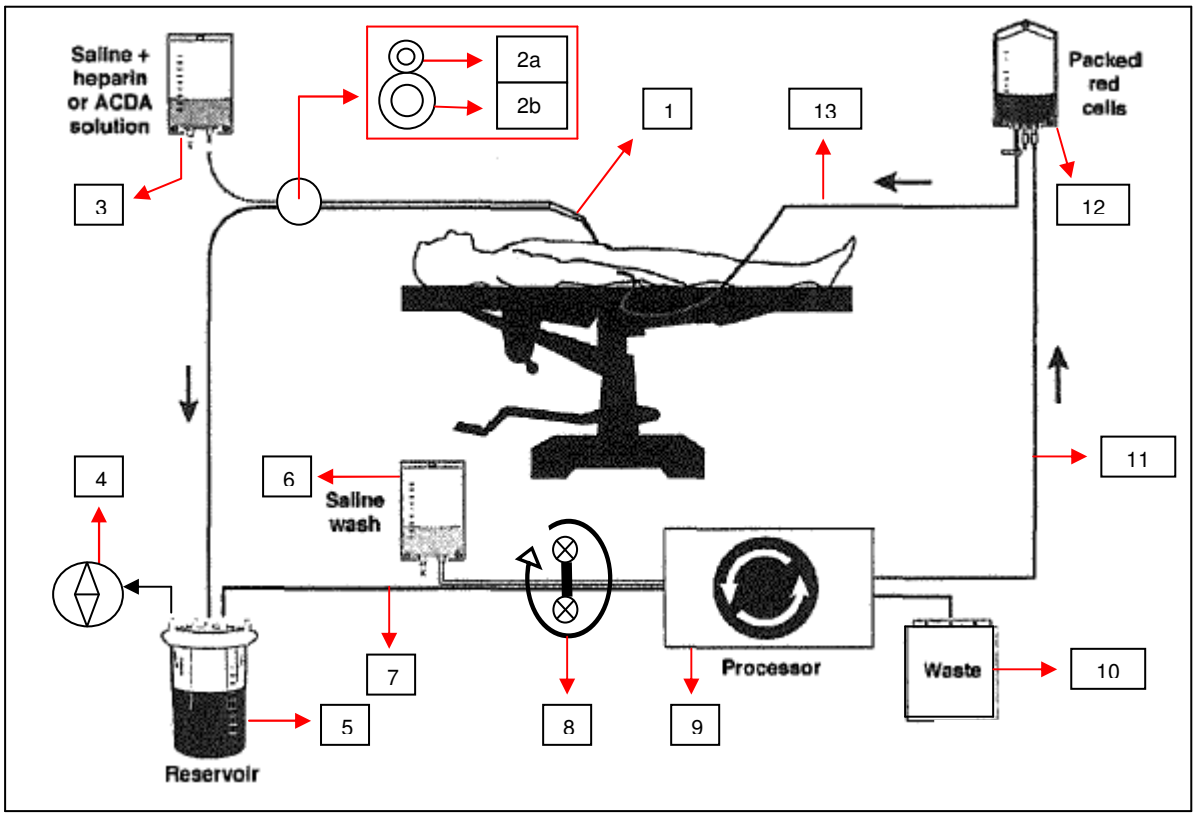



**Figure 1:** Schematic flow through an autologous cell salvage system used at Inkosi Albert Luthuli Central Hospital, Kwazulu Natal, South Africa.

The schematic flow diagram in figure 1 above represents the thirteen components required in an autologous cell salvage system (ACSS), and table 1 tabulates the components and function.

**Table 1**: Components and function in an ACSS.

| Component | Function |
|---|---|
| 1 | Represents the suction device. |
| 2 | 2a and 2b represent a double lumen tube, line 2a carries anticoagulant solution towards the suction device. Line 2b carries anticoagulated blood mixture to a collection reservoir. |
| 3 | Represents a bag with the anticoagulant solution. The anticoagulant used is often 30000 international units (IU) of high molecular weight heparin mixed with one litre 0.9% saline. Alternatively acid citrate dextrose solution A (ACD-A) is used. |
| 4 | Represents a variable regulated suction device. |
| 5 | Represents the collection reservoir that contains a micro-aggregate filter which has a pore size of 20-40µm (microns). |
| 6 | Represents the wash solution, usually 0.9% saline that is carried to the centrifugal processor. |
| 7 | Represents the line that carries unprocessed filtered blood to the centrifugal processor. |
| 8 | Represents a roller pump that controls the rate at which unprocessed blood or wash solution is pumped into the centrifugal processor. |
| 9 | Represents the centrifugal processor unit which may be a discontinuous or continuous centrifuge systems. |
| 10 | Represents the waste bag that collects effluent from the centrifuge. |
| 11 | Represents the processed blood collection line that connects the centrifuge processor to the reinfusion bag. It is also referred to as the reinfusion line. |
| 12 | Represents the reinfusion bag that collects the processed re-suspended red blood cells (RBC). |
| 13 | Represents a central or peripheral infusion line from the reinfusion bag. |

The components of an ACSS are required to accomplish four steps:

1. Collection and anticoagulation of shed blood: proper techniques should be applied in the collecting of shed blood to reduce RBC trauma. The system should be anticoagulated at all times to prevent clotting of blood in the system.

2. Filtering: blood must pass through a filter to remove large particles such as fat and fibrin. This will aid the centrifuging and washing process.

3. Centrifugation: separates low molecular weight and high molecular weight substances, and channels the low molecular weight substances into the waste container. Basically this fractionates RBC from other components of shed blood.

4. Washing: isotonic solution is introduced to carry away remaining activated coagulation factors, free hemoglobin, heparin, and proteolytic enzymes during further centrifugation. Thereafter, the RBC re-suspension can be collected for reinfusion.

## 2.3 THEORY OF OPERATION OF THE CENTRIFUGE UNIT

The centrifuge unit depends on basic principles of physics for its operation. The processing of salvaged blood into its various constituents depends firstly on the various densities (ρ) of these constituents (table 2) and secondly the centrifugal forces generated in the processing bowl (figure 2).

**Table 2**: Density range for the constituents in blood, (Reeder, 2004)

| Blood component | Constituent density range |
|---|---|
| Plasma | 1.025-1.029 ρ |
| Platelets (PLT) | 1.060-1.067 ρ |
| White blood cells (WBC) | 1.065-1.090 ρ |
| Red blood cells (RBC) | 1.085-1.097 ρ |

The constituents of blood with higher densities or mass will be subject to higher centrifugal force at higher rotational velocities.

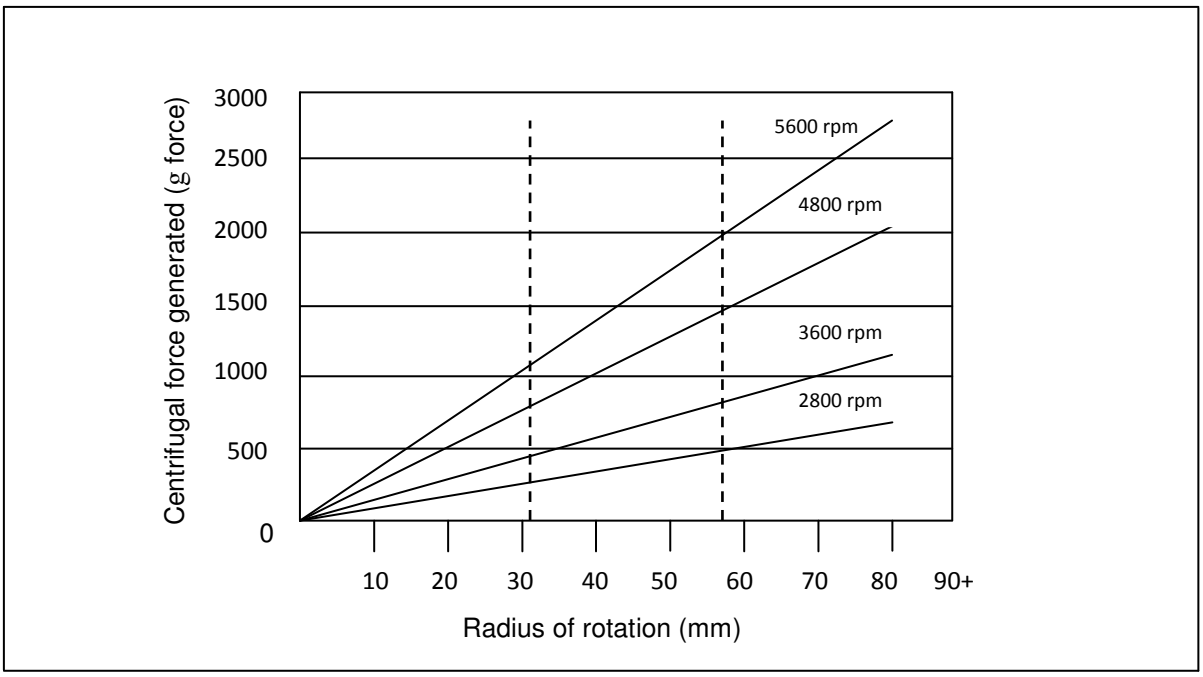



**Figure 2:** Relationship between rpm and g forces generated in the processing bowl, (Reeder, 2004).

Figure 2 illustrates that the centrifugal forces generated in the cell saver bowl is directly proportional to its rotational velocity at any given radius of rotation. Higher g forces maximise trapping of cellular constituents in the bowl. Most modern cell savers have pre-programmed rotational velocities that can be adjusted by the operator, however, this will change the quality of the final product.

As the bowl spins around its central vertical axis, progressively higher gravitational (g) forces are generated radially from the central axis (figure 3) that is expressed in the following equation (Reeder, 2004):

$$F(\mathrm{g}) = \mathrm{m}\left[\frac{\mathrm{v}^2}{\mathrm{r}}\right]$$

In the equation, the gravitational force (Fg) is in Newton's (N), "m" is the mass in kilograms (kg), "v" is the rotational velocity in meters per second ($ms^{-1}$), and "r" is the radius of rotation from the central axis in meters. With a fixed radial distance and fixed densities, the rate of

rotation or revolutions per minute (rpm) is therefore the controlling variable for centrifugal force.

## 2.4 DISCONTINUOUS AND CONTINUOUS AUTOLOGOUS CELL SALVAGE SYSTEMS

The autologous cell salvage system can be divided into either a discontinuous auto transfusion (DAT) system (figure 3 - 4), or a continuous auto transfusion (CAT) system (figure 5 - 6). The DAT system employs a conical (Latham) bowl or a cylindrical (Baylor) bowl design. The CAT system uses a double channel spiral chamber similar to that found in larger aphaeresis units. The Latham bowl has a volume of 225 millilitres (ml), the Baylor bowl 250ml, and the CAT separation chamber has a volume of approximately 30ml. Regardless of which system is used the ACSS must accomplish the four steps already mentioned on pages 4 and 5.

## 2.4a BASIC OVERVIEW OF PROCESSING SALVAGED BLOOD IN THE DAT SYSTEM

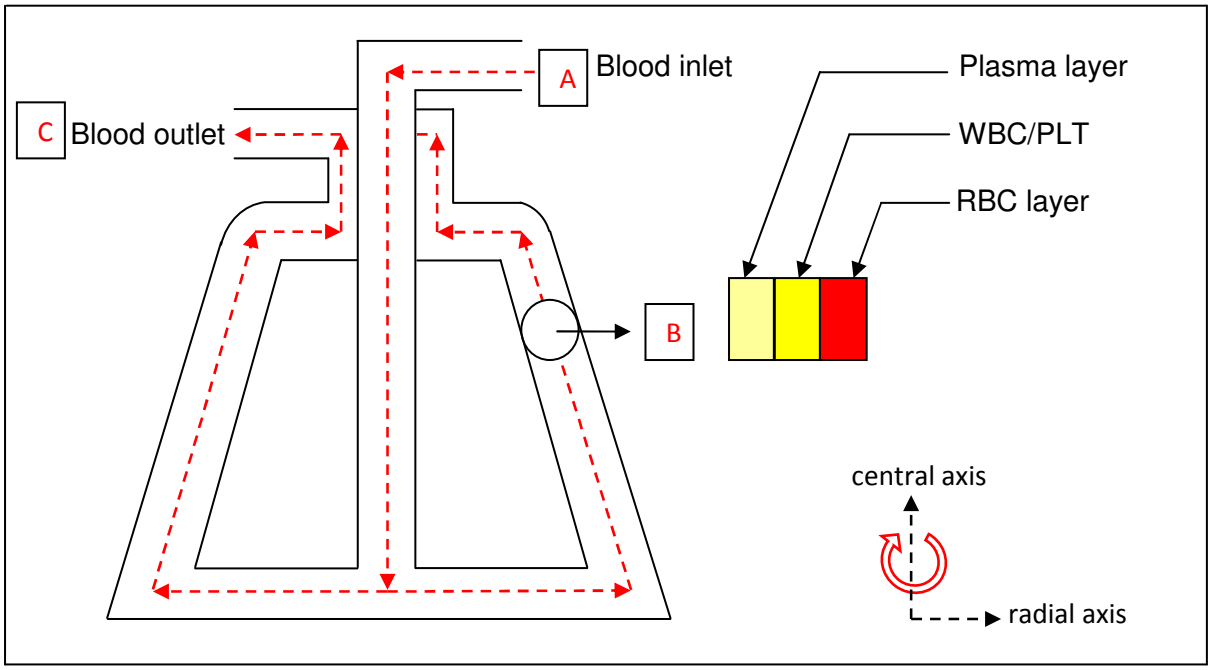



**Figure 3:** Cross sectional view of the conical (Latham) centrifuge bowl. (A) represents the blood inlet for blood and wash solution, (B) represents an exploded view of the centrifuged blood layers, that is the plasma, WBC/PLT, and RBC layers respectively, and (C) represents the blood outlet for blood and waste effluent.

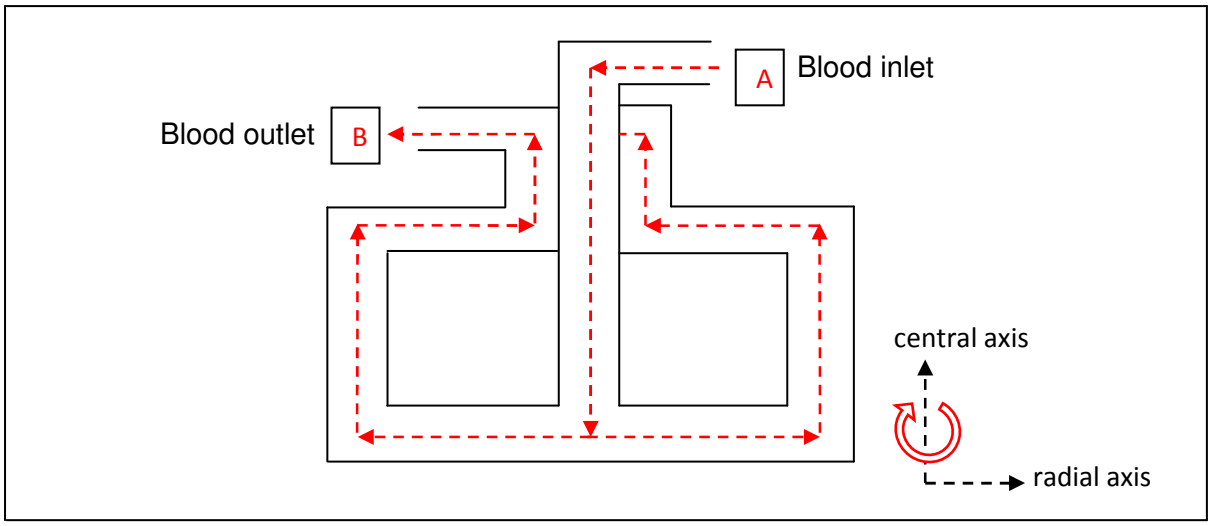



**Figure 4:** Cross sectional view of the cylindrical (Baylor) centrifuge bowl. (A) represents the blood inlet for blood and wash solution, and (C) represents the blood outlet for blood and waste effluent.

SET UP AND PRIMING

Once the ACSS has been set up as per manufacturers instructions (figure 1), 30,000 IU of high molecular weight heparin is added to 1 litre of 0.9% saline. Approximately 100ml of this anticoagulation solution is added to the reservoir to prime (wet) the micro-aggregate filter, to prevent clotting in the reservoir. The variable regulated suction device is set at negative 120 millimetres mercury (mmHg), the use of higher negative pressure can cause damage to potentially salvageable RBC.

COLLECTION OF SALVAGED BLOOD

Blood suctioned from the surgical field is mixed with anticoagulation solution and passes through the micro-aggregate filter of the reservoir where large particulate substances such as fat globules, clots and bone fragments are trapped.

FILLING AND CENTRIFUGING PHASE

The filtered blood from the reservoir is pumped into the rotating Latham (figure 3) or Baylor (figure 4) bowl at 5600rpm and 4400rpm respectively. Filtered blood enters the bowl through the blood inlet and passes through a central tube to the bottom of the bowl. Blood is then

forced centrifugally from the central axis peripherally toward the outer wall of the bowl, and the constituents of the blood separates into vertical concentric columns based on their relative density (table 2). Thus RBC are held and concentrated along the outer wall while most of the plasma, irrigating solutions, and other of the lightest constituents are progressively forced toward the centre and are displaced upward and exit through the blood outlet into the waste bag. Pumping of filtered blood into the bowl will continue until the bowl is filled with RBC, this also aids in the removal of irrigation fluids and plasma via the blood outlet. PLT and WBC will collect on the surface of the RBC pack where they can often be seen as a thin white ring, the “buffy coat.” A photo-optic sensor located near the top of the bowl detects when the bowl is filled with RBC and stops the filling phase of the bowl.

WASHING PHASE

In the washing phase normal saline is pumped into the centrifuge and between the layers of the RBC concentrate. Plasma, soluble proteins and non-erythrocyte substances that have lower densities than RBC are displaced upward and are replaced with normal saline. The displaced waste exits the blood outlet into the waste bag. Centrifugal washing removes most soluble proteins such as fibrinogen, clotting factors, albumin, thrombin antithrombin III complex, and interleukin 6. Non-erythrocyte substances include WBC, PLT, and free haemoglobin (Hb). The heparin used to anticoagulate the ACSS is also washed out.

COLLECTION PHASE

Washed RBC are re-suspended in normal saline solution are collected into a reinfusion bag. Washed re-suspended blood has a final haematocrit of approximately 55 – 70%. The entire process is repeated again if additional blood needs to be processed, therefore blood is discontinuously processed in units.

## 2.4b BASIC OVERVIEW OF PROCESSING SALVAGED BLOOD IN THE CAT SYSTEM

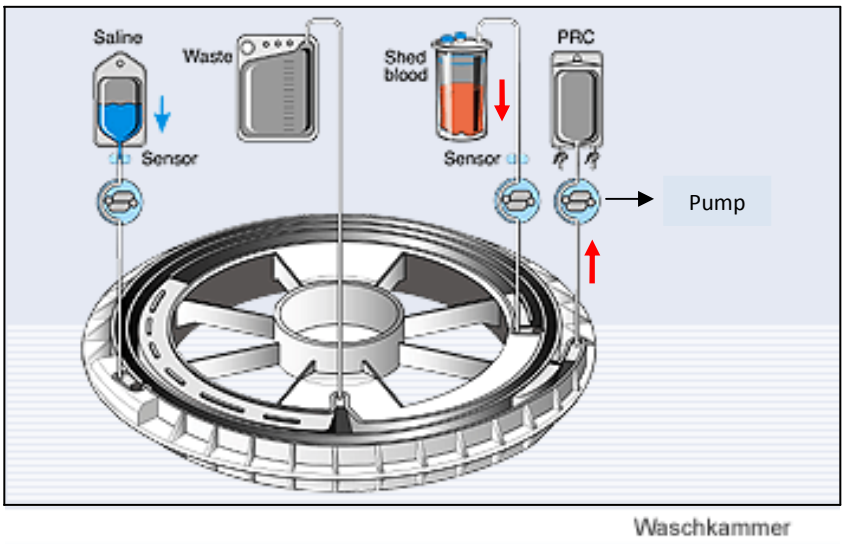



**Figure 5:** The CAT system, in which blood is centrifuged in a double channel spiral chamber, (Fresenius, n.d.).

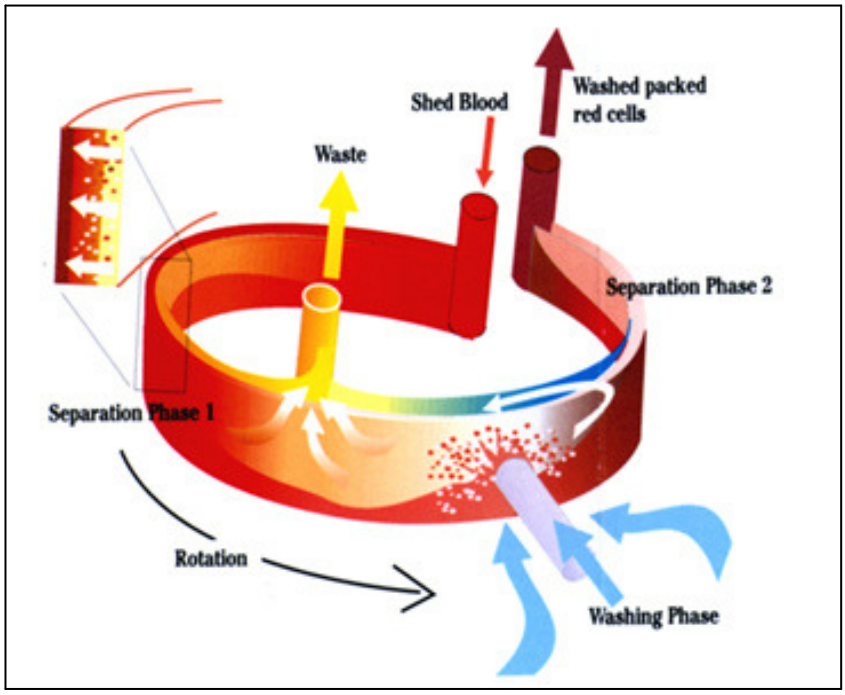



**Figure 6:** Flow path of shed blood, wash solution, waste effluent, and washed RBC's in the double channel spiral chamber of the CAT system, (Fresenius, n.d.).

In the CAT system (figure 5 – 6), setup, priming and collection of salvaged blood is identical to that of the DAT system. Filtered shed blood is suctioned from the reservoir and pumped into the inner spiral of the rotating separation chamber (1400rpm – 2400rpm). The blood is then forced centrifugally toward the outer spiral where on its way it is washed with normal saline that is added by a second pump. Plasma, soluble proteins and non-erythrocyte substances that have lower densities than RBC (table 2) leave the double spiral at its inner most point as wash effluent. The displaced waste exits into the waste bag. This represents separation phase 1. The washed concentrated RBC are centrifugally forced into the outer spiral where they are collected by a third pump into a reinfusion bag. This represents separation phase 2. The washed re-suspended RBC concentrate has a final haematocrit similar to that in the DAT system. Three steps of filling, washing, and collection are performed simultaneously by three pumps until all the blood is processed, thus blood is continuously processed. This method is therefore independent of blood volume to be processed, as in the DAT system, and the smaller priming volume may be beneficial for small volume losses such as in paediatric surgical cases.

Good practice also requires the use of blood filters of 40µm or less for the reinfusion of any salvaged blood that is processed or unprocessed.

## 2.5 HISTORIC OVERVIEW

The first successfully recorded use of cell salvage and autologous transfusion was in 1818 by a British gynaecologist named Dr James Blundell. Blundell initially became interested in transfusion as a method of treating post-partum haemorrhage after being appalled by his own helplessness to combat fatal haemorrhage during delivery (Blundell, 1818). He transfused a woman afflicted with post-partum haemorrhage, blood soaked swabs were washed in saline and then the mixture was re-infused. This practice in its time was unsurprisingly associated with high mortality. Given the knowledge of haematology in Blundell's era he opposed animal to human transfusions but was an advocate of human to human transfusions. He did, however, report the problems sucn as rapıd coagulation of the blood, infusion of air, and the dark urine passed in some patients (due to incompatible blood types) but maintained that transfusion should be used only in critically ill patients. In 1874, an English surgeon, William Highmoore, also proposed the use of autotransfusion in his article published in the Lancet in 1874. He suggested that the patient's own blood is an overlooked source which can be used to great advantage and advocated intraoperative autotransfusion

particularly in the case of post-partum haemorrhage. In 1885, a surgeon Dr John Duncan performed an amputation of a crushed limb at a late hour, and having no blood donors and only a saline solution as an imperfect alternative, chose to capture and re-infuse approximately eight ounces of a blood mixture collected while amputating the crushed limb. The transfusion of autologous blood proved successful and the patient made a full recovery.

Experimentation with cell salvage and autologous transfusion continued into the next century when it was successfully reported to be used in 1914 by a German, M. J. Theis in the treatment of ruptured ectopic pregnancy. Thereafter there were continued reports of autransfusion employed in procedures including haemothorax, ruptured spleen, and perforating abdominal injuries. In 1943, Arnold Griswald developed the concept of the first cell salvage autotransfusion device (Ashworth and Klein, 2010). Suctioned blood was collected in a bottle and then strained through a cheese cloth before being re-infused. This formed the basic principles on which modern cell salvage devices are designed today. In the 1970's an American military surgeon, Klebanoff and Bentley Laboratories developed the first commercially available cell saver machine. The system required patients needing systemic anticoagulation and was associated with a number of complications, such as haemolysis, air embolism, coagulopathy, and renal failure resulting from reinfusion of unfiltered particles (Klebanoff, 1970). As the Bentley system lost favour the Haemonetics corporation, founded by Jack Lathman in 1971, developed a discontinuous flow centrifuge system that washed salvaged blood with normal saline solution (Haemonetics Historical Timeline 2014). Fresenius later developed an alternative option, the continuous flow centrifuge system.

## 2.6 LITERATURE REVIEW

Perceptions of blood transfusions have changed as it has become appreciated that transfusions are not without risk. Stored red blood cells undergo time-dependant metabolic, biochemical, and molecular changes which eventually result in irreversible damage, defined as "storage lesions" responsible for many adverse effects of RBC transfusions (Offner, 2004). Improvement in techniques have allowed for a storage period of 42 days for homologous blood. Stored blood has been shown to accumulate cytokines and other inflammatory mediators (*ibid*). Plasma from stored RBC has been demonstrated to prime the oxidase system in neutrophils (even if leuko-reduced), delay apoptosis, and activate endothelial cells, setting the stage for neutrophil mediated tissue destruction and organ failure (*ibid*). Several investigators have identified blood transfusion as an independent risk factor for post injury multiple organ failure (*ibid*). Other investigators have observed that the

mean duration of storage of all transfused RBC was a significant predictor of post-operative pneumonia and wound infection (*ibid*). The risk of pneumonia increased by 1% per day of mean RBC storage time after controlling for other known risk factors (*ibid*).

A study was conducted by Koch et al. (2008) which examined data from patients given red cell transfusion for coronary artery bypass grafting (CABG) or heart valve surgery or both between 1998 to 2006. A total of 2872 patients received 8082 units of blood that had been stored for 14 days or less ("new blood"), and 3130 patients received 10782 units of blood that had been stored for more than 14 days ("older blood"). Patients who were given older blood had higher rates of in hospital mortality (2.8% vs. 1.7%; $p < 0.004$), intubation beyond 72 hours (9.7% vs. 5.6%; $p < 0.001$), renal failure (2.7% vs. 1.6; $p$ 0.003), and sepsis or septicaemia (4.0% vs. 2.8%; $p = 0.01$). A composite of the complications were more common in patients given older blood (25.9% vs. 22.4%; $p = 0.001$). At 1 year, mortality was significantly less in patients given newer blood (7.4% vs. 11%; $p < 0.001$). The results demonstrate that patients receiving stored blood older than two weeks had a significantly increased risk of post-operative complication as well as reduced short term and long term survival.

In a single centre retrospective study (van de Watering et al., 2006), 2732 patients between the period of 1993 until 1999 who had undergone CABG with allogenic blood use were analyzed. Endpoints were 30-day survival, hospital stay, and intensive care unit (ICU) stay. The median shelf life for all transfusions was 18 days. A total of 954 patients (34.6%) received transfusions being stored at less than 18 days (median 13 days). A total of 950 patients (34.8%) received transfusions stored longer than 18 days (median 24 days). The results revealed that patients who received blood older than 18 days compared to patients who received the same number RBC units younger than 18 days had no indication for a deleterious effect of older transfusions in patients undergoing CABG. Multivariate analyses showed that the storage time of transfused RBC in patients undergoing CABG did not correlate to 30-day survival or length of ICU stay. The univariate analyses showed strong correlation between storage time and the endpoints of 30-day survival and ICU stay. Univariate analyses also showed established risk factors such as number of RBC unit transfusions ($p < 0.001$), preoperative haemoglobin (Hb) ($p < 0.001$), duration of surgery ($p < 0.001$), and patients age ($p < 0.001$) were independently correlated with endpoints ($p < 0.001$). The investigators concluded that from their analyses no justification could be

found for the use of a particular maximum storage time for RBC transfusions in patients undergoing CABG.

In a similar retrospective study conducted (van Straten et al., 2011), retrospective data was analyzed for patients who underwent isolated CABG between 1998 and 2007. Patients were divided into 3 groups according to storage time of RBC, with the cut off point of 14 days. “Only younger blood” (n=1422), “only older blood” (n=1719), and at least 1 unit of older RBC for “any older blood” (n=2175). “Younger blood” in this study refers to RBC younger than 14 days and “older blood” refers to RBC older than 14 days. Patients in the “only older RBC” group were included in the “any older RBC”. The investigators tested the hypothesis that longer storage of transfused RBC increase the risk of early (death within 30 days postoperatively) and late mortality (death later than 30 days postoperatively) in patients who undergo CABG. Univariate and multivariate logistic regression analyses were performed for early mortality, and Cox proportional hazard regression analyses were performed for late mortality.

The results reveal that when maximum storage time was entered into the statistical model as a continuous variable for blood older than 14 days it was not identified as a risk factor for both early and late mortality. However the number of RBC and fresh frozen plasma (FFP) transfused were identified as a significant ($p$ <0.0001) risk factor for early and late mortality, and number of PLT a significant ($p$ <0.0001) risk factor for early mortality. One of the other risk factors included for both early and late mortality were preoperative Hb ($p$ < 0.0001). All the risk factors identified in the univariate statistical model were entered into the multivariate model. Once again maximum storage time of blood older than 14 days was found not to be a risk factor for early or late mortality. However the predictors for early mortality were the number of red blood cell units transfused ($p$ < 0.0001), and one of the predictors for late mortality was low preoperative Hb ($p$ < 0.0001). Van de Watering et al., (2006) also reported similar results with regard to number of RBC units transfused and preoperative Hb. The number of RBC transfusions appears to amplify the storage time of RBC thereby exerting its effects. Patients with low preoperative Hb may benefit from cell saving (CS) by decreasing the number of RBC transfusions required and improving post operative Hb. Further prospective randomized studies need to be conducted.

The automatic mode on the Haemonetics cell saver $5^+$ is not a unique mode. It has been found that different wash rates as well as different centrifuge speeds render an essential

influence on the quality of processed blood. In a study conducted by Naumenko et al., (2008), residual CPB blood was washed with 1000 ml of normal saline at a centrifuge speed of 5600 rpm (group 1) or 4350 rpm (group 2) with different washing pump speeds of 500; 800; and 1000 ml/min. The results of the data revealed that high wash pump speeds (800-1000 ml/min) and standard centrifuge speed (5650rpm) in the Haemonetics cell saver $5^+$ produced conducive results with a favourable outcome. There was perfect removal of free haemoglobin as well as maximal red blood cell concentration. Protein concentration decreased significantly ($p < 0.001$) in both groups at all pump speeds. Further studies to investigate various other changes that deviate from the manufacturers recommendations is needed to ascertain the best autologous blood protocol for individual cardiac units and procedures. Amand et al. (2002) conducted a prospective study to investigate the quality of residual CPB processed with five different cell saver machines. The results revealed a significant increase in HCT ranging from 53,7% to 68,9% ($p < 0.001$) with all ACSS, and a significant attenuation of inflammatory markers after processing residual pump blood with ACSS.

A study undertaken by Sirvinskas et al. (2005), in which patients undergoing coronary artery bypass grafts (CABG) or mitral valve replacement (MVR), were divided into three groups. Group 1 (37 patients) received residual pump blood without processing, group 2 (45 patients) did not receive residual pump blood, and group 3 (42 patients) received transfusion of residual pump blood processed by the centrifuging without washing. The results revealed that haematocrit (HCT) values 12 hours after the procedure in group 3 were higher than those in group 1 and 2 by 13.2% and 11.1% respectively ($p < 0.05$). The number of donor blood transfusions in group 3 was significantly lower (28.57%) in comparison to groups 1 and 2 (37.83% and 38.10% respectively). The rate of infective complications in group 3 was 18.1% lower ($p < 0.05$) than in group 2 and 9.2% lower than in group 1.The mean length of hospital stay in group 3 was 25.8% shorter than in group 1 ($p < 0.05$), but did not differ from group 2. Blood loss during the first 12 hours after the operations did not differ between groups. This study shows the significant benefits of processing residual pump blood and its impact on clinical outcome.

A randomized double blinded study was conducted to assess the effect of processing of shed blood during CPB on transfusion and neurocognitive function (Rubens et al., 2012). Patients undergoing CABG and/or aortic valve surgery using CPB were randomized to receive unprocessed blood or cardiotomy blood that had been processed by ICS and lipid

filtration. The unprocessed blood group consisted of 134 patients and the treatment group consisted of 132 patients. The results of the trial reported that the proportion of patients transfused was higher in the treatment group (42% vs. 36%) but did not reach statistical significance ($p = 0.27$). Overall patients in the treatment group demonstrated increased RBC (1.24 ± 2.71 vs. 0.81 ± 1.24; $p = 0.001$) units per patient and non-RBC (2.06 ± 7.70 vs. 1.12 ± 4.74; $p < 0.001$) units per patient transfusion. Blood loss from protamine administration to skin closure was no different between groups. Post operative chest tube output was significantly higher in the treatment group as opposed to the control group (control 1014 ± 420 ml vs. treatment 1133 ± 476 ml per 24 hours, $p = 0.04$). Patients were monitored intraoperatively by transcranial Doppler and they underwent neuropsychometric testing before surgery and at 5 days and three months after surgery. There was no incidence of postoperative cognitive dysfunction, quality of life, or number of emboli detected in both groups. The investigators concluded that processing of cardiotomy blood before reinfusion resulted in a paradoxical increase in blood product use in patients undergoing cardiac surgery. There is no clinical evidence of any neurologic benefit in terms of postoperative cognitive function with processed cardiotomy blood.

In contrast a review article by Lau et al. (2007), revealed that small capillary arteriolar dilations (SCADS) as a result of fat emboli, were lodged in the brain and organs of deceased patients following CPB. Djaiani et al. (2007), reported a median emboli count of 90 (range, 5 to 1531) in the cell saver and 133 (range, 18 to 1811) in the control group ($p = 0.31$) and that processing of shed blood with CS resulted in clinically significant reduction ($p = 0.03$) in postoperative cognitive dysfunction in patients older than 60 years undergoing elective CABG. Cognitive dysfunction was present in 6% of the CS group and 15% of the control group. Djaiani et al. (2007), also reported that a total of 28 patients (25%) in the cell saver group and 14 patients (12%) in the control group received fresh frozen plasma (FFP) transfusion at any time during the perioperative period ($p = 0.018$). The FFP transfused group received significantly more cell saver blood than the control group not transfused ($p < 0.0001$), but the total number of PRBC between CS and control group did not differ.

Despite 0.9% NaCl being one of the most widely used crystalloid solutions throughout the world as a resuscitation, replacement and maintenance solution, its origins of becoming known as “normal” and being brought into *in vivo* clinical practice remains obscure. The Indian blue cholera pandemic that first reached England in 1831 resulted in the development of intravenous fluid therapy (Awad, Allison and Lobo, 2008). It was William Brooke O’

Shauhgnessy who studied and treated patients afflicted with cholera that realised the need to rehydrate patients with salt water solutions *(ibid)*. Different mixes of salt solutions to closely match the composition of serum were used by physicians treating chorela patients, however all the early solutions were hypotonic. Thomas Latta in 1832 formulated a modified original solution of his own that closely resembled blood electrolyte concentrations and was more physiologic than 0.9% saline *(ibid)*. However, the solution was not adopted and a similar physiologic solution was only described 50 years later by Sidney Ringer.

The first recorded use of the term "normal saline" was a published report in the Lancet in 1888 to treat a case of "scirrhus of the pylorus", and the "normal saline" solution used had no resemblance to 0.9% saline *(ibid)*. In the late $19^{th}$ century it was realised for a fluid to be physiologic it had to be isotonic with the serum of the animal. It was Hartog Jakob Hamburger who found that a 0.9% saline solution was isotonic with a majority of warm blooded animals including man. Hamburger's credibly backed scientific evidence supporting the use of 0.9% saline in clinical practice is based solely on *in vitro* study. It remains a mystery how it came into general clinical practice as an intravenous fluid. The terms "normal" and "physiologic" are reassuring terms applied to use of 0.9% saline and have no scientific validity but may have aided its widespread use, or perhaps due to the ease, convenience and low cost of mixing salt with water *(ibid)*. Historical fallacy and misconception have resulted in widespread use of 0.9% saline, however, its use is justified involving large chloride loses due to vomiting and diarrhoea.

Investigators Varghese et al. (2007), conducted a study with the aim to compare autotransfusion devices to reduce non-infectious complications related to transfusion of long stored PRBC. A prospective randomized control *in vitro* experiment was conducted to test the hypothesis. Fifty seven outdated leucodepleted PRBC were studied for increased micro-aggregate load and electrolytes outside the physiologic range. Blood was processed and evaluated using the Haemonetics CS which is a discontinuous system, or the CATS which was operated on quality and emergency modes. Blood was processed using normal saline. The observed low calcium plasma levels have been attributed to its uptake by binding to citrate used as a conservation media. The results in table 3 reveal that $[K^+]$, $[PO_4^{3-}]$, glucose, osmolality, protein, and lactate dehydrogenase (LDH), were all significantly reduced ($p < 0.05$) after processing with both CS devices. Sodium concentration was the only electrolyte that significantly increased ($p < 0.05$). This may be attributed to the wash solution. There was no significant removal of large micro-aggregates (>17.6 µm), and these bigger particles are most likely to stay with RBC during processing due to their high specific weight

and subsequent high centrifugal forces being exerted during processing. There was significant ($p < 0.05$) removal of fragments (7.8 - 17.6 µm) by the CATS on quality mode when compared to the baseline. There was a significant ($p < 0.05$) removal of extremely small particles (<7.8 µm) when compared to baseline by the CATS in both modes. The Haemonetics CS did not prove to decrease the micro-aggregate load of PRBC. The investigators concluded that in instances where long stored PRBC have to be transfused, CS processing may be feasible to reduce clinical side effects of hyperkalaemia and microvascular obstruction secondary to cell fragments. This procedure may be useful in patients who require massive transfusion and suffer or are at risk from renal failure (Varghese et al., 2007).

