SALVAGE OF RED BLOOD CELL UNITS CONTAINING HAEMOLYSIS BY A CELL WASH PROCESS IN GEORGE BLOOD BANK

By

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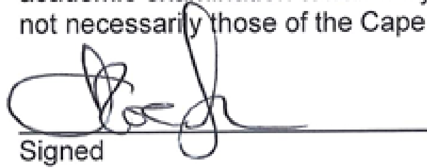
Bellville June 2023

DECLARATION

I, Charlotte Roelofse, declare that the content of this dissertation/thesis represents my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination toward any qualification. Furthermore, it represents my own opinions, not necessarily those of the Cape Peninsula University of Technology.



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ABSTRACT

Title: Salvage of red blood cell units containing haemolysis by a cell wash process in George Blood Bank

Background

Premature haemolysis in red blood cell (RBC) units received at the George blood bank became problematic. A saline wash method was successfully utilized in other countries to restore red blood cell (RBC) units containing haemolysis, to remove waste products of haemolysis. This saline wash method has not been used in South Africa.

Methods

The content of the quad bags, used for blood donation, was investigated to determine the cause of premature haemolysis. The volume of the saline, adenine, glucose and mannitol (SAGM) preservatives as well as the citrate, phosphate and dextrose (CPD) anticoagulants were verified to investigate the compliance with the package insert. The haematological autoanalyzer was used to develop a colour chart to be used with the HemoCue at George blood bank to select premature haemolysed RBC units received at the George blood bank.. The RBC units were divided into five groups: a control group which were not haemolysed nor washed (NHNW), non-haemolysed units which were washed and reconstituted in saline (NHWSI), non-haemolysed units washed in saline and reconstituted in SAGM (NHWSGM), haemolysed units washed and reconstituted in saline (HWSI) and haemolysed units washed in saline and reconstituted in SAGM (HWSGM). These units were stored in accordance with WCBS storage guidelines for a period of two weeks. All five groups were initially subjected to a full blood count (FBC) and their haemoglobin, RBC count, haematocrit, mean corpuscular volume, mean molecular haemoglobin, mean corpuscular haemoglobin concentration, white blood cell count, platelet count, RBC distribution width-standard deviation and RBC distribution width - coefficient of variation (RDW-CV) were determined as well as one and two weeks subsequently.

Results

The quad bags had inconsistent CPD volumes and were not compliant to the package inserts, whereas the SAGM volumes were compliant. The FBC results at the initial stage as well as after one and two weeks showed that the waste products of haemolysis could be removed and that the RBC remained undamaged, and retained their shape and size. The research question was answered, more cells were lost in the cell wash process for units with premature haemolysis present. About 24% of red blood cells were lost, were in red blood cell units with

no haemolysis present the cells in literature is estimated at 20%. The SAGM preserved the cells better than the 0.9% Saline, we only had one outlier that stated differently.

Conclusion

Inconsistent anticoagulant levels in the quad bags impacted on the premature haemolysis in the red blood cell units received at the George blood bank. Premature haemolysis is an ongoing challenge, and the colour chart could be useful for visual inspection of haemolysis of the RBC units. This study showed that the washing of premature haemolysis in red blood cell units is viably even when stored for up to 14 days.

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Thank you, mommy, for the help with the References. Love you.

DEDICATIONS

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ABBREVIATIONS AND ACRONYMS

AABB	American Association of Blood Banks
Autoanalyzer	Haematological autoanalyzer in Cape Town
CPB	Coronary Pulmonary Bypass
CPD	Citrate, Phosphate, Dextrose - anticoagulant
CRP	C-reactive protein
HCT	Haematocrit
Heterologous transfusion	This involves transfusion of blood and blood components from donors.
Hb	Haemoglobin
HWSGM	Haemolysis washed substituted with SAGM
HWSI	Haemolysed washed substituted with 0.9% Saline
LRBC	Leuco-reduced packed red blood cell
MCHC	Mean corpuscular haemoglobin concentration. The average concentration of haemoglobin inside a single red blood cell.
MCV	Macro cell volume
NHWSGM	Not haemolysed, washed substituted with SAGM
NHWSI	Not haemolysed, washed substituted with 0.9% Saline
PBM	Patient Blood Management
PLT	Platelet count
RBC	Red blood cell
RDW-CV	Red cell distribution width.
RDW-SD	Red cell distribution width. It measures the range in size and volume of the red blood cell.
SAGM	Saline, adenine, glucose and mannitol - preservative
SANBS	South African National Blood Service
TRALI	Transfusion associated acute lung injury
WBC	White blood cell count
WCBS	Western Cape Blood Service

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CHAPTER ONE

INTRODUCTION

1.1 Background

In 2019 when the research toward this thesis was proposed it was noticed within the Western Cape Blood Service (WCBS) that there were increasing numbers of red blood cell (RBC) and leucocyte-poor packed red blood cells (LRBC) units presenting with haemolysis. This haemolysis was found to be especially troubling when occurring upon receipt of product from headquarters at the George WCBS branch. The standard as set by the Council of Europe for haemolysis in red-packed cells is 0.8%. When the haemolysis exceeds 0.8%, units must be discarded causing unnecessary wastage of a scarce and valuable product. The cause of the increased number of RBC units with haemolysis within the WCBS has at this time been investigated, but a definite cause has not been found and thus, there was no solution to prevent or rectify the problem.

The study done by Dr Bellairs and S Sutton produced a haemolysis chart visual inspection of haemolysed units, as they also could not eliminate the prevalence of premature haemolysis (Sutton & Bellairs, 2017). In the year this research was done data was collected of the number of red cells units that had premature haemolysis present and had been discarded, this had an adverse financial implication on the WCBS, an average of 11 units per month from January to August 2021 at an approximate cost of R200 000 to R250 000 was lost at George blood bank of the WCBS. Of the RBC and LRBC units sent to George from the Cape Town Branch, 1.5% was haemolysed when it was received at the George blood bank. For the year from August 2020 to August 2021; 174 RBC and 42 LRBC units were haemolysed. An amount of 68,237 blood units were received from Cape Town in this period. The financial impact for this period alone was approximately R516,392.70 which is significant given that the WCBS is a not-for-profit organization. These numbers only represent the George branch and do not include the loss of product and cost of the remaining branches of the WCBS. The loss that implicates financial loss is not the only concern in blood transfusion as blood is a scarce commodity donated by volunteers. The loss of these units is also a loss in that the donor's intent to save a life with their donated unit, is lost. The cost of RBC and LRBC units includes the blood collection pack, transport, salaries, and post-donation safety testing of the donated blood. At the George branch of the WCBS, the processing of donated blood is limited to the separation of the blood into components, for example only RBC and plasma which is then transported to the Cape Town branch.

1.2 Research Problem

In George Branch of the Western Cape blood service blood stock, which includes red blood cell and filtered red blood cell units as well as baby bags are received from the headquarters in Cape Town. These units are used for crossmatching for our patients from various patients with different diagnosis. There was an increase of premature haemolysed units received at George Branch, which meant that these units had to be discarded and not be able to be used. This is a major problem for a Blood bank 400 km from the source of providing blood stock for our patients. This means that we will have a shortage of adequate blood to provide a service to our patients. The distance makes it very difficult for us to obtain blood easily and in a short time in dire emergencies. We use a courier service to do the transport of blood and there are only specific times for transport. Every single unit received in our blood bank is very important for our stock levels. The increase of premature haemolysis in our stock was an incident that could not be explained. Washing techniques which may remove waste products associated with haemolysis from affected units, allowing the units to be saved from being discarded would thus offer a potential solution. There is however, no such process used on the WCBS as of yet and research would be needed to confirm whether such a process would be feasible.

RBC units and LRBC units delivered to, or stored in the blood bank at times found to be haemolysed for possible reasons. These could involve processes such as donation, processing of the donated packs or transport. These processes would not in general give rise to haemolysis, unless there are defects in the process; such defects could include: Volume of preservative/anticoagulant mixture which may be out of balance with pack volume. A broken cold chain, or improper packing of units in transport hampers post clinic. Rough handling of units by processing staff, including tossing, or the aggressive shaking of units. The walk-in fridge could have cold spots that lower certain units below the accepted threshold of 1°C. These are but some of the factors that n cause unit haemolysis and cause units that are relatively fresh to have premature haemolysis and thus be deemed to be un-transfusable. Once haemolysis occurs, the unit undergoes structural changes and the associated waste products, such as free haemoglobin, permeate the RBC or LRBC. This has been shown to increase the morbidity and mortality rate in patients, and would thus increase the health burden in the country (Rapido, 2017). The decrease in microvascular density may induce vasoconstriction by scavenging nitric oxide. These units will then be discarded as the RBC are seen as unsafe for transfusion. This does not only have a financial implication for the WCBS, but also a social aspect for the donor. If donors know that their blood is being discarded this

will likely have a negative effect on the frequency of blood donations. A donor does not only donate blood, they give their time and good will to help a patient in need. Beyond this, the waste of precious resources cannot be condoned in not for profit organisations.

Washing techniques which may remove waste products associated with haemolysis from affected units, allowing the units to be saved from being discarded would thus offer a potential solution. There is however, no such process used on the WCBS as of yet and research would be needed to confirm whether such a process would be feasible.

1.3 Aim

To investigate whether anticoagulant levels were a potential reason for premature haemolysis in RBC units.

To create a colour chart specifically for the George region, to assess haemolysis in correlation with utilizing the equipment that is available in George.

To investigate the possibility of washing packed red blood cell units with saline to remove the waste products associated with haemolysis.

To investigate the possibility of storing the washed RBC units for longer than 24 hours post-wash.

1.4 Research Questions

I. Is it possible for red blood cell units with premature haemolysis to be salvaged by a cell wash method?

II. If there are different concentrations of premature haemolysis present in red blood cells, would this affect the number of cells which are lost in the saline wash process?

III. Will there be enough cells left in the red blood cell unit, after the wash process to allow for viable transfusion?

IV. If a closed wash system is used, will the unit's expiry date be extended?

V. If a closed system is used for the wash process and SAGM is added to the red blood cell units, will these units have an expiry date of at least 14 days?

1.5 Objectives

The main objective of this study was to investigate the possibility of saving donated red packed cells containing haemolysis, by washing the red blood cell units, thereby removing premature haemolysis.

The first study investigated the ratio of preservatives and anticoagulants in red blood cell units to determine whether this may be a cause of haemolysis, whilst the second study focused on

finding new, more efficient ways to measure haemolysis in a rural setting, not only for the purpose of this study, but also for future use in the blood bank.

A secondary objective of this study was to test whether the addition of SAGM instead of Saline could extend the expiry date of RBC units after the wash process was completed.

The third objective of this study was to investigate the preservatives and anticoagulants used in quad packs to determine its potential role in premature haemolysis.

Finally, the fourth objective was to determine whether washed red blood cells could be stored for 14 days using SAGM as a preservative post wash, control on the results obtained from this study.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

When red blood cells are stored over a period of time, they undergo metabolic structural changes, which are referred to as “storage lesions” (Yoshida 2019), (Bıçakçı & Olcay 2014). The glucose in the stored blood will be used by the red blood cells as fuel which leads to the levels of 2.3-diphosphoglyceate and ATP levels to decrease while the potassium levels increase. The loss of the red blood cell membrane causes haemolysis and the forming of micro particles (Chang et al., 2017). This can cause adverse blood transfusion reactions (Kim-Shapiro, Lee and Gladwin, 2011).

Washing of red blood cells (RBC), can be beneficial for patients with various diagnoses and prognoses. One example of this benefit is that the reduction in extracellular potassium, derived from storage lesions, will reduce the risk of cardiovascular incidents where blood is transfused to patients with cardiovascular disorders (Garcia-Roa 2017). Febrile and non-febrile reactions can be reduced by washing red blood cell units. By washing the units, the cytokines are removed that are produced by white blood cells (Welsby 2021). Filtering these units before washing will further reduce the incidence of febrile reactions (Simnancas-Racines 2015). The majority of literature indicates that the pack supernatant of aging units is the largest contributor to transfusion-related immunomodulation in patients, which had cardiac surgery (Lako et al., 2015). Transfusion of red blood cells have the ability to alter the immune response of the immune system in patients. Choosing prewashed products may lessen the deleterious effects and improve the outcome of all patients, including preterm infants, by washing and removing the inflammation causing substances (Lannan et al., 2013). Looking at the animal models washing of red blood cells shows blunting of pro-inflammatory response post-haemorrhage when compared to unwashed red blood cell transfusion as the micro aggregates are removed by washing and adding saline instead of preservatives (Belizaire et al., 2023). This study showed that washed cells are more beneficial than unwashed cells for cardiac patients (Keir et al., 2016), (Dave et al, 2014) and (Cholette et al., 2017). As a rule, we do not wash red cell products in South Africa, except if it is specifically requested in patients who have been presented with transfusion reactions where filtered blood is not an appropriate solution (Cardigan et al., 2020). In addition, there is no literature to indicate that this method has been tested as a method to salvage red blood cell units with haemolysis in South Africa. A study done by Cholette et al 2017 found that cardiac surgery patients who received washed RBC had a better survival rate, which was attributed to less systemic inflammation than the group who received unwashed red blood cells (Arif pp.598–602) earlier study done by Schmidt et al.,

2016 showed that by transfusing washed RBC units to patients that cannot metabolize vasodilators, the incidence of acute hypotensive transfusion reactions was reduced.

2.2 Blood transfusion in South Africa

There are currently only two Blood Services available in South Africa, namely the Western Cape Blood Service (WCBS) who solely provide blood for the Western Cape area, and the South African National Blood Service (SANBS) who provides blood for the remainder of the country. The Western Cape Blood Service is a non-profit organization, and is governed by a board of directors and a donor panel. The directors include Dr. Gregory Bellairs and Ms. Nikki du Toit. The mission of these blood services is to provide adequate and safe blood products that are cost effective, while still maintaining a high standard of quality (Bird et al., 2014).

2.3 Transfusion: Indications for the use of washed red blood cell units

Transfusion of red blood cells is primarily used in the restoration of the oxygen-carrying capacity of the body (Klein, Spahn and Carson, 2007). Red blood cells are washed for different reasons, including: to remove excess potassium, cytokines and other allergenic proteins from the supernatant of the donated unit (Schmidt 2016). In a previous study it was shown that neonates that are transfused with washed filtered red blood cell units had a better survival rate than the group that only received filtered red blood cells (Crawford et al., 2022). In patients undergoing cardiac surgery, who required top-up blood post-surgery, it has been shown that these patients recover at a better rate when they receive washed red blood cell units in comparison to those who received normal or leuco-depleted red blood cells units (Cholette, 2017). This improved recovery rate was attributed to a reduction in post-operative inflammation as well as a decreased need for post-surgery transfusions. The survival rate of patients with Acute Myeloid Leukaemia was also shown to be better after they were transfused with washed RBC units by using the Kaplan-Meier Method. The survival rate is approximately 78% in recipients who received washed transfusions versus 42% in the patients who received unwashed RBC units. At the 100-day follow-up, the survival rate of patients in the group who received washed red cell units was 100%, while the patients who received unwashed cells were 90 % (Reber 2017). Washed RBC cells are normally given to patients who present with anaphylactic reactions. This is normally observed in patients who are IgA deficient. Antibodies directed against donor proteins such as haptoglobin, C4 latex, drugs, and food may also cause anaphylactic reactions (Cardigan et al., 2020). Anaphylactic reactions associated with blood transfusions are caused by anti-IgA antibodies in the recipient that react with IgA in the donor product. In such a case, IgA-deficient blood components need to be provided, or the product must be washed to remove the IgA in the supernatant (Matthew & Anjum 2021).

In storage when blood ages the packed red cells develop storage lesions. This lesion includes leakage of chloride and potassium from the red blood cells. There is also a deplension of 2.3-diphospoglycerate and adenosine triphosphate. Other elements that are lost includes phospholipids and cholesterol. The flow of blood is slowed down with the exposure of phosphatidylserine, elaboration of lipid mediators, glutathione loss, autoxidation as well as the depletion of haemoglobin to methaemoglobin. Free haemoglobin, heme and iron, some within the microparticles may be contributors that causes significant toxicity. By removing this free haemoglobin using a wash process makes the blood safer for transfusion (Schmidt 2016).

2.4 Transfusion complications

In the Western Cape of South Africa, blood products are produced and supplied by the Western Cape Blood Service (WCBS). As such, the WCBS needs to provide the necessary mechanisms and infrastructure for the processing, testing, storing and distributing of donated units of blood while still upholding the strict quality requirements essential for such an endeavour. Blood is a scarce and valuable therapeutic tool, because it requires medically acceptable donors to donate their blood willingly, without compensation. Although it is relatively safe and easy to donate blood, blood donors tragically make up only an estimated 1% of the South African Population (Gallacher, 2022). Potassium, free haemoglobin and other metabolic waste products are released from lysed cells and are ultimately transferred to the patient's plasma upon transfusion. High levels of these waste products being transfused to patients are associated with clinical complications of the renal and cardiovascular systems (Makroo 2011). Haemolysis also means that the amount of oxygen carrying red cells of the transfused unit will be diminished (Adamczyk and Adamczyk-Sowa, 2016). To our knowledge, no studies have been published in South Africa, proposing methods for the recovery of red cell units containing haemolysis. As South Africa is a middle-income country where blood is scarce, the availability of quality donors can be a challenge as well as the transfusion itself. Taking these facts into consideration, a study examining blood recovery in South Africa would be warranted.

Transfusions also have the risk of transmitting infections, such as Hepatitis B virus, Hepatitis C virus, and human immunodeficiency viruses amongst others, from the donor to the recipient (Dwyre, Fernando and Holland, 2010).
Allergenic reactions (ATRs), which is a common complication of blood transfusion, may range from mild to severe. Mild (urticarial) reactions are presented as pruritus or hives; which normally occurs when the patient has been sensitized to antigens contained in the donor unit. These patients which normally present with hives may also develop bronchospasm stridor, hypotension or gastrointestinal symptoms. Anaphylaxis usually occurs upon the transfusion of proteins in donor plasma, to which the recipient was previously sensitized to (Hirayama, 2012).

This type of reaction is dose dependent and the antigens are soluble. Research has shown that 1% to 3% of transfusion reactions are allergenic in nature (Savage et al., 2012).

Reactions where the patient's body temperature rises at least 1°C above 37°C within 24 h after transfusion are categorized as febrile non-haemolytic transfusion reactions (FNHTR). Symptoms may involve chills, rigor and discomfort. The transfusion of leuco-depleted red blood cells has reduced the number of febrile reactions (Simancas-Racines et al., 2019). Patients who receive platelet transfusions are more likely to have this type of reaction than those receiving RBC. Febrile reactions have been linked to two main causative factors, the first associated with a release of antibody-mediated endogenous pyrogens and secondly the release of cytokines. The main cytokines involved are Interleukin -1, Interleukin- 6, Interleukin – 8 and tumour necrosis factor (Harmening 2005). Transfusion of a large number of stored blood where biochemical reactions have taken place in the units due to age, may be considered a storage lesion. Storage lesions include decreased 2, 3 diphosphoglycerate (2, 3 DPG), increased potassium and loss of the shape of the membrane of the stored RBCs. Sodium citrate, which is used in the anticoagulant, is toxic in large amounts and may thus contribute to these effects in transfusions where large volumes are transfused (Ingram et al., 2014). Traditionally, the rule of "10/30" was followed for RBC transfusion, according to which a haemoglobin (Hb) level of 10 g/dl or a haematocrit of 30% was recommended in surgical patients. Over the years, the trigger for transfusion has become more conservative or restrictive. In addition, the decision to transfuse RBCs is controlled not only on the laboratory values, but also on the objective evaluation of the patient's clinical condition and their ability to compensate.

2.5 Processing and handling of red packed cell units

2.5.1 Donations

WCBS utilizes Terumo Teruflex quad packs for blood collection. These packs are stored at temperatures between 2°C-35°C. Packs should not be used if the solutions inside the packs are not clear, and should be kept out of direct sunlight to prevent premature spoilage during storage. Packs should furthermore be discarded if any damage is visible or if it has been soiled by external substances. If the pack foil has been opened, then the packs should be used within the next 15 days. The quad packs have a shelf life of 3 years.

In the quad pack the donor donates 450ml-500 ml of blood in one of the bags with a pilot bag, where the specimens are taken from. After donating, the units will then be processed in the components section of the WCBS.

During processing of donated units in the components section, the hampers' temperature will be taken before the units will be scanned into the data control. At this time, the temperature

should be between 2 and 36°C. Then the units will be weighed and the weight will be marked on the pack.

The acceptable weight for a pack is between 741-836g. If the weight of the pack is under 741g the pack will be discarded as short bled and if the unit weighs over 836g it is over-bled and will also be discarded. Labelling of the units will now be done with blood groups if known and the component packs will be labelled with RP10, FFP or RPO's. The units will be spun down in the centrifuge to make the various components as indicated the date the units were donated and the anticipated expiry date is then placed on the pack, controlled on the product specifications and recommendations of Practice for Blood Transfusion in South Africa.

In order to produce whole blood units, packs are for 12 minutes at 4 °C at speeds of 4795g RCF and 3800 RPM, using 6000i cryofuge.

When the spinning process is completed the whole blood unit will be transferred (gently, to prevent the mixture of plasma and red cells) to the T-ACE where separation of the whole blood will take place.

The unit is placed in the T-ACE, with the tubing in the relevant position. The T-ACE separates whole blood units into different components using pressure, this involves the rupture of the fragile seals in the unit packs.