**Table 3**: Changes in [$K^+$], [$Na^+$], [$PO_4^{3-}$], [$Ca^{2+}$], glucose, osmolality, protein, and LDH after processing with CAT and DAT systems, (Varghese et al., 2007).

| Variable | Before IAT device | After HCS | After CATS (quality mode) | After CATS (emergency mode) |
|---|---|---|---|---|
| Potassium (mEq $L^{-1}$) | 52 (44; 58) | 4*† (3; 4) | 4*† (3; 5) | 17*‡ (14; 20) |
| Sodium (mEq $L^{-1}$) | 92 (87; 95) | 154*† (153; 160) | 153*† (148; 159) | 138*‡ (136; 143) |
| Phosphate (mEq $L^{-1}$) | 37 (36; 39) | 16*† (13; 18) | 13*† (10; 20) | 22*‡ (19; 25) |
| Calcium (mEq $L^{-1}$) | 0·1 (0; 0·2) | 0·1 (0; 0·2) | 0·1 (0; 0·2) | 0·1 (0; 0·2) |
| Glucose (mg $dL^{-1}$) | 369 (353; 404) | 132*† (121; 158) | 120*† (99; 149) | 214*‡ (190; 269) |
| Osmolality (mEq $L^{-1}$) | 304 (298; 310) | 287*† (285; 290) | 287*† (282; 289) | 295*‡ (293; 298) |
| Protein (g $dL^{-1}$) | 0·4 (0·4; 0·5) | 0·1*† (0·1; 0·1) | 0·1*† (0; 0·1) | 0·2*‡ (0·2; 0·3) |
| LDH (U $L^{-1}$) | 429 (376; 520) | 11*† (6; 14) | 5*†‡ (2; 7) | 75*‡ (43; 92) |

Values are median (25th, 75th percentile) of measurements with intraoperative autotransfusion devices (IAT; $n = 19$ each).
HCS, Haemonetics Cell Saver; CATS, Continuous Autotransfusion System.
*$P < 0.05$ vs. before IAT device.
†$P < 0.05$ vs. after CATS (emergency mode).
‡$P < 0.05$ vs. after HCS.

A similar study was conducted by de Vroege et al. (2007). After two weeks stored RBC showed an increase in extracellular lactate, [$K^+$] and partial pressure of carbon dioxide ($pCO_2$), while pH and bicarbonate decreased (acidosis). The investigators set out to study washing RBC with the Dideco Electa 740E CS in comparison to unwashed RBC. Twenty RBC units with a mean storage time of 36 days were used. The wash solution was 0.9% sodium chloride. Measured parameters included pH, $pCO_2$, partial oxygen pressure ($pO_2$) and oxygen saturation ($sO_2$), [$HCO_3^-$], [$Ca^{2+}$], [$Na^+$], glucose and lactate. The results showed that haematocrit (HCT) pre and post washing was (58.3 ± 4.5 vs. 57.1 ± 4.8%), the volume of blood pre and post washing is (153.8 ± 12.2 vs. 132.9 ± 12.2 ml). This translated into an 85% recovery rate of Hb after washing. The results in table 4 show that pH and $pO_2$ did not

change after processing. Glucose, $pCO_2$, $[HCO_3^-]$, and lactate were significantly reduced ($p < 0.05$). $[Na^+]$ was the only electrolyte to significantly increase ($p < 0.05$). Extremely high $[K^+]$ concentration was almost completely eliminated after washing (de Vroege et al., 2007). Varghese et al. (2007), observed similar results with $[K^+]$, glucose, lactate and $[Na^+]$ after washing. Baseline $[Ca^{2+}]$ was very low this was also observed by Varghese et al., (2007). Free Hb was not reduced and on average a small increase was found, which may be attributed to a small degree of hemolysis of unviable older RBC that do not withstand high centrifugal forces. Chloride, magnesium and phosphate which are also important electrolytes were not measured. The investigators concluded that the quality of RBC was not markedly and irreversibly affected after washing and that waste products are effectively removed. This technique may be useful where there is a risk of developing hyperkalaemia secondary to transfusion of long stored RBC, as in neonates and infants.

**Table 4**: Changes in pH, $pCO_2$, $pO_2$, $[HCO_3^-]$, $[K^+]$, $[Na^+]$, $[Ca^{2+}]$, $sO_2$, glucose, lactate, and free Hb, before and after CS processing, (de Vroege et al., 2007).

| Variable | Before washing ± SD | After washing ± SD |
|---|---|---|
| Ph | 6.36 ± 0.04 | 6.36 ± 0.05 |
| $pCO_2$ (kPa) | 8.07 ± 3.59 | 0.73 ± 0.55 # |
| $pO_2$ (kPa) | 26.92 ± 9.64 | 30.9 ± 7.25 |
| $[HCO_3^-]$ (mmol/L) | 3.36 ± 1.58 | 0.45 ± 0.13 # |
| $[K^+]$ (mmol/L) | 47.91 ± 6.78 | 1.91 ± 0.73 # |
| $[Na^+]$ (mmol/L) | 115.0 ± 6.8 | 145.0 ± 1.2 # |
| $[Ca^{2+}]$ (mmol/L) | 0 ± 0 | 0.03 ± 0.08 |
| $sO_2$ (%) | 94.0 ± 8.0 | 97.0 ± 2.0 |
| Glucose (mmol/L) | 14.0 ± 2.5 | 3.62 ± 0.9 # |
| Lactate (mmol/L) | 16.4 ± 2.1 | 7.6 ± 2.0 # |
| Free haemoglobin (g/L) | 0.93 ± 0.37 | 1.03 ± 0.40 |

SD - Standard deviation
# - significant difference compared to baseline values ($P < 0.05$)

In an *in vitro* setting Huber et al. (2013), washed ten units of PRBC with a mean age of 9 ± 5 days old, with either 0.9% NaCl or a bicarbonate-buffered haemofiltration solution (BB-HS)

using a cell saver. The results of the study (table 5) reveal that in the NaCl group, pH, $pCO_2$ and [$HCO_3^-$] significantly decreased, in contrast to the significant increase in the BB-HS group when compared to the saline group. Base excess significantly increased in the NaCl group in contrast to a significant decrease in the BB-HS group. [$K^+$] significantly decreased in both groups, whereas [$Na^+$] and [$Ca^{2+}$] increased significantly in both groups, despite saline being $Ca^{2+}$ free. [$Cl^-$] significantly increased in the NaCl group in contrast to a significant decrease in the BB-HS group. Glucose and lactate significantly decreased in both groups. Free Hb significantly increased in the NaCl group, in contrast to a significant decrease in the BB-HS group. Adenosine triphosphate (ATP) significantly increased in the BB-HS group, and osmotic resistance was significantly higher ($p < 0.05$) in the BB-HS group as compared to the NaCl group.

**Table 5**: Changes in measured variables before and after washing with 0.9% NaCl or BB-HS, (Huber et al., 2013).

| Variables measured | Baseline unwashed PRBC Mean ± SD | End of study NaCl Mean ± SD | End of study BB-HS Mean ± SD | P[a] |
|---|---|---|---|---|
| pH | 6.71 ± 0.12 | 6.57 ± 0.12 # | 6.85 ± 0.05 # | $p < 0.05$ |
| $pCO_2$ (mmHg) | 94.72 ± 12.39 | 18.03 ± 7.69 # | 157.5 ± 14.46 # | $p < 0.05$ |
| $pO_2$ (mmHg) | 50.76 ± 6.74 | 62.26 ± 9.29 # | 54.8 ± 8.98 # | $p < 0.05$ |
| Bicarbonate (mmol/L) | 11.30 ± 2.33 | 2.22 ± 1.26 # | 10.84 ± 1.82 # | $p < 0.05$ |
| Base excess(mmol/L) | -21.64 ± 3.52 | -30.15 ± 1.42 # | -7.51 ± 2.49 # | $p < 0.05$ |
| Potassium (mmol/L) | 18.35 ± 5.17 | 2.71 ± 1.18 # | 2.50 ± 1.54 # | NS |
| Sodium (mmol/L) | 123.9 ± 4.6 | 142.2 ± 1.93 # | 130.4 ± 1.84 # | $p < 0.05$ |
| Chloride (mmol/L) | 114.0 ± 2.50 | 138.0 ± 3.53 # | 103.4 ± 1.35 # | $p < 0.05$ |
| Calcium (mmol/L) | 0.16 ± 0.05 | 0.40 ± 0.07 # | 1.06 ± 0.16 # | $p < 0.05$ |
| Glucose (mmol/L) | 22.07 ± 2.42 | 7.69 ± 1.72 # | 12.72 ± 1.48 # | $p < 0.05$ |
| Lactate (mmol/L) | 15.83 ± 3.67 | 8.76 ± 2.52 # | 7.89 ± 2.23 # | NS |
| Haemoglobin (g/dL) | 22.37 ± 3.66 | 22.29 ± 2.62 | 20.43 ± 1.62 | NS |
| Haematocrit (%) | 64.27 ± 4.10 | 66.70 ± 7.43 | 61.63 ± 5.01 | NS |
| Free haemoglobin (mg/L) | 588.5 ± 221.8 | 656.4 ± 346.8 | 418.9 ± 241.8 # | $p < 0.05$ |
| LDH (U/L) | 67.1 ± 33.4 | 89.0 ± 71.2 | 48.8 ± 31.0 # | NS |
| ATP ($\mu mol.gHb^{-1}$) | 4.50 ± 0.63 | 4.55 ± 0.55 | 5.15 ± 0.40 # | $p < 0.05$ |

NS - not significant
LDH - lactate dehydogenase
# $P < 0.05$, end of study vs. baseline
$P^a$ - value at end of study, NaCl vs. BB-HS

Halpern et al. (1996), conducted an animal study with 9 mongrel dogs to evaluate the acid-base and electrolyte changes of high volume CS blood transfusion when 0.9 % saline was used as a wash solution. The Haemonetics cell saver with a 125 ml bowl was used, 8% (125ml) of the circulating blood volume was withdrawn, washed and re-transfused for each cycle. Animals underwent 15 wash cycles, blood samples were collected on every third cycle (cycles 3; 6; 9; 12; 15). A processed blood sample was collected before re-transfusion and a systemic blood sample was collected 5 min. after re-transfusion. Circulating blood volume was 1598 ± 201 ml, and by cycle twelve 96 % of the circulating volume was washed. Before CS washing, animals were volume loaded with 250 ml of 5 % dextrose in 0.45 % saline. Maintenance fluid was 50 millilitres per hour (ml/hr) 0.45 % saline. Mean systemic baseline

measured values were compared to mean washed blood values (end of cycle 12) and mean end systemic values (cycle 15). Table 6 shows the changes in the mean systemic baseline values and mean washed blood values when 0.9% saline is used as a wash solution.

**Table 6**: Changes in the mean systemic baseline values and mean washed blood values when 0.9% saline is used as a wash solution, (Halpern et al., 1996).

| Variables measured | Baseline systemic values Mean ± SD | Washed blood averaged Mean ± SD | Percentage change | *p* value |
|---|---|---|---|---|
| pH | 7.44 ± 0.08 | 7.34 ± 0.1 | - 1 | 0.005 |
| $pCO_2$ (mmHg) | 31.8 ± 10.9 | 9.8 ± 5.2 | - 69 | 0.0005 |
| Total $CO_2$ (bicarbonate: meq/L) | 19.0 ± 3.0 | 5.0 ± 2.8 | - 74 | 0.0001 |
| Potassium (meq/L) | 3.9 ± 0.56 | 1.5 ± 0.5 | - 62 | 0.0001 |
| Ionized calcium (mg/dL) | 1.44 ± 0.12 | 0.58 ± 0.2 | - 60 | 0.0001 |
| Magnesium (mg/dL) | 1.7 ± 0.2 | 0.4 ± 0.3 | - 76 | 0.0001 |
| Inorganic phosphorus (mg/dL) | 2.2 ± 1.3 | 0.9 ± 0.7 | - 57 | 0.01 |
| Sodium (meq/L) | 145.0 ± 4.0 | 151 ± 4.8 | + 4 | 0.001 |
| Chloride (meq/L) | 115.0 ± 4.0 | 145± 5.3 | + 26 | 0.0001 |
| Lactic acid (mmol/L) | 2.0 ± 0.77 | 0.8 ± 0.5 | - 61 | 0.005 |
| Total protein (g/dL) | 6.5 ± 0.96 | 1.0 ± 0.6 | - 85 | 0.0001 |
| Albumin (mg/dL) | 2.8 ± 0.37 | 0.4 ± 0.2 | - 86 | 0.0001 |
| Haemoglobin (g/dL) | 14.0 ± 1.0 | 16.5 ± 2.0 | + 20 | 0.0005 |
| Haematocrit (%) | 42.0 ± 5.0 | 48.5 ± 6.6 | +15 | 0.0005 |

The results revealed that washed blood pH was significant lower compared to baseline systemic pH, and baseline systemic pH decreased significantly ($p < 0.001$) when compared

to end systemic blood pH (7.44 ± 0.08 vs. 7.22 ± 0.12: 3 % change). Total $CO_2$ and $pCO_2$ were significantly lower in washed blood compared to baseline systemic values, but only baseline systemic total $CO_2$ decreased significantly ($p < 0.0005$) when compared to end systemic values (19 ± 3.0 vs. 7.3 ± 2.4: 62 % change).

All the electrolyte concentrations decreased significantly by more than 50% in the washed blood when compared to that of the baseline systemic blood, except for $[Na^+]$ and $[Cl^-]$ which significantly increased, the increase $[Cl^-]$ was greater than that of $[Na^+]$. Baseline systemic ionized calcium and magnesium decreased significantly when compared to end systemic values [1.44 ± 0.12 vs. 1.24 ± 0.15; ($p < 0.0005$) and 1.7 ± 1.3 vs. 1.3 ± 0.5; ($p < 0.05$)] respectively. Potassium increased insignificantly and inorganic phosphorous increased significantly ($p < 0.005$) from baseline systemic values to end systemic values (3.9 ± 0.56 vs. 4.7 ± 0.75; and 2.2 ± 1.3 vs. 5.7 ± 2.25: 157 % change) respectively despite being significantly depleted in washed blood.

The paradoxical increases were ascribed to the progressive metabolic acidosis which resulted in the intracellular to extracellular shifts of potassium and inorganic phosphorous. Hypocalcaemia may have also additionally contributed to the paradoxical potassium and inorganic phosphorous results. Chloride significantly increased ($p < 0.0001$) from baseline systemic value to end systemic value (115 ± 4 vs. 129 ± 4: 12 % change) and sodium did not change significantly. Lactic acid decreased significantly in the washed blood when compared to that of the baseline systemic blood. Total protein and albumin decreased significantly in the washed blood when compared to that of the baseline systemic blood by 85 % and 86 % respectively, and decreased significantly from baseline to end systemic values. Haemoglobin and haematocrit increased significantly in the washed blood when compared to that of the baseline systemic blood, and increased significantly from baseline to end systemic values.

The investigators concluded that the use of 0.9 % saline as a wash solution resulted in a progressive systemic (metabolic) acidosis and the depletion of total $CO_2$, $pCO_2$, electrolytes, total protein, and albumin and an increase in $[Na^+]$ and $[Cl^-]$ that are reflective of the constituents of the wash solution and the separation and wash process. Standard base excess which provides useful insight into the magnitude of the acid-base perturbations was not measured, and strong ion difference was not calculated to compare mean baseline systemic values to mean washed blood and mean end systemic values. This would have also provided more perspective.

Halpern et al. (1997) conducted a prospective randomized study to compare 0.9% normal saline with isolyte S (a balanced multi electrolyte crystalloid solution) when used as wash solution for cell saver autologous blood transfusion. Table 7 shows the changes in prewashed systemic blood and washed blood when the two different solutions are used.

**Table 7**: Changes in prewashed systemic blood and washed blood when 0.9% saline and isolyte S were used as wash solutions, (Halpern et al., 1997).

| Variables measured | Prewashed systemic blood Mean ± SD | | *p* value | Washed blood averaged Mean ± SD | | *p* value | Percent change from prewashed to washed blood | | *p* value |
|---|---|---|---|---|---|---|---|---|---|
| | Normal saline | Isolyte S | | Normal saline | Isolyte S | | Normal saline | Isolyte S | |
| pH | 7.37 ± 0.07 | 7.43 ± 0.06 | NS | 7.39 ± 0.09 | 7.39 ± 0.06 | NS | +0.3 ± 0.3 | -0.4 ± 0.3 | 0.001 |
| $pCO_2$ (mmHg) | 31.5 ± 7.0 | 31.0 ± 6.0 | NS | 9.3 ± 2.0 | 10.3 ± 3.5 | NS | -69 ± 6.0 | -67 ± 9.0 | NS |
| Bicarbonate (meq/L) | 13.6 ± 1.6 | 16 ± 2.4 | 0.05 | 4.3 ± 0.8 | 5.1± 1.4 | NS | -69 ± 4.0 | -68 ± 7.0 | NS |
| Potassium (meq/L) | 3.7 ± 0.4 | 3.6 ±0.3 | NS | 1.4 ± 0.3 | 5.0 ± 0.3 | 0.0001 | -60 ± 10 | +41 ± 8.0 | 0.0001 |
| Ionized calcium (mg/dL) | 1.3 ± 0.04 | 1.2 ± 0.06 | NS | 0.6 ± 0.1 | 0.5 ± 0.2 | NS | -55 ± 9.0 | -59 ± 14 | NS |
| Magnesium (mg/dL) | 1.5 ± 0.2 | 2.1 ± 0.2 | 0.001 | 0.6 ± 0.4 | 3.2 ± 0.2 | 0.0001 | -62 ± 23 | +58 ± 21 | 0.0001 |
| Inorganic phosphorus (mg/dL) | 4.3 ± 1.2 | 3.6 ± 1.3 | NS | 1.3 ± 0.7 | 2.1 ± 0.7 | 0.05 | -69 ± 8.0 | -37± 14 | 0.005 |
| Sodium (meq/L) | 150.0 ± 4.0 | 151.0 ± 8.0 | NS | 153.0 ± 2.0 | 147.0 ± 7.0 | 0.05 | +2.3 ± 3.0 | -3.0 ± 1.0 | 0.001 |
| Chloride (meq/L) | 125.0 ± 10 | 116.0 ± 6.0 | 0.05 | 143.0 ± 8.0 | 104.0 ± 10 | 0.0001 | +14 ± 4.4 | -10 ± 5.0 | 0.0001 |
| Lactic acid (mmol/L) | 2.7 ± 1.6 | 2.9 ± 2.0 | NS | 1.3 ± 1.0 | 1.3 ± 1.1 | NS | -54 ± 11 | -57 ± 15 | NS |
| Glucose (mg/dL) | 113 ± 38 | 104 ± 31 | NS | 23 ± 6.0 | 23 ± 15 | NS | -79 ± 5.0 | -79 ± 11 | NS |
| Total protein (g/dL) | 4.2 ± 0.7 | 4.6 ± 0.6 | NS | 0.8 ± 0.4 | 1.2 ± 0.7 | NS | -79 ± 6 | -74 ± 12 | NS |
| Albumin (mg/dL) | 2.3 ± 0.4 | 2.3 ± 0.2 | NS | 0.5 ± 0.2 | 0.6 ± 0.3 | NS | -79 ± 6.0 | - 73 ± 12 | NS |
| Haemoglobin (g/dL) | 15.8 ± 1.2 | 16.3 ± 1.3 | NS | 15.5 ± 1.7 | 15 ± 1.8 | NS | -2.0 ± 8.0 | -7.0 ± 12 | NS |
| Haematocrit (%) | 47 ± 5.0 | 49 ± 5.0 | NS | 46 ± 5.0 | 44 ± 5.0 | NS | -2.0 ± 9.0 | -9.0 ± 12 | NS |

Normal saline is associated with acid-base and electrolyte derangements when used as a wash solution. The Haemonetics cell saver with a 125 ml bowl was used; 8 % (125ml) of the circulating blood volume (approximately 1600 ml) was withdrawn, washed and re-transfused for each cycle; and animals underwent 18 wash cycles. During the study, prewashed systemic and washed blood samples were collected on every third cycle (cycles 3; 6; 9; 12; 15). In order to evaluate changes caused by the wash process itself an average of pre-washed systemic blood values and washed blood values were compared (cycles 3 to 15).

The results in table 7 reveal that in the prewashed systemic blood bicarbonate and magnesium were significantly higher in the isolyte S group as compared to the normal saline group. Pre-washed systemic blood chloride was significantly lower in the isolyte group as compared to the normal saline group. Washed blood averaged potassium, magnesium and inorganic phosphorus in washed blood were significantly higher in the isolyte S group as compared to the normal saline group. Sodium and chloride in washed blood were significantly lower in the isolyte S group as compared to the saline group. In the isolyte S group there was a significant percentage decrease in pH, sodium, and chloride, in contrast to the significant percentage increase of potassium and magnesium when compared to the normal saline group. Inorganic phosphorous decreased significantly in both groups, but was more pronounced in the normal saline group. Although statistically not significant, the percentages changes of $pCO_2$, bicarbonate and ionized calcium are clinically relevant. The investigators concluded that the use of isolyte S wash was associated with fewer acid-base and electrolyte derangements than normal saline and is recommended for high volume CS washing and transfusion (Halpern et al., 1997).

Cardiopulmonary bypass in infant and neonates often requires the use of PRBC because of the high ratio of priming volume to patient blood volume. This may result in increasing the $[K^+]$ and lactate load associated with the transfusion of stored blood. Many cardiac surgery centres use irradiated red cells because infant and neonates undergoing complex congenital heart surgery have been associated with having T-cell immunodeficiency. Irradiation inactivates any viable T-lymphocytes thus preventing the development of transfusion associated graft vs. host disease in which 90% of patients die from. Irradiation, however, weakens RBC membranes resulting in leakage of $K^+$ into the plasma causing high $K^+$ levels compared to non-irradiated stored RBC. Hyperkalaemia and hyperlactaemia have been linked to increased morbidity and mortality especially in children.

In a study conducted by Swindell et al. (2007), the investigators hypothesised that donor irradiated stored RBC processed with the CS may curtail the effects of potassium and lactate levels during neonate or infant CPB. A prospective randomized control study was conducted. The study recruited 11 patients that received CS processed blood and 11 patients that received unprocessed blood. The Dideco Electa CS was used and RBC was washed with 0.9% sodium chloride. [$K^+$] and [lactate] were compared before, during, and after CPB. Washing of irradiated RBC resulted in a significant decrease in the [$K^+$] (>20 vs. 0.8 ± 0.1 mmol/L, $p < 0.001$) and [lactate] (13.7 ± 0.5 vs. 5.0 ± 0.3mmol/L, $p < 0.001$). In contrast washing increased the [$Na^+$] (126 ± 7.0 vs. 147.6 ± 1.0mmol/L). In the CS group the prime had significantly lower [$K^+$] and [lactate] when compared to the unwashed group {[$K^+$] 2.6 ± 0.1 vs. 8.1 ± 0.4mmol/L, $p < 0.001$}; (lactate 2.6 ± 0.2 vs. 4.6 ± 0.3mmol/L, $p < 0.001$) respectively. [$Na^+$] was significantly higher in the unwashed group compared to the washed group (151.5 ± 1.0 vs. 147.5 ± 1.2mmol/L, $p = 0.02$). Immediately post CPB, the CS group had a significantly lower serum [$K^+$] than the unwashed group (3.2 ± 0.1 vs. 4.2 ± 0.2 mmol/L, $p < 0.002$). [$K^+$] above 5mmol/L have been previously shown to cause distinct electrocardiographic changes indicating hyperkalaemia, and cardiac arrhythmias have been associated with [$K^+$] of over 7.5mmol/L.

In this study 4 of the 11 patients in the in the unwashed group had [$K^+$] above 6.0mmol/L, of which 2 were associated with ventricular fibrillation at the onset of CPB. Insufficient patient numbers did not allow for statistical investigation and inference into arrhythmias. Washing of blood resulted in 4 patients becoming hypokalaemic and requiring potassium supplementation, while none required it in the unwashed group. Investigators stated that it did not elicit any electrocardiographic changes and was not detrimental and easier to manage than hyperkalaemia. Lactate increased in all patients as CPB progressed and there were no significant differences between groups. Sodium concentration, a secondary outcome in this study, was found to be significantly increased by washing irradiated RBC. Sodium levels were high throughout CPB in the washed group which was due to the wash solution used. The investigators concluded that CS washing of irradiated RBC helps prevent hyperkalaemia during CPB but does not prevent hyperlactaemia in complex congenital heart surgery (Swindell et al., 2007).

The following three interesting case studies highlight the need for choosing the correct wash solution to process blood using the CS, to achieve a satisfactory clinical objective:

Case report 1: Knichwitz et al., (2002)

A 73 year old male with end stage renal failure was to undergo elective nephrectomy. The underlying kidney tumour further caused a vena caval thrombus, which needed to be removed as well. The patient also had a rare blood type, and only 8 units of compatible PRBC were available with a storage time in the range of 28-32 days old. Preoperative HCT was 31% and decreased to 20%, therefore 2 units of PRBC were transfused and the [$K^+$] increased from 5.4 to 6.7mmol/L. Further blood transfusions were required and it was decided to wash PRBC with normal saline with the CATS CS before transfusion. The patient received 4 more units with a mean [$K^+$] of 39.6mmol/L that took 35 minutes (min) to process. The CATS allowed for a mean $K^+$ ion elimination rate of 97.3% and the processed blood had a mean [$K^+$] of 2.3mmol/L. Haematocrit increased to 33%, whereas the plasma [$K^+$] decreased from 6.7mmol/L to 5.9mmol/L during transfusion of processed PRBC. Similar observations with [$K^+$] were also made by de Vroege et al. (2007); Swindell et al. (2007); and Varghese et al. (2007). This technique has shown CS is very useful in terms of $K^+$ ion elimination in patients with end stage renal failure or patients requiring massive transfusion thus preventing hyperkalaemia. Pack red blood cells could have been washed by blood bank but this would have been time consuming and would have incurred additional costs.

Case report 2: Sohn et al., (2012)

Investigators reported a case of hyperkalaemic cardiac arrest in a 1 month 3.1Kg infant that was being operated on due to a huge haemorrhagic mass in the left posterior fossa, extended to left upper neck through jugular foramen. Before surgical intervention the HCT was 43% and serum [$K^+$] was 3.7mmol/L. Due to bleeding during the operation volume replacement consisted of 350 ml 0.9% normal saline and 1 unit of non-irradiated RBC (average unit volume 160 ml, HCT 70%). After this transfusion the patients HCT was 25%. Thereafter, operative blood loss increased and RBC that were irradiated 5 days prior to surgery were administered rapidly. Approximately 80ml of non-irradiated RBC had been transfused over 10 min., and shortly after a decreased heart rate and morphologic changes were observed on the ECG followed by cardiac arrest. The transfusion was stopped due to suspicion of transfusion induced hyperkalaemia. Blood sample analysis at this point revealed HCT of 38% and confirmed that serum [$K^+$] had increased to 14.2mmol/L. Resucitation

consisted of administration of 10 units insulin, several doses of 10 µg epinephrine, 0.1 mg atropine, 30 mg $CaCl_2$ with concomitant chest compressions. Ten min. after recovery of spontaneous circulation arterial blood sample analysis found [$K^+$] decreased substantially to 6.8mmol/L.

During the operation bleeding was uncontrollable and the patient received 28 units of RBC washed with 0.9% normal saline using the CATS cell saver, and the cell saver was not used to salvage shed blood. Blood analysis of processed blood revealed a [$K^+$] of 19.9 mmol/L and HCT of 59%, however pre-processing analysis was not done. Based on previous studies the investigators used a pre-processing [$K^+$] of 60mmol/L in irradiated units and calculated [$K^+$] was reduced by 67%. The Gem Premier 3000 used to test the processed blood operates in the range of 0.1 - 20mmol/L. Other infused fluids include 750 ml of normal saline, 640 ml of hydroxyethyl starch (HES), 21 units of fresh frozen plasma, 9 units of platelet concentrate, and 1 unit of cryoprecipitate. After using the CATS there was no ECG abnormality or cardiac arrest due to transfusion related hyperkalaemia. The patient was transferred to the ICU after the operation but demised shortly thereafter. The authors suggested that the relatively high [$K^+$] in the washed RBC could be due to three reasons. Firstly, the CS was operated on emergency mode which is characterized by faster processing and using less wash solution. Secondly, relatively older blood was used and a delay of five days in the use of these units after radiation, both of which may have lead to a high [$K^+$]. Red blood cells were stored at room temperature when not being transfused immediately and therefore may have increased haemolysis and [$K^+$].

Case report 3: Peng et al., (2006)

The investigators reported a potentially life threatening intra-operative arrhythmia caused by iatrogenic dilutional hypokalaemia resulting from rapid transfusion of re-suspended CS blood washed with normal saline during cross clamp repair of a thoracic aortic aneurysm (TAA). The 75 year old male was presented for surgery, and on the day of the procedure his baseline laboratory results showed: Hb: 14.3g/dL; HCT: 42%; PLT count: 297 per $mm^3$, [$Na^+$]: 138mEq/dL; [$K^+$]: 4.8mEq/dL; [$Cl^-$]: 101mEq/dL; [$HCO_3^-$]: 26mmol/L; blood urea nitrogen: 13mEq/dL; creatinine: 0.9mg/dL, and glucose was 118mg/dL. At initiation of cross clamping a 3mEq/kg/hr infusion of sodium bicarbonate was empirically begun to manage the anticipated cross clamp acidosis. A COBE CS was used to salvage blood. There was sustained profuse bleeding from intercostals and lumbar perforator vessels of approximately 500 ml/min. An arrhythmia developed and the patient was empirically treated with 100 mg of

lidocaine that resulted in brief resolution, 150mg of amiodarone was given which also gave a transient beneficial effect. Dysrhythmias and haemodynamic instability still remained and 50 mg of lidocaine and 2 mg of magnesium sulphate were given without any benefit. An arterial blood gas was performed and revealed a [$K^+$] of 2.2mEq/dL, this was treated by replacing 40 meq/L potassium chloride over 30 minutes and the arrhythmia resolved. Estimated blood loss was 9000ml of which 8000ml was lost during the cross clamp period. Urine output was 500ml. Total fluids administered during the procedure were 500ml of PRBC, 4000ml of CS blood, 305ml of PLT, 5000ml of Plasmalyte, and 5800ml of normal saline (approximately 2000ml via the CS). It is postulated that acute symptomatic iatrogenic hypokalaemia resulted from rapid transfusion of CS blood washed with normal saline thus producing dilutional hypokalaemia with normal acid-base status. Swindell et al. (2007), also observed a similar dilutional hypokalaemia.

From literature based on previous research Polderman and Girbes, (2004) hypothesized that patients exposed to moderate hypothermia may be at risk for electrolyte depletion during the peri-operative and postoperative periods of cardiac surgery. In this prospective controlled study 500 consecutive CPB patients (group 1) and 250 major surgical patients (group 2: control) were enrolled. Serum electrolyte levels of magnesium ($Mg^{2+}$), phosphate ($PO_4^{3-}$), potassium ($K^+$), sodium ($Na^+$) and calcium ($Ca^{2+}$) were measured. On admission all patients in group 1 received fluid infusions containing magnesium sulphate and phosphate before sample aspirations. Electrolyte levels measured 1 - 4 days before surgery for all patients were normal. During surgery all patients in group 1 received potassium supplementation (10.1 ± 4.7mmol/hr). All patients in group 1 received an average of 1 litre of cold crystalloid cardioplegia that contained electrolyte concentration (mmol/L) of: sodium 120; potassium 16; magnesium 16, calcium 1.2; and chlorine 172. Cardiac patients were cooled to temperatures between 32ºC and 34ºC, and re-warmed to 36 ºC.

The results revealed that 34% of patients in group 1 versus 8% of patients in group 2 had moderate hypokalaemia {[$K^+$] < 3.6mmol/L}, ($p < 0.001$). This was despite patients in group 1 receiving on average 16mmol of $K^+$ from cardioplegia, coupled with the $K^+$ infusion during surgery. Patients that presented with hypophosphataemia were 83% in group 1 (0.43 ± 0.22mmol/L) vs. 12% in group 2 (0.92 ± 0.32mmol/L), ($p < 0.001$). Patients that presented with hypocalcaemia were 7.8% in group 1 (1.96 ± 0.41mmol/L) vs. 5.6% in group 2 (2.12 ± 0.33mmol/L), ($p < 0.001$). Patients with hypomagnesaemia (< 0.70mmol/L) were 46% (n=228) in group 1 vs. 16% (n=40) in group 2, ($p <0.001$). Patients treated with magnesium ($Mg^{2+}$) (average amount 2.1 g) during surgery for brief arrhythmias was 76% (n=380) in

group 1 versus 2% (n=5) in group 2, ($p < 0.001$). Of the remaining 120 patients in group 1 who did not receive magnesium, 80% (n=96) had levels below 0.70mmol/L vs. 1% (n=3) in group 2, ($p < 0.001$). Urinary electrolyte excretion rate during surgery only, measured in 40 patients for group 1 vs. group 2 were significant ($p < 0.01$) as follows: [$Mg^{2+}$] (0.6 ± 0.33 vs. 0.20 ± 0.32mmol/hr), [$PO_4^{3-}$] (5.1 ± 3.0 vs. 2.2 ± 2.0mmol/hr), [$K^+$] 11.0 ± 4.8 vs. 7.2 ± 4.8mmol/hr), and [$Ca^{2+}$] (1.2 ± 0.7 vs. 0.4 ± 0.2 mmol/hr). No significant results were reported for [$Na^+$] and [$Cl^-$] was not measured which is a potential limitation of this study.
The authors concluded that patients undergoing cardiac surgery with CPB are at high risk for electrolyte depletion. The mechanism suggested for this is probably a combination of increased urinary excretion (hypothermia induced diuresis) and intracellular shift induced by a combination of intra-operative hypothermia and extracorporeal circulation. In view of this conclusion careful attention should also be given to washing solution used to process residual pump blood and further compound electrolyte depletion (Polderman and Girbes, 2004).

The SID of normal plasma is approximately 40 meq/L, The SID of intravenous fluids may therefore variably change the SID of body fluids. Researchers Morgan, Venkatesh and Hall (2002), conducted a prospective *in vitro* study based on Peter Stewart's physical chemical approach to acid-base analysis. The objective was to determine the relationship between SID of a diluting crystalloid and its metabolic acid-base effects on *in vitro* blood dilution. Three solutions of with SID of 40meq/L, 30meq/L, and 20meq/L were prepared, and two commercially available crystalloid solutions, 0.9 % saline and Hartmann's solution with a calculated SID of 0meq/L and - 4meq/L were used. Serial dilutions using each of the five solutions were carried out using well oxygenated venous blood, with blood gas analysis done before and after each dilution. Mean pre-dilution values were [Hb]: 148 ± 7g/L versus 65 ± 11g/dL after final crystalloid dilution. Mean SID and base excess (BE) were 39.2 ± 2.0meq/L and -1.0 ± 0.7meq/L respectively, and the lowest $pO_2$ was 333 torr pre dilution and 238 torr post dilution.

The results revealed that in all the haemodilutions plasma SID decreased, except for the crystalloid solution with SID equal to 40meq/L. The crystalloid solution with a SID of 40meq/L was approximately equal to the mean pre dilution plasma SID, and therefore no change occurred. In contrast BE increased during haemodilution with crystalloid SID solutions 40meq/L and 30meq/L respectively, and decreased with the remaining solutions. The investigators confirmed that small reductions in plasma SID on haemodilution is associated with a progressive metabolic alkalosis, that is quantified by an increase in BE. This is

attributable to a predominant reduction in $A_{TOT}$ (albumin and inorganic phosphate) upon haemodilution. The investigators reported that the relationship between plasma SID and whole blood BE is linearly related to diluent crystalloid SID. Linear regression revealed that SID of crystalloid producing a zero base excess/haemoglobin concentration slope during *in vitro* dilution is 23.7meq/L. Therefore infused crystalloid SID solutions less than or greater than 23.7meq/L would cause a metabolic acidosis or alkalosis respectively, *in vitro.*

In a review article Morgan and Venkatesh (2003) presented a rationale for the design of balanced crystalloid fluids using Stewarts quantitative physical chemical approach of acid base theory. In the Stewart analysis, pH and [$HCO_3^-$] are dependant variables, that are determined by three independent variables: (1) $pCO_2$, (2) SID, and (3) total concentration of non-volatile weak acid buffer ($A_{TOT}$). Infusion of a crystalloid solution modifies pH by simultaneously altering two of the three independent variables. Raising and lowering $A_{TOT}$ while clamping SID will result in a metabolic acidosis and alkalosis respectively. Lowering and raising plasma SID while clamping $A_{TOT}$ will result in a metabolic acidosis or alkalosis respectively. The SID of a crystalloid is its [$HCO_3^-$] or that part of an organic bicarbonate surrogate (anions such as lactate, gluconate, and acetate) that replace $HCO_3^-$ provided they are fully metabolized on infusion. Rapid infusion alters plasma SID towards the crystalloid SID, but also lowers $A_{TOT}$ by simple haemodilution.

The investigators concluded that a crystalloid SID of 24meq/L is “balanced” for rapid infusion (Morgan and Venkatesh, 2003). This generates a fall in plasma SID that precisely counteracts the $A_{TOT}$ dilutional alkalosis. Table 8 lists five commercially available “balanced” salt solutions (all concentrations in meq/L). Strong ion difference values assume stable plasma lactate concentrations of 2mmol/L. On inspection it seems the intention of the high SID of 50meq/L and greater in the crystalloid solutions would be to accelerate correction of metabolic acidosis, but it may over shoot resulting in a break through metabolic alkalosis. Hartmann’s solutions would be considered to be slightly alkalinisings. Water-dextrose solutions, mannitol, and 0.9 % saline have a SID of zero.

**Table 8**: Five commercially available "balanced" salt solutions (all concentrations in meq/L), (Morgan, Venkatesh and Hall, 2004).

| | Hartmann's | Plasma-Lyte 148 | Plasma-Lyte R | Normosol-R | Isolyte E |
|---|---|---|---|---|---|
| Sodium | 129 | 140 | 140 | 140 | 140 |
| Chloride | 109 | 98 | 103 | 98 | 103 |
| Potassium | 5 | 5 | 10 | 5 | 10 |
| Calcium | 4 | - | 5 | - | 5 |
| Magnesium | - | 3 | 3 | 1.5 | 3 |
| Lactate | 29 | - | 8 | - | - |
| Acetate | - | 27 | 47 | 27 | 49 |
| Gluconate | - | 23 | - | 23 | - |
| Citrate | - | - | - | - | 8 |
| Effective SID | 27 | 50 | 53 | 50 | 57 |

The overall SID in whole blood is harder to quantify because strong ions and water in crystalloids infused into the vascular tree are distributed among plasma, RBC, and interstitial fluid (where $A_{TOT}$ is negligible) in a complex way governed by Gibbs-Donnan equilibrium, laws of electrical neutrality and of chemical equilibrium. An animal model was developed to study *in vivo* acid-base effects of crystalloid SID during haemodilution (Morgan, Venkatesh and Hall, 2004). Seven groups of three Sprague-Dawley rats underwent normovolaemic haemodilution with one of seven crystalloids, with SID values ranging from 0 to 40meq/L. Final standard base excess (SBE) ranged from -8 to +7 and were directly correlated with crystalloid SID dilutions ranging from 0 to 40 meq/L, with [Hb] falling from 142 ± 17 to 44 ± 10g/L ($p < 0.0001$). A linear relationship between crystalloid SID and SBE was demonstrated. Linear regression analysis revealed that the SID of a crystalloid required to maintain neutral metabolic acid base status (SBE = 0mmol/L) after *in vivo* dilution is 24.4meq/L. This value is equal to standard state [$HCO_3^-$] when ph = 7.4 and $pCO_2$ = 40mmHg. The results of this study strongly support previous *in vitro* results (Morgan, Venkatesh and Hall, 2002). The principles are applicable in designing fluids for volume resuscitation, acute normovolaemic haemodilution, and CPB.

Omron and Omron (2009), developed a physicochemical model to project the non respiratory changes on SBE consequent to an infused volume of crystalloid solutions in common use. Clinical simulation of modelled acid-base and fluid compartment parameters was conducted in a 70-kg test participant at standard physiologic state: pH = 7.40, pCO2 = 40 mm Hg, Henderson–Hasselbalch actual bicarbonate ($[HCO_3^-]_{HH}$) = 24.5 meq/L, (SID) = 38.9 meq/L, albumin = 4.40 g/dL, inorganic phosphate = 1.16 mmol/L, citrate total = 0.135 mmol/L, and SBE = 0.1 meq/L. Simulations of multiple, sequential crystalloid infusions up to 10 litres were conducted with normal saline (SID = 0), lactated Ringer's (SID = 28), plasmalyte 148 (SID = 50), one-half normal saline + 75 meq/L sodium bicarbonate (NaHCO3; SID = 75), 0.15 mol/L NaHCO3 (SID = 150), and a hypothetical crystalloid solution whose SID = 24.5 meq/L, respectively. The induced crystalloid SID metabolic acidosis that is exactly required to balance the mitigating dilutional $A_{TOT}$ metabolic alkalosis without any deviation of SBE according to the physicochemical model is 24meq/L, or an equivalent crystalloid pre-infusion SID equal to the actual $[HCO_3^-]_{HH}$. Table 9 and figure 7 show that infusion of the hypothetical crystalloid solution with a SID = 24.5meq/L does not result in deviation from standard state as compared to other solutions in the physiochemical model.

**Table 9**: Physicochemical consequences of a 1 litre crystalloid infusion from standard state stratified by changes in SID, pH, and SBE, (Omron and Omron, 2009).

| | NS SID = 0 | SID = 24.5 | LR SID = 28 | Standard State SID = 38.9 | Plasmalyte SID = 50 | 1/2 NS + 75 mEq/L $NaHCO_3$ SID = 75 | 0.15 mol/L $NaHCO_3$ SID = 150 |
|---|---|---|---|---|---|---|---|
| SID (mEq/L) | 36.1 | 37.8 | 38.1 | 38.9 | 39.7 | 41.5 | 46.9 |
| pH | 7.370 | 7.400 | 7.404 | 7.400 | 7.429 | 7.456 | 7.528 |
| $PCO_2$ (mm Hg) | 40 | 40 | 40 | 40 | 40 | 40 | 40 |
| $[HCO_3]_{HH}$ mEq/L | 22.9 | 24.5 | 24.7 | 24.5 | 26.2 | 27.9 | 32.9 |
| SBE (mEq/L) | −1.8 | 0.1 | 0.4 | 0.1 | 2.1 | 4.0 | 9.7 |

Abbreviations: NS, normal saline; LR, lactated Ringer's; $NaHCO_3$, sodium bicarbonate; SID, strong ion difference; SBE, standard base excess; $[HCO_3]_{HH}$, Henderson–Hasselbalch actual bicarbonate; $PCO_2$, partial pressure of carbon dioxide.

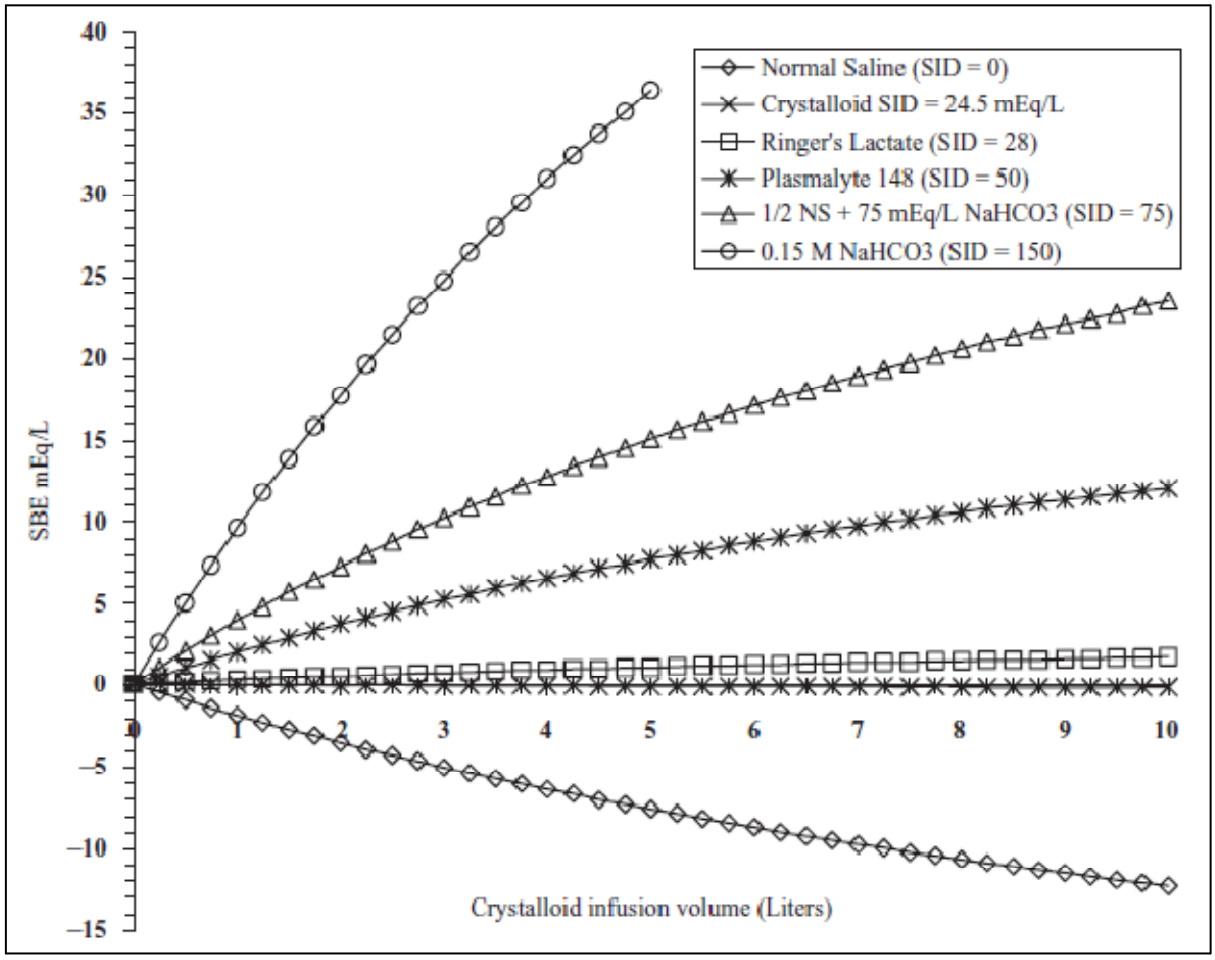



**Figure 7:** Graph illustrating standard base excess as a function of crystalloid infusion volume in litres, (Omron and Omron, 2009).

Shaw et al. (1997), conducted a retrospective cohort study to assess the association of 0.9 % saline use versus plasmalyte A (or 148), with major morbidity and clinical resource use after open abdominal surgery (elective and emergency) between the period 2005 and 2009. From the data base adult patients were selected that received exclusively 0.9 % saline (30,994 patients) or plasmalyte A (926 patients) on the day of surgery. Furthermore, only doses of 500 ml and 1000 ml were included to differentiate volume replacement from fluid for drug dilution. The primary outcome was major morbidity and the secondary outcomes were minor complications and acidosis related interventions. Outcomes were evaluated using multivariate logistic regression and propensity scoring models.

The results revealed that for the entire cohort, in hospital mortality was higher in the saline versus the plasmalyte A group (5.6 % vs. 2.9 %, $p < 0.001$). One or more major complications occurred in 33.7 % of the saline group versus 23 % in the plasmalyte A group

($p < 0.001$). Postoperative infections were more frequent in the saline group ($p < 0.006$). The use of dialysis was nearly fivefold greater in the saline group versus the plasmalyte group (4.8% vs. 1.0%, $p < 0.001$). For minor complications the model outcome revealed the odds of developing an electrolyte disturbances for potassium, sodium and magnesium were approximately 30% lower in the plasmalyte A group ($p = 0.046$). Resource utilization data revealed that patients in the saline group received more fluid than the plasmalyte A group [1976ml (SD: 1560ml) vs. 1658ml (SD: 1288ml), $p < 0.001$). Patients in the saline group underwent more tests to evaluate acidosis than the plasmalyte A group with arterial blood gases (22.9% vs. 13. 7%, $p < 0.001$) and lactic acid levels (8.0% vs. 3.3%, $p < 0.001$) respectively. Patients in the saline group had more orders for buffers (6.3% vs. 4.2%, $p = 0.02$). The saline group received more blood tansfusions than the plasmalyte A group (11.5% vs. 1.8%, $p < 0.001$) and spent more days on the ventilator [3.0 days (SD: 3.2) vs. 2.5 days (SD: 2.5)] respectively.

From the data for this cohort of patients, the saline group had higher mortality, major complications, postoperative infections, renal failure requiring dialysis, blood transfusions, longer ventilation time, and more electrolyte disturbances than the plasmalyte A group. Physicians ordered more tests (arterial blood gases and lactate levels) and more treatments (buffers, blood products, and dialysis) to investigate and manage these patients. The investigators concluded that the use of calcium free balanced crystalloid solutions on the day of major surgery was associated with a greater decrease in postoperative morbidity than 0.9 % saline (Shaw et al., 1997).

Animal experiments have shown that hyperchloremia resulting from the administration of 0.9 % saline may adversely affect renal function. Chowdhury et al. (2012), conducted a randomized double blind study to compare the effects of infusions of 0.9 % saline and plasmalyte A (or 148), on renal blood flow velocity and perfusion using magnetic resonance imaging (MRI). Twelve patients received two litres of either 0.9 % saline or plasmalyte A solution over a one hour period. MRI scanning commenced 90 min. after commencement of infusion and blood samples and weight recorded every hourly for 4 hours. Patients were permitted to pass urine as needed and in all cases at the end of the study.

The results revealed that weight changes were proportional to volume infused and urine excreted. Both infusions produced similar plasma dilutions in haematocrit, haemoglobin, and serum albumin. The time to first micturition after the start of infusion was longer in the saline group versus the plasmalyte A group (142 ± 16min. vs. 90 ± 12min. $p = 0.006$) and post

infusion urinary volume was lower in the saline group versus the plasmalyte A group (533 ± 57ml vs. 833 ± 87ml, $p$ = 0.002). Initial plasma expansion was 29% in plasmalyte A and 26% in 0.9% saline of the infused volumes, and at the end of 4 hours 14% of the plasmalyte 148 and 12 % of the 0.9 % saline infusions remained in the intravascular compartment respectively. At its peak exchange the exchange in extravascular fluid volume was 1484 ml following 0.9% saline compared with 1155ml following plasmalyte A ($p$ = 0.031). The surplus extravascular fluid in the saline group persisted in the extravascular space to the end of the study and equated to a difference of 394ml ($p$ = 0.043).

At the end of the study (240min.), the increase in weight was 1,2kg for the saline group versus 0.84 kg in the plasmalyte A group ($p$ = 0.022). After infusion of 0.9% saline chloride concentrations significantly increased and peaked at 109mmol/L and remained above the physiologic range (105mmol/L) throughout the study, in contrast to no hyperchloremia in the plasmalyte A group ($p$ < 0.0001). There was a significant fall in SID in the saline group versus the plasmalyte A group (p = 0.025) indicating an acidemia. This was an unexpected finding. According to the Stewart hypothesis infusion of plasmalyte A with a SID of 50 would result in an increase in plasma SID, the less than expected alkalinizing effect may have resulted from acetate/gluconate and chloride shifts or unexchanged gluconate elimination in the urine.

Changes in serum osmolality, sodium and potassium were similar after both infusions. There was a rise in renal artery blood velocity that lasted 14min. then returned toward baseline but not below baseline velocity for the remainder of the MRI in the plasmalyte A group. In contrast, in the saline group after 7min. there was a progressive decline in renal artery blood flow velocity. At the end of the 90min. scanning period there was a decrease in mean renal artery blood flow velocity of 3 centimetres per second, which is a 9% reduction in velocity below baseline ($p$ =0.045). After an initial rise in renal cortical perfusion in the plasmalyte A group the values returned toward but not below baseline. In the saline group there was a progressive decrease in renal cortical perfusion with a maximum reduction at 28min., equating to an 11.7% reduction from baseline perfusion ($p$ = 0.008). The investigators concluded that infusion of 0.9% saline results in reductions in renal blood flow velocities and renal cortical tissue perfusion (Chowdhury et al., 2012).

Eighteen healthy human volunteers were recruited to study the effect of intravenous Ringers lactate (RL) solution versus 0.9% NaCl solution on serum osmolality (Williams et al., 1999). The patients received 50 mg/kg over 1 hour of RL on one occasion and 0.9% NaCl on

another. The osmolality of RL is 273mOsm/L and the osmolality of 0.9% NaCl is 308mOsm/L. Serum osmolality, sodium concentration and venous blood pH were measured. Measurements were taken before infusion (T1), at the end of infusion (T2) and 1 hour after end of infusion (T3). Serum osmolality is a determinant of brain water content, and a low serum osmolality may contribute to cerebral oedema. Serum osmolality was significantly reduced ($p < 0.05$) at the end of infusion with RL, compared to an insignificant increase with 0.9% NaCl. With RL Serum osmolality returned to baseline 1 hour after the end of infusion and remained unchanged with 0.9% NaCl. There were no significant differences between sodium concentrations reported before and at the end of both infusions.

There was, however, a statistically significant difference ($p < 0.05$) for sodium concentration between RL and 0.9% NaCl (1 ± 2 vs. - 1 ± 2) at the end of infusions (T3) respectively. Whole blood pH significantly increased and decreased ($p < 0.001$) for both RL and 0.9% NaCl respectively (0.04 ± 0.04 vs. - 0.04 ± 0.04) from before infusion (T1) to the end of infusion (T2). However, with the saline infusion pH remained unchanged 1 hour after the end of infusion (T3) but with RL it insignificantly trended toward baseline. The time period before insertion of the infusion catheter to first urination was significantly longer ($p < 0.001$) with 0.9% NaCl verses RL respectively (106 ± 11min. vs. 75 ± 10min.) In the RL infusion this shorter urination time was attributed to fluid being hypotonic thus decreasing osmolality and decreasing levels of antidiuretic hormone, resulting in diuresis of hypotonic urine and returning serum osmolality to normal. The longer urination time for the saline infusion was attributed to fluid retention associated with saline.

The authors also commented that although serum chloride ion concentration was not measured, an increase from the saline infusion decreases renal blood flow and glomerular filtration rate, thereby causing urinary retention. There was a non significant trend but clinically relevant observation in which more patients with the saline infusion experienced abdominal discomfort and had a perceived difficulty with reading and mental arithmetic. However, the volunteers were not blinded and no mental state examination was conducted to verify validity to these observations (Williams et al., 1999).

Hadimioglu et al. (2008), conducted a randomized double blind study to quantify the changes in acid-base, potassium and lactate levels as a function of different crystalloid solutions during kidney transplantation to determine the ideal fluid. Three groups of 30 patients were recruited to receive either 0.9% saline, RL or plasmalyte A. The volume of fluid for each study group was 2868 ± 780ml, 2770 ± 820ml, and 2756 ± 800ml for saline, RL,

and plasmalyte A groups respectively. The results revealed that there was a significant decrease ($p < 0.05$) in pH (7.44 ± 0.5 vs. 7.36 ± 0.05), base excess (0.4 ± 3.1meq/L vs. - 4.3 ± 2.1meq/L), and bicarbonate (22.2 ± 4.4mmol/L vs. 18.2 ± 2.9mmol/L) respectively in the saline group. There was also a significant increase ($p < 0.05$) in serum chloride (104 ± 2 mmol/L vs. 125 ± 3mmol/L).

The investigators further stated that no patient in the saline group developed frank acidosis despite the hyperchloremia that took a week to return to physiologic levels. No significant changes occurred in the RL or plasmalyte A groups. Urine output increased significantly in all groups and of particular note in the saline group despite hyperchloremia, this was likely due to the improved function of the graft outweighing the hyperchloremia or due to the lack of sympathetic supply to the graft. Investigators concluded that all 3 crystalloids could be used safely for short uncomplicated renal transplants but plasmalyte A maintained the best metabolic profile (Hadimioglu et al., 2008).

Perioperative intravenous supplementation of magnesium sulphate has been highly controversial in cardiac surgical patients. Reported benefits include reduction in postoperative arrhythmia and reperfusion injury, improved coronary perfusion and increased cardiac output. Reported adverse effects include hypotension, prolonged neuromuscular block, respiratory failure, higher defibrillation energy requirements and cardiac arrest after overdose (Wilkes et al., 2002). Commercially available crystalloid solutions may or may not contain magnesium as a constituent and this is clinically relevant for cardiac surgical patients.

Wilkes and colleagues (2002), conducted a randomized control trial on 85 patients presenting for CABG, to determine whether intra-operative measurement and correction of ionized magnesium can reduce cardiac arrhythmia after CPB. The CPB circuit priming fluid was magnesium free, and intermittent cross clamping was employed with no cardioplegia being administered. The study group (n=43), received magnesium sulphate to correct ionized magnesium levels throughout CPB. In the control group (n=42) magnesium levels were measured but not corrected. Ionised and total plasma magnesium were measured at 6 specified times, before surgery, twice during CPB cold, during CPB warm, post CPB, and within an hour after surgery. Total hypomagnesemia was observed in 53% (45 patients) of all patients and was more common than ionized hypomagnesemia observed in 11% (13 patients) of all patients. The mean preoperative ionized magnesium levels in all patients was 0.50 ± 0.05mmol/L and decreased significantly ($p <0.001$) to 0.43 ± 0.05mmol/L during and

after CPB in the control group. The mean preoperative total magnesium levels were 0.69 ± 0.09mmol/L and decreased significantly ($p$ <0.001) to 0.56 ± 0.07mmol/L during and after CPB in the control group.

Thirty of the 40 patients (75%) in the control group developed ionized hypomagnesemia immediately after CPB, and all of the patients in this group were observed to have total hypomagnesemia immediately after CPB. All patients in the magnesium corrected group received magnesium sulphate supplements (mean 13.4 ± 0.9 mmol), and levels of ionized and total magnesium were 0.59 ± 0.08mmol/L and 0.94 ± 0.16 mmol/L immediately after surgery. In the control group 30% of the patients demonstrated episodes of ventricular tachycardia in the 24 hour postoperative period, compared to 7% in the magnesium corrected group. This indicated a significant reduction ($p$ < 0.01) in the risk of postoperative arrhythmia by 76% in the magnesium corrected group. The investigators concluded that the correction of ionized magnesium is associated with a reduction in postoperative ventricular arrhythmia in cardiac surgical patients (Wilkes et al., 2002).

Morgan et al. (2008), conducted a randomised single blinded study to compare the acid-base effects of a designed bicarbonate balanced priming solution (SID = 24meq/L) versus plasmalyte 148 (SID = 50meq/L) during CPB. Twenty patients were randomized equally into the plasmalyte 148 group and the bicarbonate balanced group. Plasmalyte 148 solution contained 3meq/L magnesium and the designed bicarbonate balanced fluid contained no magnesium. The cardioplegia solution contained 80meq/L magnesium, with both study groups receiving similar doses of cardiplegia. Both solutions were phosphate free.

The results revealed that just prior to termination of CPB, total magnesium was significantly lower ($p$ < 0.05) in the bicarbonate balanced fluid group as compared the Plasmalyte 148 group (1.32meq/L vs. 1.52meq/L). There were no significant changes in phosphate levels between groups prior to termination of CPB. Although not statistically compared, phosphate levels measured in the Plasmalyte 148 group and bicarbonate balanced fluid group before CPB were higher as compared prior to termination of CPB, (1.39 vs. 0.97meq/L) and (1.18 vs. 0.89meq/L) respectively. This may be a result of simple dilution of serum phosphate by the two phosphate free solutions used in the study (Morgan et al., 2008).

## 2.7 CONCLUSION

The literature review revealed that intravenous fluids are ubiquitous in all medical specialities. Peter Stewarts approach to acid-base interpretation in context to fluid therapy and CPB requires further research.

# CHAPTER THREE: MATERIALS AND METHODOLOGY

## 3.1 INTRODUCTION

The aim of the study is to analyze and quantify the quality of residual pump blood post CPB that has been washed with either an unbalanced electrolyte solution (0.9% normal saline) or a balanced electrolyte solution (Balsol®). The primary objective was to measure and compare the pH, electrolytes, metabolites, osmolality and SID of residual pump blood to the, pH, electrolytes, metabolites, osmolality and SID of processed cell saver blood, which is washed with either 0.9% normal saline or Balsol® solution. The secondary objective was to measure and compare protein levels (albumin and total protein) in residual pump blood to protein levels in processed cell saver blood, that was washed with either 0.9% normal saline or Balsol® solution. The final objective was to determine the volume, haematocrit and haemoglobin yield post cell saver processing, from the input volume of residual pump blood when washed with either 0.9% normal saline or Balsol® solution.

The study design was prospective and quantitative and *in vitro* in nature. It was conducted at Inkosi Albert Luthuli Central Hospital, Kwazulu-Natal, South Africa. The study included the first 40 patients who were undergoing elective cardiac surgery who met the inclusion criteria, and who gave informed consent to participate in the study. Patients were equally divided into the 0.9% NaCl control group (n=20) and the Balsol® interventional group (n=20).

The data collection resulted in 720 measurements per group, which included pre and post cell saver processing. Similar previous studies have shown that this sample size is adequate to compare the control to the interventional group (de Vroege et al., 2007; Varghese et al., 2007; Huber et al., 2013). The number of patients recruited and the sample size for the study has been verified by a biostatistician from the University of KwaZulu-Natal (UKZN) South Africa, and is confirmed to be sufficient to show statistical significance (Appendix A). The plan and time frame of the research process are set out in Appendix B and Appendix C respectively.

### 3.2 SELECTION CRITERIA

Inclusion criteria:

1) Age over 18 years.
2) All patients undergoing elective cardiac surgery with the use of intra-operative cell saver.
3) Pre-operative haematocrit of 30% (Hb > 10g/dL).

Exclusion criteria:

1) Pregnant patients.
2) HIV positive patients.
3) Patients with rare blood groups.
4) Patients with coagulopathies and other blood disorders.
5) Patients with endocrine or metabolic disease that may affect electrolyte balance.
6) Patients with any form of renal dysfunction or failure.

Before commencement of the study, ethical approval was obtained from the Durban University of Technology Ethics committee (Appendix D). The Department of Cardiothoracic Surgery and the Department of Perfusion granted permission for the study to be conducted (Appendix E). A letter was submitted to Inkosi Albert Luthuli Central Hospital medical manager (Appendix F) and Department of KwaZulu-Natal health provincial office recommending the study (Appendix G).

Patients who met the inclusion criteria were recruited in the cardiac ward. A letter of information and consent form, drawn up by the researcher in both English and Isizulu was presented to all patients who volunteered to participate in the study. Patients were informed of the purpose and requirements of the study. Patients were informed that their right to participate in the trial was entirely voluntary and that they were entitled to withdraw at any point without affecting their medical treatment. They were also informed that all information used in the trial would remain confidential and that any data reported in scientific journals or published would not include information identifying them as a patient in the study (refer to appendix H and I in Isizulu and English). Patients who were willing to participate signed the

consent form. All patients recruited into the study were under the consultant care of the surgeon performing the operation, who confirmed that the patient required surgery with CPB and intra-operative cell saving.

## 3.3 METHODOLOGY

**3.3.1** Anaesthesia and cardiopulmonary bypass:

When the patient was brought into theatre consent was checked and verified with the patient. The patient was placed on the operating table and the perfusionist placed monitoring devices on the patient. These included:

- Electrocardiogram to monitor heart rate and rhythm.
- $SpO_2$ monitor to measure arterial oxygen saturation.
- Non invasive blood pressure cuff, to monitor blood pressure.

An invasive arterial catheter (femoral or radial) and peripheral line was achieved under local anaesthetic. Anaesthesia is a cardio stable opiod based induction using sufenta (0.5µg/kg) and maintained with sevoflurane and oxygen. Pancuronium bromide (0.1mg/kg) and rocuronium (0.5-1mg/kg) were used as muscle relaxant to facilitate intubation. This was followed by the insertion of central venous catheter to monitor central venous pressure. Fentanyl (0.02 – 1µg/kg/min) was given for analgesia. Midazolam (0.2 – 0.5µg/kg/min) was given for sedation. Before cannulation Heparin (400 iu/kg) is administered for anticoagulation and was supplemented to maintain an activated clotting time (ACT) of more than 480 seconds (Hemocron Jnr). After the surgical procedure adrenaline (0.1µg/kg/min) was used as the inotrope of choice. After removal of the aortic cannula protamine was used to neutralized heparin in ratio of 1 mg protamine sulfate: 100iu (international units) heparin and was tested so as the activated clotting time returns to +/- 10% of baseline value. The extracorporeal circuit consisted of a standard integral hollow fibre membrane oxygenator and tubing that was primed with 1500-1800 millilitres of balanced crystalloid solution (Balsol®, (Appendix J) for both the control group (0.9% normal saline, (Appendix K) and the interventional group, and addition of 5000iu heparin. The balanced crystalloid solution (Balsol®) is the approved standard CPB priming solution for all cardiac procedures at Inkosi Albert Luthuli Central Hospital. This setup is used with the Stockert *S5* roller pump heart lung machine.

The operations were performed as per protocol with standard non-pulsatile CPB and hypothermia was maintained at 28 – 32 ºC (core) and haemodilution (HCT 20% to 30%). A standard flow rate of 2.4 L/min/m² was used. Cardio protection consisted of either cold Blood Cardioplegia (Appendix L) using the Buckberg 4:1 ratio, being four parts blood to one part cardioplegia (with the 35ml of 20% Dextrose + 1 gram Magnesium Sulphate added per 500ml), or 20ml/kg cold St Thomas II cardioplegia (Appendix M) (with addition of 10ml of 8.5% $NaHCO_3$ + 100mg lignocain per litre). Topical cooling was achieved with ice cold 0.9% saline. Maintenance fluid used during CPB was Balsol®, and will remain the same for both the control and the interventional groups. Calcium, potassium and sodium bicarbonate was administered as required during CPB to correct deficits for both groups. Weaning of CPB was performed after re-warming to a rectal temperature of at least 35 ºC, which applied to both study groups.

**3.3.2** Cell salvage procedure:

Cell savage was achieved using the Medtronic autolog cell saver machine. A standard 175ml bowl was used. The cell saver was installed identically for every patient. The blood collection reservoir was routinely primed with 100mls of 0.9% saline, with 25000iu heparin added per litre. The balanced solution used for the interventional group was Balsol® to prime the cell salvage system in the same method. This is not routine practice and was the comparative wash solution being analysed. The balanced crystalloid solution (Balsol®) is an approved priming and maintenance solution for all CPB pump circuits, however, it is not used as a wash solution and therefore requires investigation. Suction was regulated at 100 - 150mmHg for both study groups as per normal practice. Immediately after termination of CPB residual blood in the venous line was gravity drained into the venous reservoir. Residual pump blood in the CPB circuit was then flushed out via the aortic cannula with one litre balanced crystalloid solution (Balsol®) and suctioned to the cell salvage reservoir and processed with balanced or unbalanced crystalloid solution dependant on the group being studied. All shed blood that was collected intra-operatively from skin incision, and after the administration of protamine sulphate to skin closure was processed. In this study the first 20 consecutive patients were processed with balanced crystalloid solution (Balsol®) and the second 20 consecutive patients were processed with unbalanced crystalloid solution (0.9% normal saline). The input volume and processed volume was documented at the end of the cell salvage process.

**3.3.3** Blood sampling and laboratory measurements:

Immediately after termination of CPB and before collection of residual blood from the venous line, pre-processing blood samples was collected by the investigator. A 10ml blood sample was collected from the sampling manifold of the CPB circuit because it was safe, easy and convenient and is the site where all routine blood gas samples are collected routinely throughout the procedure. Blood samples drawn from this point in the procedure are independent of the patients circulating blood volume and is residual blood left in the CPB circuit. Blood samples were placed into yellow top blood collection tube and sent to the laboratory for analysis, using the Siemens Advia 1800 blood gas analyser to measure magnesium, inorganic phosphate, albumin and total protein, and the Gonotech osmometer for osmolality (see table 10 for variables to be analysed). All laboratory blood samples were processed by National Health Laboratory Services (NHLS), an accredited Health Professionals Council of South Africa (HPCSA) laboratory located on the institutional premises, where all routine blood processing is done. A 2ml blood sample was also collected for immediate analysis using the GEM® 4000 premier™ point of care blood gas analyser (see table 10 for variables to be analysed).

After processing of all bloods with the cell saver with either balanced or unbalanced crystalloid wash solution, post processing samples were collected (the same variables will be measured in the post processing samples as is listed in table 10). A 10ml sample was collected from the collection bag and placed into a yellow top blood collecting tube and sent to the laboratory for analysis. A 2ml blood sample was also collected for immediate analysis using the GEM 4000® premier™ blood gas analyser. Laboratory blood gas samples were centrifuged (1400 rpm) and the entire sample content in the collecting tubes were frozen at 20ºC in a bio-freezer and analyzed at the end of the study in a batch to negate the occurrence of errors and artefact. Blood sampling is not routine and only a small amount of blood was taken for this study and did not affect or harm the patient in any way, because all blood samples were taken from residual blood left in the CPB circuit after the procedure. Blood samples taken were only used for what the patient had consented to and was disposed of according to institutional protocol once processed.

Table 10 shows a summary of the variables that were measured before and after processing of residual pump blood using the cell saver.

**Table 10**: summary of the variables that will be measured before and after processing of residual pump blood using the cell saver.

| | **LABORATORY SAMPLE (NHLS)** | **GEM® 4000 PREMIER™ SAMPLE (THEATRE)** |
|---|---|---|
| 1 | TOTAL MAGNESIUM | pH |
| 3 | INORGANIC PHOSPHATE | SODIUM |
| 4 | ALBUMIN | POTASSIUM |
| 5 | OSMOLALITY | CHLORIDE |
| 6 | TOTAL PROTEIN | CALCIUM |
| 7 | ---------- | $PO_2$ |
| 8 | ---------- | $PCO_2$ |
| 9 | ---------- | GLUCOSE |
| 10 | ---------- | LACTATE |
| 11 | ---------- | TOTAL $CO_2$ |
| 12 | ---------- | ACTUAL BICARBONATE |
| 13 | ---------- | HAEMATOCRIT |
| 14 | ---------- | HAEMOGLOBIN |

### 3.3.4 Calculations

Strong ion difference was calculated according to the following equation, ionized magnesium was not available and was therefore excluded from the equation (Morgan et al., 2008):

$$SID = [SUM\ OF\ STRONG\ CATIONS] - [SUM\ OF\ STRONG\ ANIONS]$$

$$SID = [sodium] + [potassium] + [calcium] - [chloride + lactate]$$

Strong ion difference is expressed in meq/L therefore all variables in the equation are also expressed in meq/L (Stewart, 1981).

The GEM 4000® premier™ blood gas analyser (Appendix N) reports electrolyte and lactate concentrations in mmol/L. These values require conversion from mmol/L to meq/L in order to calculate SID. The conversions for [$Na^+$], [$K^+$], [$Ca^{2+}$], [$Cl^-$] and [lactate] was as follows:

A conversion factor of 1 was used for all univalent ions ($Na^+$, $K^+$, $Cl^-$ and $HCO_3^-$) and the metabolite lactate (Cooper, Forrest, and Cramp, 2006).

Example: 135mmol/L $Na^+$ ⟶ 135mmol/L x 1 = 135meq/L $Na^+$

A conversion factor of 2 was used for the divalent ion $Ca^{2+}$ (Wallach, 2007).

Example: 2.0mmol/L $Ca^{2+}$ ⟶ 2.0mmol/L x 2 = 4.0 meq/L $Ca^{2+}$

The GEM 4000® premier™ blood gas analyser and laboratory analysers report results for the measured variables in a specific range (Appendix N and Appendix O). In samples where the blood gas analyser reports results below the reference range of the electrode, the lower reference range was taken as the result to make a statistical analysis. The data collected for the 0.9% NaCl control group and the Balsol® interventional group are listed in Appendix P and Appendix Q respectively.

### 3.3.5 Statistical analysis

Statistical analyses were performed using STATA v13 (StataCorp, Texas, USA). The values presented in the tables are means ± SD. Two sets of analyses were performed, the changes are expressed as differences between the before (pre wash) and after (post wash) washing, and $change\ (\Delta) = post\ wash - pre\ wash$ for both NaCl and Balsol®. The within group differences were analysed using the paired Student's t-test after the Shapiro-Wilks normality test demonstrated normal distributions. Wilcoxon's matched pairs signed rank test was used to examine within group differences when normal distributions were not demonstrated. Comparison of the differences between groups (NaCl vs Balsol®) were done using Student's t test for two independent groups or Wilcoxon's rank sum test for unmatched data as appropriate. All $p$ values < 0.05 were considered statistically significant.

Box plots were used to represent before and after washing with normal saline solution and Balsol® solution. Data are expressed as medians, 25% and 75% percentile and the whiskers represent the highest and lowest values that are not outliers. A (**+**) symbol represents $p < 0.05$ for changes between before and after washing within the NaCl or Balsol® group. The (**#**) symbol represents $p < 0.05$ for a change between the NaCl solution group vs. the Balsol® solution group after washing. All data collection was performed by the principal investigator and statistical analysis was conducted with the assistance of a biostatistician.

### 3.3.6 Conclusion

In this chapter, selection criteria, methodology, calculations and statistical analysisof the study were discussed. The following chapter will discuss the results that were obtained from the study.

## CHAPTER FOUR: RESULTS

### 4.1 INTRODUCTION

This was a prospective quantitative study conducted to quantify the quality of residual pump blood post CPB that has been washed with either an unbalanced electrolyte solution (0.9% normal saline) or a balanced electrolyte solution (Balsol®). Complete sets of data were collected for both the control and interventional arms of the study.

### 4.2 DEMOGRAPHICS

Forty patients were enrolled for the study and all 40 completed follow up. The case distribution for both study groups are shown in table 11.

**TABLE 11:** Statistical summary for case distribution

| CASE | NaCl GROUP COUNT (CONTROL) | NaCl GROUP PERCENTAGE | BALSOL GROUP COUNT (INTERVENTION) | BALSOL GROUP PERCENTAGE |
|---|---|---|---|---|
| CORONARY ARTERY BYPASS GRAFT | 10 | 50% | 14 | 70% |
| DOUBLE VALVE REPLACEMENT+TRICUSPID ANNULOPLASTY | 3 | 15% | 2 | 10% |
| DOUBLE VALVE REPLACEMENT | 3 | 15% | 1 | 5% |
| REDO DOUBLE VALVE REPLACEMENT | 1 | 5% | 0 | 0% |
| MITRAL VALVE REPLACEMENT | 0 | 0% | 2 | 10% |
| REDO MITRAL VALVE REPLACEMENT | 1 | 5% | 0 | 0% |
| AORTIC VALVE REPLACEMENT | 1 | 5% | 1 | 5% |
| DISCRETE SUB AORTIC STENOSIS | 1 | 5% | 0 | 0% |
| TOTAL | 20 | 100% | 20 | 100% |

## 4.3 STATISTICAL SUMMARY OF DATA

**4.3.1** The statistical summary for the variables measured in the NaCl control group are shown in table 12 as mean ± SD. For all the variables measured there was a significant difference between post and pre wash values ($p < 0.05$).