Traceability of the unit is ensured by scanning the barcode and the information is stored on the T-ACE computer. Processing of a unit takes approximately 3 and a half minutes if there are clots present in the primary bag it may delay or stop the separation and affected units discarded.
Blood pack units will be placed in the cold room between 1˚C-6˚C after completion of production processes. The Plasma product from the above process, will be placed in the snap freezer where the plasma will be frozen solid within 45 minutes and stored at -40 °C in the freezer room (Com-W03). In Appendix D the donor questionnaire can be found.

2.5.2 Storage of blood

The Standard of practice still recommends storage of red blood cell units for up to 42 days if citrate dextrose-phosphate (CPD) is used for an anticoagulant and sodium, adenine, glucose, mannitol, and 376MOsm/l (SAGM) is used for the preservative. The red blood cell units should be stored in a good well-ventilated fridge which is maintained at a temperature between 1°C and 6°C (D'Alessandro 2010).

There is still a lack of a universally accepted standard criteria on evidence that reflects the molecular changes which occur during prolonged storage on red blood cells. Standards that are currently used for patented new additive solutions in the USA (United States of America), and recommendations of the European Counsel have two parameters, one is the level of free haemoglobin that must be below 0.8% at the end of the storage period and the second is the survival rate of the transfused cells which should have a survival rate of 75% after 24 hours. This method differs from our research, where we did full blood counts on all the units after the wash process. This method also indicates if the red blood cells in storage post wash has survived for the 2 weeks. During storage red blood cells undergo morphological changes, these include slowing down of the metabolism of these cells with a resulting decrease in adenosine triphosphate production. Due to this, acidosis will occur within the stored units, which will lead to a decrease in the concentration of 2, 3 diphospoglycerate. In addition, the cells lose their function, because membrane cation pumps will stop functioning due to lack of ATP and thus intracellular potassium will increase with a concomitant increase of sodium within the cytoplasm of cells. The decrease of the pH and the increase of potassium and lactate start in the first few hours of storage of red blood cell units. The more extreme changes valuable are morphological changes which start to occur after days or weeks within storage of red blood cells. Considering all of these morphological changes' effects the efficiency and safety of long-term storage of red blood cells. Cells stored for long periods have the potential risk of releasing exogenous toxicants in the red blood cells, the oxygen carrying capacity are then be reduced. (Bennett-Guerrero 2014). Proper storage of red blood cell units is very important to protect the cells from undergoing metabolic damage. There is a 30-minute rule which states that a unit of red packed cells which is left outside a controlled storage temperature for more than 30 minutes should be discarded and not be placed back in storage for reuse (Carson et al., 2016), (Ramirez-Arcos et al., 2013). Units that were removed from controlled storage for transfusion should also be infused within 4 hours to limit metabolic damage to cells (Brunskill 2012).

In one of the twenty-three studies done in October 2010 on the quality of RBC by the Cochrane Library, National Health Device Blood and Transplant's Transfusion Evidence Library, MEDLINE, EMCONTROL, transfusion medicine and blood bank experts. Red blood cell units contained an average of 19 bacterial contamination after exposure to temperatures higher than 4 °C ± 2°C for between 20 minutes to 42 days. Conclusion of the study showed that overall temperature exposure did not have adversely affected the quality of the RBC units or bacterial contamination (Brunskill 2012), (Damgaard et al., 2015).

2.6 Quality

Christiaan Seidl, the Quality Management Working Party Chair and Vice Medical Director from German Red Cross BDS, Frankfurt, Germany had an International Blood Transfusion (ISBT)

2023 about quality systems. He explained that quality systems provide mechanisms to ensure that safe blood is available for all transfusion requirements. A series of steps to ensure quality control need to be followed in blood transfusion. In order to achieve a quality system, the implementation should incorporate the principles of good practice and quality risk management. The first is in selecting donors then collecting and processing blood donations, testing of donor blood and samples of patients, as well as the issuing of compatible blood for transfusion. An effective quality framework is one in which activities are set up and standardized and in which outcome is constantly monitored to ensure constant improvement (ISBT, 2023). The World Health Organization said that quality Blood transfusion is a key part of modern health care. Adequate blood supply for patient's requirements and the quality of blood products for clinical use is the responsibility of the national blood program. The quality of the products must be appropriate, consistent, safe and clinically effective. The strategy in which this can be achieved is a nationally - coordinated well-organized Blood transfusion service (BTS). Donors should be voluntary non-remunerated regular blood donors that are a low - risk for transfusion transmittable diseases. All donated units should be tested for transfusion transmittable infections, blood grouping and compatibility testing. The quality system should include all aspects of traceability, from recruitment and selection of blood donors to the transfusion of the products to blood product recipients. This system should show the structure, capabilities and needs of the BTS, as well as the needs of the patients and hospitals it serves (WHO/BCT/02.02. Printed July 2002).

When a quality management system is set up the system should be identified and described and the findings that reflect the system or organization should be written down in documents. These documents are called standard operating procedures (SOPs). In order to monitor systems and constantly improve performance, a monitor system should be in place. In blood establishments where donor vigilance and product quality come together these SOPs are for personnel and organization a quality policy (Strengers, 2011).

The key elements of a quality system should include: organizational management, standards, documents, training and assessments. A quality system, external quality assessments and education and training of staff can significantly reduce risks associated with blood transfusion. All staff should understand the importance of quality and the consequences if there is failure in the quality system. The Quality Management Project (QMP) was established in 2000 under the authority of World Health Day, which was dedicated to the theme of blood safety with the slogan "Safe Blood Starts with Me – Blood Saves Lives" The project is aimed at the need to adopt the principles of quality management in all areas of blood transfusion services.

2.6.1 Patient Blood Management

The definition of patient blood management (PBM) involves multidisciplinary application of evidence-controlled surgical and medical concepts aimed at 1st screening for diagnosing and

appropriate treatment of anaemia. The second is minimizing surgical procedures and blood loss and managing coagulopathy bleeding. The 3rd is supporting the patient while the appropriate treatment is initiated to improve patient outcome (SABM 2015). PBM have the ability to reduce transfusions and safe costs (Franchini et al., 2019), (Farmer et al.,2015). In blood transfusion, blood management is very important, because of the limited availability of safe RBC units and the negative effects compromised blood can have on patients if transfused (Müller et al.,2015). Patients that are diagnosed with anaemia only need the red blood cells for the oxygen capability. Whole blood will not be transfused, because the patient does not need the volume associated with whole blood, only the red blood cells (Kashefi et al., 2018). PBM has the ability to reduce the transfusion of donor blood and reduce the cost of care. The world health organization defines anaemia as a cut-off value of 13g/d for males and 12g/dl for females as a normal haemoglobin level. Patients with haematological cancer are 40% more likely to be anaemic. In PBM it is important to appropriately treat patients. Depending on the patient's characteristics and clinical symptoms a level of <9/dl is used as a cut-off for transfusion. The Hb rises with +/- 1g/dl if one unit of RBC is transfused. An Hb of 6g/dl is an indication to transfuse blood. If the Hb of the patient is between 6-10g/dl, other factors must also be considered, such as the patient's risk of ischemia, rate and volume of blood loss, age and how seriously ill the patient is. Transfusion of patients occurs if there is acute blood loss when there is a 30% blood volume loss. The standard guidelines state an Hb level <7g/dl is sufficient in a post-operative patient, if ischemia is a risk to the organ or if hypoxia is present (Yaddanapudi & Yaddanapudi 2014). In patients with a heart condition an Hb of <8g/dl is an indication for transfusion according to the standard of practice (Lotterman and Sharma, 2023). In transfusion medicine, the decision to transfuse a patient is a well thought through process, because of transfusion-related complications, such as iron overload, viral and bacterial infections and immune injury are often experienced (Despotis, Zhang and Lublin, 2007). The half-life of red blood cells in the body is 120 days. In transfusion practice, if four units are transfused to a patient, one will be eliminated by the time the last unit is transfused, because 25% of one unit will be removed from the patient's body after 24 hours when the transfusion started. The reason for this is that aged red cells are present in amongst the red blood cells that were donated by the donor (Liumbruno 2009). Transfusing washed red blood cells units may not increase the Hb by 1 g/dl, as red blood cells are lost in the wash process, and this can cause an increase in the units transfused. (Schmidt 2016). Research has indicated that a patient will recover sooner with fewer complications when they receive their own blood either by cell savers or by autologous transfusion where the patients donate for themselves before the operation (Kelly et al., 2016). Blood management, for this reason, is important for the treatment of patients that are anaemic with as few heterologous transfusions as possible and to establish other alternatives for the treatment of these patients.

2.7 Specifications of blood components

A whole blood unit of acceptable quality contains 405-495 ml (mean 470ml) of blood, collected into 63 ml of citrate phosphate dextrose (CPD) anticoagulant and 100 ml of SAGM. A unit of red blood cell concentrate, where the buffy-coat was removed and suspended in additive solution, should have a haematocrit between 50% and 70%. Each unit should at least contain 43g haemoglobin (Hb) at the end of the preparation procedure. The RBC units should retail all of the red blood cells except for 30ml, which contains the buffy coat, which is removed. When red blood cell units are stored in SAGM the expiry date is 42 (Liumbruno 2009). Washing red blood cells with a gravity-driven system that uses 0.5ml/min of blood gives a final haematocrit of 36, 7 ± 3.4% (32.3-41.2%), n=10) the amount of haematocrit that is lost is 3.4 ± 0.7% (2.4-4.3%, n = 10), at the end of the wash process 80% of the free haemoglobin and 90% of total free protein is removed. Washing the RBC units improved the red blood cell's ability to perfuse an artificial microvascular network by 20% (Khanal 2017).

In the work instruction for components (COM-S02) for the Western Cape Blood Service is followed that describes the prevention of temperature-related unit loss as follows.

- Whole Blood units are processed into their various derived products, each of which has its storage temperatures and expiry dates.
- Packed red cell-derived units, the focus of this thesis, are stored between 2 to 6 degrees until issued via Cape Town stock control to the various blood bank locations within the Western Cape.
- To keep the units within the cold chain parameters, they are transported via insulate containers of 20 units each with standardised ice bricks, or eutectics, to keep them within the 2 to 10-degree transport temperature.
- Before departure from stock control, the temperatures of the units are recorded on the stock control computer system and recorded again upon receipt by blood bank staff, at the final destination.

Blood bank protocol states the following parameters in terms of unit temperature:

1. Red blood cell units may not be stored within the transport containers for longer than 24 hours. If this time is exceeded, the decision of use relies on whether the temperatures of the units are still below 10 degrees. The HOD will make the final decision of whether units are usable or not.
2. Units may not be left unrefrigerated for longer than 45 minutes. If units are left unrefrigerated for longer than that they will have to be discarded.

3. Any break in the cold chain is to be investigated and followed up. All fridges are consistently monitored 24/7 via two different electronic systems. Any breakdowns or fluctuations are immediately and thoroughly dealt with and corrected.

Therefore, no temperature-related haemolysis should be present. No blood received or sent will be out of range. On receipt, the unit's temperature is taken via a calibrated thermometer and recorded on the blood management system that will keep the record indefinitely. When units are sent to headquarters the temperature of the fridge in which the red cell units are stored is taken and recorded on the system. This is how the temperature is controlled. All the equipment is validated and has the appropriate certificate for calibration. All units in the blood bank are controlled this way, this includes the units that were used for this study.

The next possible reason for haemolysis to be investigated was the volume of preservatives and anticoagulants found in each quad-pack donating set. It was necessary to investigate whether they could be insufficient, or vary in amount in some or all sets. If there is not enough citrate phosphate dextrose (CPD) in the packs the packs may present with clots. This is problematic when the unit is made in components.

If there are clots in the pack, blockages may occur during transfer from the collection bag to the storage bag. The blood is transferred between the bags using a connecting tube and pressure. Clots may obstruct the tube opening, leading to increased pressure in the collection pack. This in turn will transfer the pressure to the cells in the pack, leaving the packed red blood cell unit with haemolysed cells.

Figure 2.1: Separation of whole blood in components with the T-ACE II.

2.8 Measuring of haemolysis

In a Journal Frontiers ^{51}Chronium labelled cells are used to measure the percentage of cells that survive in donated units bled from different donors. In another study, the same method was used with ^{51}Chronium, but only using autologous blood of a donor and then label 15-30ml of the donor cells and reinfuse it to the donor. The way in which the cells survival rate is checked was by taking a blood sample soon after injecting the recipient. The time includes 5 min,7.5min,10min,12.5min and 15 minutes, then only again after 24 hours. In each sample a radioactivity count number is acquired. The method used to calculate the survival time is

technetium-99(^{99m}Tc/^{51}Cr double-labelling technique. The red blood cell volume of the recipient was first evaluated by using known amounts of labelled ^{99m}Tc, this is how transfusion recovery was calculated. Using the ^{51}Chronium method testing for haemolysis in storage is easier to monitor and showed that some donors resist storage lesions better than others. Haemolysis present in units after 5 to 6 weeks of storage was 0.2-0.4% (Roussel, Buffet and Amireault, 2018).

A Study done by (Damiani et al., 2015) free haemoglobin was tested to see if there are more free haemoglobin in units that were older than 15 days than in units fresher than 10 days in storage. The method used was arterial blood samples that were taken before transfusion and one hour after transfusion. The samples were centrifuged immediately after and the plasma was stored at -70°C. The free haemoglobin was then quantified in each sample through the colorimetric assay using Drabkin's reagent (Sigma-Aldrich, Saint Louis, Missouri, USA). The way in which Sigma works is to provide a stable, dry Drabkin's Reagent, which is combined with a surfactant to prepare the Drabkin's Solution. The surfactant minimizes turbidity sometimes caused by the presence of erythrocyte stroma. This procedure is controlled by the oxidation of haemoglobin and its derivatives (except sulfhemoglobin) to methaemoglobin in the presence of alkaline potassium ferricyanide. Methemoglobin reacts with potassium cyanide to form cyanmethemoglobin, which has maximum absorption at 540 nm. The colour intensity measured at 540 nm is proportional to the total haemoglobin concentration. (Damiani et al., 2015).
In a journal PLOS ONE the HemoCue machine was used to test haemoglobin levels in children at clinics where automated haematological analyzers are not available. The HemoCue®201+ capillary blood collection method involved pricing the finger and letting the blood from the finger go directly to the microcuvette for analysis.
The HemoCue are validated, but still the reliability depends on the quality of the sample to give an accurate measurement. Collection technique is very important here. Previous studies compared HemoCue® Hb to an automated haematology analyser using venous blood samples had significantly higher Hb values than using capillary samples from healthy blood donors. The mean difference =+0.7g/dl for pregnant women (mean difference = +8) and for anaemic children (mean difference = +1.2) (Brehm, South and George, 2023). In the blood bank we could only use the HemoCue for red cell haemoglobin testing, although it was sensitive enough to read haemolysis in the serum samples. HemoCue has a machine available, the HemoCue® Plasma/Low Hb system. This little point of care machine consists of a specially designed photometer and micro cuvette for the quantity's determination of low levels of haemoglobin in plasma or serum specimens. In practise this HemoCue is used to measure haemolysis perioperative or blood salvage where blood is processed by hemofiltration or by cell savers (HaemoCue, 2018)

2.9 Full blood count

The main purpose of the full blood count for the study is to evaluate the following results, which will give us a clear indication of how the cells are preserved in storage after a wash process. The haemoglobin test (Hb) will show how much of the cells were lost during washing and in the following weeks of storage the Hb will show how well the cells are preserved in the Saline or SAGM (Pulliam et al., 2020). 10-20% of red blood cells are lost in the cell wash process which will have a decline in Hb. A red blood cell count was also performed to see if the unit has enough oxygen carrying capacity. If the cells are washed red cells are lost in this process which will also have a decline in the red blood cell count. The haematocrit was also done on the units that measure the proportions of the red blood cells in the blood. When the cells are washed the ratio between the red cells and the supernatant are lower or higher depending on the number of additive solutions that is added to the red blood cells. The haematocrit for men ranges from 41%-50% and for women, 36%-44%. When the cells are washed the serum and the additive solution is removed and the cells are resuspended in saline or SAGM this also has an influence on the haematocrit. (Zhao et al., 2024). When red blood cells are washed the white blood cells count will decline or be removed to help with patients with allergic reactions. In this study the white blood cells do not really play an important part, because the cells are wased to get rid of free haemoglobin and waste products (Pulliam et al., 2020). The mean corpuscular volume (MCV) was tested, this test measures the actual size of the cells to observe how small or big the cells are. In a study done by Reinhart et al., 2015 the red blood cells that were washed and stored in SAGM between 4ºC to 6ºC increased in size. This indicates RBC swelling during storage, although there was minor swelling in storage the cells were similar to those before storage (Reinhart et al,. 2015).

The mean corpuscular haemoglobin concentration (MCHC) checks the average amount of haemoglobin in a group of red blood cells this test was used. In a study by Reinhart et al., 2015, in the 6th -week post-wash in storage the cells swelled which had an influence on the MCHC level. This test was used in our study so that we could also investigate the size of our red blood cells in washed packs. cells in storage. The MCV level declined, because of the cells that lose their form and become smaller, whereas the MCHC increased, because of the cells that swell.

The mean corpuscular haemoglobin (MCH) test is the average amount of haemoglobin within the red blood cells.

The red cell distribution width standard deviation (RDW-SD) shows the difference in the number of cells present and the different sizes of the cells

The red cell distribution width coefficient of variation in percentage (RDW-CV). This test is performed to see if the red blood cells are of normal size and shape. Normal red blood cells are disc shaped, usually flat with a diameter of around 7.5um. There will always be red blood

cells that are not properly formed. A normal value for red blood cells that are normal is between 85% - 89%. A normal RDW-CV range will then be 11% - 15%. The RDW does not measure the red blood cells, but rather how big the variation is in size and volume. The platelet count is also part of the full blood count, the platelets are also washed out.(Cardigan et al., 2020).

2.10 Washing of red blood cells

In Blood transfusion, we aim to transfuse patients with the best products that suit their needs. Red blood cells will be washed in cases where patients have an allergy to plasma proteins. Not only would the red blood cells be washed, but the platelets too Studies suggest that washing removes 90-95% of plasma. Units below 0.05 mg/dl (equivalent to IgA deficient) depending on the method used. Washing of red blood cells also reduces the potassium levels that are beneficial for patients to reduce lung and kidney injury (Cardigan et al., 2020). In a study done by Keir et al., 2016, infant red blood cells were washed before transfusing 21 infants, this was done to see if the morbidity and mortality rate of the infant would be reduced. Unfortunately, the results showed no difference between washed and unwashed red blood cells. Studies were then done on children. Cholette et al., 2017 have done a study on 162 subjects studied, 81 was in one group. 34 subjects (17 per group) were given no blood transfusions. The subjects that did receive blood had similar storage products. The patients that were transfused at a 12-hour 1L ratio were significantly lower in the washed group. Post-operative tests indicated that the CRP was lower in subjects receiving washed blood. There was a numerical, but not a statistically significant decrease in the total blood products that was transfused. (203 versus 260). The deaths were less in the washed group than in the unwashed group. (2 versus 6). In this study it was concluded that if blood is washed for surgery patients it would reduce the inflammatory biomarkers. In a study done by Welsby et al., 2021 cytokines, chemokines and macrovesicles were removed from the supernatant of allogeneic red blood cells which was thought to help reduce adverse transfusion reactions. This study was conducted, because it is not always possible to receive washed packed cells from the blood bank. The setting in George, when washed packed cells are ordered is a timely procedure and sometimes it can take up to two days to receive the ordered product. This product needs to be ordered from Cape Town and transport needs to be taken into consideration. Part of the study was a sub-study which evaluated bedside allogeneic red cell washing, during cardiac surgery using a cell saver. The data was collected from the first 75 washed red blood cell units and given during the intervention arm of the patient’s part of the clinical trial. Samples were taken pre and post wash, centrifuged and the supernatant from the bags were aspirated and frozen at -70°C. The supernatants were batch tested for soluble CD40 ligands, cell-derived macrovesicles, chemokine ligand 5, neutral lipids and free haemoglobin. All of these are associated with transfusion reactions. The study would be feasible if 75% of units were washed per protocol. In the study 74 of the units CD40 ligand, chemokine ligand 5 and the macro-

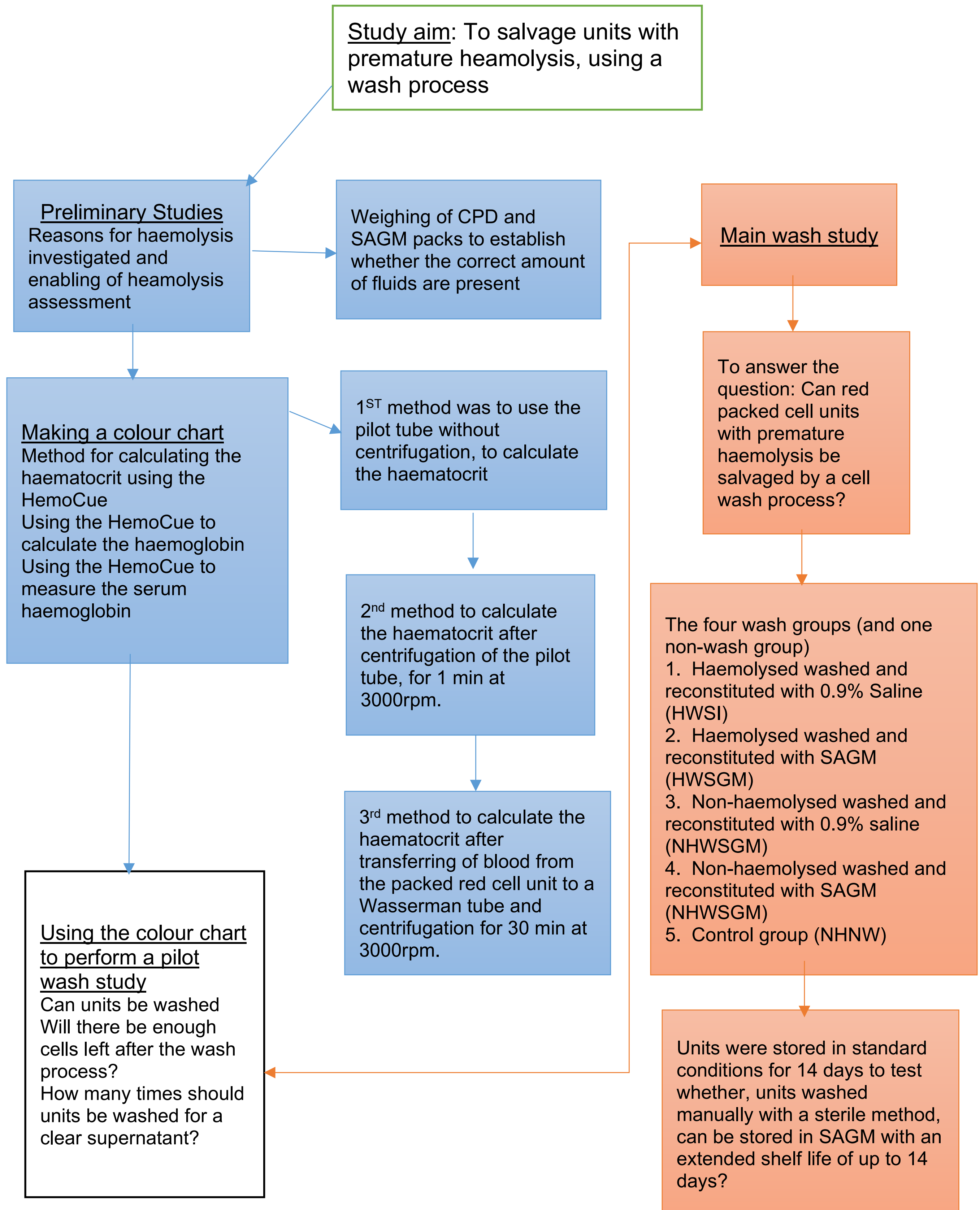
vesicles were reduced. These units had no haemolysis present before washing but after washing the free haemoglobin was higher. The conclusion was that bedside washing was feasible for 80 of the 81 patients (99%). In this study it was apparent that by washing red blood cells, harmful particles can be removed. Free haemoglobin is toxic to the body, it is best to remove it from the red blood cells before transfusion. Washing the units to remove free haemoglobin could make the units safer for transfusion and not discard the units.