**TABLE 12:** Statistical summary for the variables measured in the NaCl control group

| Variables measured | Pre - wash Mean ± SD | Post - wash Mean ± SD | Δ (Post – pre) Mean ± SD | % Δ (Post – pre) Mean ± SD | Shapiro Wilk[1] | Statistical test | *p* value |
|---|---|---|---|---|---|---|---|
| pH | 7.5 ± 0.1 | 7.7 ± 0.1 | 0.2 ± 0.1 | 2.4 ± 1.6 | 0.4 | t test (paired)[2] | < 0.001 |
| $pCO_2$ | 28.3 ± 2.9 | 6.0 ± 0.0 | -22.25 ± 2.9 | -80.5 ± 2.3 | 0.8 | t test (paired) | < 0.001 |
| $pO_2$ | 607.3 ±72.0 | 220.6 ±19.4 | -386.7 ± 68.7 | -63.3 ± 5.2 | 0.01 | Wilcoxon signed rank test[3] | = 0.0001 |
| $[K^+]$ | 4.5 ± 0.5 | 1.0 ± 0.7 | -3.5 ± 0.8 | -77.8 ± 15.6 | 0.1 | t test (paired) | < 0.001 |
| $[Na^+]$ | 132.9 ± 3.2 | 146.3 ± 1.9 | 13.4 ± 3.8 | 10.1 ± 3.0 | 0.1 | t test (paired) | < 0.001 |
| $[Cl^-]$ | 107.8 ± 3.1 | 127.4 ± 2.1 | 19.7 ± 3.0 | 18.3 ± 3.1 | 0.6 | t test (paired) | < 0.001 |
| $[Mg^{2+}]$ | 1.7 ± 0.7 | 0.29 ± 0 | -1.4 ± 0.7 | -80.4 ± 7.4 | 0.02 | Wilcoxon signed rank test | < 0.001 |
| $[Ca^{2+}]$ | 1.0 ± 0.09 | 0.1 ± 0.03 | -0.9 ± 0.1 | -88.3 ± 4.5 | 0.01 | Wilcoxon signed rank test | < 0.001 |
| $[PO_4^{3-}]$ | 0.9 ± 0.4 | 0.09 ± 0.04 | -0.8 ± 0.3 | -89.5 ± 3.0 | 0.2 | t test (paired) | < 0.001 |
| Lactate | 4.5 ± 1.8 | 1.7 ± 0.6 | -2.9 ± 1.6 | -60.2 ± 15.6 | 0.1 | t test (paired) | < 0.001 |
| Glucose | 13.4 ± 3.1 | 1.55 ± 0.7 | -11.9 ± 2.8 | -88.6 ± 4.4 | 0.3 | t test (paired) | < 0.001 |
| Osmolality | 256.9 ± 38.4 | 296.2 ± 57.5 | 39.3 ± 65.4 | 17.9 ± 30.2 | 0.03 | Wilcoxon signed rank test | = 0.01 |
| Albumin | 22.2 ± 4.1 | 10 ± 0.0 | -12.2 ± 4.1 | -53.0 ± 11.4 | 0.6 | t test (paired) | < 0.001 |
| Total Protein | 40.6 ± 9.3 | 20 ± 0.0 | -20.6 ± 9.3 | -48.1 ± 12.5 | 0.8 | t test (paired) | < 0.001 |
| Haematocrit | 27.05 ± 2.2 | 62.3 ± 6.1 | 35.3 ± 5.6 | 131.0 ± 22.2 | p<0.001 | Wilcoxon signed rank test | = 0.001 |
| Haemoglobin | 9.0 ± 0.7 | 20.7 ± 2 | 11.7 ± 1.9 | 130.9 ± 21.6 | 0.01 | Wilcoxon signed rank test | < 0.001 |
| $TCO_2$ | 22.4 ± 2.9 | * | * | | NA | NA | NA |
| Bicarbonate | 21.51 ± 2.8 | * | * | | NA | NA | NA |
| Blood Volume | 2620 ± 935.2 | 476.4 ± 222.1 | -2144 ± 768.6 | -81.8 ± 4.9 | 0.00 | Wilcoxon signed rank test | < 0.001 |
| SID | 27.1 ± 2.1 | 18.4 ± 2.2 | -8.7 ± 3.1 | -31.5 ± 9.8 | 0.2 | t test (paired) | < 0.001 |

*: incalculable by blood gas analyser, NA: not applicable

**STATISTICAL TEST:**

- SHAPIRO WILK[1] – SHAPIRO WILK W TEST FOR NORMAL DATA
- t test(paired)[2]
- Wilcoxon signed rank test[3]
- *p* values < 0.05 were considered statistically significant.

**4.3.2** The statistical summary for the variables measured in the Balsol® interventional group are shown in table 13 as mean ± SD. For all the variables measured there was a significant difference between post and pre wash values ($p < 0.05$), except for total magnesium and SID were insignificant.

**TABLE 13:** Statistical summary for the variables measured in the Balsol® interventional group

| Variables measured | Pre - wash Mean ± SD | Post - wash Mean ± SD | Δ (Post – pre) Mean ± SD | % Δ (Post – pre) Mean ± SD | Shapiro Wilk[1] | Statistical test | *p* value |
|---|---|---|---|---|---|---|---|
| pH | 7.5 ± 0.1 | 7.7 ± 0.1 | 0.2 ± 0.1 | 2.4 ± 1.6 | 0.4 | t test (paired)[2] | < 0.001 |
| $pCO_2$ | 30.15 ± 6.0 | 18.9 ± 4.9 | -11.25 ± 6.4 | -35.4 ± 18.3 | 0.9 | t test (paired) | < 0.001 |
| $pO_2$ | 582.9 ± 69.5 | 226 ± 10.8 | -356.9 ± 65.1 | -60.8 ± 4.0 | 0.1 | t test (paired) | = 0.0001 |
| $[K^+]$ | 4.2 ± 0.4 | 4.6 ± 0.3 | 0.4 ± 0.4 | 9.4 ± 10.2 | 0.9 | t test (paired) | = 0.0007 |
| $[Na^+]$ | 134.7 ± 2.2 | 125.6 ± 1 | -9.15 ± 2.1 | -6.8 ± 1.4 | 0.8 | t test (paired) | < 0.001 |
| $[Cl^-]$ | 108.8 ± 2.7 | 100.2 ± 1.4 | -8.6 ± 3.0 | -7.9 ± 2.6 | 0.9 | t test (paired) | < 0.001 |
| $[Mg^{2+}]$ | 1.4 ± 0.4 | 1.4 ± 0.03 | 0.0065 ± 0.4 | 7.8 ± 31.4 | 0.7 | t test (paired) | = 0.9 |
| $[Ca^{2+}]$ | 0.9 ± 0.1 | 0.02 ± 0.04 | -0.92 ± 0.1 | -98.0 ± 4.1 | 0.8 | t test (paired) | < 0.001 |
| $[PO_4^{3-}]$ | 0.8 ± 0.2 | 0.1 ± 0.024 | -0.73 ± 0.19 | -88.0 ± 3.6 | 0.6 | t test (paired) | < 0.001 |
| Lactate | 4.3 ± 1.8 | 1.6 ± 0.4 | -2.77 ± 1.4 | -61.5 ± 9.5 | 0.2 | t test (paired) | < 0.001 |
| Glucose | 12.1 ± 3.2 | 1.6 ± 0.7 | -10.44 ± 2.7 | -86.9 ± 4.1 | 0.5 | t test (paired) | < 0.001 |
| Osmolatility | 288.8 ± 20.6 | 272.8 ± 19.9 | -15.95 ± 29 | -5.0 ± 10.4 | p<0.001 | Wilcoxon signed rank test[3] | = 0.009 |
| Albumin | 23.2 ± 3.3 | 10 ± 0 | -13.2 ± 3.3 | -56.2 ± 5.6 | 0.03 | Wilcoxon signed rank test | < 0.001 |
| Total Protein | 40.6 ± 6.4 | 20 ± 0 | -20.65 ± 6.4 | -49.7 ± 7.6 | 0.3 | t test (paired) | < 0.001 |
| Hematocrit | 27.4 ± 2.2 | 62.6 ± 3.8 | 35.25 ± 4.7 | 130.6 ± 26.4 | 0.9 | t test (paired) | < 0.001 |
| Hemoglobin | 9.2 ± 0.9 | 20.9 ± 1.3 | 11.66 ± 1.7 | 129.4 ± 29.5 | 0.9 | t test (paired) | < 0.001 |
| $TCO_2$ | 22.9 ± 2.8 | 24.3 ± 0.5 | 1.33 ± 2.7 | 7.3 ± 13.8 | 0.6 | t test (paired) | = 0.04 |
| Bicarbonate | 22 ± 2.7 | 23.7 ± 0.6 | 1.68 ± 2.6 | 9.2 ± 14.0 | 0.8 | t test (paired) | = 0.01 |
| Blood Volume | 2612 ± 859.7 | 447.4 ± 153.9 | -2165 ± 825.4 | -81.7 ± 6.9 | 0.2 | t test (paired) | < 0.001 |
| SID | 27.6 ± 3.0 | 28.44 ± 1.5 | 0.84 ± 3.3 | 3.9 ± 13.2 | 0.1 | t test (paired) | = 0.3 |

**STATISTICAL TEST:**

- SHAPIRO WILK[1] – Shapiro Wilk W-test for normal data.
- t test(paired)[2]
- Wilcoxon signed rank test[3]
- *p* values < 0.05 were considered statistically significant.

**4.3.3** The statistical summary of the change in post NaCl wash versus the change in post Balsol® wash are shown in table 14 as mean ± SD. For all the variables measured there was a significant difference between the change in post wash values ($p < 0.05$), except for pH, [$PO_4^{3-}$], lactate, glucose, albumin, total protein, HCT, Hb and blood volume.

**TABLE 14:** Statistical summary of the change in post NaCl wash versus the change in post Balsol® wash.

| Variables measured | Post – wash NaCl Mean ± SD | Post – wash Balsol Mean ± SD | Statistical test | *p* value |
|---|---|---|---|---|
| pH | 0.2 ± 0.1 | 0.2 ± 0.1 | t test (2 group)[1] | = 0.1 |
| $pCO_2$ | -22.25 ± 2.9 | -11.25 ± 6.4 | t test (2 group) | < 0.001 |
| $pO_2$ | -386.7 ± 68.7 | -356.9 ± 65.1 | Mann-Whitney test[2] | = 0.03 |
| [$K^+$] | -3.5 ± 0.8 | 0.4 ± 0.4 | t test (2 group) | < 0.001 |
| [$Na^+$] | 13.4 ± 3.8 | -9.15 ± 2.1 | t test (2 group) | < 0.001 |
| [$Cl^-$] | 19.7 ± 3.0 | -8.6 ± 3.0 | t test (2 group) | < 0.001 |
| [$Mg^{2+}$] | -1.4 ± 0.7 | 0.0065 ± 0.4 | Mann-Whitney test | < 0.001 |
| [$Ca^{2+}$] | -0.9 ± 0.1 | -0.92 ± 0.1 | Mann-Whitney test | = 0.004 |
| [$PO_4^{3-}$] | -0.8 ± 0.3 | -0.73 ± 0.19 | t test (2 group) | = 0.2 |
| Lactate | -2.9 ± 1.6 | -2.77 ± 1.4 | t test (2 group) | = 0.8 |
| Glucose | -11.9 ± 2.8 | -10.44 ± 2.7 | t test (2 group) | = 0.1 |
| Osmolatility | 39.3 ± 65.4 | -15.95 ± 29 | Mann-Whitney test | < 0.001 |
| Albumin | -12.2 ± 4.1 | -13.2 ± 3.3 | Mann-Whitney test | = 0.8 |
| Total Protein | -20.6 ± 9.3 | -20.65 ± 6.4 | t test (2 group) | = 0.9 |
| Haematocrit | 35.3 ± 5.6 | 35.25 ± 4.7 | Mann-Whitney test | = 0.6 |
| Haemoglobin | 11.7 ± 1.9 | 11.66 ± 1.7 | Mann-Whitney test | = 0.6 |
| $TCO_2$ | * | 1.33 ± 2.7 | NA | NA |
| Bicarbonate | * | 1.68 ± 2.6 | NA | NA |
| Blood Volume | -2144 ± 768.6 | -2165 ± 825.4 | Mann-Whitney test | =0.9 |
| SID | -8.7 ± 3.1 | 0.84 ± 3.3 | t test (2 group) | < 0.001 |

*: incalculable by blood gas analyser, NA: not applicable

**STATISTICAL TEST:**

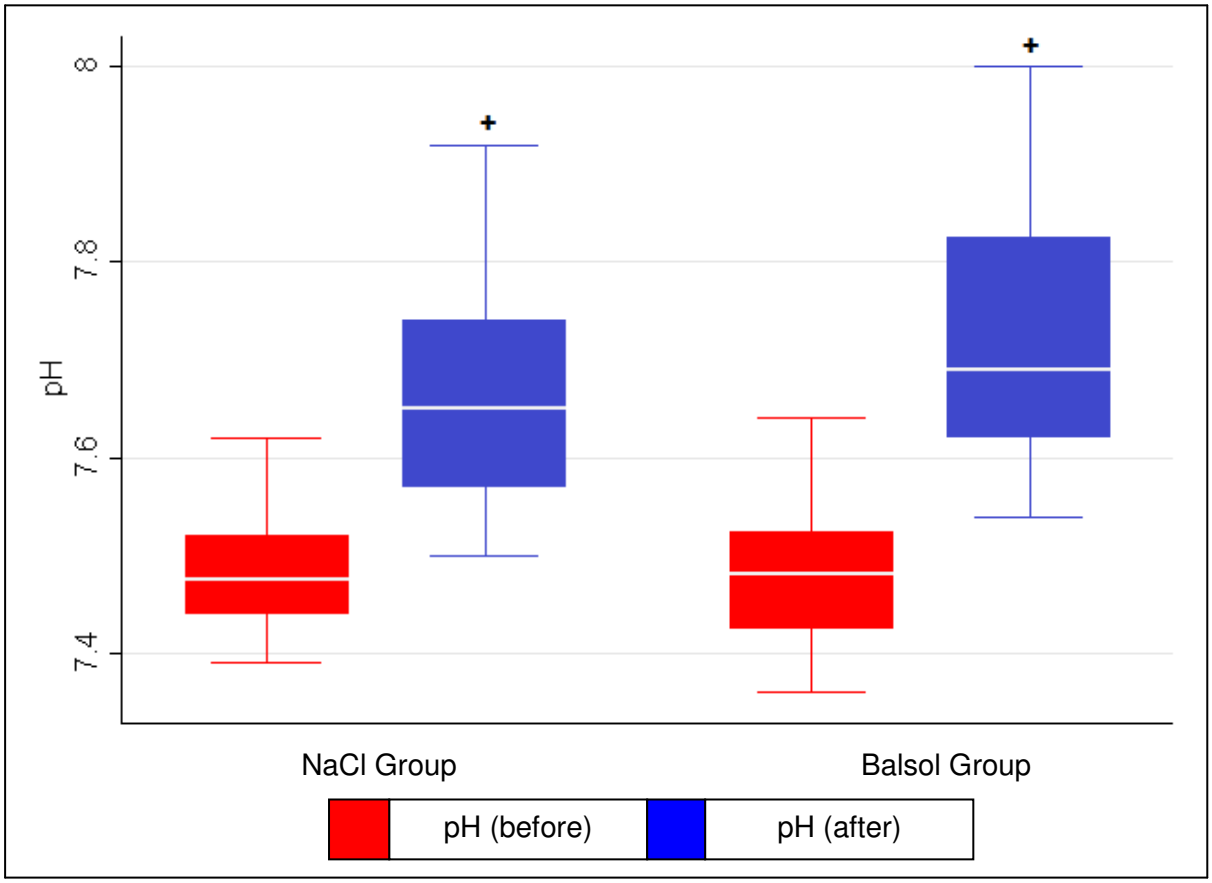
- If one of the distribution did not meet the assumption of normality, then the non-parametric test, the Mann-Whitney test was used to compare groups.
- t test (2 group)[1] – two sample t test with equal variances
- Whitney test[2] – two sample Wilcoxon rank sum (Mann-Whitney) test
- *p* values < 0.05 were considered statistically significant.

### 4.4 Changes in pH

There was a highly significant increase in pH within the NaCl and Balsol® groups after washing ($p < 0.001$) (table 15). Figure 8 illustrates the changes in pH before and after washing with NaCl and Balsol® solutions respectively.

**Table 15:** changes in pH before and after washing with NaCl and Balsol® solutions.

| Variable | NaCl Group | | P Value | Balsol Group | | P Value | Post wash – Pre wash | | P Value |
|---|---|---|---|---|---|---|---|---|---|
| | Pre wash | Post wash | | Pre wash | Post wash | | Δ NaCl | Δ Balsol | |
| pH | 7.5 ± 0.1 | 7.7 ± 0.1 | < 0.001 | 7.5 ± 0.1 | 7.7 ± 0.1 | < 0.001 | 0.2 ± 0.1 | 0.2 ± 0.1 | = 0.1 |




**+** significant change $p < 0.05$ after washing within group.

**Figure 8**: Box plot illustrating pH levels in NaCl and Balsol® groups before and after washing with NaCl and Balsol® solutions respectively.

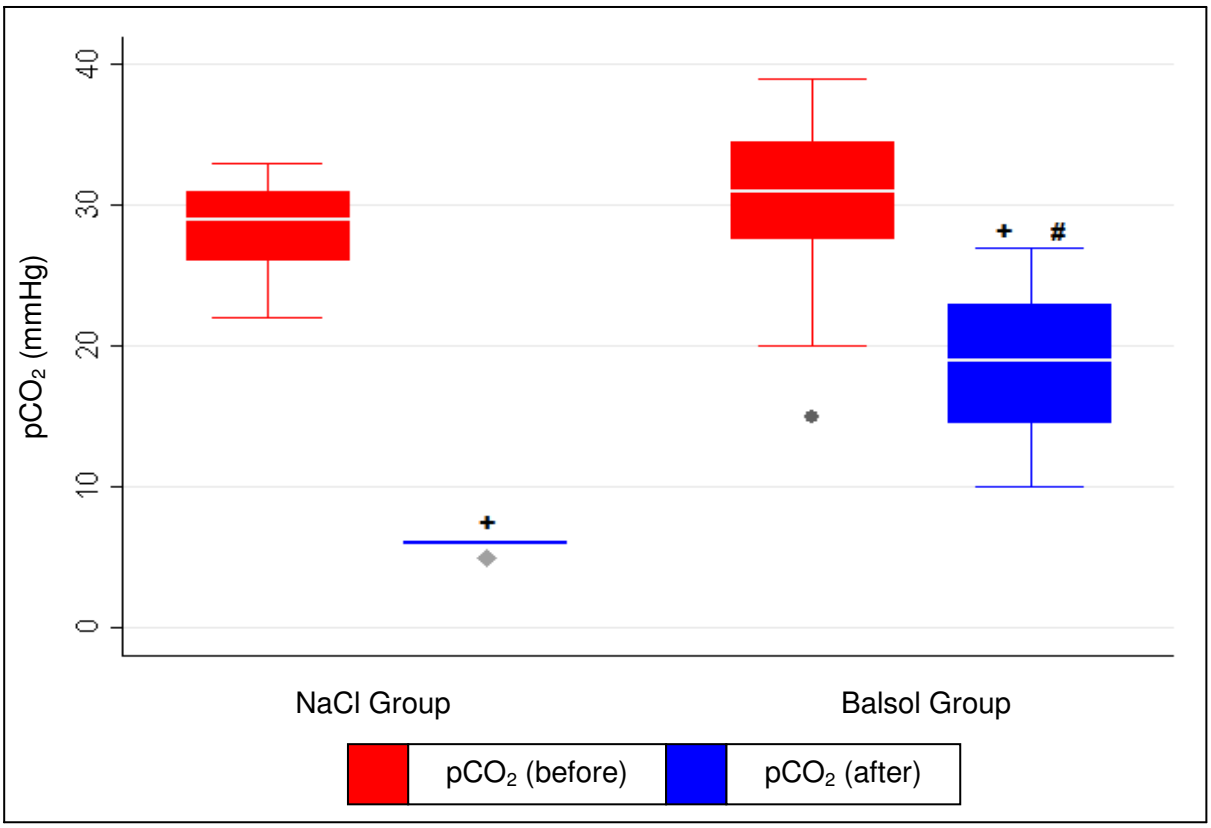
### 4.5 Changes in $pCO_2$

There was a highly significant decrease in $pCO_2$ within the NaCl and Balsol® groups after washing ($p < 0.001$), and a highly significant difference in the change between groups after washing ($p < 0.001$) (table 16). Figure 9 illustrates the changes in $pCO_2$ before and after washing with NaCl and Balsol® solutions respectively.

**Table 16:** changes in $pCO_2$ before and after washing with NaCl and Balsol® solutions.

| Variable | NaCl Group | | P Value | Balsol Group | | P Value | Post wash – Pre wash | | P Value |
|---|---|---|---|---|---|---|---|---|---|
| | Pre wash | Post wash | | Pre wash | Post wash | | Δ NaCl | Δ Balsol | |
| $pCO_2$ | 28.3 ± 2.9 | *6.0 ± 0.0 | < 0.001 | 30.15 ± 6.0 | 18.9 ± 4.9 | < 0.001 | -22.25 ± 2.9 | -11.25 ± 6.4 | < 0.001 |

* All samples in the NaCl group fell below the detectable range after washing, therefore the lower limit of the reference range was used for statistical analysis.




**+** significant change $p < 0.05$ after washing within group.
**#** significant change $p < 0.05$ after washing between groups.

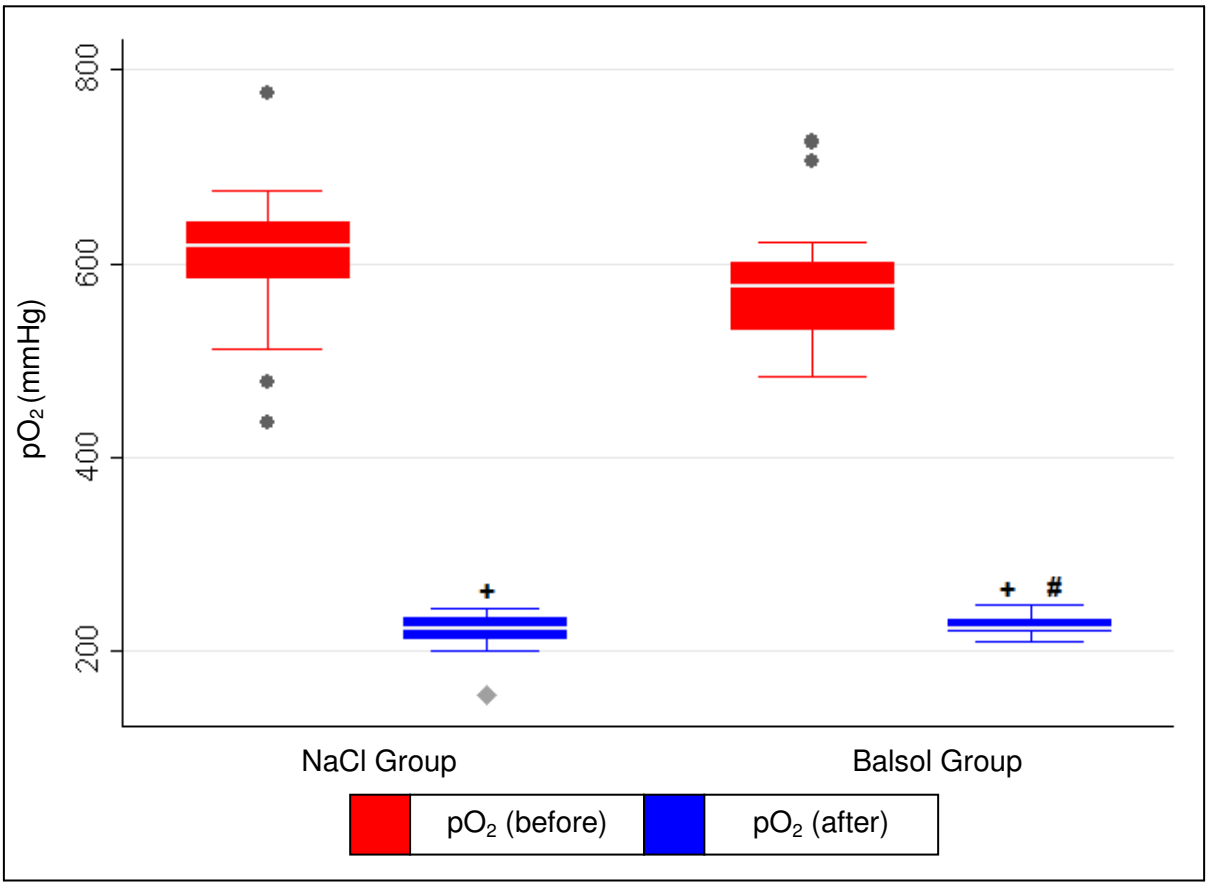
**Figure 9**: Box plot illustrating $pCO_2$ levels in NaCl and Balsol® groups before and after washing with NaCl and Balsol® solutions respectively.

### 4.6 Changes in $pO_2$

There was a highly significant decrease in $pO_2$ within the NaCl and Balsol® groups after washing ($p < 0.0001$), and a significant difference in the change between groups after washing ($p = 0.03$) (table 17). Figure 10 illustrates the changes in $pO_2$ before and after washing with NaCl and Balsol® solutions respectively.

**Table 17:** changes in $pO_2$ before and after washing with NaCl and Balsol® solutions.

| Variable | NaCl Group | | P Value | Balsol Group | | P Value | Post wash – Pre wash | | P Value |
|---|---|---|---|---|---|---|---|---|---|
| | Pre wash | Post wash | | Pre wash | Post wash | | Δ NaCl | Δ Balsol | |
| $pO_2$ | 607.3 ±72.0 | 220.6 ±19.4 | < 0.0001 | 582.9 ± 69.5 | 226 ± 10.8 | < 0.0001 | -386.7 ± 68.7 | -356.9 ± 65.1 | = 0.03 |




**+** significant change $p < 0.05$ after washing within group.
**#** significant change $p < 0.05$ after washing between groups.

**Figure 10**: Box plot illustrating $pO_2$ levels before and after washing with NaCl and Balsol® solutions respectively.

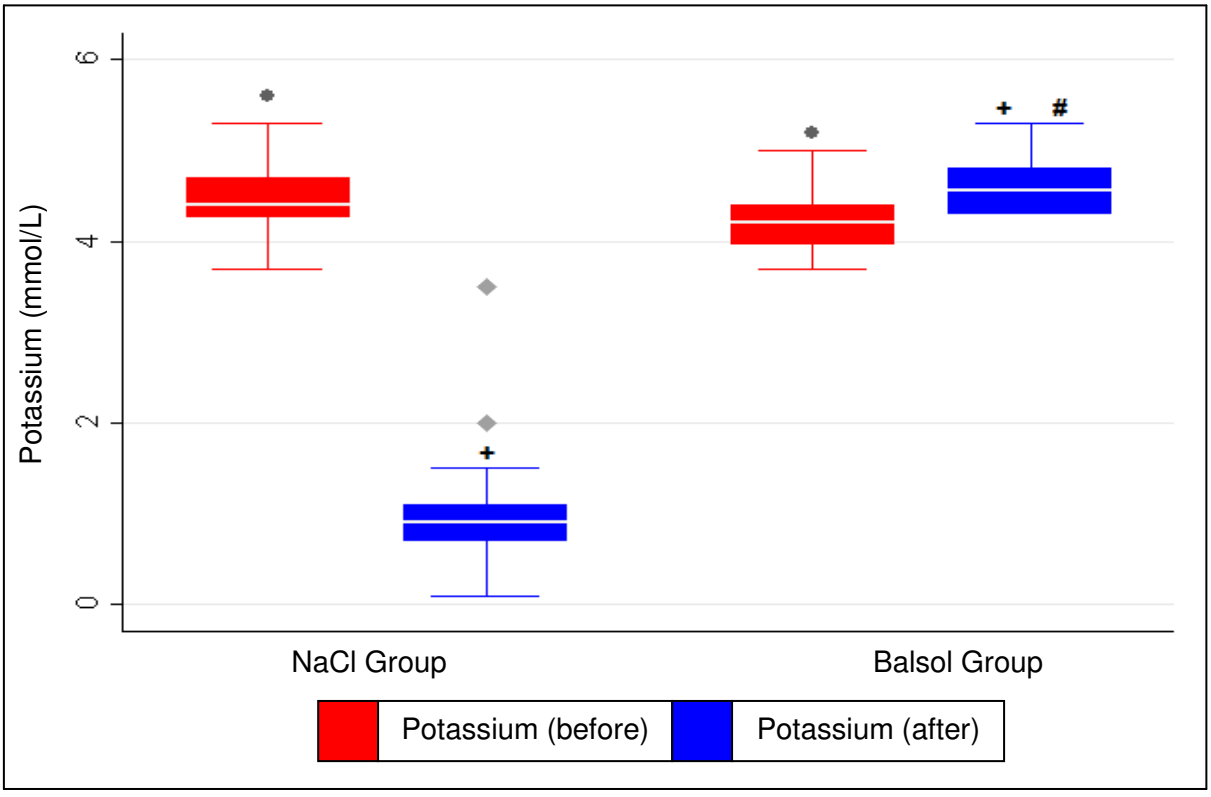
### 4.7 Changes in potassium

There was a highly significant decrease in [$K^+$] within the NaCl group after washing ($p < 0.001$), and a highly significant increase in [$K^+$] within the Balsol® group after washing ($p < 0.0007$). There was also a highly significant difference in the change between groups after washing ($p < 0.001$) (table 18). Figure 11 illustrates the changes in [$K^+$] before and after washing with NaCl and Balsol® solutions respectively.

**Table 18:** changes in [$K^+$] before and after washing with NaCl and Balsol® solutions.

| Variable | NaCl Group | | P Value | Balsol Group | | P Value | Post wash – Pre wash | | P Value |
|---|---|---|---|---|---|---|---|---|---|
| | Pre wash | Post wash | | Pre wash | Post wash | | Δ NaCl | Δ Balsol | |
| [$K^+$] | 4.5 ± 0.5 | *1.0 ± 0.7 | < 0.001 | 4.2 ± 0.4 | 4.6 ± 0.3 | < 0.0007 | -3.5 ± 0.8 | 0.4 ± 0.4 | 0.001 |

*3 samples in the NaCl group fell below the detectable range after washing, therefore the lower limit of the reference range was used for statistical analysis.




**+** significant change $p < 0.05$ after washing within group.
**#** significant change $p < 0.05$ after washing between groups.

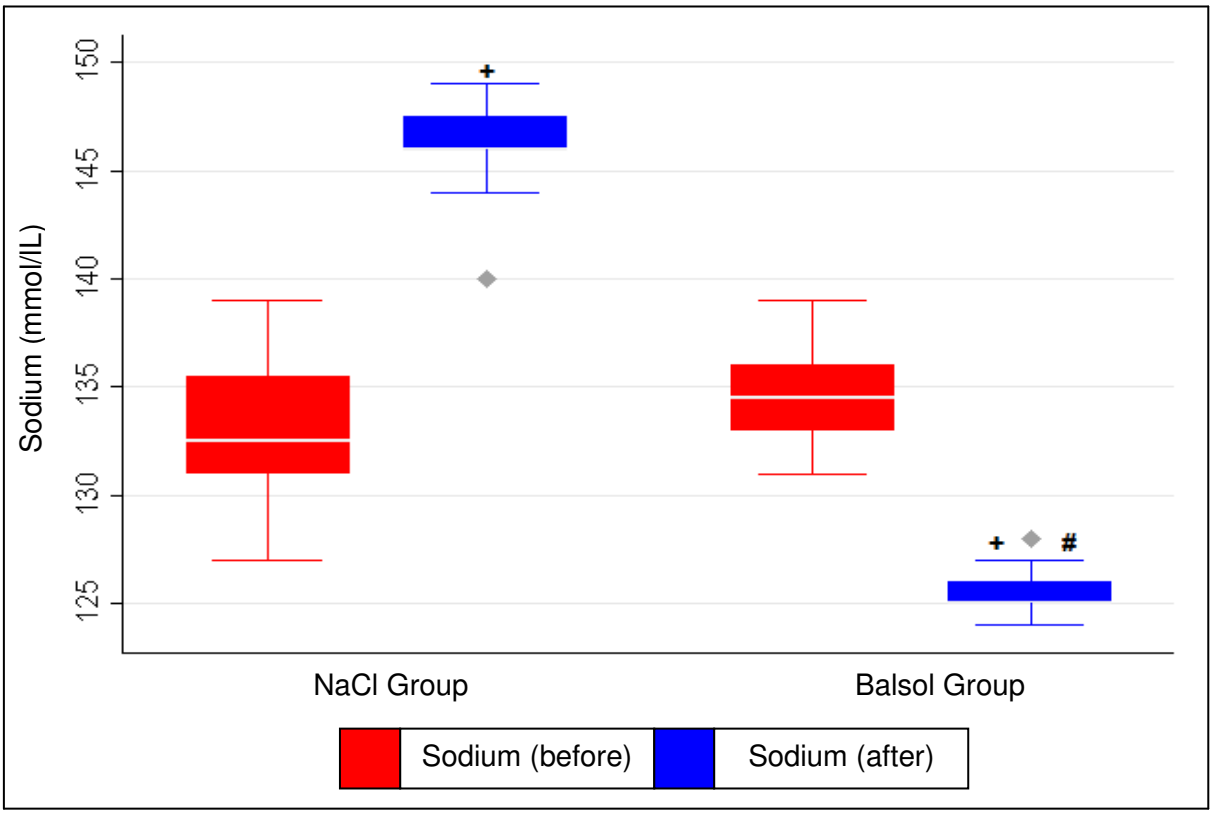
**Figure 11**: Box plot illustrating potassium levels before and after washing with NaCl and Balsol® solutions respectively.

### 4.8 Changes in sodium

There was a highly significant increase in [$Na^+$] within the NaCl group after washing ($p < 0.001$), and a highly significant decrease in [$Na^+$] within the Balsol® group after washing ($p < 0.001$). There was also a highly significant difference in the change between groups after washing ($p < 0.001$) (table 19). Figure 12 illustrates the changes in [$Na^+$] before and after washing with NaCl and Balsol® solutions respectively.

**Table 19:** changes in [$Na^+$] before and after washing with NaCl and Balsol® solutions.

| Variable | NaCl Group | | P Value | Balsol Group | | P Value | Post wash – Pre wash | | P Value |
|---|---|---|---|---|---|---|---|---|---|
| | Pre wash | Post wash | | Pre wash | Post wash | | Δ NaCl | Δ Balsol | |
| [$Na^+$] | 132.9 ± 3.2 | 146.3 ± 1.9 | < 0.001 | 134.7 ± 2.2 | 125.6 ± 1 | < 0.001 | 13.4 ± 3.8 | -9.15 ± 2.1 | < 0.001 |




**+** significant change $p < 0.05$ after washing within group.
**#** significant change $p < 0.05$ after washing between groups.

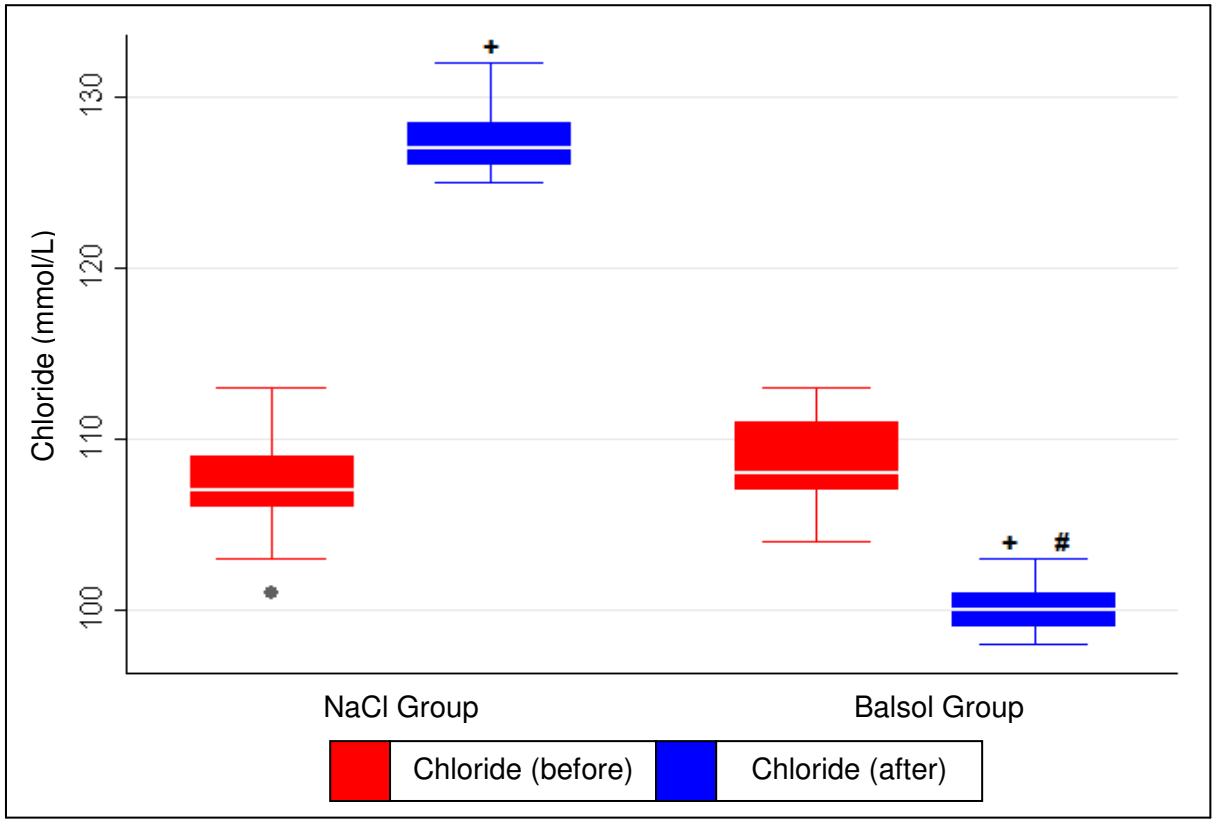
**Figure 12**: Box plot illustrating sodium levels before and after washing with NaCl and Balsol® solutions respectively.

### 4.9 Changes in chloride

There was a highly significant increase in [$Cl^-$] within the NaCl group after washing ($p < 0.001$), and a highly significant decrease in [$Cl^-$] within the Balsol® group after washing ($p < 0.001$). There was also a highly significant difference in the change between groups after washing ($p < 0.001$) (table 20). Figure 13 illustrates the changes in [$Cl^-$] before and after washing with NaCl and Balsol® solutions respectively.

**Table 20:** changes in chloride before and after washing with NaCl and Balsol® solutions.

| Variable | NaCl Group | | P Value | Balsol Group | | P Value | Post wash – Pre wash | | P Value |
|---|---|---|---|---|---|---|---|---|---|
| | Pre wash | Post wash | | Pre wash | Post wash | | Δ NaCl | Δ Balsol | |
| [$Cl^-$] | 107.8 ± 3.1 | 127.4 ± 2.1 | < 0.001 | 108.8 ± 2.7 | 100.2 ± 1.4 | < 0.001 | 19.7 ± 3.0 | -8.6 ± 3.0 | < 0.001 |




**+** significant change $p < 0.05$ after washing within group.
**#** significant change $p < 0.05$ after washing between groups.

**Figure 13**: Box plot illustrating chloride levels before and after washing with NaCl and Balsol® solutions respectively.

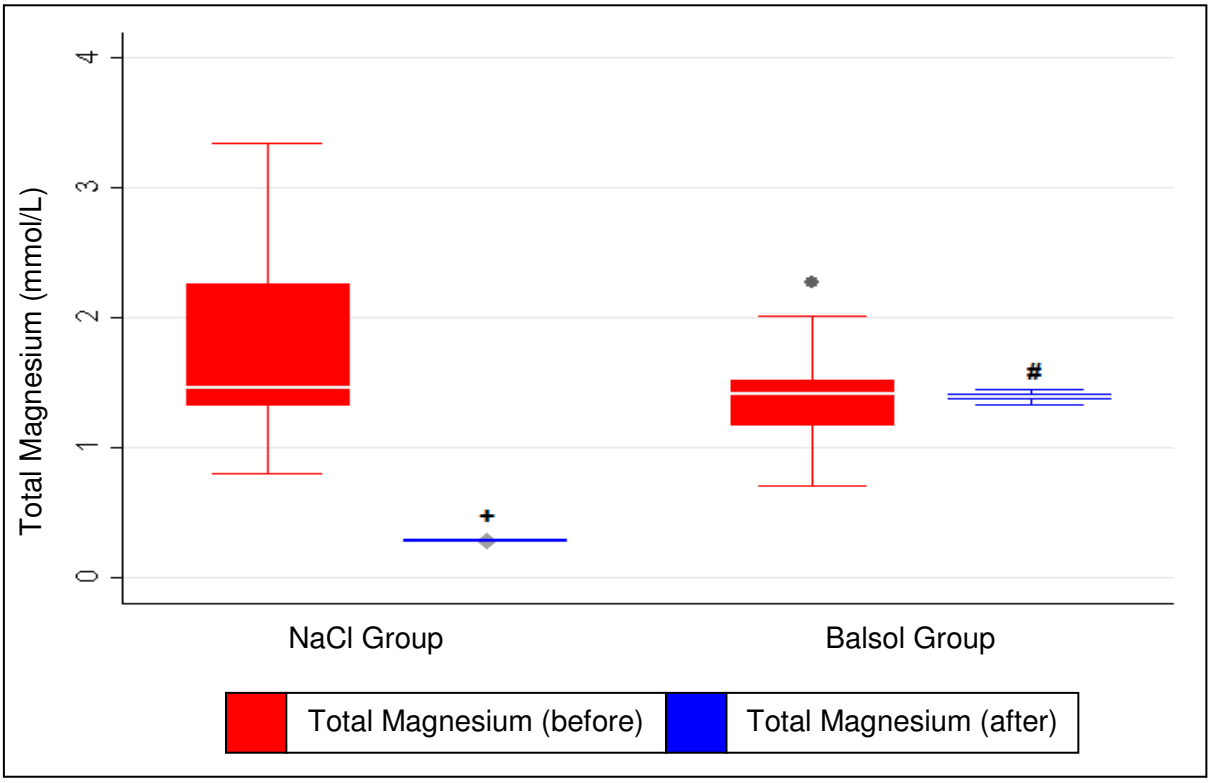
### 4.10 Changes in total magnesium

There was a highly significant decrease in total [$Mg^{2+}$] within the NaCl group after washing and also a highly significant difference in the change between groups after washing ($p < 0.001$) (table 21). Figure 14 illustrates the changes in total [$Mg^{2+}$] before and after washing with NaCl and Balsol® solutions respectively.

**Table 21:** changes in total magnesium before and after washing with NaCl and Balsol® solutions.

| Variable | NaCl Group | | P Value | Balsol Group | | P Value | Post wash – Pre wash | | P Value |
|---|---|---|---|---|---|---|---|---|---|
| | Pre wash | Post wash | | Pre wash | Post wash | | Δ NaCl | Δ Balsol | |
| [$Mg^{2+}$] | 1.7 ± 0.7 | *0.29 ± 0 | < 0.001 | 1.4 ± 0.4 | 1.4 ± 0.03 | = 0.9 | -1.4 ± 0.7 | 0.0065 ± 0.4 | 0.001 |

* All samples in the NaCl group fell below the detectable range after washing, therefore the lower limit of the reference range was used for statistical analysis.




**+** significant change $p < 0.05$ after washing within group.
**#** significant change $p < 0.05$ after washing between groups.

**Figure 14**: Box plot illustrating total magnesium levels before and after washing with NaCl and Balsol® solutions respectively.

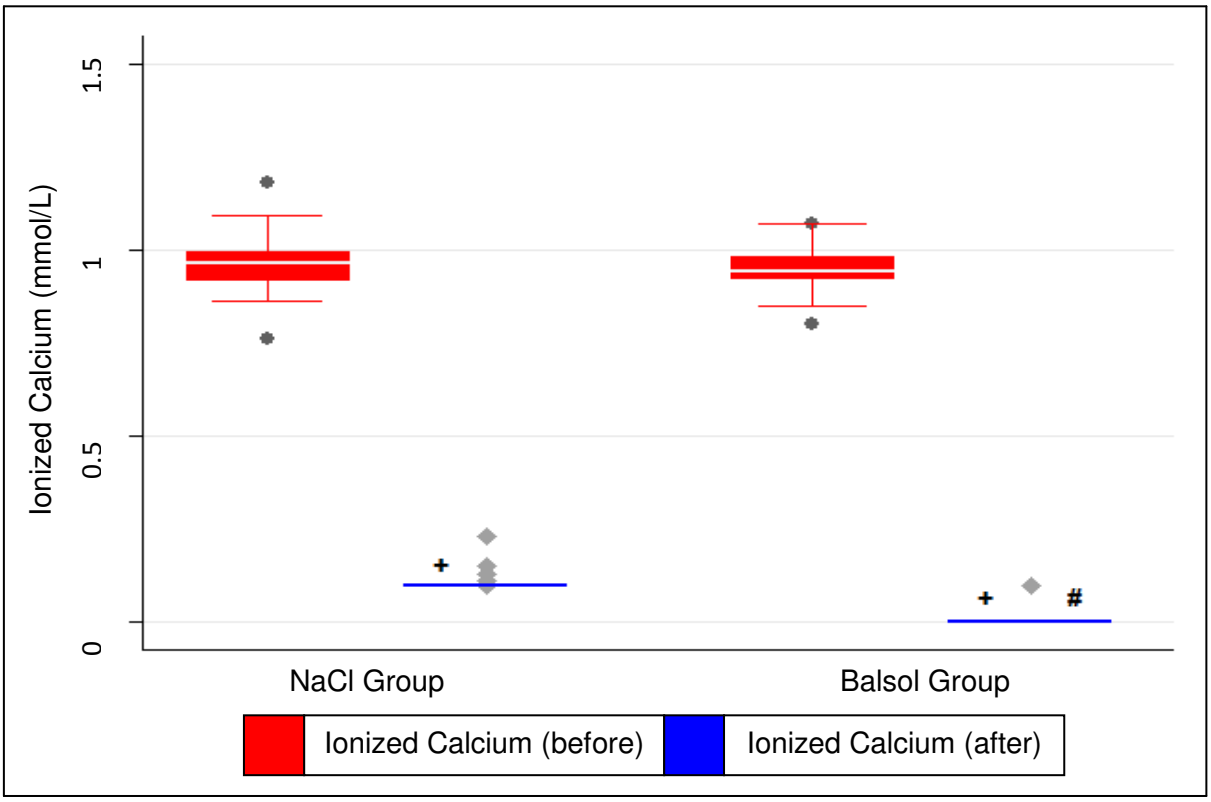
### 4.11 Changes in ionized calcium

There was a highly significant decrease in ionized [$Ca^{2+}$] within the NaCl and Balsol® groups after washing ($p < 0.001$), and a significant difference in the change between groups after washing (p = 0.004) (table 22). Figure 15 illustrates the changes in ionized [$Ca^{2+}$] before and after washing with NaCl and Balsol® solutions respectively.

**Table 22:** changes in ionized calcium before and after washing with NaCl and Balsol® solution.

| Variable | NaCl Group | | P Value | Balsol Group | | P Value | Post wash – Pre wash | | P Value |
|---|---|---|---|---|---|---|---|---|---|
| | Pre wash | Post wash | | Pre wash | Post wash | | Δ NaCl | Δ Balsol | |
| [$Ca^{2+}$] | 1.0 ± 0.09 | *0.1 ± 0.03 | < 0.001 | 0.9 ± 0.1 | *0.02 ± 0.04 | < 0.001 | -0.9 ± 0.1 | -0.92 ± 0.1 | = 0.004 |

* 12 and 16 samples in the NaCl and Balsol® groups respectively fell below the detectable range after washing, therefore the lower limit of the reference range was used for statistical analysis.




**+** significant change $p < 0.05$ after washing within group.
**#** significant change $p < 0.05$ after washing between groups.

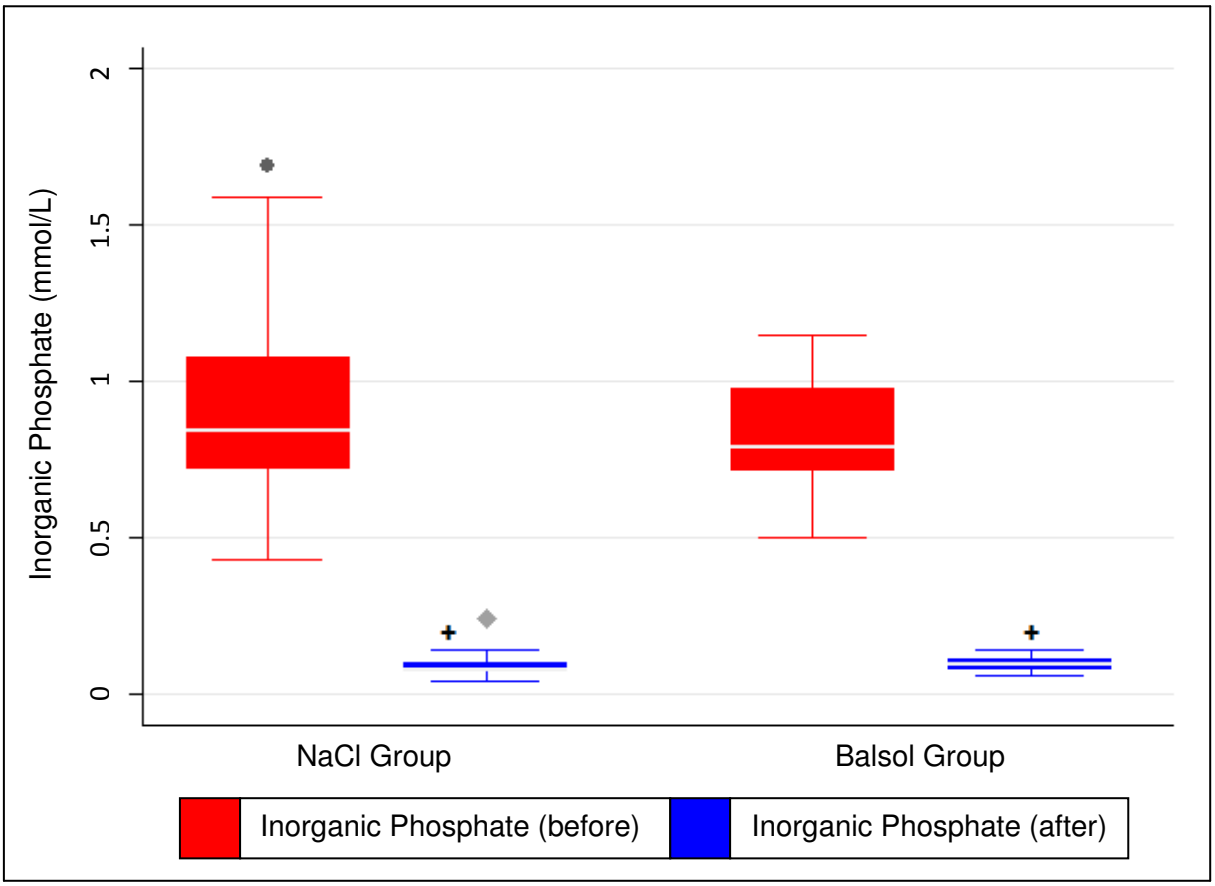
**Figure 15**: Box plot illustrating ionized calcium levels before and after washing with NaCl and Balsol® solutions respectively.

### 4.12 Changes in inorganic phosphate

There was a highly significant decrease in [$PO_4^{3-}$] within the NaCl and Balsol® groups after washing ($p < 0.001$) (table 23). Figure 16 illustrates the changes in [$PO_4^{3-}$] before and after washing with NaCl and Balsol® solutions respectively.

**Table 23:** changes in inorganic phosphate before and after washing with NaCl and Balsol® solutions.

| Variable | NaCl Group | | P Value | Balsol Group | | P Value | Post wash – Pre wash | | P Value |
|---|---|---|---|---|---|---|---|---|---|
| | Pre wash | Post wash | | Pre wash | Post wash | | Δ NaCl | Δ Balsol | |
| [$PO_4^{3-}$] | 0.9 ± 0.4 | 0.09 ± 0.04 | < 0.001 | 0.8 ± 0.2 | 0.1 ± 0.024 | < 0.001 | -0.8 ± 0.3 | -0.73 ± 0.19 | = 0.2 |




**+** significant change $p < 0.05$ after washing within group.

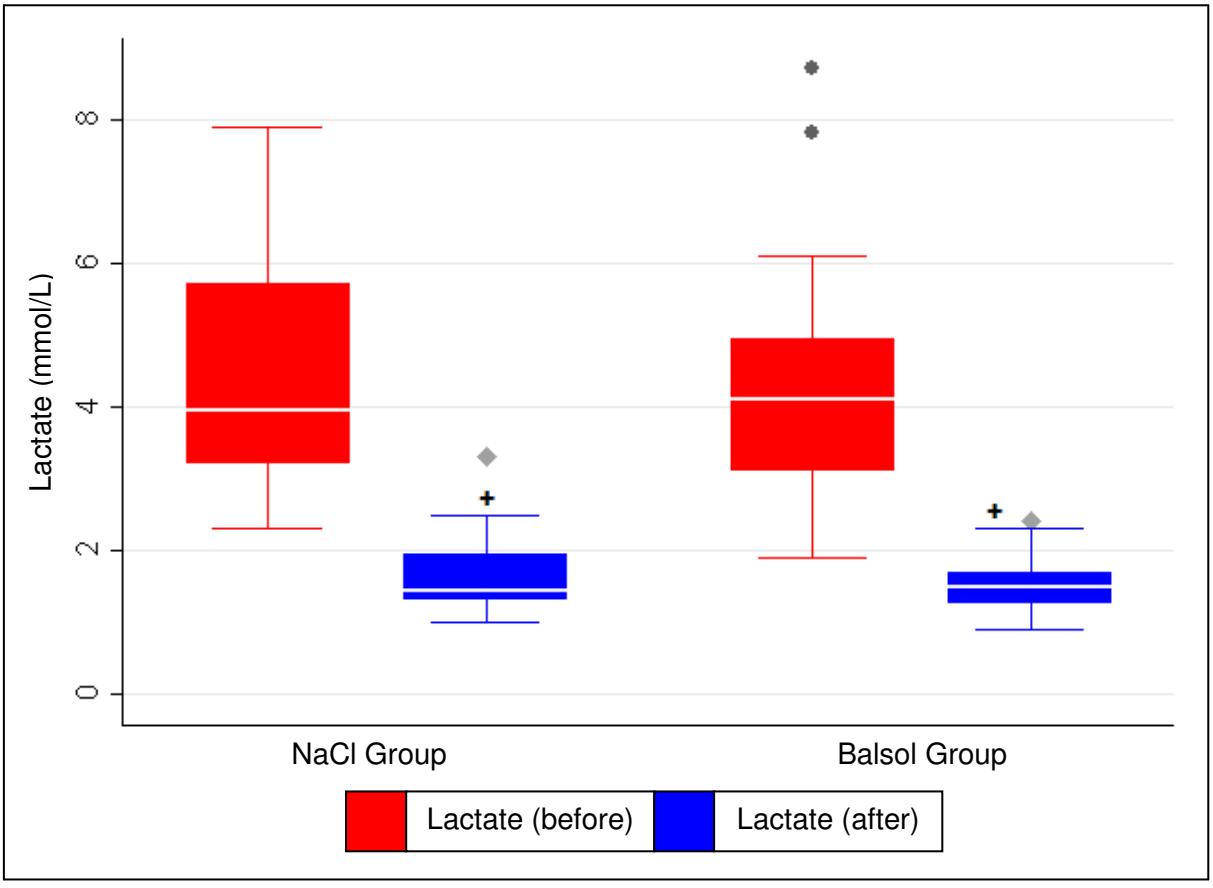
**Figure 16**: Box plot illustrating inorganic phosphate levels before and after washing with NaCl and Balsol® solutions respectively.

### 4.13 Changes in lactate

There was a highly significant decrease in lactate within the NaCl and Balsol® groups after washing ($p < 0.001$) (table 24). Figure 17 illustrates the changes in lactate before and after washing with NaCl and Balsol® solutions respectively.

**Table 24:** changes in lactate before and after washing with NaCl and Balsol® solutions.

| Variable | NaCl Group | | P Value | Balsol Group | | P Value | Post wash – Pre wash | | P Value |
|---|---|---|---|---|---|---|---|---|---|
| | Pre wash | Post wash | | Pre wash | Post wash | | Δ NaCl | Δ Balsol | |
| lactate | 4.5 ± 1.8 | 1.7 ± 0.6 | < 0.001 | 4.3 ± 1.8 | 1.6 ± 0.4 | < 0.001 | -2.9 ± 1.6 | -2.77 ± 1.4 | = 0.8 |




**+** significant change $p < 0.05$ after washing within group.

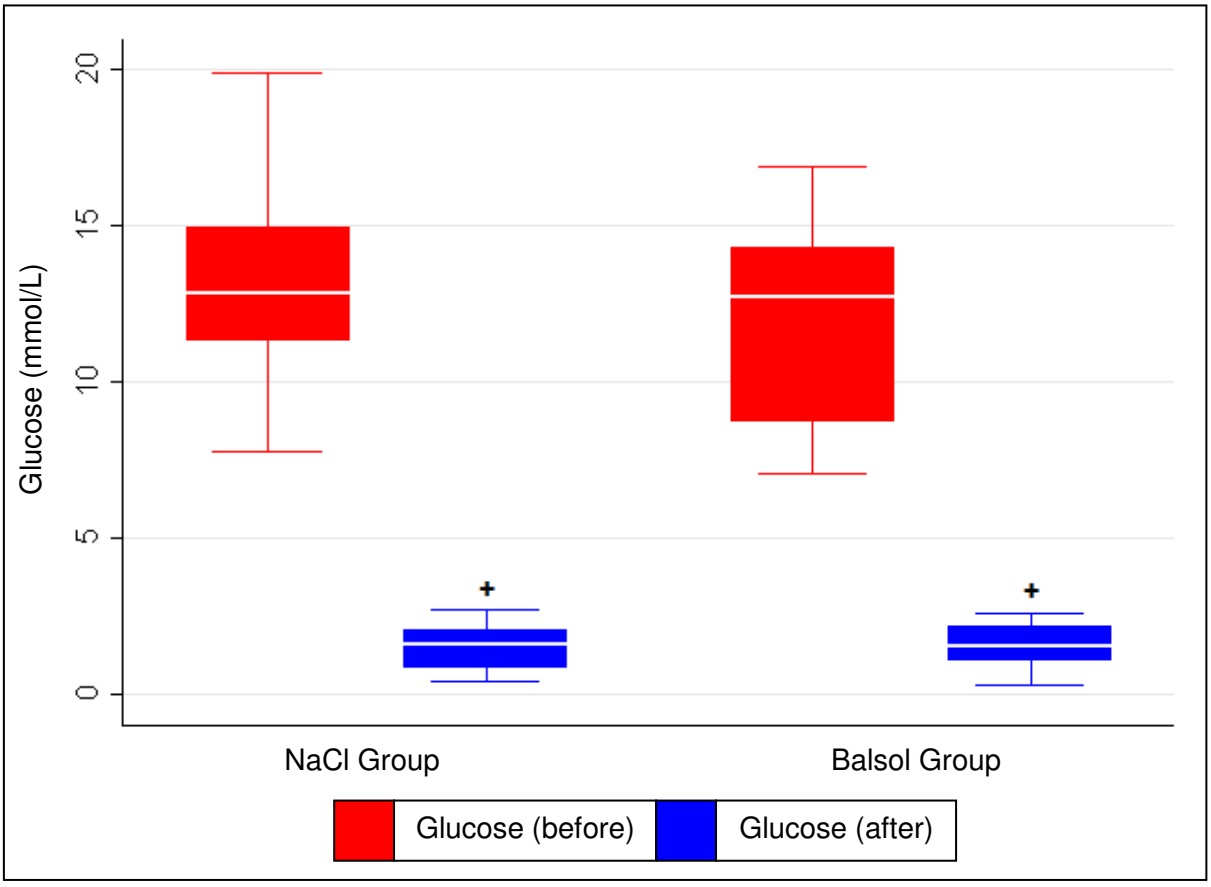
**Figure 17**: Box plot illustrating lactate levels before and after washing with NaCl and Balsol® solutions respectively.

### 4.14 Changes in glucose

There was a highly significant decrease in glucose within the NaCl and Balsol® groups after washing ($p < 0.001$) (table 25). Figure 18 illustrates the changes in glucose before and after washing with NaCl and Balsol® solutions respectively.

**Table 25:** changes in glucose before and after washing with NaCl and Balsol® solutions.

| Variable | NaCl Group | | P Value | Balsol Group | | P Value | Post wash – Pre wash | | P Value |
|---|---|---|---|---|---|---|---|---|---|
| | Pre wash | Post wash | | Pre wash | Post wash | | Δ NaCl | Δ Balsol | |
| Glucose | 13.4 ± 3.1 | 1.55 ± 0.7 | < 0.001 | 12.1 ± 3.2 | 1.6 ± 0.7 | < 0.001 | -11.9 ± 2.8 | -10.44 ± 2.7 | = 0.1 |




**+** significant change $p < 0.05$ after washing within group.

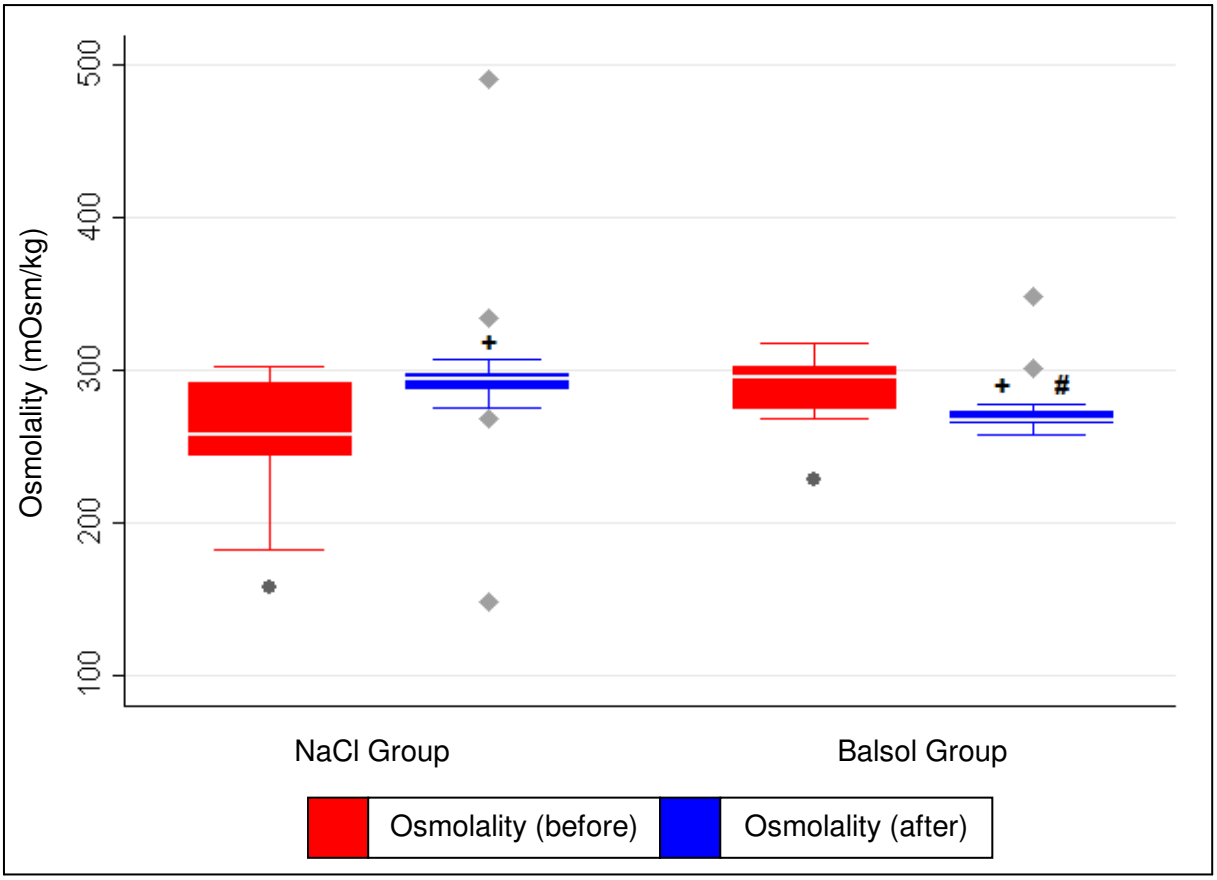
**Figure 18**: Box plot illustrating glucose levels before and after washing with NaCl and Balsol® solutions respectively.

### 4.15 Changes in osmolality

There was a significant increase in osmolality within the NaCl group after washing ($p < 0.01$), and highly significant decrease within the Balsol® group after washing ($p < 0.009$). There was also a highly significant difference in the change between groups after washing ($p < 0.001$) (table 26). Figure 19 illustrates the changes in osmolality before and after washing with NaCl and Balsol® solutions respectively.

**Table 26:** changes in osmolality before and after washing with NaCl and Balsol® solutions.

| Variable | NaCl Group | | P Value | Balsol Group | | P Value | Post wash – Pre wash | | P Value |
|---|---|---|---|---|---|---|---|---|---|
| | Pre wash | Post wash | | Pre wash | Post wash | | Δ NaCl | Δ Balsol | |
| Osmolality | 256.9 ± 38.4 | 296.2 ± 57.5 | < 0.01 | 288.8 ± 20.6 | 272.8 ± 19.9 | < 0.009 | 39.3 ± 65.4 | -15.95 ± 29 | < 0.001 |




**+** significant change $p < 0.05$ after washing within group.
**#** significant change $p < 0.05$ after washing between groups.

**Figure 19**: Box plot illustrating osmolality levels before and after washing with NaCl and Balsol® solutions respectively.

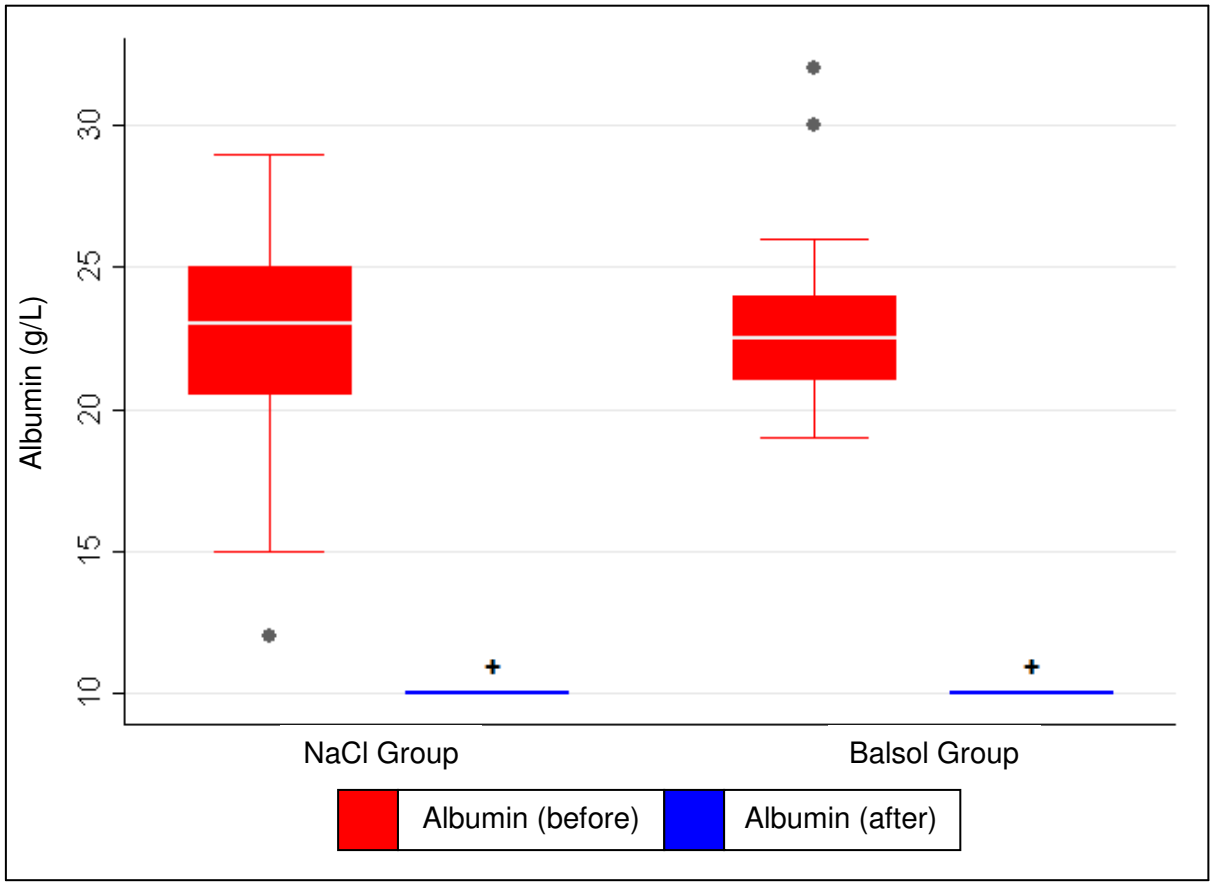
### 4.16 Changes in albumin

There was a highly significant decrease in albumin within the NaCl and Balsol® groups after washing ($p < 0.001$) (table 27). Figure 20 illustrates the changes in albumin before and after washing with NaCl and Balsol® solutions respectively.

**Table 27:** changes in albumin before and after washing with NaCl and Balsol® solutions.

| Variable | NaCl Group | | P Value | Balsol Group | | P Value | Post wash – Pre wash | | P Value |
|---|---|---|---|---|---|---|---|---|---|
| | Pre wash | Post wash | | Pre wash | Post wash | | Δ NaCl | Δ Balsol | |
| Albumin | 22.2 ± 4.1 | *10 ± 0 | < 0.001 | 23.2 ± 3.3 | *10 ± 0 | < 0.001 | -12.2 ± 4.1 | -13.2 ± 3.3 | = 0.8 |

* All samples in the NaCl and Balsol® groups fell below the detectable range after washing, therefore the lower limit of the reference range was used for statistical analysis.




+ significant change $p < 0.05$ after washing within group.

**Figure 20**: Box plot illustrating albumin levels before and after washing with NaCl and Balsol® solutions respectively.

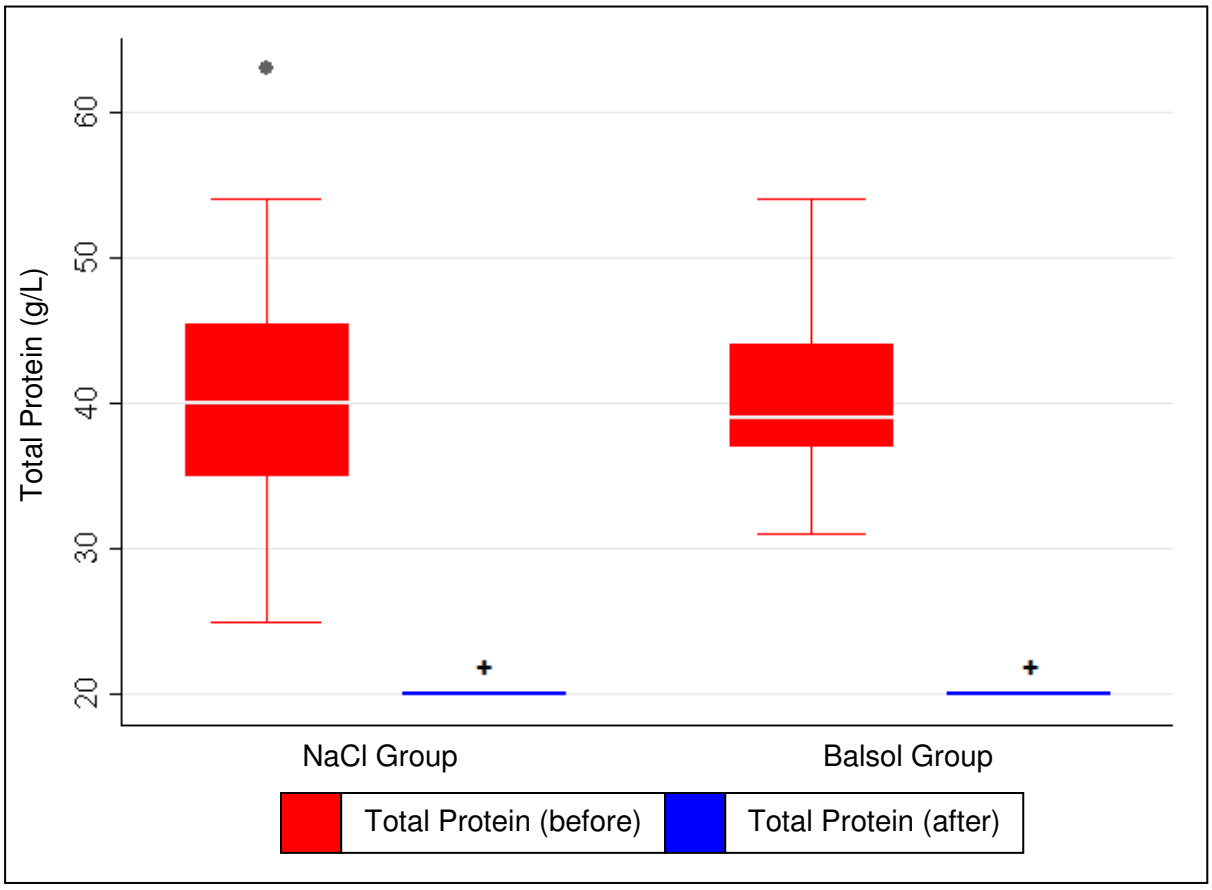
### 4.17 Changes in total protein

There was a highly significant decrease in total protein within the NaCl and Balsol® groups after washing ($p < 0.001$) (table 28). Figure 21 illustrates the changes in total protein before and after washing with NaCl and Balsol® solutions respectively.

**Table 28:** changes in total protein before and after washing with NaCl and Balsol® solutions.

| Variable | NaCl Group | | P Value | Balsol Group | | P Value | Post wash – Pre wash | | P Value |
|---|---|---|---|---|---|---|---|---|---|
| | Pre wash | Post wash | | Pre wash | Post wash | | Δ NaCl | Δ Balsol | |
| Total protein | 40.6 ± 9.3 | *20 ± 0 | < 0.001 | 40.6 ± 6.4 | *20 ± 0 | < 0.001 | -20.6 ± 9.3 | -20.65 ± 6.4 | = 0.9 |

* All samples in the NaCl and Balsol® groups fell below the detectable range after washing, therefore the lower limit of the reference range was used for statistical analysis.




+ significant change $p < 0.05$ after washing within group.

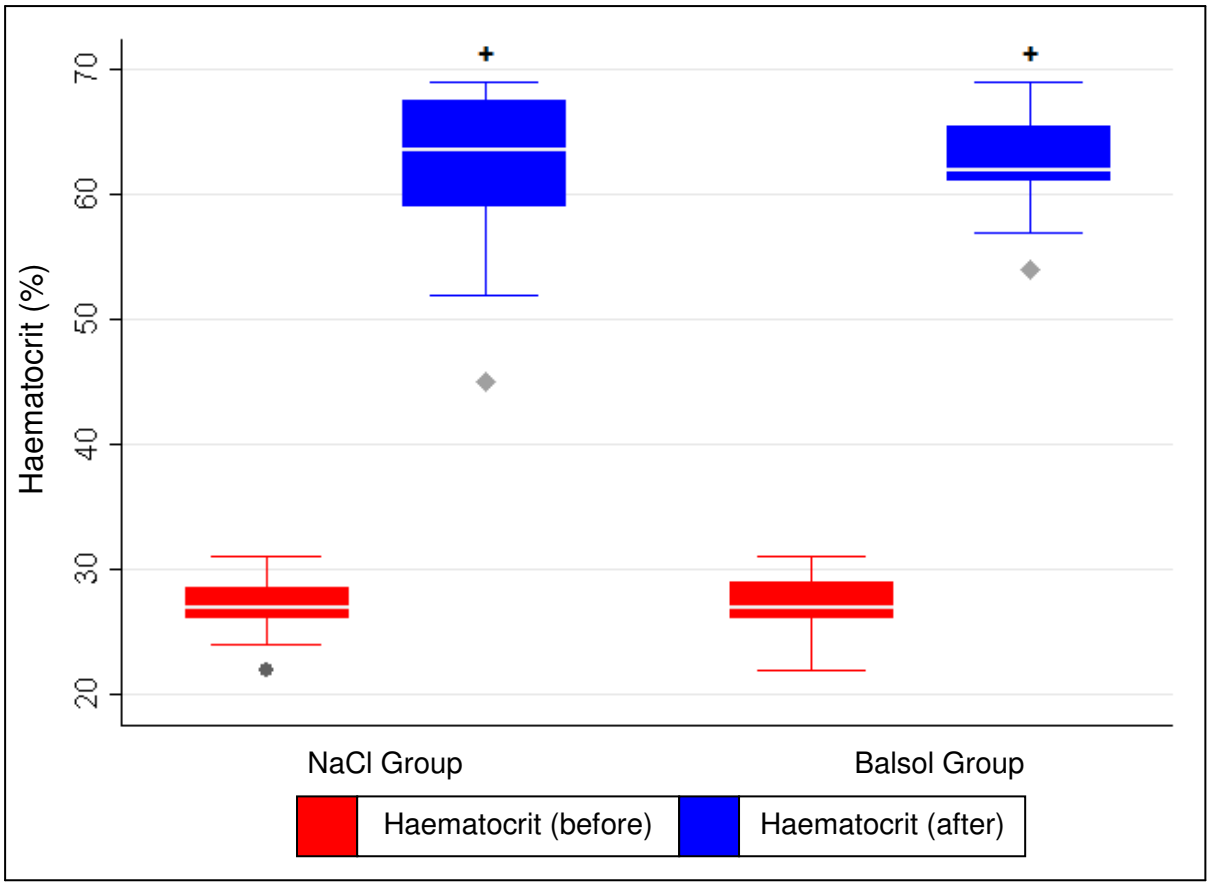
**Figure 21**: Box plot illustrating total protein levels before and after washing with NaCl and Balsol® solutions respectively.

### 4.18 Changes in haematocrit

There was a highly significant increase in haematocrit within the NaCl and Balsol® groups after washing ($p < 0.001$) (table 29). Figure 22 illustrates the changes in haematocrit before and after washing with NaCl and Balsol® solutions respectively.