In a study done by Proffitt et al., 2018 automated cell washers compared to manual cell washing were paired. The automated cell washer in this study was the ACP device. The manual washing method had different combinations of wash and storage solutions. The aim of the study was to see if washed red blood cells using a manual method could permit a storage time of more than 24 hours. Red blood cells that were 14 days old were pooled, split and washed in one of five ways. The 1st method was using the ACP215 and storing the units in SAGM. The second was a manual washing method and stored in saline. The third method was a manual wash process with saline and stored in SAGM. The 4th method was a manual wash method using saline-glucose and storing of red blood cells in SAGM. The last method was a manually washed method and the red blood cells were stored in SAGM. There were additional units that were pooled and split, washed manually or with the ACP215 and irradiated on the 14th day. All units were sampled 14 days after washing and stored at $4 \pm 2°C$. The results of this study showed that the removal of plasma proteins was better using the manual method. Washing with a manual method had less red cell loss than using the ACP215cell washer. All the units post wash had less than 0.8% haemolysis present after 14 days in storage. In this study the units were preserved better in SAGM after manually washing the red blood cells, here the adenosine triphosphate, glucose, lactate and pH was better than storage in saline. In this study the shelf life of manually washed red blood cells was suggested could be stored for 14 days if SAGM was used as a storage solution instead of saline. This study was very helpful for initiating this research project as in George there is no automated cell washer. The Cobe 2991 cell washer was controlled in Cape Town and in this study, equipment available to in George blood bank was used to save the cells that presented with premature haemolysis. In the case where there are a lot of units with premature haemolysis, it would be an advantage to be able to store these units for longer than 24 hours after the manual wash process. This will give an extended expiry 0 and help with stock rotation not to have an increase in expired product at the Branch.

CHAPTER THREE

METHODOLOGY

3.1 Study design



The research design employed in this thesis was a quasi-experimental study. The purpose of the study was to investigate red blood cell units (RBC) with premature haemolysis in the supernatant that we receive as blood stock at the George branch from the head office in Cape Town. During production whole blood units are labelled with expiry dates according to the units' date of donation. This date serves to monitor the age of the unit, as within the 42-day expiry period, provided that the cold chain and storage parameters are met, little to no haemolysis should be present within all post-processed red cell products (García-Roa et al., 2017). The temperature of the units leaving Cape town have a stock document number and the temperature of the units will be recorded on that document. When blood arrives in George as stock, the temperature is taken again, to make sure that the unit's transport temperature was in range keeping the cold chain standards. The temperature for blood in transport must be between 2 ºC to 10 ºC. The cold chain was not compromised so a further investigation followed. In previous studies it was found that older blood units may start to have free haemoglobin in their supernatant. The units received from Cape Town at George blood bank were checked to see if the units were near their expiry date. The units that were received at the blood bank were still in their first week post donation, this could not have been the cause for premature haemolysis. Going forward the preservative, saline, adenine, glucose, mannitol (SAGM) and the anticoagulant, citrate phosphate dextrose (CPD) in blood donation quad packs were tested. These elements are an important part of the blood donation pack it is a source of nutrients for the red cells during the duration of storage. Roughly a hundred empty, newly opened quad packs with preservatives and anticoagulants were weighed to assess whether any variation in the volume of CPD or SAGM was possible. After weighing a single set of 4 packs, the CPD and SAGM packs were removed and weighed separately. A simple calculation which included weighing an empty blood pack first and then subtracting the weight of the bag from bag weighted gave the grams of the liquid in the bag. In Figure 3.4 it is demonstrated how the packs were weighted. Table 4.2 shows the weight of the empty bag (28g), the pack weight is then subtracted from the scale (-28g) to only weigh the liquid in the pack. Further investigation then followed, then followed, to see, if possible, to salvage red blood cell units with premature haemolysis using a wash method.

The equipment available for the study was limited to the HemoCue. Originally Pathcare, an independent pathology laboratory, was contacted to perform the serum haemoglobin test, but the test is only available at their laboratory in Cape Town. The HemoCue was thus the only equipment for measuring the serum haemoglobin and the red blood cell haemoglobin. After the haemolysed units were grouped according to their colour, using a colour chart, it was important to calculate the haematocrit for each unit. These units' haematocrit and amount of haemolysis were calculated mathematically. These methods to quantify haematocrit and haemolysis in George was standardised as follows: First a preliminary study was done, were;

16 units were sent to Cape Town for a full blood count to derive standardized values for haemoglobin and haematocrit of haemolysed units. To control for differences between haemolysed and non-haemolysed units, this was done for 11 haemolysed and 5 non-haemolysed samples. The haematological autoanalyzer in use at Cape Town headquarters is the Beckman Coulter Access®. In order to create a colour chart in George the HemoCue handheld instrument was used to measure the Haemoglobin (Hb) contained in red blood cells and the serum of units. As full blood counts are not possible in George, other required blood measures would need to be determined using other methods.

3.1.1 Standardizing the haematocrit result

There were 3 methods used in George to try and reach the same haematocrit measurement as performed in Cape Town on the haematological autoanalyzer. The first method is shown in figure 3.1.

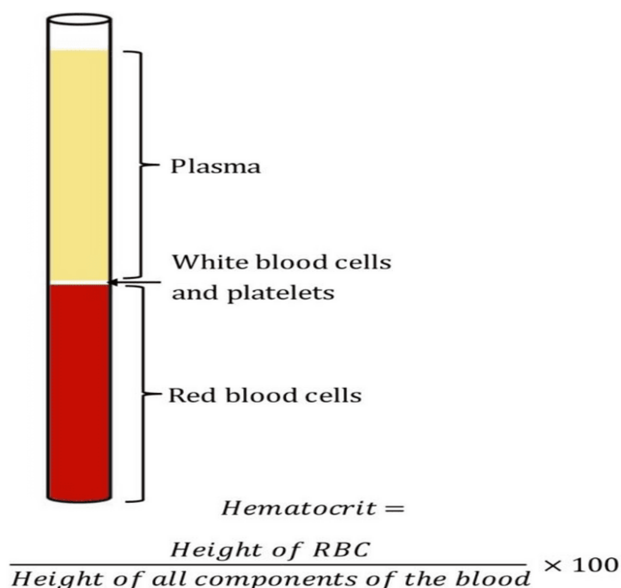


Figure 3.1: The illustration and formula to calculate the haematocrit by (Mondal and Lotfollahzadeh, 2022)

Three methods were tested to find the result that matched the haematological autoanalyzer results exactly or closest to it. The first method employed was to calculate the haematocrit by using the pilot tube of a unit containing haemolysis in the supernatant. The second method was to spin the pilot tube at 3000rpm for 30 minutes. The third method used was to take blood from the sample bag and transfer it into two test tubes. The one test tube was for the haematological autoanalyzer to perform a full blood count and the other test tube was for the manual calculation of the haematocrit. The three methods were compared and there were deviations in all 3 methods from the results of the full blood count. It was very difficult to obtain

the same results for all three of the methods. The third method was used for haematocrit results for calculation of haemolysis present in a red blood cell unit. The level of haemolysis was also calculated, using the 3 different methods used for determining haemoglobin. The 3 methods were then compared to the haematological autoanalyzer. The results were compared to find the exact same or closest result to the autoanalyzer. Method 3 was used for the haemoglobin; this result was also used in calculating the percentage haemolysis in the units. The serum haemoglobin test using the HemoCue was the only equipment and test, available to calculate serum haemoglobin.

These results were then used in creating a new colour chart. The new colour chart is the presentation of the percentage of haemolysis present in the unit. The amount of haemolysis in the supernatant will be presented by a specific colour. With a colour chart standardized using only the equipment available at the George WCBS. It will be possible to more accurately evaluate units with haemolysis present in the supernatant. With this chart decisions can be made on the units to be used or need to be discarded.

3.1.2 Washing of the cells (COM-28)

The RBC units are washed with 0.9% saline as this is similar to intracellular fluid of erythrocytes. 0.9% Saline contains a solution of sodium chloride and water. The 0.9% NaCl solution is seen as isotonic solution. The intracellular and extracellular fluids are said to be in osmotic equilibrium across the cell membrane and there will be no influx or efflux of water. The osmotic pressure is dependent on the difference between the concentrations of non-diffusible irons on each side of the membrane (Lotterman and Sharma, 2023).
0.9% saline was docked to a transfer bag with a sterile docking device so that no air can enter the bag. A volume of 250ml of Saline was moved to the transfer bag and sealed. The transferred bag, which contained the saline, was sterile docked to the RBC unit. A plastic clip to prevent the liquid from mixing separated the saline transfer bag and the RBC bag. The plastic clip was removed and the saline in the transfer bag was transferred to the RBC. The plastic clip was then sealed off the transfer bag so that the saline red cell mixture does not enter the transfer bag when the bag was spun down in the centrifuge. The RBC has been mixed to make sure that the saline was well re-suspended in the packed red cell. The unit was placed in the centrifuge and spun at 2000 rpm for 5 minutes. This method was for the wash process to separate the red cells and the saline. After the spun process. The unit was placed in a manual press and the supernatant (which was 0.9% saline and broken red cells) was taken off. After the previous process, the empty bag was sealed off and discarded. The wash process was repeated until the supernatant was clear. This process was done in George. A manual wash process was followed and no cell washer was used.

3.1.3 Adding the preservative and Saline to the washed cells

After the preliminary wash stud a preliminary test was done to see if the units will survive better in Saline or SAGM as a preservative post wash, because this was our second research question for the study. In order to test this theory, 7 of the units were substituted with 130ml Saline 0.9%, while the other units were reconstituted with 130ml SAGM. This test was done to see if the expiry date can be extended when the units are resubstituted with saline or SAGM. Before washing and after washing full blood counts were done on these units to assess the amount and condition of cells contained within the packs. After the red blood cell packs were washed, they were weighed after the first and the second wash. When cells are washed ± 20% of red blood cells are lost in the process (Vörös et al., 2018). It is important to see if there will be sufficient cells left after washing units containing haemolysis. If a red blood cell unit contains free haemoglobin it means that there are already broken cells in the pack which will be washed out and this means more cells could be lost if haemolysis is present before the wash process. After this preliminary study was completed, the main study was subjected to the same research as the preliminary study.

In the main study 8 of the haemolysed units were substituted with 0.9% Saline. 10 of the haemolysed units were substituted with SAGM. Then there were the control groups, the 11 red blood cell units were not haemolysed, after washing 5 of them were substituted with 0.9% Saline and the other units were substituted with SAGM. The last group of 5 units were not haemolysed and were not washed, they were left in the fridge to see the normal degradation of red blood cells in storage. The rule is if units are washed the units need to be transfused within 24 hours. In the case where there are a lot of haemolysed units, it would help a lot if these units can be kept for even just a week not to get wasted. Specimens for a full blood count were taken before washing, after washing and every week after washing for two weeks. The blood specimens were collected out of a specimen bag. This bag was attached to the main red blood cell bag to not contaminate the units between testing periods. The testing bags were also sterile docked so there would be no contamination in the blood pack that had to be left for 2 weeks for testing. The data that came out of the testing period was used to see how the cells behave after washing and if this would be a viable product for transfusion. Data were compared between the units that were substituted with 0.9% Saline and those substituted with SAGM to see if it is possible to extend the store period after washing the haemolysis out of the packs. The data will also help evaluate red-packed cell units that need to be washed for patients that need washed-RBC with IgA antibodies.

3.1.4 Preliminary wash study

This wash study was done before the main study to make sure the main wash study was viable. This study also assisted in refining the methodology for the wash study. The centrifuge that

was used for the wash study was the Terumo Scientific 6000i cryofuge. The units with different levels of haemolysis present were selected for this study. The first objective of this wash study was to determine whether the waste products of haemolysis could be removed from a pack by a cell wash process. The theory behind this is that if the haemolysed cells and waste products are removed from a unit, it could be safe for transfusion as the free haemoglobin which is toxic to the body would be eliminated (Rapido, 2017). After 12 units were selected by the varied percentages of haemolysis, they were washed with 0.9% Saline. First, 12 units were washed as a trial to see if sufficient cells would be left over and intact after washing. For this purpose, 10 units which were haemolysed and 2 units which were not haemolysed were used. The 2 not-haemolysed units were included to serve as a control group for this experiment. As part of the study the amount of wash cycles compared to the amount of haemolysis present in red packed cells was also tested. Some of the units that had a higher amount of haemolysis were not clear after 2 wash cycles of the wash process and had to be washed for a third time. In this experiment, it was important to see how many times units with a varied level of haemolysis needed to be washed to result in a clear supernatant.

3.1.5 The main wash study

After completing the preliminary wash study, the main study followed. Red blood cell units used in the main study were randomly selected, with the only criteria for inclusion being that the units should be less than 10 days old. The inclusion criteria did not consider numerical alphabetical, gender or phenotypic preferences. The identification and selection of haemolysed units was done using the previously created colour chart and determining the haematocrit and the percentage of haemoglobin on them as per the preliminary study. The study included 18 units presenting with haemolysis, while 11 not-haemolysed units were selected as the control group. This group had no haemolysis and would also undergo the wash process. After selection, all selected units except the 5 units were washed, this units were used to compare results to the washed cells to see the difference in cell destruction in storage. After washing 8 of the haemolysed units were substituted with 0.9% Saline. The other 10 units were reconstituted with SAGM. Similarly, in the 11 non-haemolysed red blood cell units 5 were substituted with 0.9% Saline and the other 6 units were reconstituted with SAGM, after the wash process. These washed and reconstituted units were then placed in the fridge and stored for a period of 2 weeks to determine whether reconstitution in saline or SAGM would allow for prolonged storage. The last group of 5 units were non-haemolysed and not washed, they were left in the fridge to see the normal degradation of red blood cells in storage. The non-haemolysed units washed was a control group for units washed with premature haemolysis. The current standard operation procedure states that if units are washed the units need to be transfused within 24 hours. Specimens for a full blood count analyses were taken before and after washing as well as every week after washing for two weeks. The blood specimens were

collected out of a specimen bag that was attached to the main red blood cell bag, in order to prevent contamination of the units between testing periods. The testing bags were also sterile docked so there would be no contamination in the blood pack that had to be left for 2 weeks for testing. To evaluate the red blood cells the full blood count was done before the wash process and after the wash process.

3.2 Statistical analysis

Statistical analysis of data was performed using Graphpad Prism version 5 for Windows, Graphpad Software, California, USA. Data was expressed as means and standard error of the mean, with normality being tested using the Kolmogorov-Smirnov test. Datasets were found to be normally distributed and therefore, significance between groups was tested using the One-Way ANOVA with a Bonferroni Multiple Comparison analysis post hoc test. In cases where different time points of the same experimental group were compared, Repeated Measures ANOVA with Bonferroni Multiple comparison analysis post hoc test was employed. The unpaired student t-test (two-tailed) was used to perform a statistical analysis in cases where only two groups of data were compared. Where a p-value of 0.05 or less was obtained using ANOVA or t-test analysis, a significant difference was reported. Microsoft Excel 2016 was used for data collation and preparation of graphs.

3.3 Ethical Consideration

Ethical approval was sought and obtained from the Research Ethics Committee, (CPUT/HWREC 2019/A1), Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology. Human Blood donated by donors was used. Consent is obtained for research on a valid donor questionnaire, filled in by all donors before the donation of blood. The donor questionnaire will be added at the end as appendix D. The Ethics Approval letter is in Appendix C.

CHAPTER FOUR

RESULTS

4.1 Determination of potential reasons for Haemolysis in packs

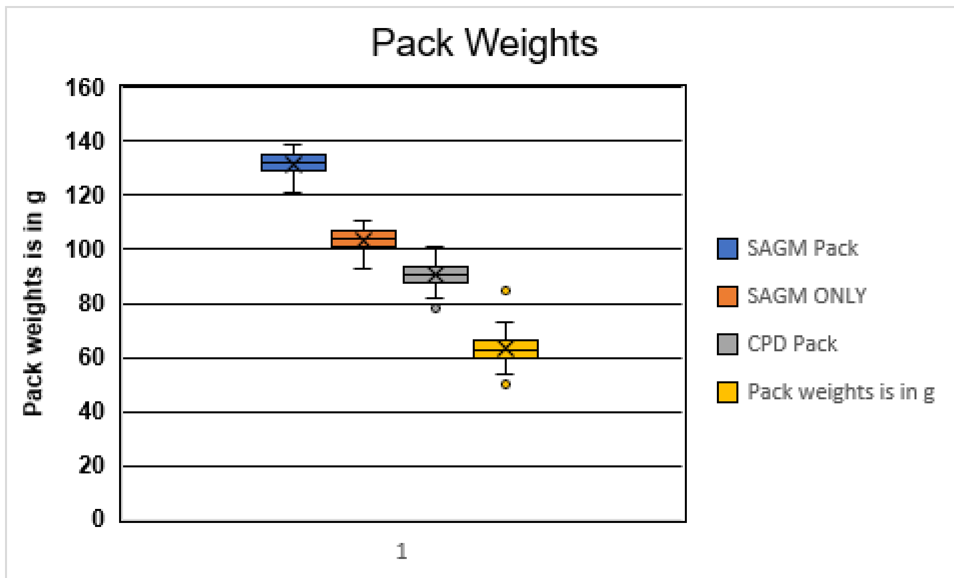
The first potential reason that was investigated was to check the date doners were bled to produce the units. This was done to ensure that units received from Cape town was not near to their expiry date (Aalaei et al., 2019). Units closer to their expiry dates would be expected to have a higher incidence of haemolysis. The 22 units of blood that included RBC and LRBC received from Cape Town stock control was still in the first week post-donation and is not expected to have any haemolysis present in the supernatant (Aalaei et al., 2019).

4.2 Weighing of pack additives to ensure uniform production standards

In Figure 4.1 The pack weights of the CPD and SAGM of 102 packs measured has been provided. The calculation for SAGM and CPD was xg=(x-y), x = total weight – y (28g). The weight of the CPD varied the most and could be the result of the premature haemolysis, but it should be considered that there are a lot of potential reasons for premature haemolysis from the time of donation until the process of component production is completed. Appendix A.1 the process was demonstrated in more detail with pictures that includes figure A.1 – A.4

4.3 Pack weights

Figure 4.1 shows the weight of the CPD and SAGM liquid of 102 pre-donated quad bags, this was to observe if the quantities of the CPD and SAGM are similar for all the bags. 102 SAGM bags were weighted to compare the amount of SAGM in each bag. The SAGM liquid in the pack did not vary a lot and was mostly the same as with the instructions of the package insert. The graph also shows the SEM and mean of the 102 bags that was weighed. The results of the graph show that the CPD varied the most with QV > 5%.



Average	131.3235294	103.3039216	90.90196078	63.28431373
SDEV	3.948083292	3.939616546	4.317841879	5.275344203
SEM	0.390918497	0.390080164	0.427530053	0.52233691

Figure 4.1: Pack weights. The chart shows that the CPD varied the most with QV > 5%.