**Table 29:** changes in haematocrit before and after washing with NaCl and Balsol® solutions.

| Variable | NaCl Group | | P Value | Balsol Group | | P Value | Post wash – Pre wash | | P Value |
|---|---|---|---|---|---|---|---|---|---|
| | Pre wash | Post wash | | Pre wash | Post wash | | Δ NaCl | Δ Balsol | |
| HCT | 27.05 ± 2.2 | 62.3 ± 6.1 | < 0.001 | 27.4 ± 2.2 | 62.6 ± 3.8 | < 0.001 | 35.3 ± 5.6 | 35.25 ± 4.7 | = 0.6 |




**+** significant change $p < 0.05$ after washing within group.

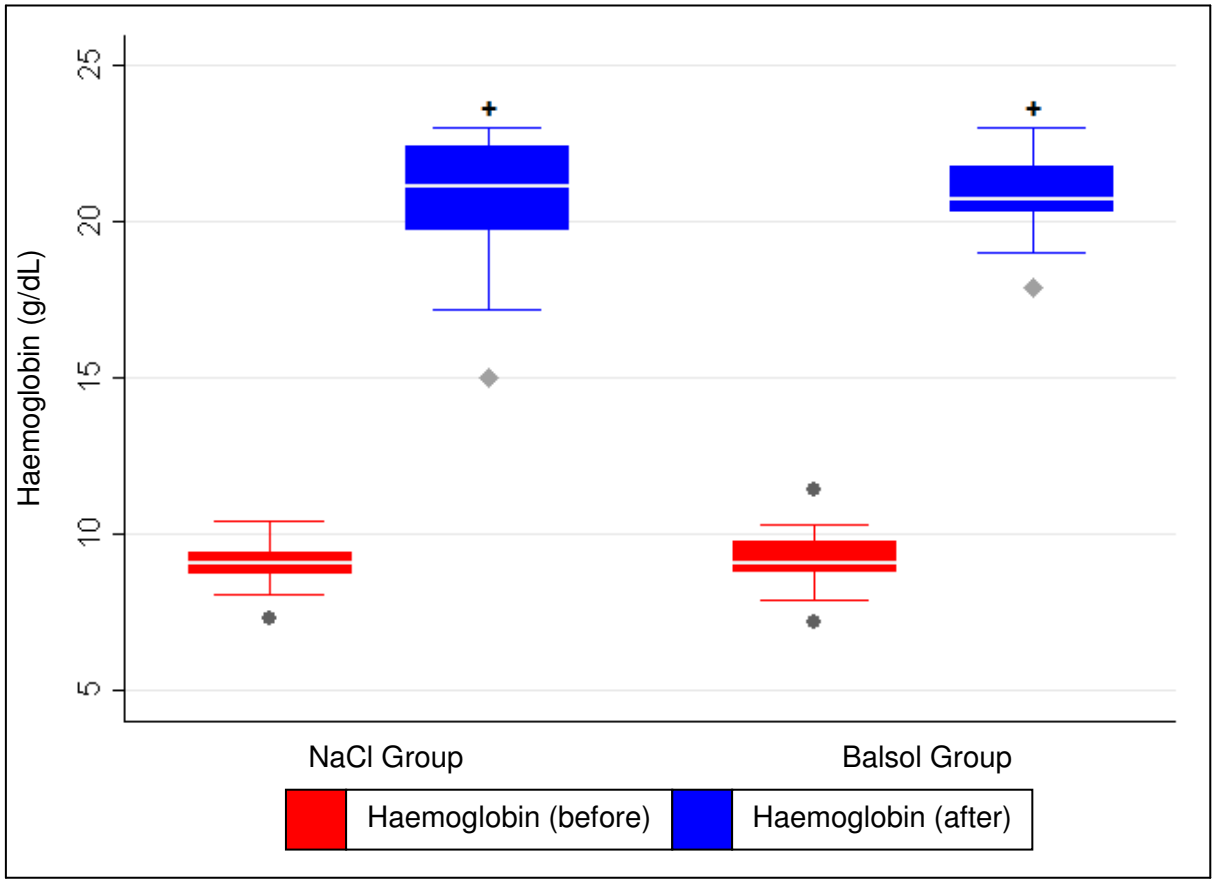
**Figure 22**: Box plot illustrating haematocrit levels before and after washing with NaCl and Balsol® solutions respectively.

### 4.19 Changes in haemoglobin

There was a highly significant increase in haemoglobin within the NaCl and Balsol® groups after washing ($p < 0.001$) (table 30). Figure 23 illustrates the changes in haemoglobin before and after washing with NaCl and Balsol® solutions respectively.

**Table 30:** changes in haemoglobin before and after washing with NaCl and Balsol® solution.

| Variable | NaCl Group | | P Value | Balsol Group | | P Value | Post wash – Pre wash | | P Value |
|---|---|---|---|---|---|---|---|---|---|
| | Pre wash | Post wash | | Pre wash | Post wash | | Δ NaCl | Δ Balsol | |
| Hb | 9.0 ± 0.7 | 20.7 ± 2 | < 0.001 | 9.2 ± 0.9 | 20.9 ± 1.3 | < 0.001 | 11.7 ± 1.9 | 11.66 ± 1.7 | = 0.6 |




**+** significant change $p < 0.05$ after washing within group.

**Figure 23**: Box plot illustrating haemoglobin levels before and after washing with NaCl and Balsol® solutions respectively.

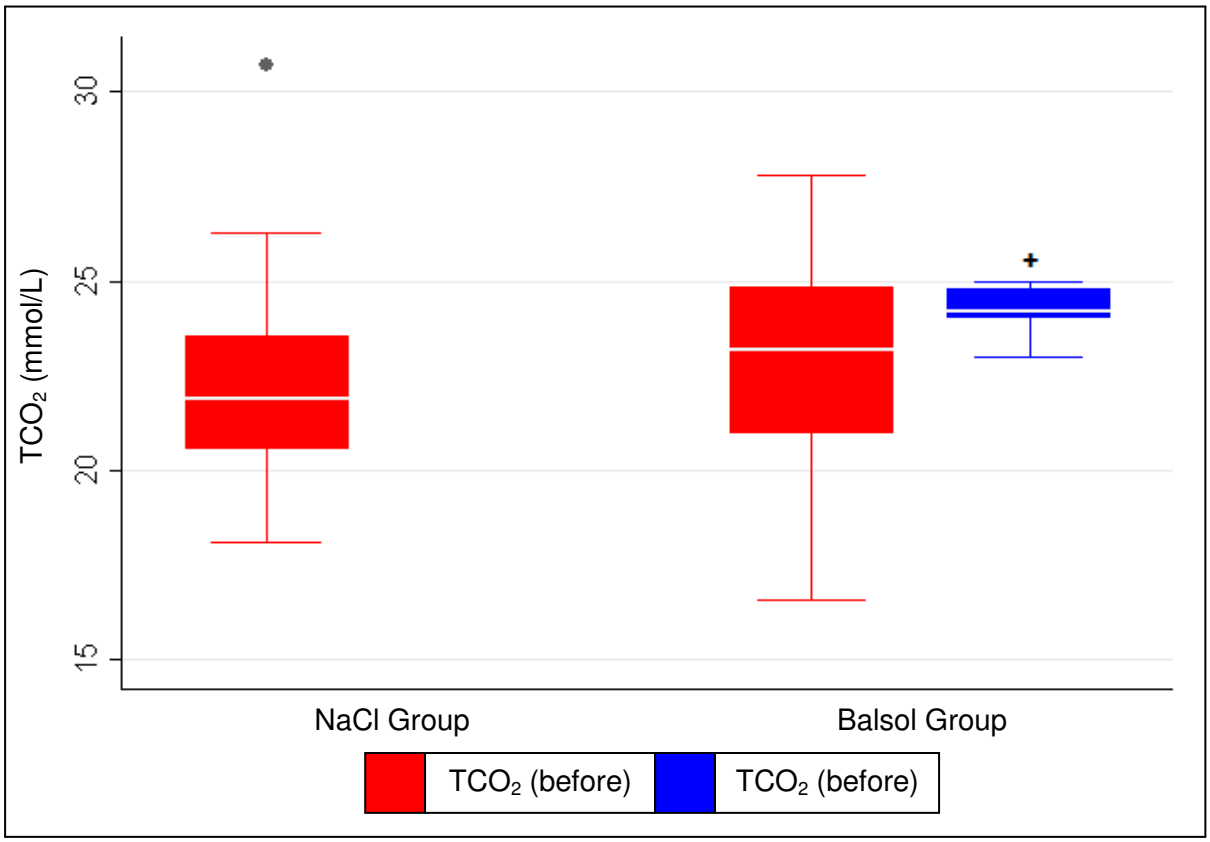
### 4.20 Changes in $TCO_2$

Post wash samples in the NaCl group were incalculable by blood gas analyser. There was a significant increase in $TCO_2$ within the Balsol® group after washing (p = 0.04) (table 31). Figure 24 illustrates the changes in $TCO_2$ before and after washing with NaCl and Balsol® solutions respectively.

**Table 31:** changes in $TCO_2$ before and after washing with NaCl and Balsol® solutions.

| Variable | NaCl Group | | P Value | Balsol Group | | P Value | Post wash – Pre wash | | P Value |
|---|---|---|---|---|---|---|---|---|---|
| | Pre wash | Post wash | | Pre wash | Post wash | | Δ NaCl | Δ Balsol | |
| $TCO_2$ | 22.4 ± 2.9 | * | NA | 22.9 ± 2.8 | 24.3 ± 0.5 | = 0.04 | * | 1.33 ± 2.7 | NA |

* - incalculable by blood gas analyser.
NA – not applicable.




+ significant change $p < 0.05$ after washing within group.

**Figure 24**: Box plot illustrating $TCO_2$ levels before and after washing with NaCl and Balsol® solutions respectively.

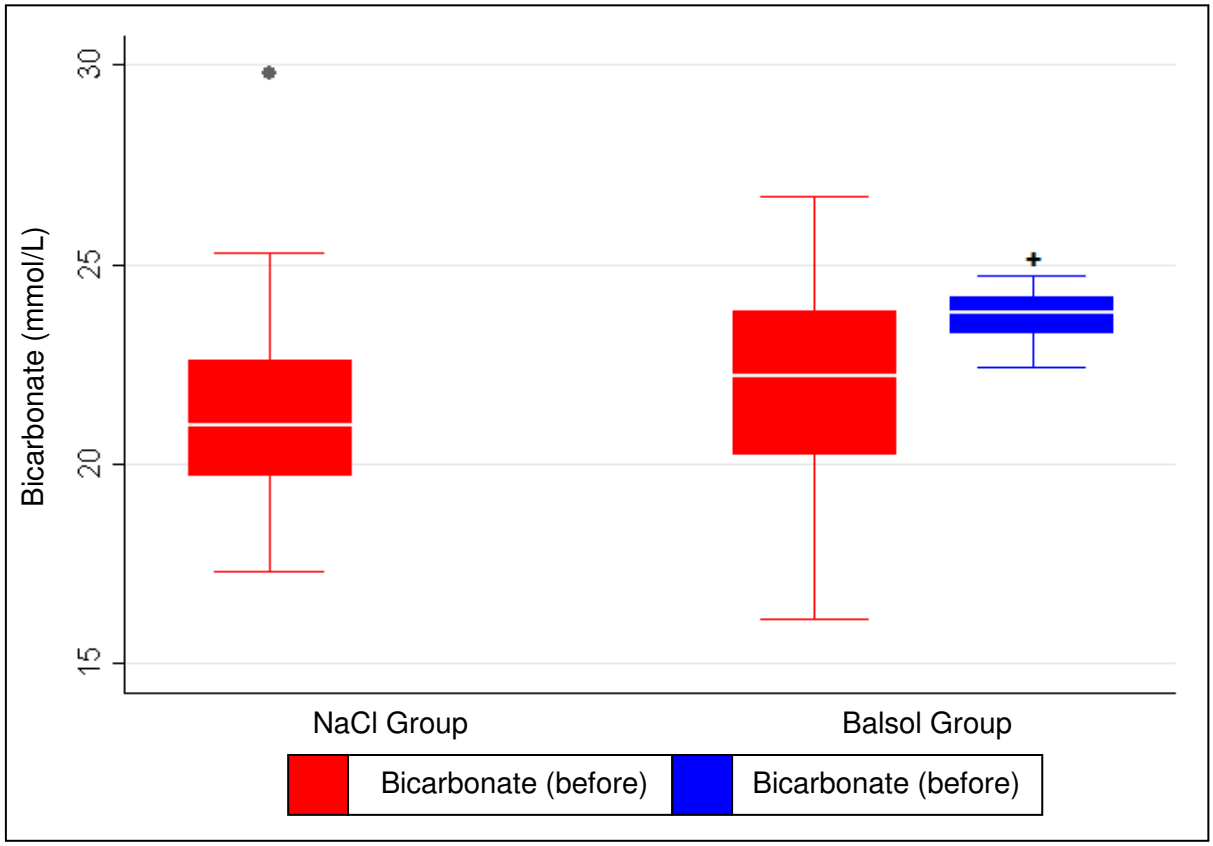
### 4.21 Changes in bicarbonate

Post wash samples in the NaCl group were incalculable by blood gas analyser. There was a significant increase in bicarbonate within the Balsol® group after washing (p = 0.01) (table 32). Figure 25 illustrates the changes in bicarbonate before and after washing with NaCl and Balsol® solutions respectively.

**Table 32:** changes in bicarbonate before and after washing with NaCl and Balsol® solutions.

| Variable | NaCl Group | | P Value | Balsol Group | | P Value | Post wash – Pre wash | | P Value |
|---|---|---|---|---|---|---|---|---|---|
| | Pre wash | Post wash | | Pre wash | Post wash | | Δ NaCl | Δ Balsol | |
| $HCO_3^-$ | 21.51 ± 2.8 | * | NA | 22 ± 2.7 | 23.7 ± 0.6 | = 0.01 | * | 1.68 ± 2.6 | NA |

* - incalculable by blood gas analyser.
NA – not applicable.




**+** significant change $p < 0.05$ after washing within group.

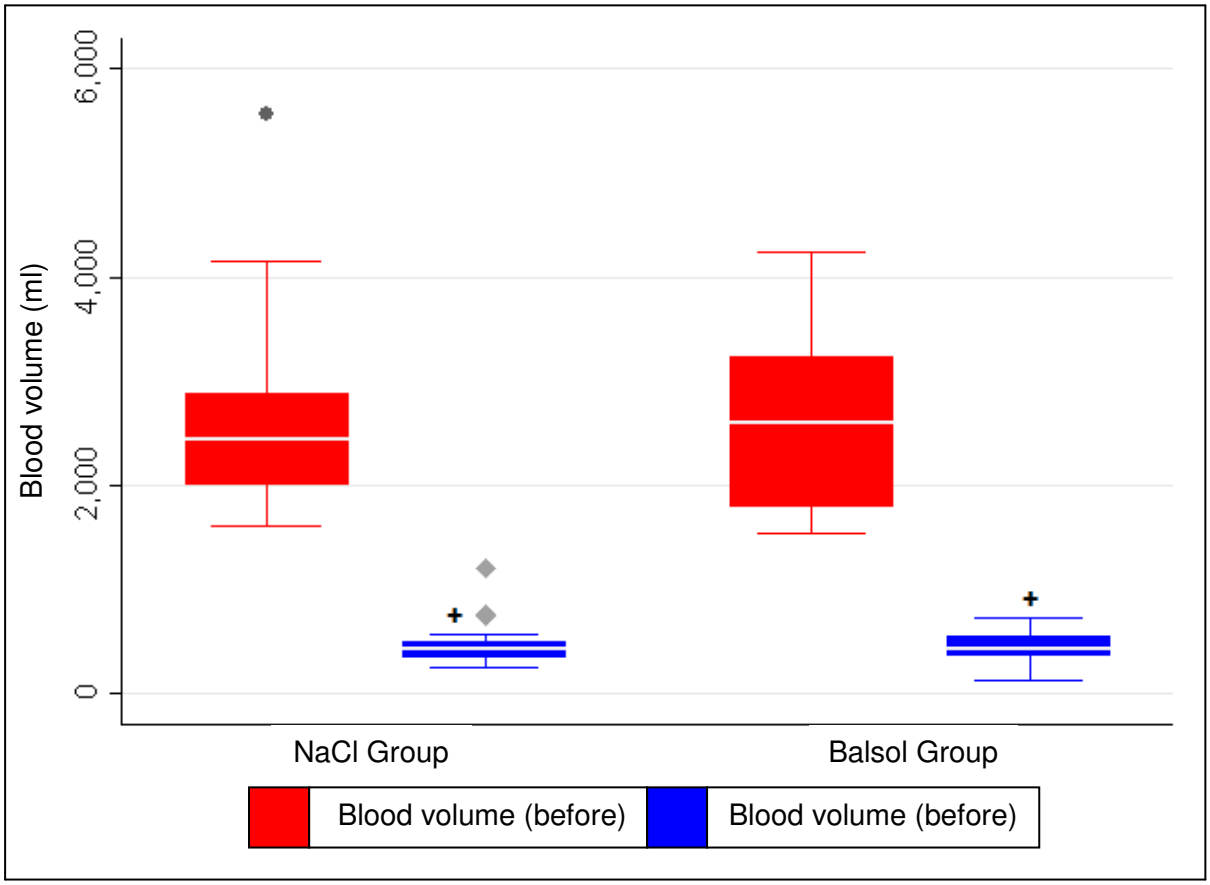
**Figure 25**: Box plot illustrating bicarbonate levels before and after washing with NaCl and Balsol® solutions respectively.

### 4.22 Changes in blood volume

There was a highly significant decrease in blood volume within the NaCl and Balsol® groups after washing ($p < 0.001$) (table 33). Figure 26 illustrates the changes in blood volume before and after washing with NaCl and Balsol® solutions respectively.

**Table 33:** changes in blood volume before and after washing with NaCl and Balsol® solution.

| Variable | NaCl Group | | P Value | Balsol Group | | P Value | Post wash – Pre wash | | P Value |
|---|---|---|---|---|---|---|---|---|---|
| | Pre wash | Post wash | | Pre wash | Post wash | | Δ NaCl | Δ Balsol | |
| Blood volume | 2620 ± 935.2 | 476.4 ± 222.1 | < 0.001 | 2612 ± 859.7 | 447.4 ± 153.9 | < 0.001 | -2144 ± 768.6 | -2165 ± 825.4 | = 0.9 |




**+** significant change $p < 0.05$ after washing within group.

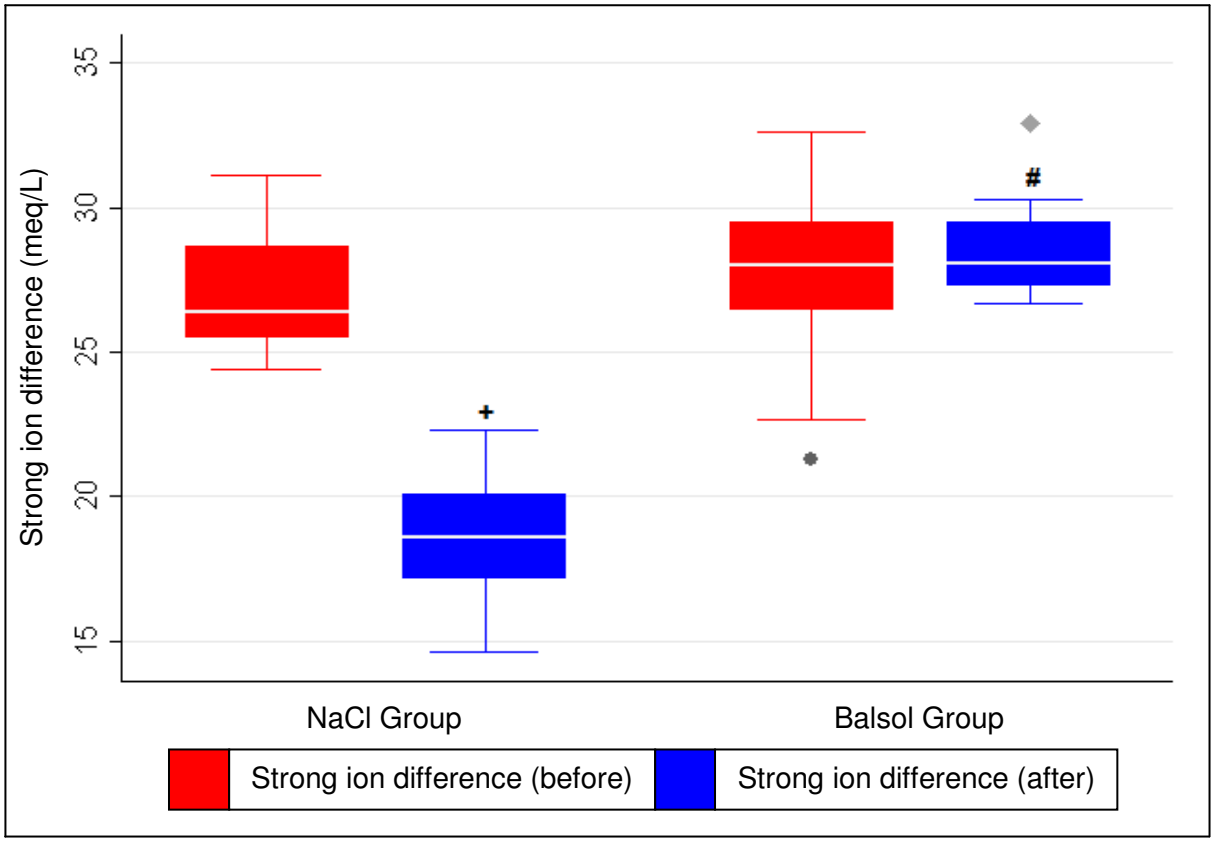
**Figure 26**: Box plot illustrating blood volume before and after washing with NaCl and Balsol® solutions respectively.

### 4.23 Changes in strong ion difference

There was a highly significant decrease in SID within the NaCl group after washing ($p < 0.001$). There was also a highly significant difference in the change between groups after washing ($p < 0.001$) (table 34). Figure 27 illustrates the changes in SID before and after washing with NaCl and Balsol® solutions respectively.

**Table 34:** changes in SID before and after washing with NaCl and Balsol® solutions.

| Variable | NaCl Group | | P Value | Balsol Group | | P Value | Post wash – Pre wash | | P Value |
|---|---|---|---|---|---|---|---|---|---|
| | Pre wash | Post wash | | Pre wash | Post wash | | Δ NaCl | Δ Balsol | |
| SID | 27.1 ± 2.1 | 18.4 ± 2.2 | < 0.001 | 27.6 ± 3.0 | 28.44 ± 1.5 | = 0.3 | -8.7 ± 3.1 | 0.84 ± 3.3 | < 0.001 |




**+** significant change $p < 0.05$ after washing within group.
**#** significant change $p < 0.05$ after washing between groups.

**Figure 27**: Box plot illustrating strong ion difference levels before and after washing with NaCl and Balsol® solutions respectively.

## 4.24 STRONG ION DIFFERENCE CALCULATIONS

### 4.24a NaCl control group

$$SID_{\text{pre wash}} = [\text{sum of strong cations}] - [\text{sum strong anions}]$$
$$= [(\text{sodium} + \text{potassium} + \text{calcium}) - (\text{chloride} + \text{lactate})]$$
$$= [(132.9 \pm 3.2) + (4.5 \pm 0.5) + (2.0 \pm 0.18) - (107.8 \pm 3.1) + (4.5 \pm 1.8)]$$
$$= 27.1 \pm 2.1$$

$$SID_{\text{post wash}} = [\text{sum of strong cations}] - [\text{sum strong anions}]$$
$$= [(\text{sodium} + \text{potassium} + \text{calcium}) - (\text{chloride} + \text{lactate})]$$
$$= [(146.3 \pm 1.9) + (1.0 \pm 0.7) + (0.2 \pm 0.06) - (127.4 \pm 2.1) + (1.7 \pm 0.6)]$$
$$= 18.4 \pm 2.2$$

$$\Delta SID = SID_{\text{post wash}} - SID_{\text{pre wash}}$$
$$= 18.5 \pm 2.2 - 27.1 \pm 2.1$$
$$= -8.7 \pm 3.1$$

### 4.24b Balsol® interventional group

$$SID_{\text{pre wash}} = [\text{sum of strong cations}] - [\text{sum strong anions}]$$
$$= [(\text{sodium} + \text{potassium} + \text{calcium}) - (\text{chloride} + \text{lactate})]$$
$$= [(134.7 \pm 2.2) + (4.2 \pm 0.4) + (1.8 \pm 0.2) - (108.8 \pm 2.7) + (4.3 \pm 1.8)]$$
$$= 27.6 \pm 3.0$$

$$SID_{\text{post wash}} = [\text{sum of strong cations}] - [\text{sum strong anions}]$$
$$= [(\text{sodium} + \text{potassium} + \text{calcium}) - (\text{chloride} + \text{lactate})]$$
$$= [(125.6 \pm 1.0) + (4.6 \pm 0.3) + (0.04 \pm 0.08) - (100.2 \pm 1.4) + (1.6 \pm 0.4)]$$
$$= 28.44 \pm 1.5$$

$$\Delta SID = SID_{\text{post wash}} - SID_{\text{pre wash}}$$
$$= 28.44 \pm 1.5 - 27.6 \pm 3.0$$
$$= 0.84 \pm 3.3$$

# CHAPTER FIVE: DISCUSSION

## 5.1 INTRODUCTION

Allogeneic blood is a scarce and life saving resource, but its prescription is also not without risk. Stored red blood cells undergo time-dependant metabolic, biochemical, and molecular changes which eventually result in irreversible damage, defined as “storage lesions” responsible for many adverse effects of RBC transfusions (Offner, 2004). Allogeneic blood transfusions have been associated with febrile, anaphylactic, and haemolytic transfusion reactions, transfusion related acute lung injury, and although rare, risk of viral, bacterial, and parasitic infections as well (Kuppurao and Wee, 2010).The introduction of autologous cell salvage systems to cardiac surgery has decreased the demand for the scarce and precious allogeneic blood resource, as well as its associated risks. There is also evidence that merely centrifuging residual CPB pump blood without washing can improve clinical outcomes by reducing the rate of infective complications, length of hospital stay (Sirvinskas et al, 2005), and reduced postoperative cognitive dysfunction (Djaiani et al., 2007). Manufacturers of autologous cell salvage systems recommend the use of normal saline for the processing of salvaged blood. A study conducted by Shaw et al. (1997), revealed that patients receiving exclusively normal saline on the day of surgery had higher mortality, major complications, postoperative infections, renal failure requiring dialysis, blood transfusions, longer ventilation time, and more electrolyte disturbances than those receiving balanced crystalloids. Healthy volunteers receiving 0.9% saline infusions showed decreased urine output (Chowdhury et al., 2012; Williams et al., 1999). Patients undergoing cardiac surgery with CPB are also at higher risk for electrolyte depletion (Polderman and Girbes, 2004). Therefore, the use of normal saline as a wash solution for autologous cell salvage systems requires further investigation.

In this prospective investigation in a series of 40 patients undergoing elective cardiac surgery with CPB, the first 20 patients were allocated to the NaCl control group and the second 20 were allocated to the Balsol® interventional group. Residual blood from the CPB circuit was processed with either 0.9% NaCl or Balsol® solution. The primary objective was to measure and compare the pH, electrolytes, metabolites, osmolality and SID of residual pump blood to the, pH, electrolytes, metabolites, osmolality and SID of processed cell saver blood. The secondary objective was to measure and compare protein levels (albumin and

total protein) in residual pump blood to protein levels in processed cell saver blood. The final objective was to determine the volume, haematocrit and haemoglobin yield post cell saver processing, from the input volume of residual pump blood.

## 5.2 DICSUSSION

In this study there was a significant increase in pH within the NaCl and Balsol® groups after washing ($p < 0.001$) (table 15) (figure 8), and no significance difference between groups. In a study conducted by de Vroege et al. (2007), 20 RBC units with a mean age of 36 days were washed with 0.9% NaCl and there was no change in pH (6.36 ± 0.05). In an animal study conducted by Halpern et al. (1996), washing blood with 0.9% NaCl and re-infusing blood after every wash cycle resulted in a significant decrease in pH. Halpern et al. (1997), conducted a similar animal study to compare washing blood with either 0.9% normal saline or isolyte S and re-infused washed blood after every wash cycle. They found that the pH increased with 0.9% NaCl by 0.3% in the mean averaged washed blood when compared to the mean prewashed systemic blood. In study conducted by Huber et al. (2013), ten units of PRBC with a mean age of 9 days were washed with either 0.9% NaCl or BB-HS. The results revealed a significant decrease and increase in pH from baseline after washing with 0.9% NaCl and BB-HS respectively, with pH being significantly higher at the end of washing with BB-HS. In the present study the contrasting increase in pH in the NaCl group may be as a result of the 0.9% NaCl wash solution depleting $TCO_2$. The result is a shift of bicarbonate and hydrogen ions to carbonic acid and carbon dioxide and water within the bicarbonate buffering system in each wash cycle (Kacmarek, Stoller and Heuer, 2014). The increase in pH in the Balsol® group is a result of the Balsol® solution already containing 27mmol/L of bicarbonate ions, thus shifting the bicarbonate and hydrogen ions to carbonic acid and carbon dioxide and water within the bicarbonate buffering system in each wash cycle (Kacmarek, Stoller and Heuer, 2014).

The infusion of 0.9% NaCl and the associated acidosis has also been explained as a dilutional acidosis of plasma bicarbonate by large volume infusions of non bicarbonate solutions (Morgan and Venkatesh 2003). In the present study there was a highly significant decrease in $pCO_2$ within the NaCl group ($p < 0.001$) below the detectable range of the electrode (< 6mmHg), and a less pronounced but significant decrease in $pCO_2$ within the Balsol® group ($p < 0.001$) after washing. The change between the NaCl and Balsol® groups revealed that $pCO_2$ in the NaCl group significantly decreased twice as much when compared

to the Balsol® group ($p < 0.001$) (table 16) (figure 9). Total carbon dixide and bicarbonate were incalculable by the blood gas analyser within the NaCl group. This was probably due to the high rate of depletion of bicarbonate by the non bicarbonate 0.9% NaCl solution. In contrast there was a significant increase in $TCO_2$ ($p < 0.04$) (table 31) (figure 24) and bicarbonate ($p < 0.01$) (table 32) (figure 25) within the Balsol® group after washing, due to Balsol® solution containing 27mmol/L of bicarbonate. This may also explain the maintenance of $pCO_2$ in the Balsol® group resulting in a shift of bicarbonate and hydrogen ions to carbonic acid and carbon dioxide and water within the bicarbonate buffering system after each wash cycle (Kacmarek, Stoller and Heuer, 2014).

After washing PRBC with 0.9% NaCl, de Vroege et al. (2007), observed a significant decrease of ≈ 90% in both $pCO_2$ and bicarbonate. Huber et al. (2013), observed a significant decrease (≈ 80%) and increase (≈ 70%) in $pCO_2$ from baseline after washing PRBC with 0.9% NaCl and BB-HS respectively, with $pCO_2$ being significantly higher at the end of washing with BB-HS. Huber et al. (2013), also revealed a significant decrease (≈ 80%) in bicarbonate from baseline after washing with 0.9% NaCl and a less pronounced but significant decrease (≈ 4%) in bicarbonate after washing BB-HS, with bicarbonate being significantly higher at the end of washing with BB-HS. The increase in $pCO_2$ and higher bicarbonate with Huber and colleagues can be attributed to BB-HS containing 35mmol/L of bicarbonate. Huber et al. (2013), also demonstrated that BE significantly improved from baseline after washing with BB-HS as compared to 0.9% NaCl. In an animal study Halpern et al. (1996), observed a significant decrease of ≈ 70% in $pCO_2$ and bicarbonate between mean baseline systemic values and mean averaged washed blood values when 0.9% NaCl was used as a wash solution. Halpern et al. (1997), also demonstrated that when compared to washing with normal saline, isolyte S resulted in significantly higher bicarbonate levels in systemic averaged prewashed blood.

There was a highly significant decrease in $pO_2$ within the NaCl and Balsol® groups after washing ($p < 0.0001$), and a significant difference in the change between groups after washing ($p = 0.03$) (table 17) (figure 10). In contrast after washing PRBC with 0.9% NaCl de Vroege et al. (2007), reported a non significant increase in $pO_2$. Huber et al. (2013), reported a significant increase in $pO_2$ at the end of washing with 0.9% NaCl and a significant but marginal increase after washing with BB-HS, with the increase being significantly greater with 0.9% NaCl. This increase in $pO_2$ with saline washing that Huber and co-workers

reported can possibly be due to the Bohr effect, in which a decrease in pH decreases haemoglobins affinity for oxygen. In the present study, a high $pO_2$ greater than 500mmHg was expected on termination of CPB for both control and interventional groups. The decrease in $pO_2$ is possibly due to oxygen diffusing into the atmosphere during the collection and washing process, because the ACSS is open to the atmosphere.

In a study conducted by Polderman and Girbes, (2004), it was revealed that 34% patients requiring CPB developed a significant moderate hypokalaemia (potassium < 3.6 mmol/L, $p < 0.001$) despite receiving on average 16mmol/L of potassium from cardiopleagia and potassium infusion supplementation (10.1 ± 4.7 mmol/hr) during surgery. The present study reported a highly significant decrease in $[K^+]$ within the NaCl group after washing ($p < 0.001$), and a highly significant increase in $[K^+]$ within the Balsol® group after washing ($p < 0.0007$). There was also a highly significant difference in the change between groups after washing ($p < 0.001$) (table 18) (figure 11). Varghese et al. (2007), and de Vroege et al. (2007), both reported that potassium was significantly reduced when washing PRBC with 0.9% NaCl. Huber et al. (2013), also reported significantly reduced potassium with both 0.9% NaCl and BB-HS. Swindell et al. (2007), demonstrated that washing irradiated PRBC resulted in CPB circuits for neonates and infants having lower potassium loads than circuits that received unwashed blood. Immediately after CPB serum potassium was significantly lower in the washed blood group compared to the unwashed group. Swindell and co-workers also reported washing blood resulted in 4 patients becoming hypokaelemic during CPB, but commented that it was easier to manage than hyperkalaemia.

The animal study conducted by Halpern et al. (1996), demonstrated that continuous washing with 0.9% NaCl and re-infusion of blood resulted in a significant decrease in mean washed blood average potassium as compared to baseline systemic values. Halpern et al. (1997), also demonstrated a significantly higher potassium level in the mean washed blood average when isolyte S was used as a wash solution as opposed to 0.9% NaCl. A case report by Knichwitz et al. (2002), demonstrates that in an *in vivo* setting the use of 0.9% NaCl as a wash solution is very useful in terms of $K^+$ ion elimination in patients with end stage renal failure or patients requiring massive transfusion, thus preventing hyperkalaemia. Sohn et al., (2012) also confirmed in an *in vivo* setting that the use of CS with 0.9% NaCl as a wash solution is useful in preventing ECG abnormality or cardiac arrest due to massive transfusion related hyperkalaemia. In contrast Peng et al. (2006), reported that in washing massive amounts of exsaguinated blood with 0.9% NaCl and immediate transfusion thereafter produced an iatrogenic dilutional hypokalaemia.

The results of the present study confirm the results of several other investigators, and demonstrates that washing residual pump blood in the CPB circuit with 0.9% NaCl depletes potassium concentrations in CS blood (Halpern et al., 1996; Knichwitz et al., 2002; Sohn et al., 2012; Peng et al., 2006), and washing blood with a balanced electrolyte solution results in potassium in CS blood being relatively unchanged (Halpern et al., 1997).

In previous investigations by Varghese et al. (2007), de Vroege et al. (2007), Huber et al. (2013), and Swindell et al. (2007), all reported significant increases in sodium after washing PRBC with 0.9% NaCl solution. The animal study conducted by Halpern et al. (1996), demonstrated that continuous washing with 0.9% NaCl and re-infusion of blood resulted in a significant increase in mean washed blood average sodium as compared to baseline systemic values. Halpern et al. (1997), also demonstrated a significantly higher sodium level in the mean washed blood average when 0.9% NaCl was used as a wash solution compared to isolyte S.

The results of the present study report a highly significant increase in sodium within the NaCl group after washing ($p < 0.001$), and a highly significant decrease in sodium within the Balsol® group after washing ($p < 0.001$). There was also a highly significant difference in the change between groups after washing ($p < 0.001$) (table 19) (figure 12). These observations support the results of previous investigators demonstrating that 0.9% NaCl increases sodium levels, whereas balanced solutions like Balsol® and isolyte S decrease sodium levels.

The infusion of 0.9% NaCl into healthy volunteers is reported to result in a significant decrease in pH (Williams et al., 1999), and an elevated chloride concentrations and a significant fall in SID (Reid et al., 2003; Chowdhury et al., 2012) indicating an hyperchloraemic acidemia. Volunteers were also reported to have a significantly longer time to first micturition (Williams et al., 1999; Chowdhury et al., 2012) and a significantly decreased urine output (Reid et al., 2003; Chowdhury et al., 2012). Significant extravascular fluid retention (Chowdhury et al., 2012) and increased weight (Reid et al., 2003; Chowdhury et al., 2012) were also reported. Magnetic resonance imaging in healthy volunteers revealed infusion of 0.9 % saline results in significant reductions in renal blood flow velocities and renal cortical tissue perfusion (Chowdhury et al., 2012). Hadimioglu et al. (2008), conducted a randomized double blind study to investigate the function of different crystalloid solutions during kidney transplantation to determine the ideal fluid. The volume of fluid for each study group was 2868 ± 780ml, 2770 ± 820ml, and 2756 ± 800ml for saline, RL, and plasmalyte A

groups, respectively. The results revealed that there was a significant decrease in pH, base excess, and bicarbonate respectively in the saline group. There was also a significant increase in serum chloride, and hyperchloraemia took a week to return to physiologic levels. No significant changes occurred in the RL or plasmalyte A groups. A literature review revealed that patients undergoing major surgery with a lower chloride load had greater urine output, and in a separate study patients had significantly lower concentrations of kidney specific markers of injury (Yunos et al., 2010). A hyperchloraemic acidosis may also result in a secondary extracellular shift of potassium, increasing the prevalence of hyperkalaemia (Yunos et al., 2010).

The results in the present study revealed a highly significant increase in [$Cl^-$] within the NaCl group after washing ($p < 0.001$), and a highly significant decrease in [$Cl^-$] within the Balsol® group after washing ($p < 0.001$). There was also a highly significant difference in the change between groups after washing ($p < 0.001$) (table 20) (figure 13). Huber et al. (2013), reported after washing PRBC with 0.9% NaCl and BB-HS there was a significant increase and decrease in [$Cl^-$] from baseline respectively, with [$Cl^-$] being significantly lower after washing with BB-HS. The animal study conducted by Halpern et al. (1997), also demonstrated that after washing with 0.9% NaCl and isolyte S, [$Cl^-$] increased and decreased respectively in mean washed blood average, with [$Cl^-$] being significantly higher in the 0.9% NaCl group. Halpern et al. (1996), also reported that continuous washing with 0.9% NaCl and re-infusion of blood resulted in a significant increase in mean washed blood average chloride as compared to baseline systemic values. The results of the present study also demonstrated that washing blood with 0.9% NaCl and Balsol® solution significantly increased and decreased [$Cl^-$] respectively. This highlights the clinical relevance of a balanced electrolyte solution for washing salvaged blood to curtail hyperchloraemia and ameliorate its deleterious effects.

A study was conducted by Wilkes et al. (2002), in which 85 patients underwent CABG with intermittent cross clamping being employed and with no cardioplegia being administered. The results revealed that total hypomagnesemia was observed in 53% of all patients and was more common than ionized hypomagnesemia that was observed in 11% of all patients. In the control group ($Mg^{2+}$ restricted) mean preoperative total magnesium levels decreased significantly during CPB and all patients in this group were observed to have total hypomagnesemia immediately after CPB. Morgan et al. (2008), reported that patients who received a magnesium free priming solution requiring CPB had significantly lower total

magnesium just prior to termination of CPB, despite the cardioplegia solution containing 80 meq/L magnesium.