The lack of enough anticoagulants may lead to clots. In the process of making components, the blood is pressured out of one bag into the pilot bag, a clot can narrow the passage in the pilot tube and the cells may haemolyse if pressure is added to them as red blood cells are very fragile.

4.4 Testing different methods to measure haematocrit

The haematological autoanalyzer is the general method used to test for the haematocrit. In this study, three different methods were used to find similar or identical test results, as what would have been gathered if the haematological autoanalyzer was used. Although all these methods were used, it was difficult to find a haematocrit result exactly the same as obtained by the haematological autoanalyzer. The spun segment method was found to have the closest proximity of results, in terms of similarity, as obtained by the haematological autoanalyzer.

4.5 Haematocrit determination

Figure 4.2 illustrates haematocrit determination, in graph format, the different results obtained by using different methods. In this graph it illustrates the different methods used to determine the haematocrit that was compared to the haematological autoanalyzer.

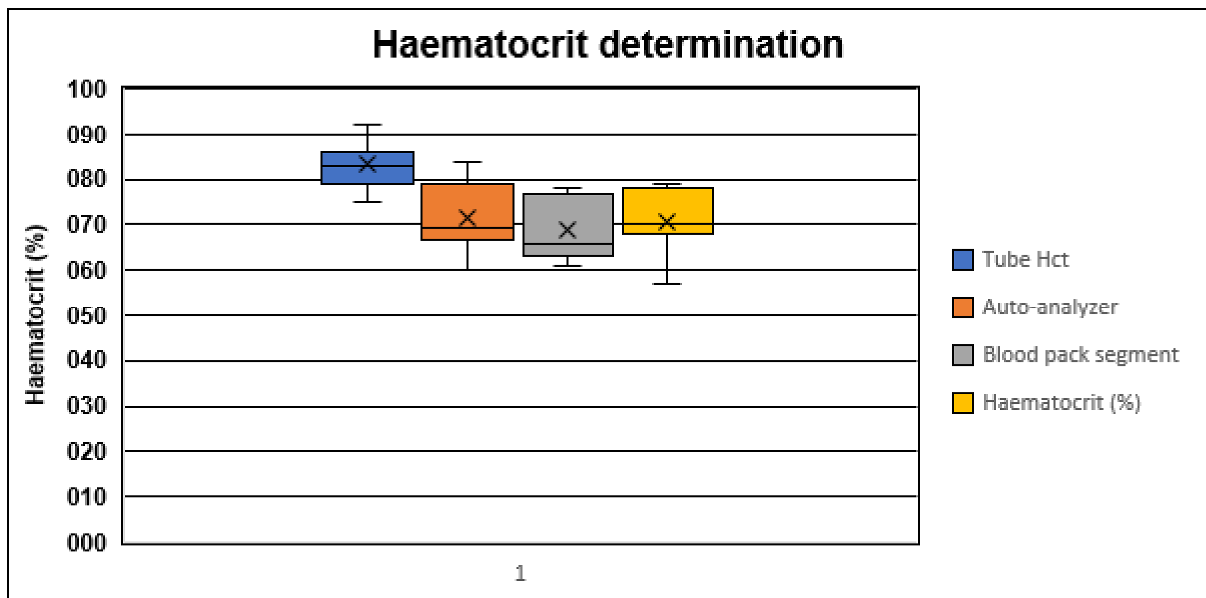


Figure 4.2: The graph illustrates the different results that were obtained using different methods to calculate the haematocrit results. These methods included the tube technique results, the haematological autoanalyzer results and the segments haematocrit results.

Evidently, as shown in the figure above, it was almost impossible to obtain the same haematocrit results as the autoanalyzer using the three different methods. There is a significant difference between the other methods used and the tube haematocrit P-value < 0.05.

4.6 Results of percentage of haemolysis

The percentage haemolysis was calculated using the haematocrit, haemoglobin result and the serum haemoglobin result. All the different methods to calculate the haematocrit had an influence on the parentage haemolysis present in the supernatant. The spun segment results and the serum haemoglobin results were used for the calculation of the haematocrit. This result with the serum Hb was used to calculate the percentage haemolysis. In George, a standard method for calculating the haematocrit was difficult, but using the pre-spun segments for the haematocrit could be used with the serum haemoglobin results to create a colour chart that was similar to the one that was done by Dr Bellairs and S. Sutton. The charts that were used as a guideline for the colour of free haemoglobin and the percentage haemolysis can be seen in Appendix A figure A.5 as well as another chart done at the Canadian Blood Service figure A.6. In Appendix A figure A.7 and figure A.8 shows in pictures the use of the HemoCue to do the serum haemoglobin and it shows the packs with different degrees of haemolysis in the supernatant. In Appendix A figure A.9 – A.12 is a demonstration to calculate the haematocrit with the 3 different methods.

4.7 Percentage of haemolysis in a graph

The results in figure 4.3 illustrates how the percentage of haemolysis differs from one method to another, whilst using the same units.

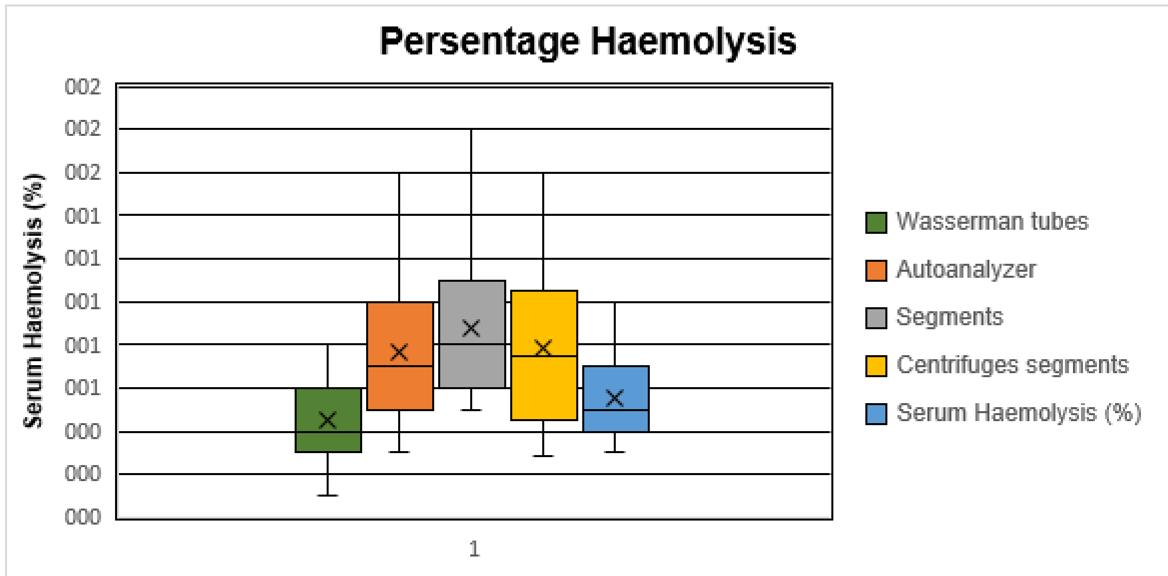


Figure 4.3: Difference in results for the serum haemolysis done with the Wasserman tube method and the other methods of using a segment from the pack and using a spun segment which was compared to the autoanalyzer. The p-value showed a p-value < 0.05 which means there was a significant difference between the tube method done to measure serum haemolysis and the haematological autoanalyzer result for serum haemolysis.

The p-value < 0.05 shows a significant difference between the Wasserman tube method and the other methods used to calculate the percentage haemolysis**.**

4.8 Comparison between the haematocrit of haemolysed and not-haemolysed units

The haematocrit results of units with and without premature haemolysis were compared, to observe if there were a difference in results. If the Hb and Hct results were not on par the wash process would not have taken place. If the unit's Hb or Hct is too low then the unit will not fit the criteria according to the standard of practice for transfusable products. The specific standard is written in the literature review. This would have shown that the units will not be able to be saved with a wash process and needs to be discarded. In Appendix A figure A.13, the calculation for percentage haemolysis was illustrated. In Appendix A figure A.14 and A.15 shows the colour charts that were used as guidelines to create my own colour chart. In Appendix A figure A.16 and A.17 shows the different colours and percentage haemolysis that is used as a colour chart in George blood bank.

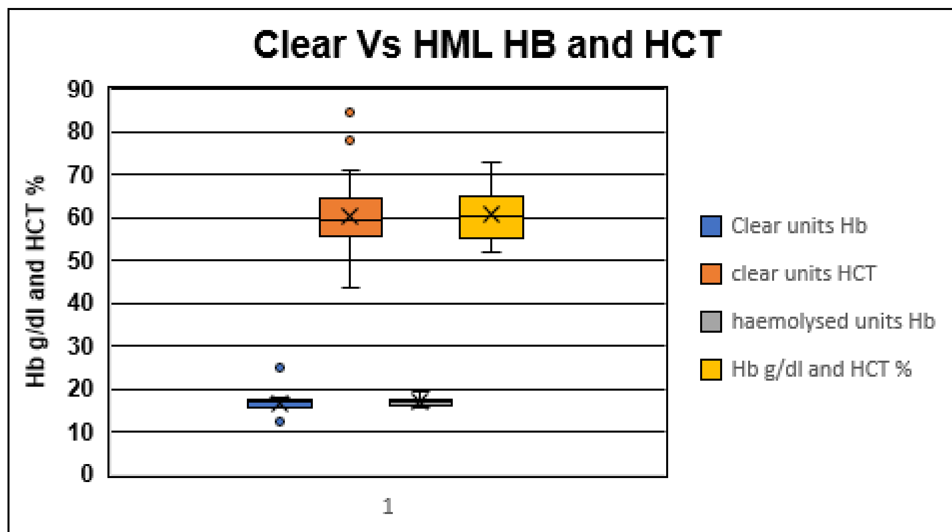


Figure 4.4: Comparing the haemoglobin for the haemolysed and non-haemolysed units in the box and whiskers chart here is clear that the haemolysis present did not have an impact on the haemoglobin and the haematocrit results.

4.9 Washing of red packed cell units

Quantifying cell loss in the wash process was important, if the haemolysed units had too much broken cells, washing them can result in an RBC with very little blood inside. A unit with too little cells would be discarded and the whole purpose of the study would be in vain. The amount of cell washes had to be established for the amount of haemolysis present in an RBC unit, to ensure removal of toxic waste, without loss of too many cells. It was therefore important to confirm the number of washes and percentage of haemolysis present in the supernatant to find a clear supernatant as an end product. Most of the packed red blood cells presenting with haemolysis were washed twice. The cells washed with premature haemolysis had an average cell loss of 24% in this study. The percentage of haemolysis in the units that were washed had no more than 3% haemolysis present in the packed red blood cells. A cell wash system with fresh non-haemolysed cells will have a cell loss of 10-20% (https://reference.medscape.com/drug/washed-red-blood-cells-999508). The result was seen as acceptable and the main study was started. In appendix B the table depicts units washed twice and for a third time, together with cells lost. The units that were selected for examining the number of times the units should be washed for a clear supernatant had a percentage haemolysis between 0.6% and 0.8%. With a high percentage haemolysis, the cells had to be washed 3 times for a clear supernatant result. In Appendix A figure A.18 and A.19 a visual presentation can be viewed of the different percentage of haemolysis and the number of times it should be washed. These units lost 27% of their cells in the wash process. In the study these units were excluded, because the percentage of cells lost were deemed too high to allow for a transfusable product.

4.10 Introduction to the main study's wash process

After the pilot study was done to make sure it is possible to wash units presenting with haemolysis the main study was done. In the main study, there were 5 groups. The first group was Not haemolysed washed and suspended with 0.9% Saline (NHWSI). The second group was Not haemolysed washed and suspended with SAGM (NHWSG). The third group was haemolysed washed and suspended in 0.9% Saline (HWSI). The fourth group was haemolysed washed and suspended with SAGM (HWSGM). The last wash group was the control group, this group was not haemolysed not washed (NHNW). Haemolysed units and units not haemolysed were compared with each other. One group was resuspended after washing with SAGM as preservative and the other group only with 0.9% Saline. These units included red-packed (ARBC) cells and Leuco-depleted red-packed cells (LRBC). In Appendix A figure A.20, the different equipment used for the wash process can be seen. In table A.1 the different wash groups were illustrated. This is to make it easier to understand the different groups used in this study. In Appendix B the different research groups with their results in graph format is illustrated from figure B.1 – B.61.

4.11 Pre-wash results for the 5 research groups

In the study before the units were washed, the full blood count was done on all the groups. This was to see if there is a significant difference between the group with haemolysis present and the group without haemolysis present in the supernatant. The full blood count results showed no significant difference between the study groups although the one group had haemolysis present before the wash process. Although the group with haemolysis had lysed red blood cells the cell had no significance in the RDW results that showed that the cells were not in stress before they were washed. In George blood bank we had a few incidences were units had clots and had to be discarded. This units were sent to reference lab for investigation the results concluded that there were no other anomalies that contributed to the clots that was found in the packs. The WCBS also had a period where they did full blood counts on all the donors to make sure the donors were healthy. In this period there were still units that had clots in the pack as well as premature haemolysis.

Table 4.1: Comparison of the full blood count measurements for the five groups compared to the control group

	NHNW	NHWSI	NHWSGM	HWSI	HWSGM
HGB	22.42	19.02	20.76	19.68	21.74
RBC	7.53	6.54	7.01	7.03	7.25
HCT	71.02	67.03	63.48	67.03	71.83
MCV	94.48	95.88	98.34	95.98	99.36
MCH	29.88	29.24	29.62	28.275	30.07
MCHC	31.62	30.46	30.12	29.55	30.27
WBC	0.850	1.22	0.960	0.770	0.610
PLT	7.80	9.00	5.40	7.00	6.70
RDW-SD	46.74	47.7	51.48	50.05	48.3
RDW-CV	14.68	13.88	15.06	15.35	14.03

The 5 groups were compared with each other and the control group. The control group is known as the group with no haemolysis that was not washed (NHNW) and stored in a temperature control blood fridge between 2 and 6 ºC. The first group in blue, not haemolysed washed and suspended in 0.9% saline (NHWSI) will always be portrayed in blue. The 2ND group in yellow, not haemolysed washed and suspended in SAGM (NHWSGM) will always be in yellow. The 3rd group in green, haemolysed washed and suspended in 0.9% saline (HWSI) will always be in green. The 4 groups in pink, haemolysed, washed and suspended in SAGM (HWSGM) will always be in pink. The control group will only be black (NHNW). The full blood count was done on the units: haemoglobin (HGB), red blood cell count (RBC), haematocrit (HCT), mean corpuscular volume (MCV), mean molecular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell count (WBC), platelet count (PLT), red blood cell distribution width-standard deviation (RDW-SD), red blood cell distribution width - coefficient of variation (RDW-CV). There were no significant differences ($p > 0.05$) between the groups.

Table 4.2: Comparison of the full blood count measurements post-wash for the five groups compared to the control group.

POST-WASH	NHNW	NHWSI	NHWSGM	HWSI	HWSGM	p-value	Significant
HGB	22.42	16.02	16.24	13.47	14.24	p-value < 0.01 NHWSI vs NHNW p-value < 0.01 NHWSGM vs NHNW p-value < 0.001 HWSI vs NHNW p-value < 0.001 HWSGM vs NHNW	Very Very Highly Highly
RBC	7.53	5.48	5.48	4.7	4.83	p-value < 0.01 NHWSI vs NHNW p-value <0.001 NHWGM vs NHNW p-value < 0.001 HWSI vs NHNW p-value < 0.001 HWSGM vs NHNW	Very Highly Highly Highly
HCT	71.02	51.96	54.92	46.28	48.24	p-value < 0.001 NHWSI vs NHNW p-value < 0.01 NHWSGM vs NHNW p-value < 0.001 HWSI vs NHNW p-value < 0.001 HWSGM vs NHNW	Highly Very Highly Highly
MCV	94.48	95.34	100.27	99.4	99.78	p-value > 0.05	No
MCH	29.88	29.2	30.03	28.93	29.49	p-value > 0.05	No
MCHC	31.62	30.6	29.97	29.04	29.58	p-value <0.05 HWSI vs NHNW	Significant
WBC	0.85	0.35	2.78	0.19	0.75	p-value > 0.05	No
PLT	7.8	5.4	2	2.25	2.8	p-value > 0.05	No
RDW-SD	46.74	46.84	49.98	49.58	48.99	p-value > 0.05	No

The full blood count was performed on all the groups, as well as the control group that did not go through the wash process. The control group represents the normal storing of cells without any premature haemolysis or cell washing. The control group is first with no haemolysis present in the supernatant and no wash process. (NHNW). The 2nd group not haemolysed washed and substituted with 0.9 % saline (NHWSI). The 3rd group is not haemolysed washed suspended in SAGM. The 4th group is haemolysed washed and suspended in 0.9% saline (HWSI) the 5th group haemolysed washed and suspended in SAGM (HWSGM) and the last group the not haemolysed not wash group.

4.12 Post-wash full blood count results for the research groups

Post-wash full blood count results showed that the haemoglobin results had a very significant difference for the HGB results between the NHWSI, NHWSGM and the NHNW with p-value < 0.01. There was a highly significant difference for the HGB results between the HWSI, HWSGM and the NHNW results. The reason for this can be that the units that already had haemolysis inside the pack lost more cells because the haemoglobin already leaked out of the cells and the debris of the cells was washed out. This is a good indication that washing the cells removes the waste products out of the packs, leaving a good transfusable product.

There was a very significant difference in the RBC results between the group NHWSI and the NHNW group. The groups NHWSGM, HWSI, HWSGM had a highly significant difference from the NHNW group in the RBC results. It is no clear why the first group NHWSI only had a very significant difference while the other groups had a highly significant difference in red blood cell count. It can be that the red blood cell count for this specific group may have been higher and with the wash process, less cells were lost.

The HCT results had almost the same pattern as the RBC and HBG results. The groups NHWS, HWSI and HWSGM had a highly significant difference from the NHNW group. The HCT results for group NHWSGM had a very significant difference from NHNW. The HGB, RBC

AND HCT blood tests had the most significant differences from the control group. The reason for this is, is that about 20% of cells are lost if fresh non-haemolysed packed cells are washed, but in this study, there is also 2 groups that had premature haemolysis already present in the supernatant, in the pre-study before the main study the stats showed that about 24% of cells were lost when different amounts of free haemoglobin units were washed. The haematocrit also had a significant difference, because although 130ml of saline or
SAGM were added after washing the number of cells lost had an impact on the haematocrit that is the ratio between the cells and the fluid.
The MCHC had a significant difference between the HWSI and NHNW group. This was the only group that had a significant difference in the post-wash results.

Table 4.3: Comparison of the full blood count measurements 1-week post-wash for the five groups compared to the control group.

1 WEEK POST-WASH	NHNW	NHWSI	NHWSGM	HWSI	HWSGM	p-value	Significant
HGB	27.4	15.72	17.12	14.45	15.83	p-value < 0.01 HWSI vs NHNW p-value < 0.05 HWSGM vs NHNW	Very Significant
RBC	7.02	5.17	5.75	5.11	5.42	p-value < 0.05 NHWSI vs NHNW p-value < 0.01 HWSI vs NHNW p-value <0.05 HWSGM vs NHNW	Significant Very Significant
HCT	67.1	53.3	57.47	50.09	53.56	p-value < 0.5 NHWSI vs NHNW p-value < 0.001 HWSI vs NHNW p-value < 0.5 HWSGM vs NHNW	Significant Highly Significant
MCV	95.62	99.84	99.93	99.21	98.62	p-value > 0.05	
MCH	29.68	30.56	29.75	28.86	29.23	p-value > 0.05	
MCHC	31.02	30.6	28.42	29.03	29.54	p-value > 0.05	
WBC	1.16	0.1	0.05	0.98	0118	p-value > 0.05	
PLT	4.6	5	2.67	4	1.67	p-value > 0.05	
RDW-SD	46.62	46.14	48.8	49.7	49.13	p-value > 0.05	
RDW-CV	13.7	12.58	13.15	14.1	13.66	p-value > 0.05	

This groups were compared with themselves and with the control group. The control group was not washed and not haemolysed and only stored in normal conditions with the units that were washed. The full blood count was taken every week for all the groups. This result was an indication on how well the cells are preserved in 0.9% Saline or SAGM after they were washed. In this table both the haemolysed group as well as the not haemolysed group was included. The abbreviations for the groups, not haemolysed washed and suspended in saline (NHWSI) and the second group, not haemolysed washed and suspended in SAGM (NHWSGM) the third group haemolysed washed and suspended in 0.9% saline (HWSI) and the last group, haemolysed washed and suspended in SAGM (HWSGM).

4.13 Comparison of units 1-week post wash

In the week post-wash, the HGB results showed a very significant difference between the HWSI group and the NHNW group. It also showed a significant difference for the group HWSGM vs the NHNW group. Only the units with premature haemolysis had a significant difference. It seems that the haemolysed units stored in SAGM was preserved better in the first week, because this group had a less significant difference from the control group (NHNW) one week in storage.
The red blood cell count had a significant difference between the NHWSI, NHWGM and the NHNW group. There was also a highly significant difference between the HWSI group and the NHNW group. Looks that the cells already haemolysed is fragile and in storage with 0.9% saline as the preservative they do not survive that well. The groups NHWSI and the group HWSGM
The HCT results had a significant difference between the groups NHWSI, HWSGM and the NHNW group. There was a highly significant difference between the group HWSI and the NHNW group. The results indicate that SAGM preserve the cells better post wash than the saline. Although the RDW results did not have significant differences in the group, it indicates we can store the cells post wash for 2 weeks, this only looks viable for the groups with no premature haemolysis, but the groups with premature haemolysis, would be recommended for one-week post wash storage. A follow up study would be recommended were the oxidative stress test would give a much better physiological picture.

Table 4.4: Comparison of the full blood count measurements 2-week post-wash for the five groups compared to the control group.

2nd week post-wash	NHNW	NHWSI	NHWSGM	HWSI	HWSGM	p-value	Significant
HGB	20	14.5	16.62	14.51	14.42	p-value < 0.001 NHWSI vs NHNW p-value < 0.01 NHWSGM vs NHNW p-value < 0.001 HWSI vs NHNW p-value < 0.001 HWSGM vs NHNW p-value < 0.05 NHWSGM vs HWSGM	Highly Very Highly Highly significant
RBC	6.71	4.8	5.6	4.97	4.95	p-value < 0.001 NHWSI vs NHNW p-value < 0.05 NHWSGM vs NHNW p-value < 0.001 HWSI vs NHNW p-value < 0.001 HWSGM vs NHNW	Highly Significant Highly Highly
HCT	66.78	49.32	50.86	57.3	50.45	p-value < 0.001 NHWSI vs NHNW p-value < 0.05 NHWSGM vs NHNW p-value < 0.001 HWSI vs NHNW p-value < 0.001 HWSGM vs NHNW	Highly Significant Highly Highly
MCV	99.94	102.64	102.34	102.9	102.02	p-value > 0.05	Not significant
MCH	29.96	30.26	29.7	29.33	29.13	p-value > 0.05	
MCHC	29.96	29.88	29.02	28.45	28.59	p-value > 0.05	
WBC	0.38	0.01	0.01	0.05	0.11	p-value > 0.05	
PLT	3.2	1.8	1.8	1.78	1.4	p-value > 0.05	
RDW-SD	48.04	46.58	49.8	51.76	59.59	p-value > 0.05	
RDW-CV	13.12	12.46	13.22	14.11	13.28	p-value > 0.05	

Comparison of units 2 weeks post wash. The full blood count results were done for all groups the 2nd week in storage. The 5 groups included, 1st group not the control group not haemolysed not washed (NHNW) the 2nd group haemolysed washed and suspended 0.9% saline (NHWSI), the 3rd group not haemolysed washed and suspended in SAGM (NHWSGM) the 4th group haemolysed washed and suspended in 0.9% saline (HWSI) the 5th group haemolysed washed and suspended in SAGM (HWSGM).

4.14 Comparison of units 2 weeks post wash

The p-value was interpreted as p-value>0.05 no significant difference, p-value<0.05 significant difference, p-value< 0.01 very significant p-value< 0.001 highly significant. The stats comparison between the post-wash and week 1 and 2 had no significant differences between the weeks of storage. The stats for week 1 vs week 2 post-wash results were also not included, because there were no significant differences.

Table 4.5: The full blood count results for the group not haemolysed washed suspended in 0.9% saline (NHWSI).

Tests	Bartlett's p-value		NHWSI - SIGNIFICANT YES/NO AND INTENSITY		
			Pre-wash vs Post wash	Pre-wash vs 1st week	Pre-wash vs 2nd week
WBC	p-value>0.05	no significance	Significant	very significant	very significant
RBC	p-value<0.01	very significant	No	significant	very significant
HGB	p-value<0.01	very significant	No	No	very significant
HCT	p-value<0.05	significant	No	No	Significant
MCV	p-value<0.05	significant	No	No	No
MCH	p-value<0.05	significant	No	No	No
MCHC	p-value<0.05	significant	No	No	No
PLT	p-value> 0.05	not significant	No	No	No
RDW-ST	p-value< 0.001	highly significant	No	No	No
RDW-CV	p-value<0.05	significant	No	significant	Significant

Bartlett's p-value is shown here for the full blood count's different tests that was performed on the group not haemolysed washed and suspended in saline. This table also includes the p-values for the pre-wash till the second week in storage. The p-value with different values have different interpretations for example p-value>0.05 no significant difference, p-value<0.05 significant difference, p-value<0.01 very significant difference and p-value<0.001 highly significant.

4.15 The interpretation of the full blood count for NHWSI

The white blood cell (WBC) results for the group not haemolysed washed and suspended in saline had a significant difference in white blood cell results p-value < 0.05. Comparing the pre-wash and post-wash results there was a very significant difference, this means that a high percentage of white blood cells were washed out of the product. A product with reduced leucocytes attenuates or eliminates the inflammatory response. Leuco-reduced products have shown to lower the incidence of virus transmission, immunosuppression alloimmunization and inflammation as all of these contribute to mortality and morbidity. The pre-wash and week 1 in storage had a very significant difference. This could mean that the white blood cells do not store well in saline. The results for pre-wash and 2nd week in storage were the same as the first week, the results also had a very significant difference.

The red blood cell count showed no significant difference between the pre-wash and the post-wash, this could mean that for this group there was not a lot of red cell loss in the wash process. This group had no premature haemolysis that could also contribute to the fewer cells lost in the wash process. The pre-wash compared to the 1st week in storage had a significant difference, this can be that some of the cells had some stress and lysed in storage or the saline is not a good preservative for post storage of washed red cells. The pre-wash and the second week in storage had a very significant difference, the reason for this is the same as for the post-wash 1 week in storage.

The p-value for the haemoglobin showed a very significant difference. Comparing the pre-wash and the post-wash there were no significant difference, there were not a significant difference for the red blood cells for the pre-wash to the post wash and this is the same for the

haemoglobin, if less red blood cells are lost the haemoglobin result will also not decline that much because their results are linked. The pre-wash haemoglobin result and the 1st week in storage had no significant difference. The second week in storage compared to the pre-wash had a very significant difference, this could be that the cells are now breaking down and are not preserved well enough in the saline.

The haematocrit results had no significant differences for the pre-wash and the post-wash. The haematocrit results for the pre-wash and the 1st week in storage also had no significant difference. In the second week of storage the haematocrit had a significant difference from the pre-wash. It can be that the red blood cells are lysing and this can influence the haematocrit.

The MCH, MCV, MCHC, PLT and the RDW-ST had no significant differences between the pre-wash until the second week in storage. The RDW-CV had a significant difference between the pre-wash and the 1st week in storage as well as the 2nd week in storage. This result indicates that the size of the cells changed, as cells go through oxidative stress they damage and haemolysis takes place, because there is a loss in membrane integrity, which releases haemoglobin and other intracellular proteins.

Table 4.6: Full blood counts for the group not haemolysed washed and suspended in saline, adenine, glucose and mannitol (SAGM).

	p-value		NHWSGM - SIGNIFICANT YES/NO AND INTENSITY		
			Pre-wash vs Post wash	Pre-wash vs 1st week	Pre-wash vs 2nd week
WBC	p-value>0.05	Not significant	No	No	No
RBC	p-value>0.05	Not significant	highly significant	very significant	very significant
HGB	p-value>0.05	Not significant	very significant	Significant	very significant
HCT	p-value<0.05	Significant	No	No	No
MCV	p-value>0.05	Not Significant	No	No	Significant
MCH	p-value>0.05	Not significant	No	No	No
MCHC	p-value<0.01	Very Significant	No	No	No
PLT	p-value>0.05	Not significant	No	No	No
RDW-ST	p-value>0.05	Not significant	No	No	No
RDW-CV	p-value>0.05	Not significant	No	No	No

Bartlett's p-value is included in the table for the specific test of the full blood count. The p-value for the different tests from the pre-wash till the second week in storage had a significant difference. The post-wash until the 2nd week in storage was not included, because there were no differences for it.

4.16 The results for NHWSGM

The full blood count was done on the group not haemolysed washed and suspended in SAGM. Bartlett's p-value for the group's white blood cell count was p-value > not significant. The white blood cell count did not have any significant differences from the pre-wash till the second week in storage in SAGM. The red blood cell p-value for the group was not significant. The result between pre-wash and post-wash had a highly significant difference. As stated, before red cells are lost in the wash process this can be the reason for the drop in red blood cells. The pre-wash and the 1st as well as the 2nd week in storage had a very significant difference. The

haemoglobin p-value for this group had no significant difference. The pre-wash and the post-wash had a very significant difference. The pre-wash and 1st week in storage had a significant difference. The pre-wash and the 2nd week in storage had a very significant difference in storage. The result is mostly the result of cells being lost in the wash process and it influences the haemoglobin concentration. The 2nd week in storage with very significant differences can be a result of the cells with oxidation stress.

The overall Bartlett's p-value of the haematocrit shows a significant difference. The results between the pre-wash and the post-wash, 1st and 2nd week in storage had no significant difference in the haematocrit results. In the mean corpuscular volume only, the pre-wash and the second week in storage had a significant difference. This result can be that the cells swelled in storage after the wash process. Only Bartlett's p-value for the MCHC had a very significant difference. The pre-wash till the second week in storage had no significant difference, this means that the cells held their shape and form. The RDW-ST and the RDW-CV had no significant differences between the pre-wash until the 2nd week in storage. This confirms that the cells were stored in SAGM better than in saline. There was no swelling or braking of cells in storage.

Table 4.7: The full blood count results for the group haemolysed washed and suspended in 0.9% Saline (HWSI).

Tests	p-value	HWSI – SIGNIFICANT YES/NO AND INTENSITY		
		Pre-wash vs Post wash	Pre-wash vs 1st week	Pre-wash vs 2nd week
WBC	p-value<0.001	No	Significant	Significant
RBC	p-value>0.05	highly significant	highly significant	highly significant
HGB	p-value<0.05	highly significant	highly significant	highly significant
HCT	p-value>0.05	highly significant	highly significant	highly significant
MCV	p-value<0.05	No	No	No
MCH	p-value>0.05	No	No	No
MCHC	p-value>0.05	No	No	No
PLT	p-value>0.05	Significant	No	very significant
RDW-ST	p-value>0.05	No	No	No
RDW-CV	p-value>0.05	No	No	No

Each week Bartlett's p-value is shown. All the tests from pre-wash till the 2nd week in storage was documented with the intensity of significance.

4.17 Full blood count results for group haemolyzed washed suspended in saline (NHWSI)

The Bartlett's p-value had a highly significant difference. This is an indication that the WBC wash washed out, but between the weeks of storage there were significant differences. The pre-wash and the post-wash for the Benforoni stats between the pre-wash and the post wash

showed no significant difference. The pre-wash and the 1st and 2nd week in storage post-wash had a significant difference in white blood cells. The red blood cell counts with Bartlett's p-value showed no significant differences. All three groups had a highly significant difference this include the pre-wash from the post-wash, 1st week in storage as well as the 2nd week in storage. This units had already lysed red blood cells that was washed out. The Bartlett's p-value for the haemoglobin (HGB) had a significant difference. In the pre-wash and the post-wash there were a highly significant difference. This can be of the cells washed out in the wash process and because there were already cells lysed pre-wash more cells were washed out so the concentration of the haemoglobin was much less. Between the 1st week in storage and 2nd week in storage there were also a highly significant difference. The Bartlett's p-value for the group had no significant difference. In the groups pre-wash and post wash there were a highly significant difference. In the was process you lose a lot of cells and when the same amount of preservative is added after the wash process the haematocrit is lower, because it is the percentage cells to volume of supernatant. Between the pre-wash and the 1st week in storage and 2nd week in storage there were a highly significant difference. The MCH, MCV and the MCHC had no significant differences between the groups from pre-wash till the second week in storage. The platelet count had a significant difference between the pre-wash and the post-wash. Between the pre-wash and the 1st week in storage there were no significant differences. In the pre-wash and the 2nd week in storage there was a very significant difference. The platelets did not survive that long in saline as a preservative. The pre-wash for the RDW-ST and the RDW-CV had no significant difference from the pre-wash till the 2nd week in storage. This means that the red blood cells did not lose their shape and were well preserved in the saline. This is an indication that the cells can be stored for 2 weeks after the wash process.

Table 4.8: Full blood count results for the group haemolysed washed and suspended in SAGM (HWSGM).

Tests	p-value		HWSGM - SIGNIFICANT YES/NO AND INTENSITY		
			Pre-wash vs Post wash	Pre-wash vs 1st week	Pre-wash vs 2nd week
WBC	p-value<0.001	Highly significant	No	No	No
RBC	p-value<0.01	Very significant	highly significant	highly significant	highly significant
HGB	p-value>0.05	Not significant	highly significant	highly significant	highly significant
HCT	p-value>0.05	Not significant	highly significant	highly significant	highly significant
MCV	p-value<0.05	Significant	No	No	No
MCH	p-value>0.05	Not significant	No	No	No
MCHC	p-value>0.05	Not significant	No	No	very significant
PLT	p-value<0.001	Highly significant	Significant	very significant	very significant
RDW-ST	p-value>0.05	Not significant	No	No	No
RDW-CV	p-value>0.05	Not significant	No	No	No

Bartlett's p-value is calculated for each result of the full blood count. The p-values are as follows: p>0.05 no significant difference, p<0.05 significant difference, p-value 0.01 very significant and the last p-value 0.001 highly significant. The weeks from pre-wash till the 2nd week in storage were compared and the intensity of significance stated.

4.18 Full blood count results for the group haemolysed washed and suspended in SAGM

Bartlett's p-value for the white blood cells had a highly significant difference. In the pre-wash till the second week in storage there were no significant differences.

As with the other groups the red blood cells were washed out and Bartlett's p-value for the group had a highly significant difference. The p-value for the pre-wash until the second week in storage had a highly significant difference. In this group as well as the HWSI there were already lysis in the red blood cell before the wash process that was washed out with healthy cells and leads to a decline in the red blood cell count. The HGB Bartlet's p-value shows no significant differences. The pre-wash until the 2nd week in storage had a highly significant difference.

The p-value for the haematocrit had no significant differences. Between the pre-wash until the second week in storage there was a significant difference. The p-value for the MCV result had a significant difference. The pre-wash until the second week in storage had no significant differences. The MCH had no significant differences in p-value. There were no significant differences for the pre-wash till the second week in storage as well. Only the MCHC results between the pre-wash and the second week in storage had a highly significant difference. For this reason, it is better to only store the haemolyzed washed cells for one-week post-wash for a safe product. The RDW-SD and RDW-CV showed no significant differences. The cells were well preserved in the SAGM; they kept their form and volume. It is possible to keep the cells after washing for 2 weeks, but because of the MCHC result it would be safer to only store them for 1 week.

Table 4.9: The full blood count was performed on the control group not haemolysed not washed (NHNW).

Tests	p-value		NHNW - SIGNIFICANT YES/NO AND INTENSITY		
			Pre-wash vs Post wash	Pre-wash vs 1st week	Pre-wash vs 2nd week
WBC	p-value>0.05	Not significant	No	No	No
RBC	p-value>0.05	Not significant	No	No	No
HGB	p-value<0.001	Highly significant	No	No	No
HCT	p-value>0.05	Not significant	No	No	No
MCV	p-value<0.05	Significant	No	No	No
MCH	p-value>0.05	Not significant	No	No	No
MCHC	p-value>0.05	Not significant	No	No	No
PLT	p-value<0.01	Very significant	No	No	No
RDW-ST	p-value>0.05	Not significant	No	No	No
RDW-CV	p-value>0.05	Not significant	No	No	No

Bartlett's p-value for each test was done. The significant differences from the pre-wash till the second week in storage was also documented. The p-value results are interpreted as p-value > 0.5 not significant, p-value<0.5 significant, p-value <0.01 very significant and the p-value <0.001 very significant

4.19 Full blood count results for the group not haemolysed not washed (NHNW)

The control group wash not washed and had no premature haemolysis. In the WBC, RBC results it shows that there were no significant differences between the time they were stored till the second week in storage. The HGB had a highly significant difference in this group, the pre-wash until the 2nd week in storage had no significant differences. The MCV had a significant difference, this can be that the donors selected could have had a variant of cell sizes when they donated. In the pre-wash until the second week in storage there were no significant differences. The platelet count had a very significant difference, this can be that platelets do not survive in storage well. The RDW-SD and RDW-CV had no significant differences. This concludes if blood is donated and stored without washing and premature hemolysis the cells are reserved well.

The post-wash results for all the groups and all the tests from the 1st week in storage and 2nd week in storage had no significant differences.

CHAPTER FIVE

DISCUSSION

5.1. Background

In the period from 2017 to 2020, there was an increase in premature haemolysis in the red-packed cell units received at George Blood Bank. There was no specific indicator tor premature haemolysis. Some of these units were still within their first week after donation. In the following weeks small studies were preformed to see if the root cause of the premature haemolysis could be found and eliminate to save our precious red packed cell units. The first aim was to see if the amount of the preservative and the anticoagulant is as prescribed in the product specification leaflet. The firs of the small studies were done to investigate the volume of the Saline, Adenine, Glucose and Mannitol (SAGM) the preservative or the anticoagulant Citrate, Phosphate and Dextrose (CPD) were constant and at correct levels as stated in the package insert of the distributor. The packs were weighed to determine whether there were any discrepancies in the weight of the SAGM and the CPD by using the package insert for a reference. If the CPD is too little this can cause clots in the donated pack. In the process of making components of a whole blood, clots can stop the flowing of the red blood cells from the primary bag to the secondary bag. In George, components are made by using a Terumo blood component extractor (T-ACE) The T- ACE machine uses an automatic press system that presses the cells out of the primary bag (buffy coat bag for making platelets) into the secondary bag that will be the red blood cell unit (RBC) as an end product. Any extra pressure inflicted on the red blood cells that are transferred to the secondary bag, may cause haemolysis in the bag. This bag is the red packed cells that we receive as stock in George blood bank. Any clots in the bag can hider the flow and causes extra pressure, which will have haemolysis as a result (Rapido, 2017).

The second aim was to see if it possible to make a colour chart, presenting free haemoglobin within a red packed cell unit with the corresponding colour in the supernatant. Dr Bellairs and S Sutton made a colour chart using specialized equipment. This colour chart was used as a reference to make a colour chart in George Blood Bank. In George specialized equipment is not available and alternative methods had to be used to create a colour chart. Percentage of haemolysis had to be calculated mathematically. For this mathematically equation, the haematocrit, serum haemoglobin and haemoglobin for red cells are mandatory. In George the option for serum haemoglobin tests is not available. The HemoCue was used to obtain the serum and the red cell haemoglobin results. Using the HemoCue to calculate the percentage haemoglobin had its obstacles. For accurate results, a full blood count was done at headquarters and the manual results obtained from the HemoCue was compared to the results.

Three sets of tests were done to find a standardized test for haemoglobin, haematocrit and percentage haemolysis for our setting. The findings show that specimens that were spun and taken from the blood bag were most similar to the tests done with a full blood count in Cape Town. Our laboratory was the only laboratory in the Western Cape Blood Service that used this method to calculate the haemoglobin, haematocrit and the percentage of haemolysis present in units with premature haemolysis. Research done by Morris and co-workers (2001), demonstrated that the spectrophotometers that was used did not manage to provide accurate measurements for haemoglobin in low concentrations. The HemoCue haemoglobin system provided accurate measurements of lower-scale haemoglobin concentrations. This study also confirmed accurate results using the HemoCue for plasma haemoglobin and haemoglobinuria in severe intravascular haemolysis (Morris, Pont and Lewis, 2001). Morris also said that in instances where there are limited resources this is a reliable method. In a later study done by Adam et al.,2012 they did a comparison between the haematological autoanalyzer result and the HemoCue machine. This study disagreed on reliability to produce the same results for the haemoglonin results using different methods. This study was comparing the capillary test, HemoCue and haematological autoanalyzer for a haemoglobin test, pre donation. The HemoCue has a variant of ± 1.0 g/dl. For the use of people donating this could be a problem as in their results the HemoCue haemoglobin result was 12.70, the capillary result was 12.87g/dl and the autoanalyzer result was 11.53g/dl (Adam et al., 2012). In this study we had the same difficulties. The comparison between the HemoCue and the haematological autoanalyzer also had a ±1.0g/dl variant for the red cell haemoglobin result.

A haemolysis colour chart was made using only the equipment that was available to us. This was done to enable staff to determine with greater ease, which units are still safe to be issued, although there is slight haemolysis present in an RBC unit, According to Dr Bellairs and S. Sutton (2017) their colour chart could be utilized as an indicator of which units are still suitable for transfusion for the whole of the western cape blood service. The blood that we receive in George is transported to us and processed in Cape Town. By creating our own colour chart, it is possible to optimize a colour chart for our requirements at the George Blood Bank.

Using the colour chart designed for this study, samples were selected to be included in the wash study.

The third aim was to establish if it is possible to save the units received in George Blood bank with premature haemolysis by using a cell was procedure. To evaluate this process and make a decision on washing these units a full blood count was done on all the study groups till the second week in storage.

The fourth aim was to see if it is possible to extend the expiry date of these units from 24hours after wash to two weeks post wash. The reason for this aim was if we have a lot of premature

haemolysed units, we might not be able to issue all these units within 24 hours after the wash process and then at the end we might lose them in anyway, not because of haemolysis, but of a short expiry time. For this aim the units was kept after the wash process for 2 weeks in storage to evaluate the results and see if the units could be stored for two weeks using different agents for resubstituting the units post wash. Saline and SAGM was used and evaluated for storage agent in this study.

The red blood cell count (RBCC) in all the groups had a significant difference between the washed groups except for the control group. This result was expected as some red blood cells get lost during the washing process, in this scenario the patient might need another unit for an effective transfusion. The literature states that up to 20% of red blood cells are lost through the wash process. The percentage of cells lost in the study may be more than 20% which is the norm for cells lost in the preparation of washed-packed red blood cells for patients with allergic reaction as the cells that were used in the study were already presented with haemolysis in the supernatant.