In a prospective controlled study conducted by Polderman and Girbes, (2004), 500 consecutive CPB patients and 250 major surgical patients were enrolled. The results revealed that patients treated with magnesium (average amount 2.1 g) during surgery for brief arrhythmias was 76% in the CPB group versus 2% in the major surgical group ($p < 0.001$). The remaining patients in the CPB group who did not receive magnesium, 80% had levels below 0.70mmol/L vs. 1% in the major surgical group ($p < 0.001$). Urinary electrolyte excretion rate of magnesium during surgery was significantly greater in the CPB patients. Magnesium was significantly reduced after washing with 0.9% NaCl (Halpern et al., 1996). After washing with 0.9% NaCl and isolyte S, magnesium decreased and increased respectively, in the washed blood average and was significantly higher with isolyte S (Halpern et al., 1997).

The present study reported a highly significant decrease in total magnesium within the NaCl group ($p < 0.001$), with all the samples falling below the detectable range of the electrode (< 0.29mmol/L) after washing. There was no significant change in the Balsol® group after washing but a highly significant difference in the change between groups after washing ($p < 0.001$) (table 21) (figure 14). The results of this study highlight the clinical relevance of a balanced electrolyte wash solutions for maintaining magnesium levels in processed residual pump blood.

Both 0.9% NaCl and Balsol® solutions used in the present study are calcium and phosphate free. The results of the present study revealed there was a highly significant decrease in ionized [$Ca^{2+}$] within the NaCl and Balsol® groups after washing ($p < 0.001$), and a significant difference in the change between groups after washing ($p = 0.004$) (table 22) (figure 15). After washing 12 and 16 samples in the NaCl and Balsol® groups respectively fell below the detectable range of the electrode (< 0.10mmol/L). There was a highly significant decrease in [$PO_4^{3-}$] within the NaCl and Balsol® groups after washing ($p < 0.001$) (table 23) (figure 16). Varghese et al. (2007), observed a significant reduction in phosphate concentration after washing outdated PRBC with 0.9% NaCl. Animal studies conducted by Halpern et al. (1996), demonstrated that washing with 0.9% NaCl resulted in significant reductions in ionized [$Ca^{2+}$] and [$PO_4^{3-}$].

Halpern et al. (1997), also reported in an animal study after washing with 0.9% NaCl and isolyte S, ionized calcium decreased in the washed blood average with no significant difference between solutions. Inorganic phosphorous also decreased in the washed blood average, with the decrease being significantly greater in the 0.9% NaCl group, this is because isolyte S solution contains 1 mEq/L phosphate. Huber et al. (2013), reported after washing PRBC with 0.9% NaCl and BB-HS that calcium significantly increased from baseline with both solutions. The increase being significantly greater with BB-HS. Polderman and Girbes, (2004) reported that 7.8% of patients requiring CPB presented with hypocalcaemia versus 5.6% undergoing major surgery ($p < 0.001$), and 83% of patients requiring CPB presented with hypophosphataemia versus 12% undergoing major surgery ($p < 0.001$). Urinary electrolyte excretion rate of [$Ca^{2+}$] and [$PO_4^{3-}$] during surgery was significantly greater in patients requiring CPB.

In the present study there was a highly significant decrease in lactate within the NaCl and Balsol® groups after washing ($p < 0.001$) (table 24) (figure 17). Halpern et al. (1996), de Vroege et al. (2007), Huber et al. (2013), and Swindell et al. (2007), also observed a similar significant decrease in the lactate load after washing PRBC with 0.9% NaCl. After washing PRBC with 0.9% NaCl Varghese et al. (2007), reported a significant decrease in LDH, while Huber et al. (2013), reported a non significant increase in LDH after washing with 0.9% NaCl and a significant decrease in LDH after washing with BB-HS.

The results of the present study reveal a highly significant decrease in glucose within the NaCl and Balsol® groups after washing ($p < 0.001$) (table 25) (figure 18). Varghese et al. (2007), and de Vroege et al. (2007), also observed similar significant decreases after washing PRBC with 0.9% NaCl. Huber et al. (2013), reported a significant decrease in glucose from baseline after washing with PRBC with 0.9% NaCl and BB-HS, with glucose being significantly higher after washing with BB-HS. The energy source for erythrocytes is glucose, and glycolysis is anaerobic producing 2 ATPs and lactic acid (Rudmann, 2005). Huber et al. (2013), reported significantly higher ATP levels when washing PRBC with BB-HS. Phosphate is required for the formation of ATP, and the present and previous studies have demonstrated that phosphate is significantly decreased after washing salvaged blood with 0.9% NaCl (Halpern et al., 1996; Halpern et al., 1997) and in patients requiring CPB (Polderman and Girbes, 2004). Adenosine triphosphate is necessary to maintain RBC $Na^+/K^+$ ATPase membrane pump function, RBC-plasma lipid exchange, Hb function, RBC integrity and deformability (Rudmann, 2005). Washed RBC are re-suspended in a milieu that

is significantly depleted of phosphate and glucose which can impede its proper function, it would be ideal to use wash solutions that do not deplete electrolytes and glucose required for optimal RBC function. It would also be an advantage if processed blood is not administered immediately and stored temporarily.

Serum osmolality is a determinant of brain water content, and a low serum osmolality may contribute to cerebral oedema (Williams et al., 1999). In the present study 0.9% NaCl and Balsol® solution have an osmolality of 308mOsm/L and 273mOsm/L respectively. In this investigation there was a significant increase in osmolality within the NaCl group after washing ($p < 0.01$), and highly significant decrease in osmolality within the Balsol® group after washing ($p < 0.009$), which was equal to the osmolality of Balsol® solution. There was also a highly significant difference in the change between groups after washing ($p < 0.001$) (table 26) (figure 19).

An investigation with healthy volunteers conducted by Williams et al. (1999), reported a significant decrease in serum osmolality with RL infusion. The time to first micturition was significantly shorter in healthy volunteers receiving RL (Williams et al., 1999), Hartmann's solution (Reid et al., 2003) and plasmalyte 148 (Chowdhury et al., 2012) compared to those that received 0.9% NaCl. Infusions that decreased serum osmolality results in decreased secretion of antidiuretic hormone (ADH), thus increasing urine output in order to return serum osmolality to normal (Williams et al., 1999; Reid et al., 2003; Chowdhury et al., 2012). No significant changes in osmolality were reported with 0.9% NaCl by Reid et al. (2003), and Chowdhury et al. (2012), during the course of infusion when compared to Hartmann's and plasmalyte 148 solution respectively, which may support the evidence that hyperchloraemia has deleterious effects on glomerular filtration rate. In contrast Varghese et al. (2007), observed a significant decrease in osmolality after washing outdated PRBC with 0.9% NaCl.

The present investigation reported a highly significant decrease in albumin within the NaCl and Balsol® groups after washing ($p < 0.001$) (table 27) (figure 20). All samples in the NaCl and Balsol® groups fell below the detectable range of the electrode (< 10g/L) after washing. There was also a highly significant decrease in total protein within the NaCl and Balsol® groups after washing ($p < 0.001$) (table 28) (figure 21). All samples measuring total protein in the NaCl and Balsol® groups fell below the detectable range of the electrode (< 20g/L) after washing. Varghese et al. (2007), demonstrated that washing PRBC with 0.9% NaCl significantly reduced protein concentration. In an animal study the investigators also reported

a significant decrease (≈ 80%) in both albumin and total protein after washing with 0.9% NaCl (Halpern et al., 1996), and confirmed similar decreases in a second animal study (Halpern et al., 1997). Naumenko et al. (2008) washed residual CPB blood at two different centrifuge speeds with three different wash pump speeds, and reported that protein concentration was significantly reduced both with centrifuge speeds and all wash pump speeds. The results of the present study are consistent with the observations of previous investigators (Halpern et al., 1996; Halpern et al., 1997; Varghese et al., 2007; Naumenko et al., 2008).

The present study reported a highly significant increase in haematocrit within the NaCl and Balsol® groups after washing ($p < 0.001$) (table 29) (figure 22). There was also a highly significant increase in haemoglobin within the NaCl and Balsol® groups after washing ($p < 0.001$) (table 30) (figure 23). The observations of a significant increase in HCT and Hb after processing residual pump blood in this investigation are consistent with reports by Amand et al. (2005), and Naumenko et al. (2008). Sirvinskas et al. (2005), also reported that centrifuging residual CPB pump blood without washing can significantly reduce the number of donor blood transfusions, decrease the rate of infective complications and decrease the length of hospital stay. There was a highly significant decrease in blood volume (≈ 81%) within the NaCl and Balsol® groups after washing ($p < 0.001$) (table 33) (figure 26).

After termination of CPB the SID may vary significantly according to the type of priming and maintenance solution used, the constituents of cardioplegia administered, and the factors that dictate Gibbs-Donnan equilibrium. A physicochemical model developed by Omron and Omron (2009), demonstrated that for a crystalloid infusion to result in a neutral metabolic acid-base status it must have a SID of 24.5mEq/L or the equivalent pre-infusion actual bicarbonate concentration. In an *in vitro* investigation Morgan, Venkatesh and Hall (2002), demonstrated after serial dilutions of whole blood with crystalloids ranging in SID from - 4mEq/L to 40mEq/L, plasma SID and whole BE were linearly related to diluents crystalloid SID. Linear regression analysis revealed a crystalloid diluent SID of 23.7mEq/L resulted in neutral acid-base status. Morgan, Venkatesh and Hall (2004), confirmed in an animal model that a crystalloid diluents SID of 24.4mEq/L resulted in neutral acid base status. These investigators have demonstrated that infusion of a crystalloid solution with a SID of

< 24mEq/L or > 24mEq/L will result in a metabolic acidosis or alkalosis respectively if pH = 7.40, pCO2 = 40 mm Hg.

The present study reported a highly significant decrease in SID within the NaCl group after washing ($p < 0.001$), and there was no significant change in SID after washing in the Balsol® group. There was also a highly significant difference in the change between groups after washing ($p < 0.001$) (table 34) (figure 27). In this investigation the CPB circuit was primed with Balsol® solution and was also used as the only replacement solution used during CPB. Washing haemodiluted residual pump with the same solution resulted in a re-suspended RBC concentrate having significantly reduced levels of calcium and inorganic phosphorous, but, with a SID equivalent to that of residual pump blood. This was in contrast to the significantly reduced SID after washing blood with 0.9% NaCl. It may be also tempting to speculate that the SID of the re-suspended RBC concentrate which is slightly higher than 24mEq/L would have a slightly alkalinizing effect, that would combat a metabolic acidosis on infusion. Further prospective investigations will be required to test this hypothesis.

# CHAPTER SIX: CONCLUSION

The results of this prospective *in vitro* study demonstrate that there was a highly significant difference in the variables measured within and between the control and interventional groups when washing residual pump blood with 0.9% NaCl or Balsol® solution respectively. In the interventional group osmolality and essential electrolytes such as potassium, sodium, chloride and total magnesium were more reflective of the constituents of the Balsol® solution after washing, when compared to the control group. Ionized calcium and inorganic phosphate were virtually completely washed out in both control and interventional groups due to both solutions not containing them. Lactate, glucose, albumin and total protein were all significantly reduced after washing in both arms of the study and is consistent with previous studies.

Although pH was significantly increased in both control and interventional groups, the mechanism for the change in pH is highly reflective of 0.9% NaCl being a non bicarbonate solution and Balsol® being a bicarbonate solution. The calculation of SID is also a useful indicator in the prediction of the role of electrolytes on acid-base changes (Omron and Omron, 2009), and will also help design better wash solutions for salvaged blood (Morgan and Venkatesh, 2003). Centrifuging and washing haemodilute residual pump blood results in RBC being concentrated, however, the concentrated RBC are re-suspended in a solution that is very reflective of the wash solution used. In order for RBC to remain as effective and viable as they were *in situ*, the wash solution should replicate normal blood osmolality and plasma electrolyte profile.

This investigation concludes that the balanced electrolyte solution Balsol® used for washing residual CPB blood results in a re-suspended RBC concentrate with an osmolality and electrolyte profile that is superior compared to washing blood with 0.9% NaCl solution.

## LIMITATIONS

Firstly, processed blood was not transfused immediately to the patients and no *in vivo* observations could be made in terms of acid-base, osmolality and electrolyte changes. Secondly, the re-suspended RBC concentrate may have also undergone time dependant changes in the interim between being processed and re-transfusion, which were not investigated. Thirdly, other important variables like osmotic fragility, free Hb and ATP levels

were not measured due to financial and logistical constraints. Finally, ionized magnesium was not available at the research laboratory for measurement and was therefore excluded from the SID calculation.

## STRENGTHS

The strength of this study lay in the use of the pump prime solution also being also used to process residual pump blood, thus ensuring the continuity of the use of a single balanced electrolyte solution. Applying Peter Stewart's SID formula and concept also allowed the investigation unique insight into comparing residual pump blood with processed cell saver blood.

## RECOMMENDATIONS

From the results of this of this investigation it is recommended that other balanced electrolyte solutions employed with cell saving machines for processing residual pump blood in cardiac surgery be further researched. It is recommended that further investigations be conducted to address the limitations found in this investigation. It is also recommended that the use of balanced electrolyte solutions with cell saver in other specialities such as vascular, orthopaedic and neurosurgery be investigated.

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## APPENDIX A: STATISTICIANS LETTER

UNIVERSITY OF
KWAZULU-NATAL

20 February 2013

TO WHOM IT MAY CONCERN

This is to confirm that Mr Krishnan Pillay has consulted with me on his research proposal for the M Tech (Clinical Technology). We have discussed the research proposal and the study is statistically sound and viable.

The statistical methodology will include paired t-tests on readings taken pre- and post-operation for the 0.9% saline group and the Balsol group to determine whether there is any change in the components that will be measured.

There will also be a comparison between the between the 0.9% saline group and the Balsol group for the differences between the pre- and post-operation measurements for the components. This comparison will be done using t-tests for two independent groups.

The statistical software that will be used will be SPSS or SAS version 9.3.

Yours faithfully,

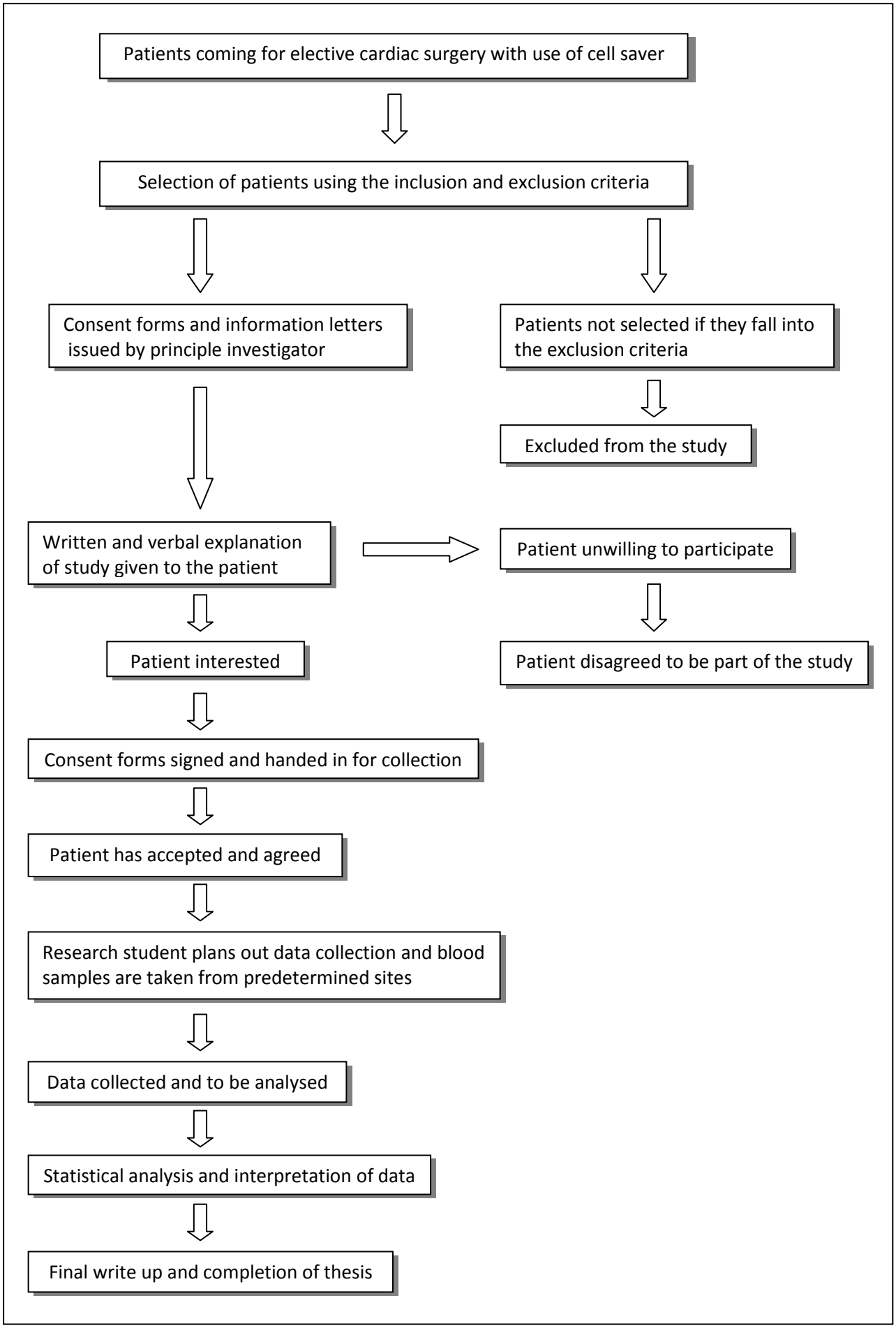
[redacted]

Professor GB Matthews

School of Mathematics, Statistics and Computer Science,

**Westville Campus**
**Postal address:** Private Bag X54001, Durban, 4000, South Africa
**Telephone:** +27(0)31 2603011 **Facsimile:** +27(0)31 2601009

## APPENDIX B: FLOW CHART SUMMARISING ACTIVITIES OF RESEARCH PROCESS



## APPENDIX C

**Time frame**

**January – April 2015 (4 months)**

- Introduction of proposal
- Review of Literature & Methodology
- Background
- Submission of proposal

**May 2015 (1 month)**

- Consent to notify patients of study
- Consent of cardiac staff and collection
- Selection of patients (study population)

**June – October 2015 (6 months)**

- Collection of blood samples and other related data
- Write up of dissertation: introduction, literature review, methodology
- Analyzing and presentation of results

**November – December 2015 (2 months)**

- Final write up and corrections
- Submission of dissertation for examination

# APPENDIX D

**Institutional Research Ethics Committee**
Faculty of Health Sciences
Room MS 49, Mansfield School Site
Gate 8, Ritson Campus
Durban University of Technology

P O Box 1334, Durban, South Africa, 4001

Tel: 031 373 2900
Fax: 031 373 2407
Email: lavishad@dut.ac.za
http://www.dut.ac.za/research/institutional_research_ethics

**www.dut.ac.za**

28 August 2015

Mr K Pillay
P O Box 41669
Rossburgh
4072

Dear Mr Pillay

Application for Amendment of Approved Research Proposal

**Comparative *in vitro* analysis of a balanced electrolyte solution versus an unbalanced electrolyte solution, for processing of residual pump blood using cell saver for patients undergoing elective cardiac surgery**

I am pleased to inform you that your application for amendment to the title and population group of your research proposal have been Approved.

Yours Sincerely

[redacted]
Professor PN Sibya
Deputy Chairperson: IREC

DUT DURBAN UNIVERSITY OF TECHNOLOGY
2015 -08- 28
INSITUTUTIONAL RESEARCH ETHICS COMMITTEE
P O BOX 1334 DURBAN 4000 SOUTH AFRICA

## APPENDIX E

INKOSI ALBERT LUTHULI CENTRAL HOSPITAL

HEALTH
KwaZulu-Natal

800 Bellair Road, Mayville, 4091
Private Bag X03, Mayville, 4058
Tel: 031 - 240 1000
Email: @ialch.co.za
www.kznhealth.gov.za

25/02/2013

Durban University of Technology
Department of Biomedical and Clinical Technology
Faculty of Health Sciences

To Whom It May Concern

**Re: Conduction of post graduate research (Masters)**

This letter serves to confirm that Krishnan Pillay will be conducting research for the fulfillment of his master's degree in the Department of Perfusion Cardio-thoracic surgery. Patients undergoing cardiopulmonary bypass with the use of intra-operative cell salvage will be recruited for this study.

[redacted]

Name: [redacted]

Designation: H.O.D PERFUSION

[redacted]

Designation: CONSULTANT CARDIO-THORACIC SURGERY

**APPENDIX F**

24 June 2015

Dept of Health

Dr M Joshua

Medical Manager (IALCH)

**RE: Conduction of post graduate research (Masters)**

The department of cardiothoracic surgery/perfusion has recommended that the study entitled: Comparative analysis of Balsol versus 0.9 % normal saline for processing of residual pump blood using cell saver for patients undergoing elective cardiac surgery. This research will be conducted by Mr. Krishnan Pillay in the department of perfusion.

Please find attached copy of departmental approval to the Durban University of Technology.

---

Mr. V Mathai

Control perfusionist

Department of perfusion

## APPENDIX G

INKOSI ALBERT LUTHULI CENTRAL HOSPITAL

HEALTH
KwaZulu-Natal

800 Bellair Road, Mayville, 4091
Private Bag X03, Mayville, 4058
Tel: 031 - 240 2509
Email: vikeshmat@ialch.co.za
www.kznhealth.gov.za

24 June 2015

Research office
KZN Department of Health
330 Langalibalele Street
Natalia Building
Pietermaritzburg
3200
Email- hrkm@kznhealth.gov.za

Attention: Researcher

RE: Conduction of post graduate research (Masters)

The department of cardiothoracic surgery/perfusion has recommended that the study entitled: Comparative analysis of Balsol versus 0.9 % normal saline for processing of residual pump blood using cell saver for patients undergoing elective cardiac surgery. This research will be conducted by Mr. Krishnan Pillay in the department of perfusion.

Please find attached copy of departmental approval together with research protocol submitted to the Durban University of Technology.

Mr. V Mathai

Control perfusionist
Department of perfusion


## LETTER OF INFORMATION

**Title of the Research Study:** Comparative analysis of balanced electrolyte solution versus an unbalanced electrolyte solution, for processing of residual pump blood using cell saver for patients undergoing elective cardiac surgery.

**Principal Investigator/s/researcher:** Krishnan Pillay (B-Tech. Clinical Technology)

**Co-Investigator/s/supervisor/s:** Supervisor: Prof J.K Adam (D-Tech. Clinical Technology)
Co-supervisor: Mr M. J. Mohapi (M Ed UKZN, B-Tech. Clinical Technology)

**Brief Introduction and Purpose of the Study:** Good day Sir/Madam my name is Krishnan Pillay and I am studying for a Masters degree at the Durban University of Technology. You are invited to be one of the 40 volunteers for a research study. The information in this letter will help you understand what the research is about and how it will benefit the quality of the operation. If there are any questions or concerns, which are not clearly explained in this letter, do not hesitate to ask the investigator, supervisor, or co-supervisors. The purpose of this study is to determine whether the approved solutions, 0.9% normal saline crystalloid solution, or Balsol® a balanced crystalloid solution is more beneficial when used to process left over blood after your procedure.

**Outline of the Procedures:** Thirty patients will be recruited for this study, fifteen consecutive patients will be allocated into the 0.9% normal saline group and fifteen will be allocated into the Balsol® group. Four blood gas samples will be taken for each patient. A 2ml and a 10ml sample will be taken of the residual pump blood (pre-processing sample), and a 2ml and 10ml sample will be taken after processing (post processing sample). The samples will be processed using a blood gas analyser in theatre and also sent to the hospital laboratory for processing and analysis. Important chemical levels that affect how your heart functions will be analysed in this study include potassium, sodium, calcium, magnesium, phosphate, chlorine, pH, albumin, osmolality, total protein, haematocrit, haemoglobin, glucose and lactate. These samples will be discarded after analysis and will not be used for any other purpose. Other routine data that is documented for the operation will be used for this study as well.

**Risks or Discomforts to the Participant:** There are no risks or side effects involved. Blood samples will be taken from blood left over from after your procedure and therefore you will not endure any discomfort or pain.

**Benefits:** The new information gained from this study will help improve the quality of processed blood, and decrease the exposure to bank blood and save cost for the patient and hospital. Bank blood is a scarce and expensive resource and is not without its own risks.

**Reason/s why the Participant May Be Withdrawn from the Study:** Your participation in this research is completely voluntary. You may withdraw at any time and this will not affect your routine treatment.

**Remuneration:** There will be no form of remuneration. Participation is voluntary.

**Costs of the Study:** You will not be asked to cover any cost relating to the study.

**Confidentiality:** All the information collected will be kept confidential. You will be allocated a number and all your details will be recorded under that number. This means that anyone who looks at my records will not be able to trace it to you. Data that may be reported in the scientific journals or published will not include information that will identify you as a patient in this study. This is done to protect your privacy.

**Research-related Injury:** There will be no research-related injury as the products being researched are approved products routinely used in all cardiac procedures. Also all blood samples are from residual blood left over after the procedure and not from your blood circulation directly.

**Persons to Contact in the Event of Any Problems or Queries:**
Please contact the researcher (073176010), my supervisor (031 373 5291) or the Institutional Research Ethics administrator on 031 373 2900. Complaints can be reported to the DVC: TIP, Prof F. Otieno on 031 373 2382 or dvctip@dut.ac.za.

**CONSENT**

**Statement of Agreement to Participate in the Research Study:**

- I hereby confirm that I have been informed by the researcher, _____ (name of researcher), about the nature, conduct, benefits and risks of this study - Research Ethics Clearance Number: _____,
- I have also received, read and understood the above written information (Participant Letter of Information) regarding the study.
- I am aware that the results of the study, including personal details regarding my sex, age, date of birth, initials and diagnosis will be anonymously processed into a study report.
- In view of the requirements of research, I agree that the data collected during this study can be processed in a computerised system by the researcher.
- I may, at any stage, without prejudice, withdraw my consent and participation in the study.
- I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to participate in the study.
- I understand that significant new findings developed during the course of this research which may relate to my participation will be made available to me.

| _____ | _____ | _____ | _____ |
|---|---|---|---|
| **Full Name of Participant** | **Date** | **Time** | **Signature / Right Thumbprint** |

I, _____ (name of researcher) herewith confirm that the above participant has been fully informed about the nature, conduct and risks of the above study.

| _____ | _____ | _____ |
|---|---|---|
| **Full Name of Researcher** | **Date** | **Signature** |
| _____ | _____ | _____ |
| **Full Name of Witness (If applicable)** | **Date** | **Signature** |
| _____ | _____ | _____ |
| **Full Name of Legal Guardian (If applicable)** | **Date** | **Signature** |


**Isihloko sesifundo ngocwaningo:** Ucubungulo ngokuqhathanisa i-Balsol® kanye ne-0.9% normal saline uma kusetshenzwa ngegazi elikwi-residual pump kusetshenziswa i-cell saver kuziguli ezichitshilelwa imithambo yazo yegazi enhliziyweni ngokuhleliwe.

**Umcwaningi Omkhulu:** Krishnan Pillay (B-Tech. Clinical Technology)

**Abaqaphi bocwaningo**: Omkhulu - Prof Adam (D-Tech. Clinical Technology)
Abasizi bakhe - Mr M. J. Mohapi (M Ed UKZN, B-Tech. Clinical Technology)

**Isingeniso kanye nenhloso yocwaningo:** Sawubona, Igamalami ngingu – Krishnan Pillay ofundela iziqu zeMasters degree esikhungweni sase Durban University of Technology. Ngingathanda kakhulu uma ungaba womunye abangamashumi amathathu (40) abazoba yingxenye yocwaningo. Ulwazi oluqukethwe kulencwandi luzokusiza ukwazi kabanzi mayelana nalolucwaningo nokuthi lingayikhuphula kanjani izinga lokuhlinzwa kweziguli.Uma kukhona imibuzo ongayiqondi kahle kulencwadi, ungabuza Umcwaningi noma Abaqhaphi balolucwaningo. Injongo yalolucwaninga ukubheka ukuthi ngabe ingqxubevange yamanzi evumelikile, eye-0.9% normal saline noma eye-Balsol®, iyiphi enomthelela omuhle kakhulu ekuhluzeni igazi elikumshini we-cardiopulmonary bypass circuit emva kokuhlukaniswa ngesikhathi sokuhlinzwa enhliziyweni.

**Uhlelo locwaningo**: Iziguli ezingamashumi amathathu zizomemelwa kulesifundo, eziyishumi nanhlanu zizoba kwingxenye ye-0.9% normal saline ezinye eziyishumi nanhlanu zibe kwinxenye ye-Balsol®. Kuzothathwa isampulana yegazi kane kozozonke iziguli. Amasampula angu 2ml ne 10ml azothathwa ku-residual blood pump (isampuli kungakasetshenzwa) bese kuphindwe kuthathwe amanye amasampula emvakokusetshenzwa kwegazi elikikwi-residual blood pump. Amasampula wonke azohlolwa acutshungwe ngomshini we-blood gas analyser osegumbini lokuhlinzela aphindwe ayiswe nasegumbini yokuhlola amasampula esibhedlela. Izinto ezibalulekile ezizocutshungulwa ezinomthelela ekusebenzeni kwenhliziyo nemithambo yegazi zibandakanya potassium, sodium, calcium, magnesium, phosphate, chlorine, pH, albumin, osmolality, total protein, haematocrit, haemoglobin, glucose kanye ne-lactate. Lamasampula elikumshini we-cardiopulmonary bypass circuit emva kokuhlukaniswa ngesikhathi sokuhlinzwa enhliziyweni.

**Uhlelo locwaningo**: Iziguli ezingamashumi amathathu zizomemelwa kulesifundo, eziyishumi nanhlanu zizoba kwingxenye ye-0.9% normal saline ezinye eziyishumi nanhlanu zibe kwinxenye ye-Balsol®. Kuzothathwa isampulana yegazi kane kozozonke iziguli. Amasampula angu 2ml ne 10ml azothathwa ku-residual blood pump (isampuli kungakasetshenzwa) bese kuphindwe kuthathwe amanye amasampula emvakokusetshenzwa kwegazi elikikwi-residual blood pump. Amasampula wonke azohlolwa acutshungwe ngomshini we-blood gas analyser osegumbini lokuhlinzela aphindwe ayiswe nasegumbini yokuhlola amasampula esibhedlela. Izinto ezibalulekile ezizocutshungulwa ezinomthelela ekusebenzeni kwenhliziyo nemithambo yegazi zibandakanya potassium, sodium, calcium, magnesium, phosphate, chlorine, pH, albumin, osmolality, total protein, haematocrit, haemoglobin, glucose kanye ne-lactate. Lamasampula azi lase-blood bank kanye nokonga kwezimali zesiguli nesibhedlela.

**Ilungelo leziguli lokungenela lolucwaningo**:
Ukungenela lolucwaningo akuphoqelekile, unganqaba ukuba ungenele noma usimise noma yinini uma usungenelile. Ukuyeka kwakho kulolucwaningo ngeke kuvimbele ukuthola usizo lokelashwa ngokujwayelekileyo.

**Inkokhelo**
Ayikho inkokhelo ozoyithola ngokungenela lolucwaningo. Ukungenela akuphoqiwe.

**Izindleko zocwaningo**
Akukho zindleko ezikhokhwa nguwe kulolucwaningo.

**Imfihlo**
Imininingwane yakho yonke yalolucwaningo ngeke idalulwe kumuntu izogcinwa iyimfihlo. Kuzoba khona inombolo ozonikwa yona ekuzofakwa kuyo yonke imininingwane yakho. Lokho kusho ukuthi noma ngubani ongabuka lemiqulu angeke akwazi ukuthi nguwe obungenele. Ulwazi lonke kanye nemiphumela etholwe emva kwalolucwaningo ezofakwa kuzincwadi zochwepheshe noma ezincwadini zokuqhakambisa ngeke idalule noma iveze ukuthi isiguli esithize besizimbandakanye nalolucwaningo.

**Ukulimala ngesikhathi socwaningo**
Angeke kube khona ukulimala ngesikhathi socwaningo njengoba kuzosetshenziswa amakhambi asemthethweni asetshenziswayo njalo kuziguli ezinezidingo ezihlukene zenhliziyo. Namasampula athathwayo yilawo asuke esele esemshini wokuphampa emvakohlinzo akathathwa emithanjeni yakho ngqo.

**Abantu ongaxhumana nabo uma unenkinga noma unemibuzo**:
Thintana noMcwaningi omkhulu (073176010), Umqhaphi wocwaningo omkhulu: Prof Adam (031 373 5291) noma ihhovisi lesikhungo elangemele ucwaningo ku 031 373 2900. Izikhalo zingabikwa kwi-DVC: TIP, Prof F. Otieno on 031 373 2382 or dvctip@dut.ac.za.

**Isitatimende sokuzivumela ukungela isifundo socwaningo**

- Mina ngiyazivumela ukuthi ngazisiwe umcwaningi , _____ (Igama lomcwaningi), mayelana nesimo, nangendlela, nokuzotholakal Kanye nokungaba yingozi kulesisifyndo – Research Ethics Clearance Number: _____.

- Ngiphinda ngathola, ngafunda ngase ngiyaqonda mayelana nencwadi yolwazi ngalesisifundo.

- Ngiyazi ukuthi imiphumela yalesisifundo, kumbandakanya imininingwane yami yobulili, iminyaka, nosuku lokuzalwa, amagama ami kanye nesifo esingiphethe ngeke kadalulwa emqulwini yalesisifundo.

- Ekwazisweni izidingo zalesisifundo, ngiyavuma ukuthi Umcwaningi angathatha imininingwane ifakwe iphinde isetshenziswe ngokwezinga lama-computer.

- Ngingasihoxisa noma inini lesisivumelwano ekungeneleni lesisifundo ngaphandle kokuba sengcupheni yokungelashwa.

- Ngibile nesikhathi esanele sokufundisia, ngabuza nemibuzo, ngakhoke ngiyazivumela ngokukhululeka ukungenela lesisifundo.