The haemoglobin (HGB) results for the wash groups also had a significant difference between the wash groups except for the control group. There was a significant difference between the wash groups' haemoglobin count post-wash except for the control group which did not have any significant differences in the storage procedure for 2 weeks. The haemoglobin where the cell was lost in the wash process had a direct influence on the haemoglobin results (Bennett-Guerrero., et al 2014).

The haematocrit (HCT) results for all the wash groups also had a significant difference between the wash groups except the control group. Most of the washed group except for the not-haemolysed washed and substituted with SAGM and the control group had no significant differences between the pre-, post-wash, week 1 and week 2 in storage. The haematocrit was influenced by the cells that were lost and the percentage of cells left and the amount of fluid in the bag. The cells that were washed and substituted with SAGM had the same amount of flued substituted as with the original component making of a whole blood. In this instance where the not-haemolysed units substituted with SAGM had no significant difference, the units with haemolysis and substituted with SAGM had a significant difference, this can be, because of the cells that was already lysed were washed out. If cells are washed all the fragmented cells will be removed and if the concentration of premature haemolysis is high, more cells will be lost and the haematocrit will be lower than in a pack that did not have premature haemolysis. Although the units were substituted with the same amount of SAGM the units with no premature haemolysis will have a higher concentration haematocrit. The haematocrit is the ratio between the cells and the plasma in the body (Reinhart et al., 2015), (Mancini et al., 2012).

The mean corpuscular volume (MCV) measures the actual size of the cells. How large or how small the cells are. The mean corpuscular volume of the cells had no significant differences

between the control group and the wash groups. The groups pre-wash, post-wash, 1st week in storage and the 2nd week in storage also showed no significant differences in results. This is a good indication for the wash process, this means that the cells could hold their shape and not deteriorate. All the cells were still the same shape before the wash process and after the 2nd week in storage for all the groups. In a study done by Reinhart et al., 2015 they washed units and stored them for 6 weeks in the first and second weeks of storage there were also no significant differences for the cells in storage. Damaged cells can be an effect of the wash process and can influence the MCV test result. In Reinhart's research only in the 6th week, they experienced that the MCV declined.

The mean corpuscular haemoglobin (MCH) test is the average amount of haemoglobin within the red blood cells. The mean corpuscular haemoglobin was done on all the wash groups and the control group, there were no significant differences between the wash group and the control group. There were also no significant differences between the pre-wash and the post-wash, week 1 and week 2 in storage. This means that the haemoglobin distribution stayed the same although the cells that had haemolysis present were washed out, there was no significant difference from the control group.

The mean corpuscular haemoglobin concentration (MCHC) this test checks the average amount of haemoglobin in a group of red blood cells. The MCHC also had no significant differences between the wash groups and the control group. The only significant difference that was present was the pre-wash and the 2nd week in storage for the not-haemolysed washed and substituted with SAGM. In the study by Reinhart et al., 2015 in the 6th-week post-wash in storage the cells swelled which had an influence on the MCHC level. The MCV level declined, because of the cells that lose their form and become smaller, whereas the MCHC increased, because of the cells that swell. In this study, the cells were only stored post-wash for 2 weeks and had no significant differences between the group, which is good news that the expiry date might be extended after the cells were washed and reconstituted with SAGM.

The platelet count (PLT) for the wash groups and the control group had no significant differences. The group HWSGM (haemolysed, washed and substituted with SAGM) had significant differences between the pre-wash and the post-wash, 1st week in storage as well as the 2nd week in storage post-wash. The platelet count had a significant difference in the wash group HWSI (haemolysed, washed and substituted with 0.9%saline) pre-wash and post-wash. There were also significant differences between the pre-wash and 2nd week in storage post-wash. The wash groups may have no significant differences, because in the making of the components the buffy coat is removed from the whole blood, in the wash procedure there would have been little platelets left in the red-packed cells. In the groups that had already haemolysis present prior to the wash process. The platelet counts in that specific group had a significant difference between the pre- and post-wash as well as in the weeks of storage. In an article of Medscape, it is stated that if red-packed cells are washed 33% of the platelet

products are lost. The functionality of the platelets that are left in the red-packed cell may be altered and provide a suboptimal response. In these red-packed cells already present with haemolysis prior to washing can have an influence on the platelet functionality in storage which can lead to the platelet's increased deterioration.

The red cell distribution width standard deviation (RDW-SD) shows the difference in the number of cells present and the different sizes of the cells. The 2 different wash groups and the ones with haemolysis present and those without haemolysis present had no significant differences from the control group. The different groups from the pre-wash till the 2nd week in storage also had no significant differences.

The red cell distribution width coefficient of variation in percentage (RDW-CV). This test is performed to see if the red blood cells are of normal size and shape. Normal red blood cells are disc-shaped, usually flat with a diameter of around 7.5um. There will always be red blood cells that are not properly formed. A normal value for red blood cells that are normal is between 85% - 89%. A normal RDW-CV range will then be 11% - 15%. The RDW does not measure the red blood cells, but rather how big the variation is in size and volume.

The white blood cell count (WBC) showed no significant differences between the wash groups except a very significant difference between the NHWSI and the NHNW after 1 week of storage. The NHWSGM also had a very significant difference from the control group (NHNW). The HWSI also had a highly significant difference from NHNW. The last group HWSGM had a highly significant difference from the NHNW. There were no significant differences between the pre-wash and post-wash. The significant differences were only between the pre-wash and week 1 of the NHWSI. There was also a significant difference between the pre-wash and the second week in storage, post wash.

The limitation of this thesis was that we had no specialized equipment to perform these tests. We had to work around this and try to find a way to get a standardized method to work out the percentage of the haemoglobin and the haematocrit. The method where the pilot tube was spun for 30 minutes at 3000rpm was the nearest to the autoanalyzer result. In the end, we used the HemoCue to work out the serum haemoglobin as the HemoCue was the only equipment we had. The private laboratories could not do serum haemoglobin for us, as their serum haemoglobin tests are also performed in Cape Town. The university was closed and when the university opened there was limited space for students to perform tests. In future if we need to do manual calculations of the haematocrit a cuvet and HemoCue that is specifically for serum haemoglobin can be used.

In the wash study the intention was to see whether the waste products associated with haemolysis could be removed from packed red cells. Current SOP's make provision for washing of packs for patients with allergies. In our case it can be argued that there were more waste products to be washed out, because of the presence of haemolysis. The wash method used for allergic patients with IgA antibodies to remove plasma proteins states that the unit will

only be washed once. In the current study it was necessary to determine the amount of wash cycles needed to produce a clear supernatant in units containing different amounts of free haemoglobin. Depending on the amount of haemolysis present the packs used in our study had to be washed twice and sometimes for a 3rd time to be able to have a clear supernatant. Investigating the results after the wash process it seems plausible to be able to save the units with a wash process. This is controlled mainly by the full blood count results of washed packs in our study. In a study done by Pulliam et al, they washed older RBC units 14 days old to remove the storage lesions from the packs (Pulliam et al., 2020). These RBC units accumulate biochemical and structural changes known as red-blood cell storage lesions. In this study washing the cells decreased the accumulation of the storage lesions which is present in older stored units. Storage lesions cause an increase in morbidity and mortality in patients receiving these units. Since the study was done in George there was no literature on previous studies done on RBC units presenting with haemolysis that were washed to remove the waste products. According to WCBS SOPs, units are discarded if any visible haemolysis is present. The second part of our study question was whether it is possible to extend the expiry date for these units by adding SAGM as a preservative to the units after washing? Evaluating the full blood count done on the 4 wash groups and the control group it is apparent that the RBC units may be able to be stored for two weeks. The tests performed to validate potential storage of washed units was done for two weeks and in our study appeared feasible. More tests are recommended to confirm this. A research article done by Cardigan et al., 2020 states if the red-packed cells are washed with the COBE 2991 which is an open system the expiry date is 24 hours after the RBC unit's seal was broken. The average day that the red-packed cells were washed was on the 9th day after donation. There were 19 service wash solutions used. 10 of the solutions were saline with glucose which is recommended as an ACP215 wash solution and 3 wash with a red cell additive solution. Post-wash solutions included suspending the red-packed cells in saline, five suspended glucose with saline and eight in additive solution. When red-packed cells are washed with the COBE 2991 the expiry date of the washed-packed cells is 24 hours as this is an open system. The 24 hours starts after the seal was broken off the red-packed cell when the washing process started. If the ACP215 system is used with haemonetics the maximum shelf life is 14 days, as this is a closed wash system (Cardigan et al., 2020). More tests need to be done in the future, to confirm our findings. We hope to conduct further biochemical analysis, but due to limited laboratory space in the Covid-19 lockdown, this was not possible. Although the samples were taken to perform the oxidative stress tests it was not possible to perform them. In future studies in laboratories with limited specialized equipment, it would be very helpful if there could be a HemoCue cuvet available that measures serum haemoglobin levels, which is calibrated for this method. This will be interesting to see if the serum haemoglobin tests will vary a lot from this study. In potential future research, it would be advisable that more studies are conducted to measure the

oxidative stress developed in washed and unwashed RBC units that are being stored. These studies could then also include tests such as potassium levels, cytokines and LDH levels. If confirmatory tests show positive outcomes, it is possible that clinical guidelines could be amended to limit the wastage of blood due to haemolysis.

Therefore, the main objective was to try and save the red blood cell units with premature haemolysis and not to fail the project by discarding it.

The main study had 5 groups. The first group was not-haemolysed, washed and reconstituted with 0.9% saline (NHWSI). The second group was not-haemolysed, washed and reconstituted with SAGM (NHWSG). The third group was haemolysed washed and reconstituted with 0.9% saline (HWSI). The fourth group was haemolysed, washed and reconstituted with SAGM. The last group or 5th group wash is not haemolysed and not washed (NHNW) also referred to as the control group.

5.2 Findings to the investigation for potential reasons for haemolysis

The amount of Citrate Phosphate Dextrose should be 63ml for a collection of 450ml of whole blood. The ratio for CPD to whole blood is 1:7 (Cober et al., 2001). The amount of SAGM should be 100ml for 450ml of whole blood as a preservative (D'Amici et al., 2012). The results inferred a significant difference in the CPD results of the units, as the amount of CPD fluctuated significantly between the bags. This can be the reason for premature haemolysis in the red blood cells. It is important to have the right amount of CPD per 500ml blood if not otherwise clots form in the donated bag.

5.3 Procedure in creating a colour chart

Out of the 3 methods used to calculate the haematocrit results compared to the autoanalyzer, was the spun segment, which gave similar results. The same methods were used to calculate the haemoglobin results. 3 methods were also used, the nearest to the autoanalyzer were the spun segment. The formula to calculate the percentage haemolysis needs the haematocrit result, serum haemoglobin result and the red cell haemoglobin result. Using only the HemoCue the colour chat was created. This colour chart compared to the one Dr Bellairs and S Sutton created. This chart is very useful when units present with premature haemolysis to decide to save the unit or discard it. Another chart where also used to select units that represents the percentage of haemolysis present for the amount of g/dl free haemoglobin (Jaeger 2021)

5.4 The findings of units washed with 0.9% Saline

The full blood count results showed promising results. As expected, red blood cells were lost and influenced the red blood cell count, haematocrit and the haemoglobin results. In literature

it states that +- 20% of cells is lost in the cell wash process. The other results showed that the platelets and white blood cells are reduced in the wash process; this leaves a safer product for transfusion, because all the waste products have been removed. White blood cells in a packed red cell cause reaction and can lead to inflammation. Although there was red cell loss, there were still enough cells left for a patient to be transfused after two cycles of saline washing, in normal washing procedures. The volume of a unit of red cell concentrate is approximately 300-350 ml (including the additive solution) and the haematocrit is between 0.55 and 0.70 l/l. (Professional Education, 2021). The aim is to be able to wash out the haemolysed units leaving them after washing as a transfusable product was confirmed. Future studies are needed to implement this procedure and limit product loss.

5.5 Storage of units with premature haemolysis

When evaluating the results of the full blood count done on the pre-wash units, it is apparent that the stats showed no significant differences between the units presenting with premature haemolysis and the units without haemolysis. This means that the free haemoglobin was in the pack, but did not influence the haemoglobin results. Although the units were haemolysed, they were not under stress, because the RDW-CV and RDW-SD had no significant differences. The results do show that the units washed that had premature haemolysis had significant differences in storage sooner than the 2 groups that were washed and not haemolysed before the wash process. For this reason, it is advisable to only store the units for one-week post-wash and not 2 weeks (COM-28)

CHAPTER SIX

CONCLUSION

The findings showed that there was a significant difference in the amount of Citrate, Phosphate and Dextrose (CPD) in the pre-donation bags. This irregularity between the quad bags could be the cause of premature haemolysis in recently processed units into components.
The study results showed that it is possible to wash out premature haemolysis from a donated red packed cell. This means that units can be saved and not discarded unnecessary. This will save our concern for stock levels at a blood bank far from the source that provides us with blood. Although this study has shown that it is possible to wash out free haemoglobin from donated red blood cells it is important to do further testing to confirm this conclusion.

Evaluating the results of the full blood count done from pre-wash till the second week in storage it is possible to keep the units for two weeks in storage post wash and not only for 24 hours as stipulated in the work in struct ion. Although our findings suggested it is safe to keep the units in storage for two weeks, it is recommended that more test are performed to confirm these findings.
The study additionally created a standardised colour chard calibrated for the region, which can be used to visually assess haemolysis, given the lack of equipment to measure haemolysis.

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APPENDICES

APPENDIX A: CHAPTER 3 ADDITIONAL INFORMATION

A.1 Weighing of packs

Approximately 100 quad packs were weighed to ensure that the CPD and SAGM levels in the packs were as prescribed in the manufacturers leaflet. First, the quad packs without any blood contained therein were weighed as shown in figure A.1 below. This was to determine whether all the bags weighed the same so that the SAGM and the CPD would then be of the same volume. Most of the quad pack's pre-donation weighed 330g.

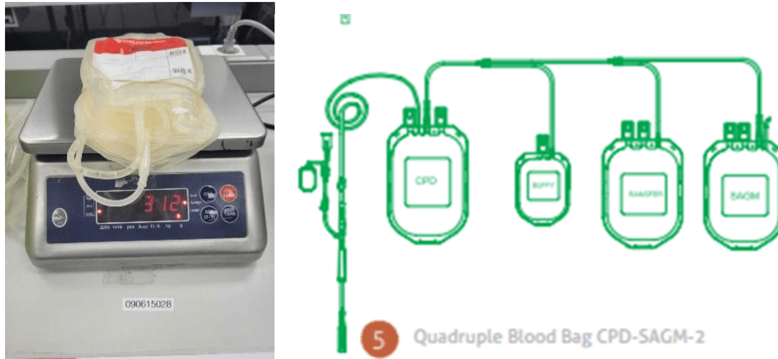

Figure A.1: Empty quad pack

Figure A.2: How to take off the weight of the empty bag

Figure A.2 is an illustration of how the weight of the CPD and the SAGM bags were measured. On the scale illustrated, the weight of the satellite packs was first measured and then eliminated from the component weight by pressing the TARE button.
Both the CPD bags were weighed and the SAGM bags were weighed in this manner.

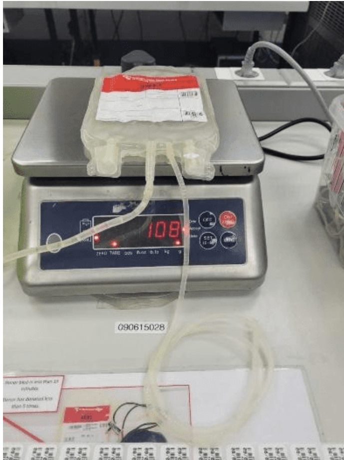

Figure A.3: The method that was used to weigh the SAGM

Figure A.3 illustrates the weighing of SAGM packs only to determine the volume inside.

Figure A.4: The method of how the CPD were weighted

Figure A.4 illustrates the weighing of CPD packs only to determine the volume inside.

A.2 Measurement of Haemoglobin

A colour chart that utilized the degree of discolouration of each haemolysed unit's supernatant would indicate the degree of haemolysis of each implicated packed cell unit.

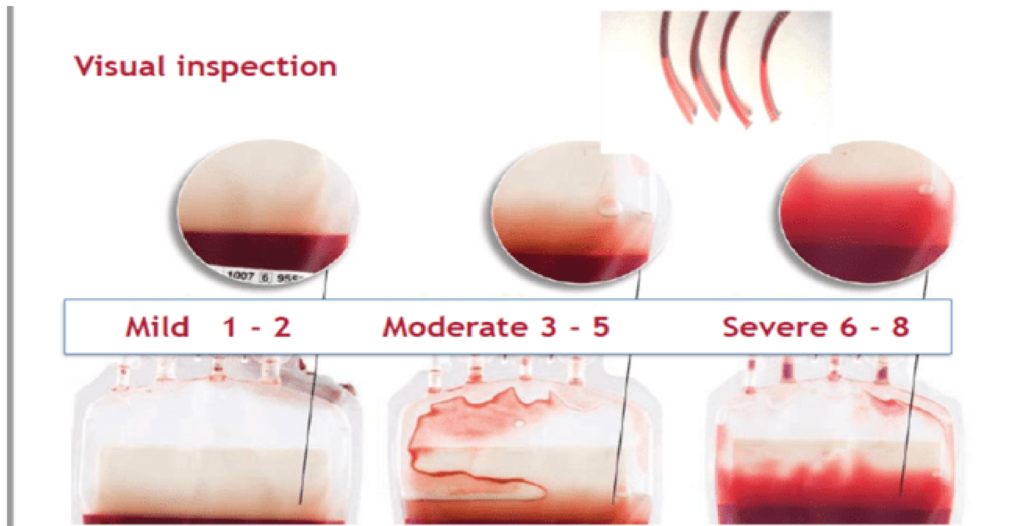

Figure A.5: The visual assessment guide of the Canadian Blood Services and the Sutton & Bellairs (2017) presentation was utilised to visually assess the percentage of haemolysis in RBC units (Canadian Blood Services, 2009; Sutton & Bellairs, 2017)

After the congress in 2017, the colour chart in Figure A.5 was used in the Western Cape blood bank to evaluate units containing haemolysis. The colour chart is used to aid the technologists to visually inspect the presence of Haemolysis. In this study, the colour of the supernatant on this chart was used, to aid in determining which units could be selected, as well as to group the units with similar supernatant together without the aid of serum haemoglobin (Hb), red blood cell Hb or the percentage haemolysis. The units were grouped in order of haemolysis colour present in the supernatant, ranging from light pink to dark red.

Since the George Branch did not have the specialized equipment to mathematically determine the percentage of haemolysis, alternative approaches were investigated.

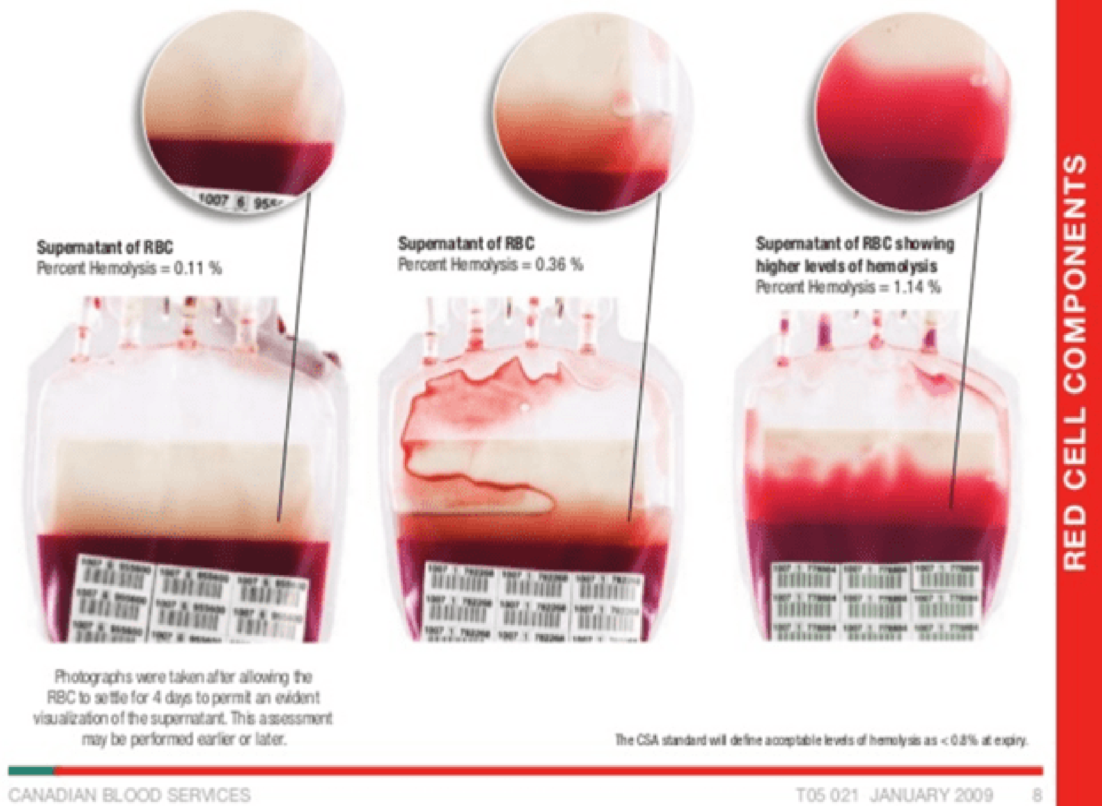

Figure A.6: Visual assessment guide for assessing units containing haemolysis and amount of haemolysis present

This visual assessment guide shown in Figure A.6, which was obtained from the Canadian blood service, helped to group the units according to the colour haemolysis and percentage haemolysis present. Two methods were used to create a colour chart, namely visual comparison and calculation. After the grouping of haemolysed units according to their colour was completed, it was then important to calculate the haematocrit for each unit.

A 2.1.1 Measurement of Haemoglobin via HemoCue

The headquarters in Cape Town have an automated system, namely the QWALYS EVO, that has the ability to do full blood counts. At the George Blood Bank, however, we only have the HemoCue to perform the Haemoglobin (Hb) of red blood cells and the serum haemoglobin of the packs.

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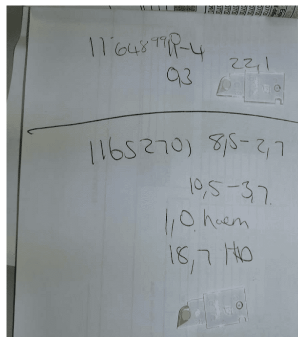


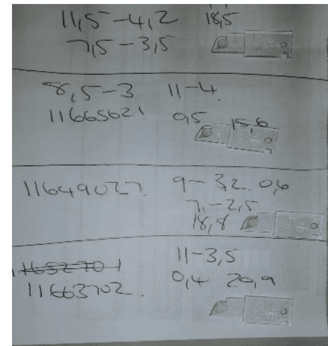
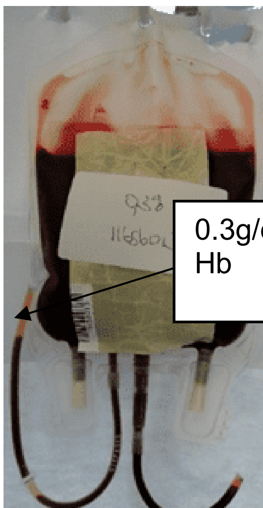
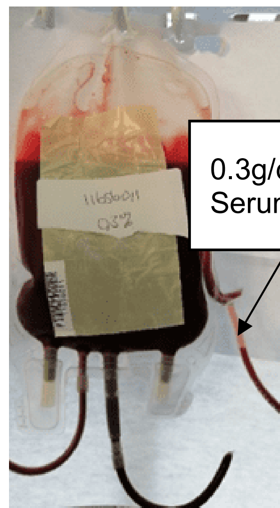
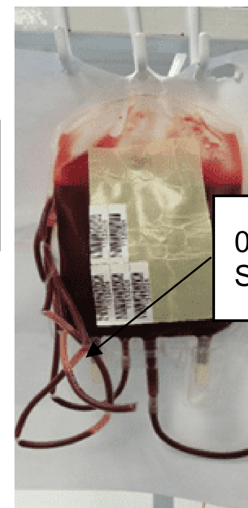


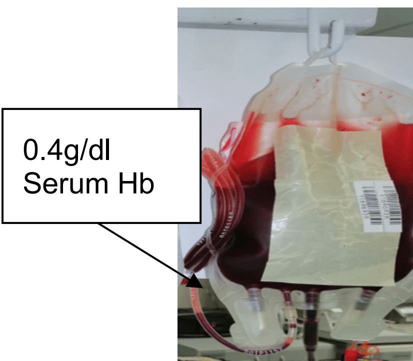
Figure A.7: A picture of a HemoCue instrument, as employed in this study, as well as an example of a calculation of results for serum Hb as measured with the HemoCue

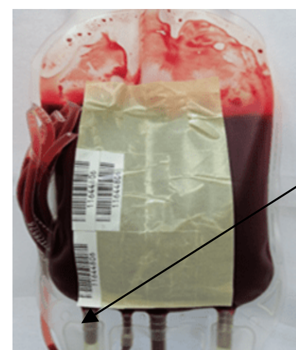
The blood haematocrit and the serum haemoglobin were both tested on the HemoCue. When the supernatant is darker, it means that the amount of free haemoglobin is increased. The darker the supernatant, the more free-haemoglobin is present in the red blood cell unit as shown in Figure A.7. Units with the same serum Hb were sorted together and compared to ensure that the colour correspond with the colour chart.











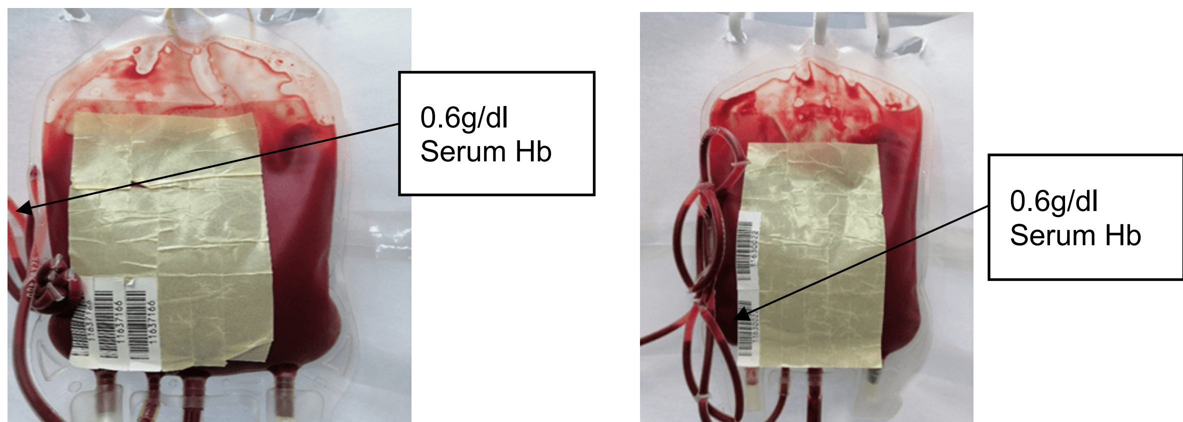


Figure A.8: Pictures of red cell concentrate units with haemolysis visible in the supernatant as received at the George blood bank. These pictures show the difficulty in visually determining the difference in the level of haemolysis between packs visually

In Figure A.8 units with the same serum haemoglobin levels were paired together. Units which were paired together had 0.3g/dl, 0.4g/dl and 0.6g/dl serum haemoglobin in the supernatant. This was to illustrate how the colour would present increasingly darker in the supernatant when the serum haemoglobin elevated. This discovery was helpful in creating a custom colour chart for the George Blood Bank. The segments of the red blood cell units were used for testing the serum haemoglobin.

The full blood counts could not be done at the George Blood Bank, as the haematocrit had to be worked out mathematically. It was important to calculate the haematocrit to be able to work out the percentage of haemolysis present in the unit. The first 16 units were sent to Cape Town for a full blood count that was performed on the QWALYS EVO as a standard that had to be worked out for the haemolysed units. 11 of these units had haemolysis and the other 5 were non-haemolysed units. This was also to compare the red blood cell count for haemolysed and non-haemolysed red blood cell units to see if there was a difference.

2.1.2 Standardizing the haematocrit result

There were 3 methods done at the George Blood Bank to try and reach the same haematocrit done in Cape Town on the haematological autoanalyzer.

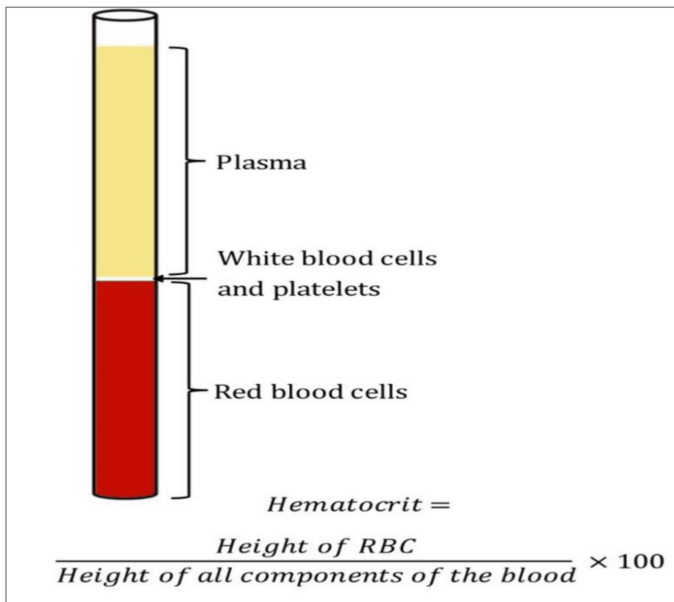
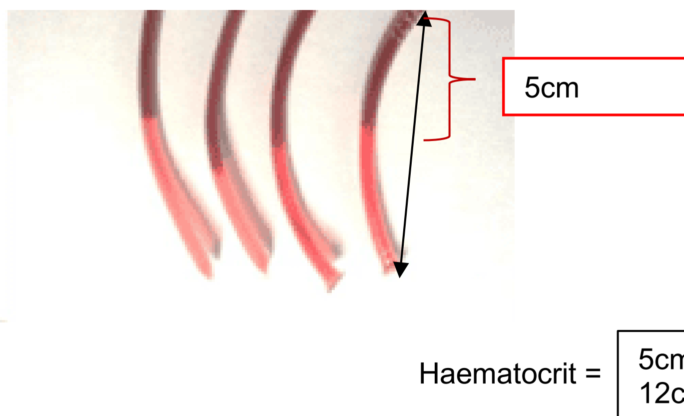


Figure A.9: The illustration and formula to calculate the haematocrit by (Mondal and Lotfollahzadeh, 2021)

Previously it was stated that in George we do not have the equipment to do full blood counts, Figure A.9 shows the method that was used in George to mathematically work out the haematocrit for the units.



Haematocrit = $\frac{5cm}{12cm}$ X100 = 41.7%

Figure A.10: The figure illustrates the first method that was used to mathematically calculate the haematocrit using the pilot tube of a red blood cell unit with haemolysis present in the supernatant

This method did not completely correlate with the automated haematocrit result.

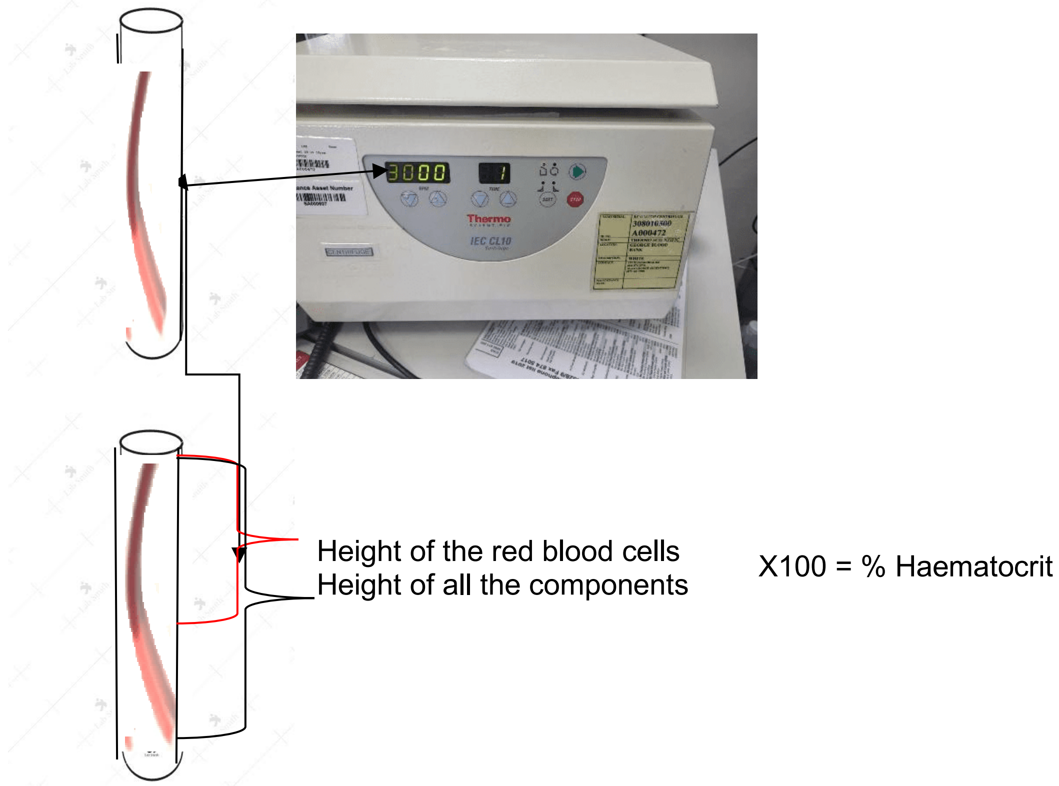
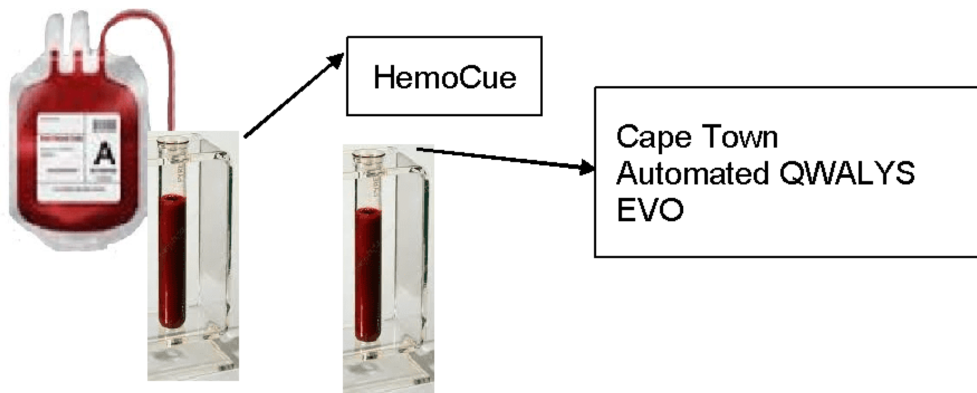
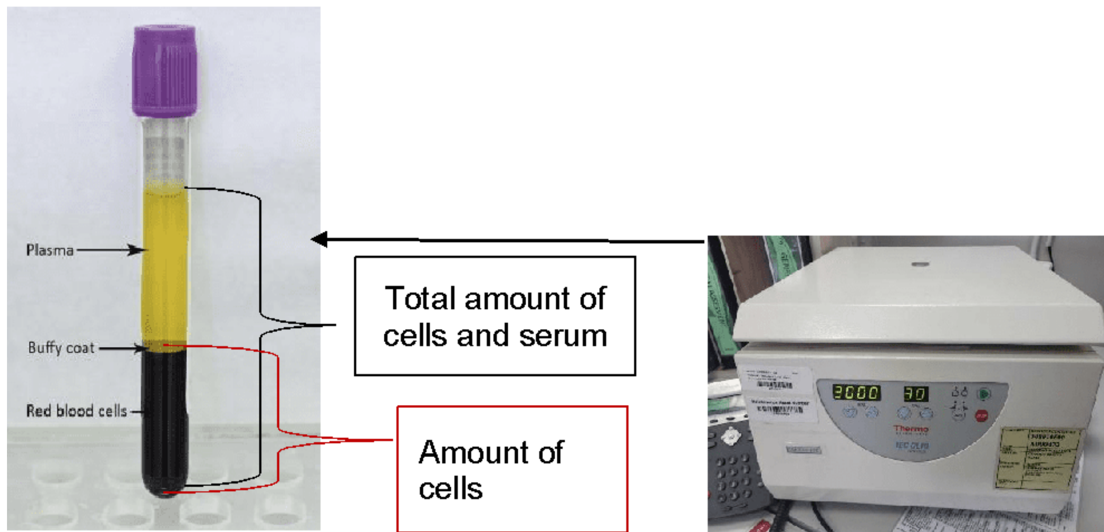


Figure A.11: Second method used to calculate the haematocrit

In Figure A.11 a second method was used which included a method by spinning the pilot tube at 3000 rpm for 1 minute. The result of this haematocrit was also not comparable to the automated haematocrit.





Height of the red blood cells / Height of all components of blood X100 = % haematocrit

Figure A.12: Third method that was used to calculate the haematocrit

Figure A.12 consisted of taking blood from the sample bag and transferring it into two test tubes, one for the HemoCue and another one for the QWALYS EVO used in Cape Town.

The three methods were compared. Although there were almost none of these 3 methods that were exactly like the automated full blood count result, the last method's results were the closest.
Using the method by Sawant et al., 2007 the percentage haemolysis could be calculated for these units.

A.2.1.3 Calculating Haemolysis using 3 haematocrit methods

The percentage of haemolysis in RBC units were calculated as follows:
(100-Hct) x plasma Haemoglobin (gdl^{-1})/Total Hb (gdl^{-1})
The formula (Sawant et al., 2007)

Figure A.13: The formula for calculating percentage haemolysis

In Figure A.13 the method illustrated was used to work out the percentage of haemolysis for the red blood cell units. In Table 4.3 the number of haemolysis was also worked out for the 4 different methods used to work out the haematocrit. In the table, it is apparent that the haematocrit influenced the amount of haemolysis calculated. A haemolysis chart was created for George to guide us so that we do not discard units that are still safe to use. Table A.4 shows the difference in the percentage of haemolysis worked out using the methods previously stated.

The colour chart was also necessary for the study because we do not know what causes haemolysis. At the time when the thesis was done there were no solutions to eliminate wastage, because of haemolysis. The colour chart was also necessary for the study as this was a guideline to select the units for the washing process. The units were first selected with the colour chart and groups of the same colour haemolysis in the plasma were grouped together. These units' haematocrit and amount of haemolysis were worked out mathematically.

Results:

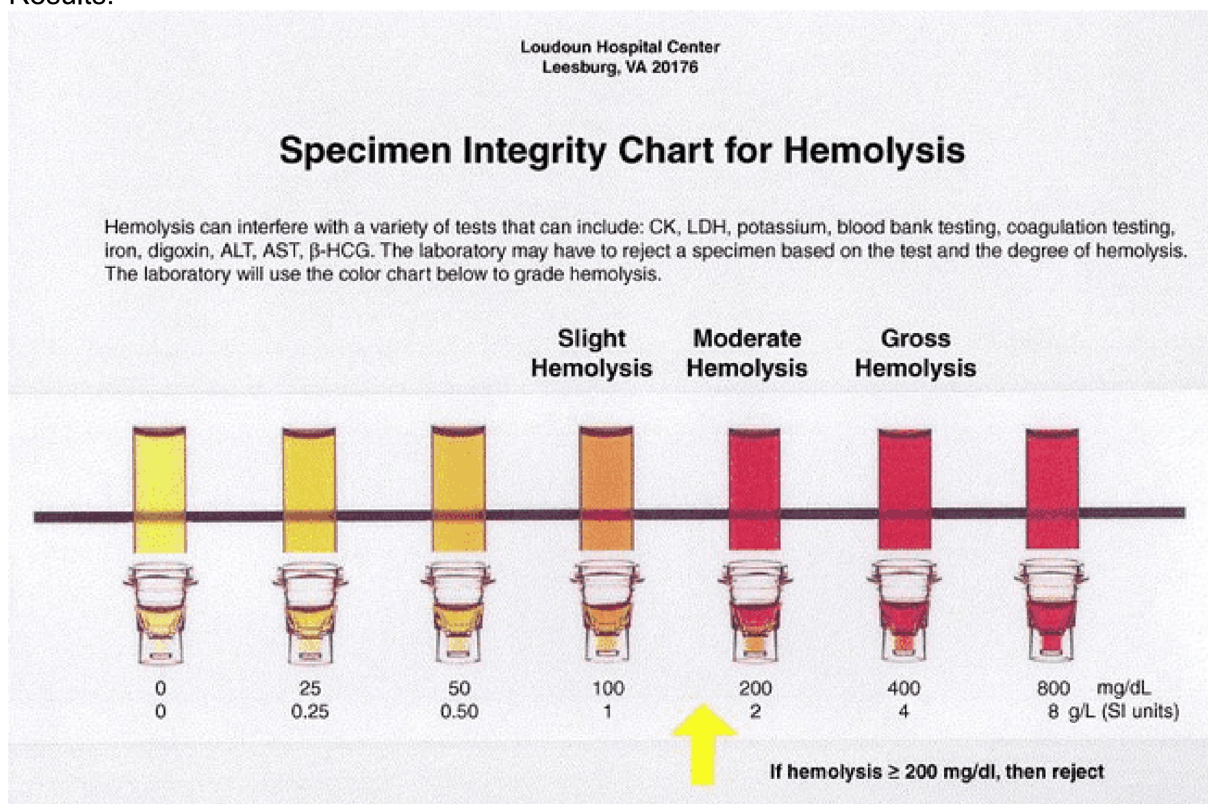


Figure A.14: Haemolysis chart done (Ni et al., 2020) this chart is an assessment of haemolysis in a blood specimen which affects test results. Haemolysis in a test sample can give false positive or negative results. This chart was used as a reference in the illustration of the amount of free haemoglobin g/dl in a test tube and the colour it presents.