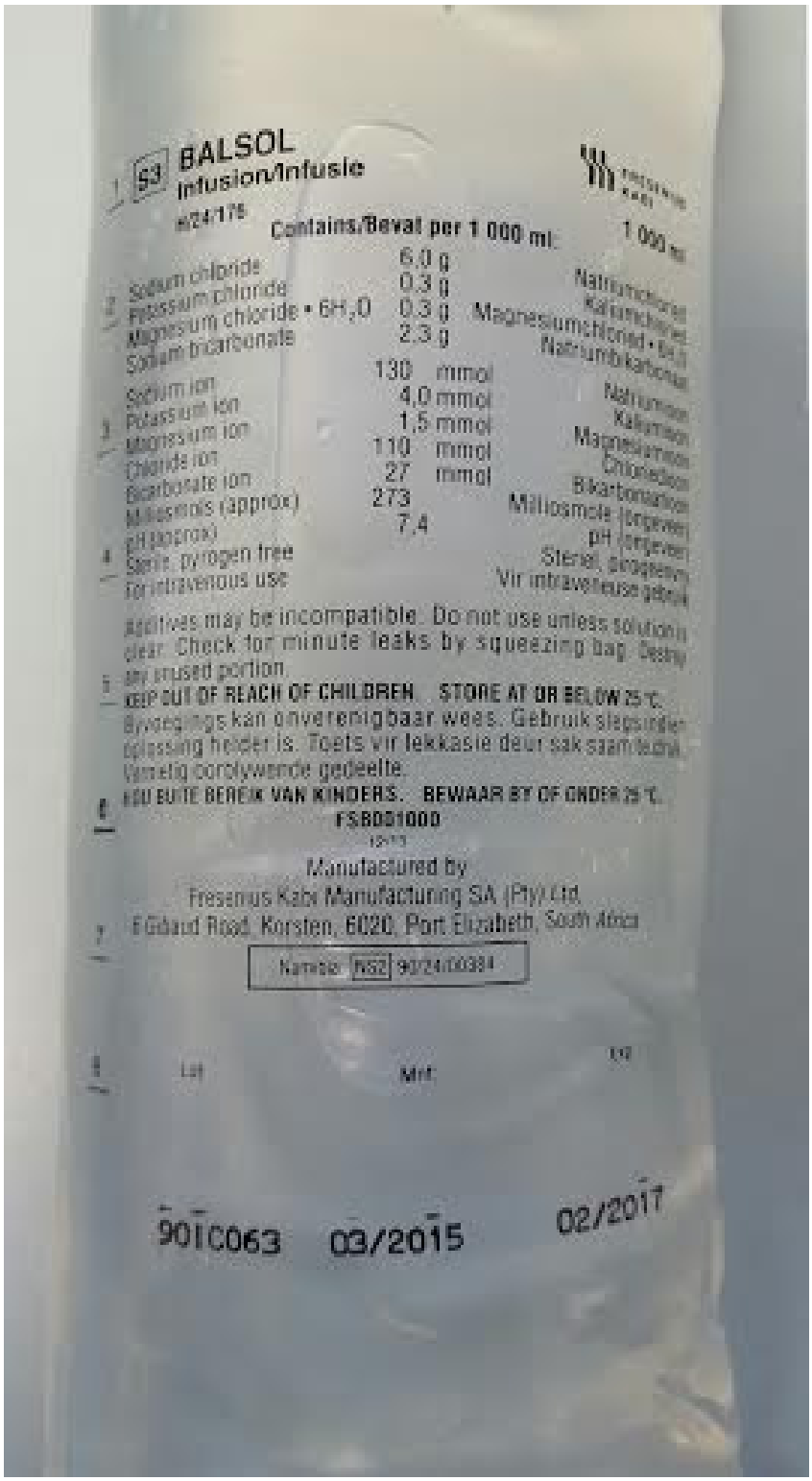
- Ngiyaqonda ukuthi izinto ezibalulekile ezingatholakala ngalolucwaningo lwalesisifundo ngiyokwaziswa ngalo.

| _____ | _____ | _____ | _____ |
|---|---|---|---|
| **Amagama Omngeneli** | **Usuku** | **Isikhathi** | **Sayina / Faka isithupha** |

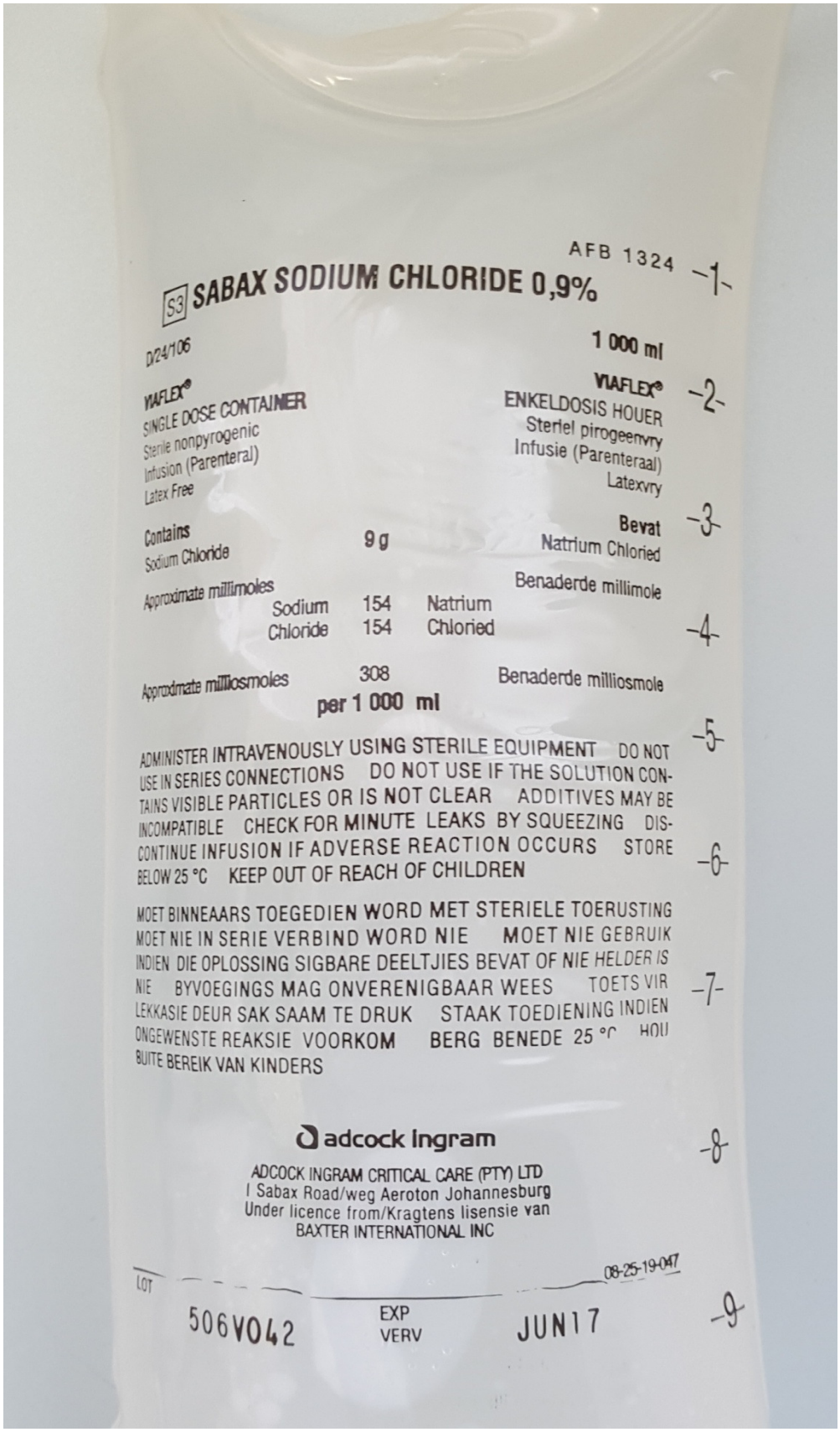
Mina, _____ (igama lomcwaningi) ngiyagcizelela lapha ukuthi lomngeneli ongaphezulu wazisiwe kabanzi ngesimo, impatho kanye nobungozi obungenzeka kulesisifundo.

| _____ | _____ | _____ |
|---|---|---|
| **Amagama Omcwaningi** | **Usuku** | **Sayina** |
| _____ | _____ | _____ |
| **Amagama kafakazi (uma kunesidingo)** | **Usuku** | **Sayina** |
| _____ | _____ | _____ |
| **Amagama omzali osemthethweni (uma kudingeka)** | **Usuku** | **Sayina** |

**APPENDIX J**


BALSOL
Infusion/Infusie
Contains/Bevat per 1 000 ml:
Sodium chloride 6,0 g
Potassium chloride 0,3 g
Magnesium chloride • 6H2O 0,3 g
Sodium bicarbonate 2,3 g
Sodium ion 130 mmol
Potassium ion 4,0 mmol
Magnesium ion 1,5 mmol
Chloride ion 110 mmol
Bicarbonate ion 27 mmol
Milliosmols (approx) 273
pH (approx) 7,4
Sterile, pyrogen free
For intravenous use
Additives may be incompatible. Do not use unless solution is clear. Check for minute leaks by squeezing bag. Destroy any unused portion.
KEEP OUT OF REACH OF CHILDREN. STORE AT OR BELOW 25 °C.
Byvoegings kan onverenigbaar wees. Gebruik slegs indien oplossing helder is. Toets vir lekkasie deur sak saam te druk. Vernietig ongebruikte gedeelte.
BUITE BEREIK VAN KINDERS. BEWAAR BY OF ONDER 25 °C.
FSB031000
Manufactured by
Fresenius Kabi Manufacturing SA (Pty) Ltd.
6 Gibaud Road, Korsten, 6020, Port Elizabeth, South Africa
Lot
Mnf
9010063 03/2015 02/2017

**APPENDIX K**


AFB 1324
S3 SABAX SODIUM CHLORIDE 0,9%
D/24/106
1 000 ml
VIAFLEX®
SINGLE DOSE CONTAINER
Sterile nonpyrogenic
Infusion (Parenteral)
Latex Free
VIAFLEX®
ENKELDOSIS HOUER
Steriel pirogeenvry
Infusie (Parenteraal)
Latexvry
Contains
Sodium Chloride
9 g
Bevat
Natrium Chloried
Approximate millimoles
Benaderde millimole
Sodium 154 Natrium
Chloride 154 Chloried
Approximate milliosmoles 308
Benaderde milliosmole
per 1 000 ml
ADMINISTER INTRAVENOUSLY USING STERILE EQUIPMENT DO NOT USE IN SERIES CONNECTIONS DO NOT USE IF THE SOLUTION CONTAINS VISIBLE PARTICLES OR IS NOT CLEAR ADDITIVES MAY BE INCOMPATIBLE CHECK FOR MINUTE LEAKS BY SQUEEZING DISCONTINUE INFUSION IF ADVERSE REACTION OCCURS STORE BELOW 25 °C KEEP OUT OF REACH OF CHILDREN
MOET BINNEAARS TOEGEDIEN WORD MET STERIELE TOERUSTING MOET NIE IN SERIE VERBIND WORD NIE MOET NIE GEBRUIK INDIEN DIE OPLOSSING SIGBARE DEELTJIES BEVAT OF NIE HELDER IS NIE BYVOEGINGS MAG ONVERENIGBAAR WEES TOETS VIR LEKKASIE DEUR SAK SAAM TE DRUK STAAK TOEDIENING INDIEN ONGEWENSTE REAKSIE VOORKOM BERG BENEDE 25 °C HOU BUITE BEREIK VAN KINDERS
adcock ingram
ADCOCK INGRAM CRITICAL CARE (PTY) LTD
1 Sabax Road/weg Aeroton Johannesburg
Under licence from/Kragtens lisensie van
BAXTER INTERNATIONAL INC
LOT
08-25-19-047
506V042
EXP
VERV
JUN17
-1-
-2-
-3-
-4-
-5-
-6-
-7-
-8-
-9-

**APPENDIX L**

SCHEDULING STATUS: S3

PROPRIETARY NAMES AND DOSAGE FORM:

# Medsol Cardioplegic Induction Solution
# Medsol Cardioplegic Maintenance Solution
# Medsol Cardioplegic Reperfusion Solution

COMPOSITION:

| Content | Maintenance Solution g/500 ml | Induction Solution g/500 ml | Reperfusion Solution g/500 ml |
|---|---|---|---|
| Sodium Chloride | 0,735 | 0,777 | |
| Potassium Chloride | 1,375 | 3,757 | 1,12 |
| Sodium Citrate, $2H_20$ | 0,785 | 0,832 | 3,10 |
| Citric Acid Monohydrate | 0,098 | 0,104 | 0,385 |
| Sodium Biphosphate $2H_20$ | 0,075 | 0,079 | 0,275 |
| Tris(hydroxymethyl)-aminomethane (Tham) | 4,28 | 4,548 | 4,28 |
| L-Aspartate (as sodium salt) | | | 4,9 |
| L-Glutamate (as sodium salt) | | | 5,35 |

**PHARMACOLOGICAL CLASSIFICATION:**
A 24 - Mineral substitutes, electrolytes.

**PHARMACOLOGICAL ACTION:**
The primary action of cardioplegic solution is dependent on the concentration of the potassium ion ($K^+$) resulting in depolarization of the cardiac muscle and cessation of contractile function. The presence of buffers in combination with $K^+$ counteracts the intracellular acidosis resulting from cardiac arrest.
Energy substrates such as glucose and gluconeogenic precursor amino acids are included to provide cellular energy during the phase of anaerobic metabolism associated with cessation of contractile function.

**INDICATIONS:**
For blood cardioplegic cardiac bypass procedures.

**SCHEDULING STATUS:** S2

**PROPRIETARY NAME (and dosage form):**
ST THOMAS' HOSPITAL CARDIOPLEGIC SOLUTION II
(Solution)

**COMPOSITION:**
**Each 1 000 ml contains:**

| | |
|---|---|
| Calcium Chloride Dihydrate | 0,175 g |
| Sodium Chloride | 6,42 g |
| Potassium Chloride | 1,19 g |
| Magnesium Chloride Hexahydrate | 3,25 g |
| Water for Injections | QS |

**ST THOMAS' HOSPITAL CARDIOPLEGIC SOLUTION II** is mixed aseptically with 10 ml of 8,5% sodium bicarbonate before use. 3,6 ml of a 50% glucose injection may be added if required.

The final measured osmolality of the solution after the addition of glucose and sodium bicarbonate is approximately 323 milliosmoles/kg.

**PHARMACOLOGICAL CLASSIFICATION:**
A.24 (Mineral substitutes, Electrolytes).

**INDICATIONS:**
Use as a cardioplegic solution.

**CONTRA-INDICATIONS:**
It is recommended that cardioplegia is not administered to patients currently on Amioderone therapy. Amioderone should be discontinued for a minimum of six weeks prior to cardiac surgery.

**WARNINGS:**
1. Solutions containing sodium should be used with great care in patients with congestive heart failure, severe renal insufficiency, and in clinical states in which there exists oedema with or without sodium retention.
2. Solutions containing potassium ions should be used with great care in patients with hyperkalemia, severe renal failure and in conditions in which potassium retention is present.
3. Solutions containing calcium ions should not be administered through the same administration set as blood anticoagulated with calcium chelators, e.g. Anticoagulants, Citrate, Glucose or Citrate Phosphate Glucose, because of the likelihood of coagulation.
4. The intravenous administration of these solutions can cause fluid and/or solute overloading, resulting in dilution of serum concentrations, overhydration, congested states and/or pulmonary oedema.
5. Additives may be incompatible. Consult with pharmacist, if necessary, to ensure compatibility of prescribed additives. When introducing additives, use aseptic technique. Mix thoroughly when additives have been introduced.
6. Do not store solutions containing additives for more than 24 hours. Do not administer unless the solution is clear and the container is intact.

**DOSAGE AND DIRECTIONS FOR USE:**
Before use mix with 10 ml of 8,5% sodium bicarbonate and if required 3,6 ml of 50% glucose injection. An induction dose of 10 to 20 ml/kg of ST THOMAS' HOSPITAL CARDIOPLEGIC SOLUTION II should be infused into the coronary circulation immediately after aortic clamping in order to effect rapid electro-mechanical arrest. Thereafter multidose cardioplegia 5 to 10 ml/kg, is reinfused every 20 to 30 minutes throughout the ischaemia cross clamp period. The solution is infused under positive pressure, and it is recommended that the infusion pressure (measured at the aortic root) should not exceed 100 mm Hg. The solution should be infused at a temperature of 4 °C to 10 °C. It is recommended that an in-line cardioplegic filter of at least 0,8 micron is used.

**SIDE-EFFECTS AND SPECIAL PRECAUTIONS:**
Reactions which may occur because of the solution or the technique of administration include febrile response, extravasation and hypervolemia.

**Pregnancy:**
Safety in pregnancy has not been established.

**IDENTIFICATION:**
A clear, colourless solution.

**PRESENTATION:**
ST THOMAS' HOSPITAL CARDIOPLEGIC SOLUTION II is available in 1 000 ml Viaflex® containers and 1 000 ml Vacoliter® containers.

**STORAGE INSTRUCTIONS:**
Store below 25 °C. After mixing, the solutions should be refrigerated (2 °C to 8 °C) and the unused portion discarded after 24 hours. **Keep out of reach of children.**

**REGISTRATION NUMBER:** Z/24/2

**NAME AND BUSINESS ADDRESS OF THE APPLICANT:**
ADCOCK INGRAM CRITICAL CARE (PTY) LTD
1 New Road, Erand Gardens, Midrand, 1685
Viaflex® and Vacoliter® are Registered Trademarks of BAXTER INTERNATIONAL INC.

**DATE OF PUBLICATION OF THIS PACKAGE INSERT:**
February 1992

08-19-03-111

Master Print 60449

# GEM Premier 4000
# Technical Specifications

## Dimensions and Weight

**Analyzer**
H: 18 in, W: 12 in, D: 15 in, Wt: 44 lbs

**PAK**
H: 6.75 in, W: 10 in, D: 8 in, Wt: 8 lbs

## Sample Volume

| | |
|---|---|
| 150 µL | BG*/Hct/Lytes**/Glu/Lac/CO-Ox or any subset of the menu that includes CO-Ox |
| 95 µL | BG/Hct/Lytes/Glu/Lac or any subset of this menu |
| 65 µL (micro mode) | BG/Hct/Lytes/Glu/Lac (capillary only) |

*BG = pH, $pCO_2$, $pO_2$
**Lytes = $Na^+$, $K^+$, $Ca^{++}$, $Cl^-$

## Sample Type

Heparinized whole blood

## Time to Results

| | |
|---|---|
| All tests without CO-Ox: | 70 seconds from sample introduction |
| All tests with CO-Ox: | 95 seconds from sample introduction |
| Sample capacity: | 75 - 450 tests |

## Measurement Methodology

| | |
|---|---|
| Amperometric: | $pO_2$, Glu, Lac |
| Potentiometric: | pH, $pCO_2$, $Na^+$, $K^+$, $Ca^{++}$, $Cl^-$ |
| Conductivity: | Hct |
| CO-Ox: | Optical measurement following chemical lysing of the whole blood sample |

## Interface Protocols

ASTM or HL7 enables data transmission to a laboratory, hospital or third-party information management system.

## Measured Analytes

| Analytes | Unit | Measured Range* |
|---|---|---|
| pH | n/a | 6.80 – 8.00 |
| $pCO_2$ | mmHg | 0 – 150 |
| $pO_2$ | mmHg | 0 – 800 |
| $Na^+$ | mmol/L | 100 – 200 |
| $K^+$ | mmol/L | 0.1 – 20.0 |
| $Ca^{++}$ | mmol/L | 0.10 – 5.00 |
| $Cl^-$ | mmol/L | 40 – 170 |
| Glu | mg/dL | 4 – 750 |
| Lac | mmol/L | 0.1 – 20.0 |
| Hct | % | 15 – 75 |
| tHb | g/dL | 5.0 – 23.0 |
| $O_2Hb$ | % | -10.0 – 110.0 |
| COHb | % | -10.0 – 110.0 |
| MetHb | % | -10.0 – 110.0 |
| HHb | % | -10.0 – 110.0 |

* The measured ranges reflect actual numeric value ranges the system can report.

## Derived (calculated) Parameters

| | |
|---|---|
| $tCO_2$ | $sO_2$ |
| BE(B) | $sO_2$(c) |
| BE(ecf) | $HCO_3^-$ std |
| tHb(c) | $HCO_3^-$ (c) |
| $Ca^{++}$ (7.4) | A-a$DO_2$ |
| Anion gap | $paO_2/pAO_2$ |
| P/F Ratio | RI |
| $pAO_2$ | $CcO_2$ |
| $CaO_2$ | a-v$DO_2$ |
| $CvO_2$ | $Q_{sp}/Q_t$ (est) |
| P50 | $Q_{sp}/Q_t$ |
| $O_2$cap | Hct(c) |

# APPENDIX O

NATIONAL HEALTH LABORATORY SERVICE
Practice No. 5200296

ACADEMIC COMPLEX BUSINESS UNIT
Chemical Pathology Department
Private Bag X03, Mayville, 4058
800 Bellair Road, Mayville, 4058
Tel: +27 (0)31 2402557/68/69

02 November 2015

Dear Krishnan

Please find information as requested.

| Analyte | Instrument | Analytical range |
|---|---|---|
| Magnesium | Siemens Advia 1800 | 0.29 - 2.06 mmol/L |
| Inorganic Phosphate | Siemens Advia 1800 | 0 - 6.46 mmol/L |
| Albumin | Siemens Advia 1800 | 10 - 60 g/L |
| Total Protein | Siemens Advia 1800 | 20 - 120 g/L |
| Osmolality | Gonotech osmometer | 0 - 2500 mOsm/kg |

Regards,

[redacted]

**Seshini Nagesur**
Laboratory Manager
Chemical Pathology (IALCH)
Tel: 031 2402570 | Fax: 031 2402576
seshini.nagesur@nhls.ac.za | www.nhls.ac.za

## APPENDIX P 0.9% NaCl WASH SOLUTION GROUP (n=20) – DATA COLLECTION SHEET

| VARIABLE | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ph | B | 7.48 | 7.51 | 7.62 | 7.42 | 7.55 | 7.57 | 7.50 | 7.62 | 7.46 | 7.43 | 7.46 | 7.45 | 7.50 | 7.47 | 7.45 | 7.43 | 7.52 | 7.52 | 7.42 | 7.39 |
| | A | 7.50 | 7.72 | 7.87 | 7.64 | 7.84 | 7.53 | 7.72 | 7.79 | 7.55 | 7.92 | 7.68 | 7.54 | 7.54 | 7.76 | 7.66 | 7.63 | 7.59 | 7.61 | 7.70 | 7.59 |
| $pCO_2$ (mmHg) | B | 29.0 | 25.0 | 29.0 | 31.0 | 28.0 | 26.0 | 25.0 | 22.0 | 27.0 | 26.0 | 30.0 | 29.0 | 29.0 | 31.0 | 26.0 | 31.0 | 25.0 | 31.0 | 33.0 | 32.0 |
| | A | <6 | <6 | <6 | <6 | <6 | <6 | <6 | <6 | <6 | <6 | <6 | <6 | <6 | <6 | <6 | <6 | <6 | <6 | <6 | <6 |
| $pO_2$ (mmHg) | B | 596.0 | 618.0 | 618.0 | 657.0 | 676.0 | 776.0 | 646.0 | 633.0 | 578.0 | 578.0 | 589.0 | 512.0 | 478.0 | 611.0 | 646.0 | 638.0 | 436.0 | 613.0 | 625.0 | 621.0 |
| | A | 233.0 | 236.0 | 221.0 | 215.0 | 211.0 | 229.0 | 236.0 | 211.0 | 200.0 | 210.0 | 219.0 | 154.0 | 228.0 | 236.0 | 236.0 | 243.0 | 225.0 | 222.0 | 233.0 | 213.0 |
| $[K^+]$ (mmol/l) | B | 4.20 | 3.90 | 3.90 | 4.70 | 4.40 | 4.30 | 5.60 | 5.30 | 4.30 | 4.10 | 3.70 | 5.10 | 4.30 | 4.50 | 4.70 | 4.60 | 4.90 | 4.40 | 4.50 | 4.40 |
| | A | 0.90 | <0.10 | 2.0 | 0.70 | 1.50 | 0.80 | 1.0 | 0.70 | 1.0 | 1.10 | <0.10 | 3.50 | <0.10 | 0.90 | 1.0 | 0.80 | 0.90 | 0.70 | 1.10 | 1.30 |
| $[Na^+]$ (mmol/l) | B | 131.0 | 131.0 | 138.0 | 131.0 | 134.0 | 131.0 | 127.0 | 134.0 | 136.0 | 135.0 | 136.0 | 135.0 | 132.0 | 139.0 | 133.0 | 129.0 | 131.0 | 136.0 | 131.0 | 129.0 |
| | A | 148.0 | 147.0 | 140.0 | 146.0 | 146.0 | 146.0 | 146.0 | 146.0 | 148.0 | 149.0 | 148.0 | 145.0 | 147.0 | 148.0 | 144.0 | 146.0 | 146.0 | 147.0 | 147.0 | 146.0 |
| $[Cl^-]$ (mmol/l) | B | 109.0 | 105.0 | 109.0 | 105.0 | 107.0 | 106.0 | 107.0 | 109.0 | 113.0 | 109.0 | 111.0 | 109.0 | 107.0 | 112.0 | 106.0 | 101.0 | 107.0 | 113.0 | 107.0 | 103.0 |
| | A | 126.0 | 127.0 | 126.0 | 130.0 | 127.0 | 125.0 | 127.0 | 130.0 | 125.0 | 131.0 | 128.0 | 127.0 | 128.0 | 129.0 | 126.0 | 125.0 | 127.0 | 132.0 | 127.0 | 125.0 |
| $[Mg^{2+}]$ (mmol/l) | B | 1.35 | 1.55 | 1.33 | 1.35 | 1.44 | 1.36 | 1.17 | 1.54 | 2.22 | 2.30 | 2.63 | 2.80 | 1.32 | 2.50 | 3.34 | 0.80 | 1.48 | 0.82 | 1.20 | 1.53 |
| | A | <0.29 | <0.29 | <0.29 | <0.29 | <0.29 | <0.29 | <0.29 | <0.29 | <0.29 | <0.29 | <0.29 | <0.29 | <0.29 | <0.29 | <0.29 | <0.29 | <0.29 | <0.29 | <0.29 | <0.29 |
| $[Ca^{2+}]$ (mmol/l) | B | 0.98 | 0.92 | 0.76 | 0.99 | 1.00 | 0.98 | 0.97 | 1.04 | 1.09 | 0.95 | 0.93 | 0.95 | 0.91 | 0.90 | 1.18 | 0.99 | 1.03 | 0.86 | 0.96 | 0.87 |
| | A | <0.10 | <0.10 | 0.23 | <0.10 | 0.10 | 0.11 | <0.10 | 0.15 | <0.10 | <0.10 | <0.10 | 0.10 | <0.10 | 0.10 | 0.13 | <0.10 | 0.10 | <0.10 | <0.10 | <0.10 |

**APPENDIX P** **0.9% NaCl WASH SOLUTION GROUP (n=20) – DATA COLLECTION SHEET**

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $[PO_4^{3}]$ (mmol/l) | B | 0.43 | 0.85 | 0.59 | 0.76 | 0.94 | 0.57 | 0.83 | 0.43 | 1.59 | 1.10 | 0.90 | 1.52 | 1.24 | 0.90 | 1.06 | 1.69 | 0.81 | 0.68 | 0.79 | 0.83 |
| | A | 0.06 | 0.07 | 0.10 | 0.07 | 0.06 | 0.08 | 0.08 | 0.04 | 0.14 | 0.10 | 0.08 | 0.24 | 0.12 | 0.08 | 0.10 | 0.10 | 0.10 | 0.07 | 0.08 | 0.11 |
| Lactate (mmol/l) | B | 2.70 | 5.10 | 4.40 | 6.60 | 2.30 | 3.20 | 2.80 | 3.10 | 4.0 | 7.60 | 5.70 | 3.40 | 3.90 | 7.90 | 7.70 | 3.50 | 3.20 | 3.30 | 4.30 | 5.70 |
| | A | 1.10 | 1.40 | 1.50 | 1.40 | 1.20 | 1.40 | 1.20 | 1.0 | 1.90 | 1.80 | 2.0 | 3.30 | 1.60 | 2.30 | 2.50 | 1.30 | 1.40 | 1.30 | 1.70 | 2.0 |
| Glucose | B | 11.20 | 19.50 | 19.9 | 17.0 | 10.30 | 12.90 | 13.70 | 9.0 | 12.40 | 14.30 | 12.70 | 10.70 | 14.30 | 11.40 | 15.50 | 14.40 | 11.90 | 7.80 | 16.20 | 12.80 |
| | A | 1.10 | 2.30 | 1.50 | 1.50 | 0.80 | 1.70 | 1.70 | 0.90 | 0.80 | 0.40 | 2.10 | 0.80 | 2.70 | 1.70 | 2.70 | 2.0 | 1.50 | 0.40 | 2.40 | 2.0 |
| Osmolality | A | 296 | 183 | 243 | 253 | 260 | 246 | 257 | 221 | 158 | 263 | 303 | 290 | 287 | 263 | 246 | 299 | 245 | 235 | 285 | 295 |
| | B | 290 | 282 | 296 | 269 | 298 | 334 | 491 | 149 | 297 | 275 | 285 | 307 | 299 | 298 | 298 | 289 | 296 | 292 | 290 | 289 |
| Albumin | B | 22 | 24 | 18 | 27 | 29 | 21 | 21 | 18 | 26 | 23 | 24 | 26 | 23 | 20 | 15 | 24 | 26 | 12 | 21 | 24 |
| | A | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 |
| Total Protein | B | 39 | 39 | 32 | 46 | 63 | 52 | 37 | 29 | 43 | 44 | 42 | 54 | 46 | 35 | 25 | 40 | 40 | 26 | 35 | 45 |
| | A | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 |
| HCT (%) | B | 28.0 | 26.0 | 24.0 | 31.0 | 27.0 | 26.0 | 27.0 | 24.0 | 26.0 | 27.0 | 26.0 | 29.0 | 27.0 | 29.0 | 28.0 | 29.0 | 28.0 | 22.0 | 31.0 | 26.0 |
| | A | 69.0 | 62.0 | 59.0 | 52.0 | 61.0 | 58.0 | 68.0 | 59.0 | 67.0 | 65.0 | 65.0 | 58.0 | 65.0 | 61.0 | 69.0 | 64.0 | 68.0 | 45.0 | 68.0 | 63.0 |
| Hb (g/dL) | B | 9.20 | 8.80 | 8.10 | 10.30 | 9.10 | 8.70 | 9.10 | 8.10 | 8.60 | 8.90 | 8.70 | 9.50 | 9.0 | 9.50 | 9.30 | 9.50 | 9.30 | 7.30 | 10.40 | 8.70 |
| | A | 22.90 | 20.70 | 19.80 | 17.20 | 20.40 | 19.30 | 22.60 | 19.60 | 22.30 | 21.70 | 21.60 | 19.40 | 21.50 | 20.30 | 23.0 | 21.30 | 22.50 | 15.0 | 22.60 | 21.0 |

**APPENDIX P** **0.9% NaCl WASH SOLUTION GROUP (n=20) – DATA COLLECTION SHEET**

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $TCO_2$ (mmol/l) | B | 22.50 | 20.70 | 30.70 | 21.10 | 25.40 | 24.60 | 20.30 | 23.30 | 20.0 | 18.10 | 22.20 | 21.10 | 23.50 | 23.60 | 18.90 | 21.60 | 21.20 | 26.30 | 22.40 | 20.40 |
| | A | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| $HCO_3^-$ (mmol/l) (Actual) | B | 21.60 | 19.90 | 29.80 | 20.10 | 24.5 | 23.80 | 19.50 | 22.60 | 19.20 | 17.30 | 21.30 | 20.20 | 22.60 | 22.60 | 18.10 | 20.60 | 20.40 | 25.30 | 21.40 | 19.40 |
| | A | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Pre - wash Vol.(ml) | | 4152 | 2570 | 1913 | 2480 | 3109 | 2151 | 2021 | 2536 | 1782 | 2019 | 2407 | 2850 | 1882 | 3584 | 2313 | 2927 | 1981 | 5564 | 1611 | 2555 |
| Post - wash Vol.(ml) | | 500 | 458 | 285 | 436 | 576 | 504 | 407 | 347 | 248 | 264 | 339 | 319 | 477 | 752 | 395 | 464 | 426 | 1210 | 351 | 769 |

## APPENDIX Q BALSOL WASH SOLUTION GROUP (n=20) – DATA COLLECTION SHEET

| VARIABLE | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ph | B | 7.45 | 7.42 | 7.44 | 7.40 | 7.36 | 7.47 | 7.43 | 7.58 | 7.51 | 7.64 | 7.52 | 7.49 | 7.55 | 7.45 | 7.53 | 7.49 | 7.53 | 7.42 | 7.50 | 7.38 |
| | A | 7.61 | 7.59 | 7.54 | 7.69 | 7.56 | 7.87 | 7.69 | 7.68 | 7.63 | 7.81 | 7.75 | 7.70 | 7.85 | 7.69 | 7.58 | 8.00 | 7.78 | 7.68 | 7.87 | 7.84 |
| $pCO_2$ (mmHg) | B | 31.0 | 37.0 | 31.0 | 39.0 | 37.0 | 32.0 | 29.0 | 20.0 | 29.0 | 15.0 | 29.0 | 34.0 | 23.0 | 33.0 | 30.0 | 35.0 | 25.0 | 36.0 | 26.0 | 32.0 |
| | A | 23.0 | 24.0 | 27.0 | 20.0 | 27.0 | 13.0 | 19.0 | 19.0 | 23.0 | 15.0 | 17.0 | 19.0 | 14.0 | 20.0 | 25.0 | 10.0 | 16.0 | 20.0 | 13.0 | 14.0 |
| $pO_2$ (mmHg) | B | 706.0 | 504.0 | 543.0 | 520.0 | 584.0 | 501.0 | 575.0 | 514.0 | 484.0 | 727.0 | 563.0 | 581.0 | 724.0 | 577.0 | 566.0 | 597.0 | 605.0 | 588.0 | 577.0 | 621.0 |
| | A | 238.0 | 221.0 | 221.0 | 222.0 | 218.0 | 229.0 | 222.0 | 216.0 | 228.0 | 235.0 | 223.0 | 222.0 | 248.0 | 214.0 | 232.0 | 233.0 | 230.0 | 248.0 | 210.0 | 210.0 |
| $[K^+]$ (mmol/l) | B | 4.20 | 5.20 | 3.90 | 3.90 | 5.0 | 4.60 | 3.70 | 3.80 | 4.70 | 4.20 | 3.90 | 4.0 | 4.20 | 4.30 | 4.0 | 4.20 | 4.10 | 4.20 | 4.0 | 4.50 |
| | A | 4.30 | 4.90 | 4.30 | 4.50 | 4.90 | 4.70 | 4.90 | 4.50 | 4.30 | 4.50 | 4.70 | 4.60 | 4.30 | 4.40 | 4.30 | 4.80 | 4.70 | 4.30 | 4.80 | 5.30 |
| $[Na^+]$ (mmol/l) | B | 134.0 | 132.0 | 135.0 | 134.0 | 132.0 | 136.0 | 136.0 | 139.0 | 132.0 | 135.0 | 136.0 | 134.0 | 133.0 | 136.0 | 138.0 | 131.0 | 133.0 | 138.0 | 134.0 | 136.0 |
| | A | 125.0 | 124.0 | 125.0 | 124.0 | 125.0 | 127.0 | 126.0 | 126.0 | 126.0 | 126.0 | 127.0 | 125.0 | 125.0 | 128.0 | 125.0 | 126.0 | 125.0 | 126.0 | 125.0 | 125.0 |
| $[Cl^-]$ (mmol/l) | B | 108.0 | 108.0 | 107.0 | 107.0 | 108.0 | 106.0 | 109.0 | 113.0 | 104.0 | 113.0 | 110.0 | 106.0 | 111.0 | 107.0 | 108.0 | 105.0 | 112.0 | 111.0 | 109.0 | 113.0 |
| | A | 101.0 | 101.0 | 99.0 | 100.0 | 100.0 | 101.0 | 99.0 | 102.0 | 99.0 | 99.0 | 102.0 | 100.0 | 101.0 | 98.0 | 99.0 | 103.0 | 101.0 | 101.0 | 98.0 | 99.0 |
| $[Mg^{2+}]$ (mmol/l) | B | 1.19 | 1.32 | 0.93 | 0.71 | 1.42 | 2.01 | 1.19 | 2.27 | 1.45 | 1.87 | 1.53 | 1.51 | 1.42 | 1.48 | 1.14 | 1.46 | 1.15 | 0.88 | 1.54 | 1.18 |
| | A | 1.36 | 1.39 | 1.37 | 1.40 | 1.36 | 1.39 | 1.42 | 1.45 | 1.42 | 1.43 | 1.42 | 1.33 | 1.43 | 1.37 | 1.34 | 1.39 | 1.33 | 1.41 | 1.38 | 1.39 |
| $[Ca^{2+}]$ (mmol/l) | B | 0.94 | 0.93 | 0.96 | 0.99 | 0.96 | 1.04 | 0.92 | 0.96 | 1.00 | 0.98 | 0.89 | 0.94 | 1.07 | 0.94 | 0.86 | 0.85 | 0.92 | 0.98 | 0.93 | 0.80 |
| | A | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | 0.10 | <0.10 | 0.10 | 0.10 | 0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 |

**APPENDIX Q BALSOL WASH SOLUTION GROUP (n=20) – DATA COLLECTION SHEET**

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $[PO_4^{3}]$ (mmol/l) | B | 1.15 | 0.92 | 1.09 | 0.94 | 0.73 | 1.12 | 1.00 | 0.95 | 1.03 | 0.72 | 0.61 | 0.50 | 0.77 | 0.81 | 0.73 | 0.71 | 0.51 | 0.94 | 0.56 | 0.74 |
| | A | 0.11 | 0.10 | 0.14 | 0.13 | 0.07 | 0.10 | 0.12 | 0.06 | 0.06 | 0.10 | 0.09 | 0.07 | 0.12 | 0.11 | 0.09 | 0.07 | 0.08 | 0.08 | 0.12 | 0.08 |
| Lac. (mmol/l) | B | 3.10 | 2.10 | 6.10 | 1.90 | 3.90 | 8.70 | 5.50 | 3.70 | 2.40 | 5.0 | 4.80 | 4.70 | 2.70 | 4.60 | 3.10 | 3.70 | 4.30 | 3.30 | 4.90 | 7.80 |
| | A | 1.30 | 1.20 | 2.20 | 1.20 | 1.60 | 2.40 | 1.90 | 1.30 | 1.20 | 1.60 | 1.70 | 1.70 | 1.0 | 1.50 | 1.30 | 0.90 | 1.50 | 1.50 | 1.70 | 2.30 |
| Glu. | B | 11.60 | 16.40 | 14.20 | 8.20 | 13.50 | 14.40 | 16.20 | 8.20 | 13.30 | 12.10 | 8.10 | 9.20 | 9.90 | 12.60 | 13.60 | 12.80 | 7.10 | 7.40 | 16.90 | 15.30 |
| | A | 2.60 | 2.50 | 2.20 | 1.10 | 1.30 | 1.80 | 2.50 | 0.30 | 2.40 | 2.20 | 0.90 | 1.10 | 1.50 | 1.50 | 2.0 | 1.0 | 0.70 | 1.0 | 2.0 | 1.60 |
| Osmo. | A | 269 | 305 | 275 | 229 | 313 | 279 | 297 | 273 | 288 | 298 | 298 | 271 | 295 | 309 | 272 | 298 | 281 | 307 | 318 | 300 |
| | B | 266 | 264 | 275 | 263 | 302 | 267 | 267 | 267 | 262 | 258 | 265 | 348 | 266 | 268 | 268 | 265 | 261 | 278 | 271 | 275 |
| Alb. | B | 24 | 19 | 23 | 26 | 23 | 22 | 21 | 21 | 32 | 22 | 22 | 24 | 24 | 26 | 23 | 22 | 20 | 30 | 19 | 21 |
| | A | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 |
| T.Prot. | B | 42 | 33 | 37 | 48 | 37 | 41 | 39 | 44 | 54 | 38 | 38 | 39 | 44 | 50 | 39 | 42 | 33 | 51 | 31 | 33 |
| | A | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 |
| HCT (%) | B | 30.0 | 27.0 | 29.0 | 27.0 | 29.0 | 26.0 | 26.0 | 27.0 | 31.0 | 26.0 | 24.0 | 27.0 | 29.0 | 26.0 | 30.0 | 28.0 | 27.0 | 34.0 | 26.0 | 22.0 |
| | A | 61.0 | 62.0 | 64.0 | 62.0 | 65.0 | 61.0 | 62.0 | 54.0 | 61.0 | 67.0 | 62.0 | 57.0 | 64.0 | 58.0 | 63.0 | 67.0 | 68.0 | 59.0 | 69.0 | 66.0 |
| Hb (g/dL) | B | 9.90 | 8.90 | 9.80 | 9.0 | 9.70 | 8.80 | 8.50 | 9.10 | 10.30 | 8.80 | 7.90 | 9.10 | 9.50 | 8.70 | 10.10 | 9.20 | 9.0 | 11.40 | 8.80 | 7.20 |
| | A | 20.30 | 20.60 | 21.30 | 20.70 | 21.50 | 20.30 | 20.50 | 17.90 | 20.40 | 22.20 | 20.70 | 19.0 | 21.10 | 19.40 | 21.10 | 22.40 | 22.80 | 19.70 | 23.0 | 22.10 |

**APPENDIX Q BALSOL WASH SOLUTION GROUP (n=20) – DATA COLLECTION SHEET**

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $TCO_2$ (mmol/l) | B | 22.50 | 25.10 | 22.10 | 25.40 | 22.0 | 24.30 | 20.10 | 19.40 | 24.0 | 16.60 | 24.60 | 26.90 | 20.80 | 23.90 | 26.0 | 27.80 | 21.70 | 24.50 | 21.10 | 19.90 |
| | A | 23.80 | 23.70 | 23.90 | 24.80 | 25.0 | 24.20 | 23.50 | 23.0 | 24.90 | 24.40 | 24.10 | 24.10 | 24.80 | 24.80 | 24.20 | 25.0 | 24.30 | 24.20 | 24.20 | 24.30 |
| $HCO_3^-$ (mmol/l) (Actual) | B | 21.50 | 24.0 | 21.10 | 24.20 | 20.90 | 23.30 | 19.20 | 18.80 | 23.10 | 16.10 | 23.70 | 25.90 | 20.10 | 22.90 | 25.10 | 26.70 | 20.90 | 23.40 | 20.30 | 18.90 |
| | A | 23.10 | 23.0 | 23.10 | 24.20 | 24.20 | 23.80 | 22.90 | 22.40 | 24.20 | 23.90 | 23.60 | 23.50 | 24.40 | 24.20 | 23.40 | 24.70 | 23.80 | 23.60 | 23.80 | 23.90 |
| Pre - wash Vol.(ml) | | 2329 | 3807 | 1545 | 1560 | 3442 | 1709 | 2267 | 3016 | 2871 | 3058 | 4054 | 2605 | 2006 | 1720 | 2594 | 2620 | 4237 | 1863 | 1533 | 3404 |
| Post - wash Vol.(ml) | | 464 | 415 | 447 | 396 | 345 | 346 | 511 | 685 | 631 | 508 | 724 | 623 | 325 | 386 | 352 | 136 | 368 | 591 | 192 | 502 |