	Measured free Hgb (g/L)						
	0	2.2	3.4	4.5	6.7	8.0	19.4
total Hgb (g/L)/HCT of unit/1% free Hgb cutoff (g/L)							
130/39%/2.1	0%	1.03%	1.60%	2.11%	3.14%	3.75%	9.10%
170/51%/3.5	0%	0.60%	0.98%	1.30%	1.93%	2.31%	5.60%
220/66%/6.5	0%	0.34%	0.53%	0.70%	1.04%	1.24%	3.00%
	Percent hemolysis [100 − HCT(unit)] × free plasma Hgb (g/L)/total Hgb (g/L) = % hemolysis Red = above 1% hemolysis cutoff Green = below 1% hemolysis cutoff						

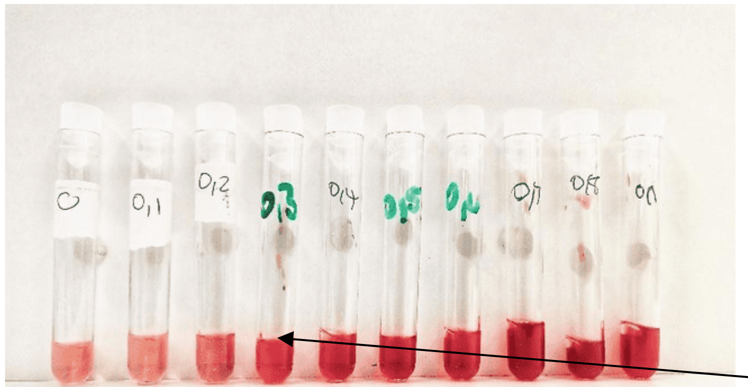
Figure A.15: This chart represents the percentage of haemolysis present for a specific amount of g/dl free haemoglobin (Jaeger 2021)

A.2.1.4 Developing a colour chart for George WCBS

Using the guidelines of Brittany Jaeger 2021 and Dugan et.al 2007 the colour chart for George was completed. All the results were compared: the haematocrit, serum haemoglobin and the percentage haemolysis were compared. After having all the results for the haematocrit and the percentage haemolysis it was very difficult to make a colour chart as done previously by previous authors as these results differ with different methods. This chart was done using mainly the serum Hb as this was the only non-variable result that we had. The HemoCue was the only equipment that we have to do the serum Hb. In the future the serum Hb may be used to evaluate a unit in George as well a comparison to the colour chart only done for George.

Figure A.16: After testing multiple units of red packed cells with haemolysis in the supernatant the first colour chart was created for George Blood Bank. The colour chart was created by using

the HemoCue instrument and by mathematically calculating the free haemoglobin in the supernatant



Here different units as in the first colour chart were used to see if the colour would be the same as the first one if compared. This colour chart would be used as a method for saving or discarding units and for the selection of the washing process. The second chart for 0.3g/dl has the same colour as the first chart.

Figure A.17: This is the second haemolysis chart that was done in George Blood bank using the HemoCue. New units were used to create the second colour chart. The results were compared to be certain the colour represents the percentage free haemoglobin in the supernatant. The first and the second colour had to have the same colour for the g/dl free haemoglobin in the supernatant

Previously the HemoCue was also used for the serum haemoglobin tests by Janatpour 2004 and he concluded that the HemoCue serum haemoglobin results were higher than with the Photometer system and the tetramethyl-benzidine chemical method. Although a higher result had been obtained by this method Janatpour 2004 says in his research that it is a reliable objective method to measure plasma haemoglobin for quality control and validation process, which is also faster, easier and reliable. A haemolysis chart is done to evaluate units to be washed by using the methods available in George's blood bank.

A.2.1.5 Experimental wash process, before the main study

The units with different amounts of the percentage of haemolysis present were used in the wash proses. The first part of the study was to see if the haemolysis could be removed by a cell wash process. The second result that we wanted to see was how many times a unit of blood needed to be washed to give a clear supernatant. In the normal wash method, the present with no haemolysis and the wash cycle is only once to remove the plasma proteins and leukocytes from the packed red blood cell. The theory behind this is that if the haemolysed cells and waste products are removed from this unit, it could be safe for transfusion as free haemoglobin is toxic to the body. The units were also weighed to make sure there are enough cells left after washing for the transfusion of a full red-packed cell. In table 4.5 the weight of the units can be seen after each wash. The units were selected by the percentage of haemolysis and then were washed.

First, 12 units were washed as a trial to see if enough cells would be left after washing. 10 units were haemolysed and 2 units were non-haemolysed and were used as a control group. Some of the units that had more haemolysis present were not clear after the 2nd wash and had

to be washed again. The end product aims to have a clear supernatant without any cell degradation products.

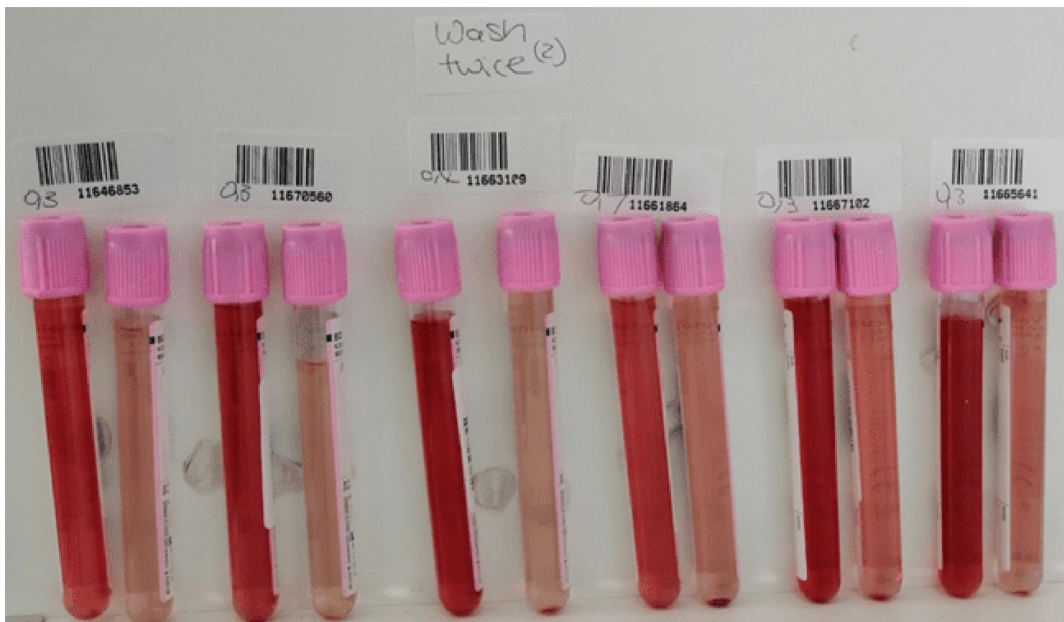


Figure A.18: This is a visual presentation of red blood cell units with 0.3g/dl free haemoglobin in the supernatant before they were washed and the second test tube the result of the red blood cell units after being washed twice with 0.9% saline

In figure A.18 shows units that contain mostly 0.3g/dl free serum haemoglobin in the supernatant. They were washed twice and the supernatant was clear enough for a safe transfusion.

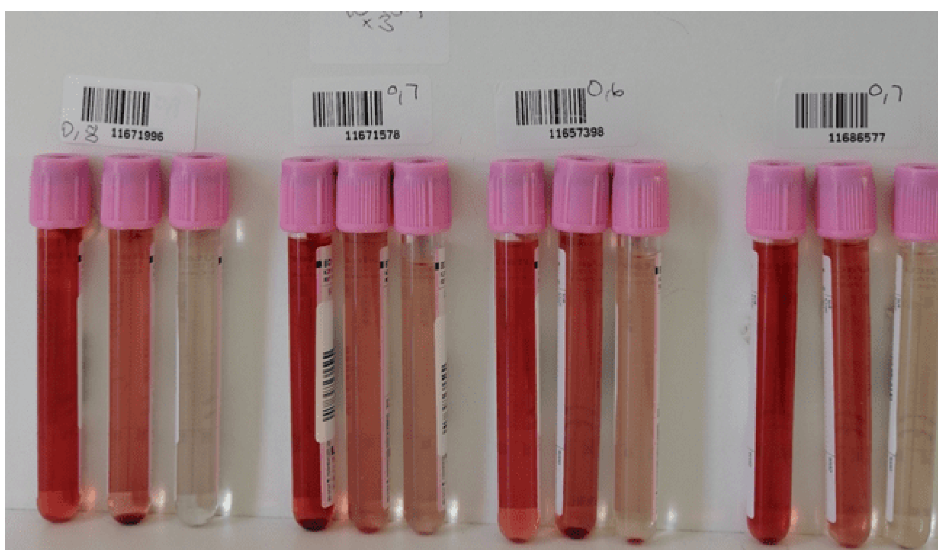


Figure A.19: Is the illustration of red blood cell units with 0.6 to 0.8g/dl free haemoglobin, the last test tubes show the supernatant after the units were washed 3 times and present a clear supernatant

The figure A.19 shows that units containing more than 0.6 g/dl or more haemoglobin need to be washed 3 times to get a clear supernatant in the unit. It is important to remove all the waste materials from the units. In this picture, it was illustrated that if the serum haemoglobin is between 0.6g/dl to 0.9g/dl of free haemoglobin it is safer to wash the units 3 times for a better result. In this trial wash process, we proceeded to the main study as all our questions were answered and we could proceed with the main study.

After the units were washed 7 of the units were substituted with 130ml Saline this included the non-haemolysed units and the other 5 units were substituted with SAGM. If units were washed and the supernatant is subtracted the cells in the bag are very thick. In the washing method, Saline as a norm will be added. In this study SAGM instead of Saline was added to try and extend the expiry date of the washed cells. Before washing and after washing the full blood counts were also done on these units. This trial was also done to see how many times it would take to wash the units with a certain amount of haemolysis present in the red-packed cells. The Question we also wanted to answer was, will there be enough cells left if haemolysed units were washed? After the trial, the answer was, there will be enough cells left after washing to transfuse a full red blood cell unit. This was done by weighing the unit before washing and after the first wash and again after the second wash. These units were then weighed for the last time after the saline and the SAGM had been infused.

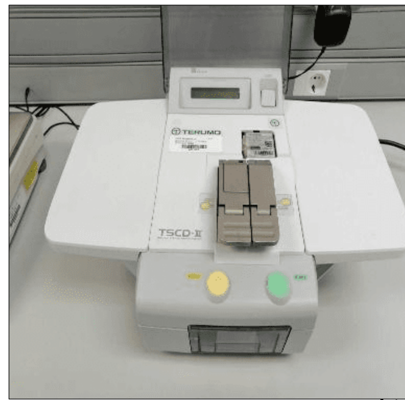
A.2.2 The main study's wash process

The main study followed. In the main study, the units were only washed once to see how the units survive after washing and if the expiry date could be extended. Red-packed cell units used in the main study and all the previous trial studies were randomly selected; the only criteria were that the units should be less than 10 days old. The inclusion criteria selection was not controlled on discrimination numerical, Alphabetical, Gender or phenotypic preferences. The selection of haemolysed units was the same as for the trial test by using the colour chart and doing the haematocrit and the percentage of haemoglobin on them. 18 units with haemolysis in the supernatant were used. 11 non-haemolysed units were selected and 5 units were selected as the control group. This group had no haemolysis and would also not go through the wash process. After selection, these units were washed.

Table A.1: The 4 washed groups and the group not washed.

18 haemolysed units		11 not-haemolysed units		5 Not haemolysed and not washed.
8 units were washed and reconstituted with 0.9% Saline HWSI	10 Units washed and reconstituted with SAGM. HWSGM	5 not-haemolysed reconstituted with 0.9% Saline. NHWSI	6 Units were haemolysed and reconstituted with SAGM NGWSGM	5 Units haemolysed not washed NHNW

The table represent the 5 groups and the number of units that was washed in 0.9% saline and the preservative added to the units in storage, as well as the control group that was not washed or haemolysed.

A. B. 

C.

Figure A.20: Equipment used in the washing method. A. Terumo centrifuge, B. Terumo docking device, C. hand press

In Figure 3.20 from the left is the Terumo Centrifuge used in washing the units. The Terumo docking device (sterile welder). This is a sterile procedure to weld the pilot tubes together without breaking the seals. This helps to prevent bacterial contamination in the washing process. The litre saline bags were also attached to this method. The hand press third from the left was used to take the supernatant off the bag that was washed.

Appendix B: Chapter 4 additional information

Table B.1: Pack weights of the SAGM and CPD without the 28 grams which the empty bag weighs. This table shows the different volumes that was inside the pack for the preservative and the anticoagulant.

NR	SAGM	`-28g	CPD	-28g	NR	SAGM	-28g	CPD	-28g
1	136	108	92	64	52	133	105	98	70
2	131	103	90	62	53	134	106	95	67
3	132	104	95	67	54	133	105	89	61
4	137	109	85	57	55	135	107	97	69
5	135	107	98	70	56	131	103	85	57
6	131	103	93	65	57	131	103	93	65
7	132	104	88	60	58	132	104	88	60
8	128	100	88	60	59	128	100	88	60
9	132	104	84	56	60	132	104	84	56
10	136	108	84	56	61	136	108	84	56
11	130	102	95	66	62	130	102	95	66
12	129	101	84	56	63	129	101	84	56
13	133	105	89	61	64	133	105	89	61
14	133	105	91	63	65	133	105	91	63
15	129	101	88	60	66	129	101	88	60
16	131	103	86	58	67	131	103	86	58
17	135	107	78	50	68	135	107	78	50
18	128	100	93	65	69	128	100	93	65
19	134	106	92	85	70	134	106	92	85
20	127	99	93	65	71	127	99	93	65
21	131	103	89	61	72	131	103	89	61
22	122	94	89	61	73	122	94	89	61
23	132	104	94	66	74	132	104	94	66
24	130	102	94	66	75	130	102	94	66
25	128	100	89	61	76	128	100	89	61
26	134	106	96	68	77	134	106	96	68
27	130	102	99	71	78	130	102	99	71
28	129	101	84	56	79	129	101	84	56
29	135	107	90	62	80	135	107	90	62
30	135	107	97	69	81	135	107	97	69
31	136	108	84	56	82	136	108	84	56
32	137	109	93	65	83	137	109	93	65
33	130	102	91	63	84	130	102	91	63
34	132	104	91	63	85	132	104	91	63
35	124	96	97	69	86	124	96	97	69
36	130	102	92	64	87	122	94	92	64
37	131	103	90	62	88	125	97	91	63
38	135	107	93	65	89	134	106	97	69
39	130	102	98	68	90	132	104	91	63

NR	SAGM	-28g	CPD	-28g	NR	SAGM	-28g	CPD	-28g
40	133	105	91	63	91	125	97	89	61
41	132	104	94	66	92	135	107	94	66
42	133	105	89	61	93	127	99	91	63
43	133	105	92	64	94	137	109	93	65
44	131	103	93	65	95	136	108	91	63
45	130	102	93	65	96	138	110	93	65
46	129	101	101	73	97	130	102	91	63
47	130	102	91	63	98	133	105	90	62
48	125	97	92	64	99	130	102	94	66
49	130	102	93	65	100	136	108	91	63
50	128	100	94	66	101	134	104	93	65
51	126	98	94	66	102	130	102	95	67
AVERAGE WEIGHT SAGM			AVERAGE WEIGHT CPD						
131.30g			91.14g						

Table B.2: Washing of the cells and the number of cells lost

Unit number	Product	Weight before wash	1st wash	2nd wash	Cells lost	Substitute with 130ml
1	ARBC	335g	317g	244	0.27g	130ml
2	LRBC	255g	227g	181g	0.29g	130ml
3	ARBC	313g	287g	235g	0.25g	130ml
4	ARBC	351g	327g	265g	0.25g	130ml
5	ARBC	352g	327g	271g	0.23g	130ml
6	ARBC	343g	328g	320g	0.06g	130ml
7	ARBC	333g	312g	254g	0.24g	130ml
8	ARBC	344g	317g	222g	0.35g	130ml
9	LRBC	308g	279g	229g	0.26g	130ml
10	ARBC	370g	344g	296g	0.20g	130ml
11	ARBC	334g	272g	224g	0.33g	130ml
12	ARBC	346g	270g	262g	0.24g	130ml
13	ARBC	364g	333g	271g	0.26g	130ml
14	ARBC	331g	307g	245g	0.26g	130ml
15	ARBC	313g	287g	224g	0.28g	130ml

The cells were weighed after each wash to calculate the number of cells lost. After the wash process the red blood cell units were suspended with 130ml saline or 130ml SAGM.

Table B.3: Washing of units 3 times and the number of cells lost

Unit Nr.	Product	Weight Pre-wash	Post wash 1	Post wash 2	Post wash 3	Cells lost after 3 washes	Units were substituted with 130ml of 0.9% Saline
16	ARBC	325g	258g	272g	232g	0.28g	130ml
17	ARBC	315g	240g	244g	214g	0.32g	130ml
18	ARBC	325g	269g	273g	248g	0.24g	130ml
19	ARBC	327g	268g	276g	247g	0.24g	130ml
20	ARBC	411g	350g	364g	298g	0.27g	130ml

B.1: RBC count pre-wash for the 5 groups

These units include haemolysed units and non-haemolysed units before the units were washed to see how the red blood cell count (RBC) differs. Looking at the table there is no significant difference between the haemolysed and non-haemolysed units.
The p-value is 0.3512 which is more than $p<0.05$ and indicates no significant differences between the 5 groups.

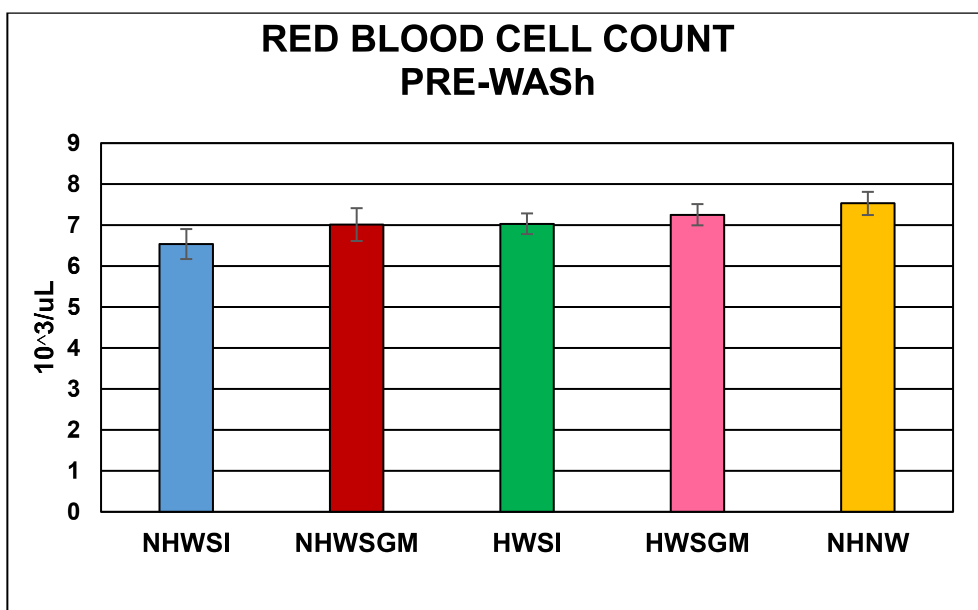


Figure B.1: This figure depicts the red blood cell count for the groups subjected to the wash process, before the cell wash process

Looking at the pre-wash groups there is no significant difference. Data is presented as a Mean and SEM, because the p-value is $p>0.05$ the results indicate no significant difference between the groups.

B.2 Red blood cell count for the 5 groups post-wash

These include units that were haemolysed washed with 0.9% saline, the one group was suspended in 0.9% saline and the other group suspended with SAGM. The other units included units presenting with no haemolysis that was washed with 0.9%saline one group was suspended in 0.9% saline and the other group with SAGM. The last group was not washed and not haemolysed this was the control group.

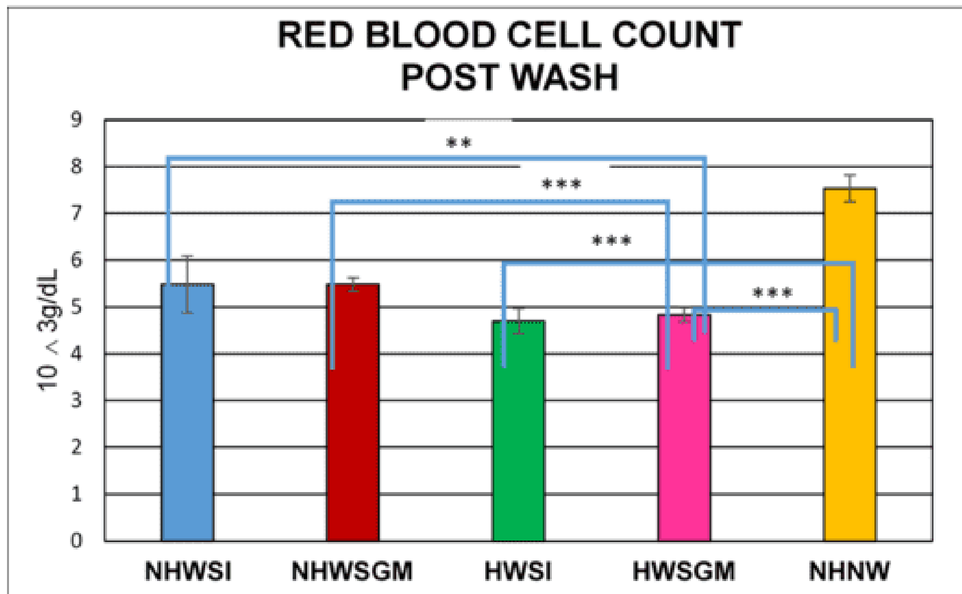


Figure B.2: This figure depicts the red blood cell count for the groups subjected to the wash process, directly after washing. ** P<0.01, *** P<0.001

The post wash results show that all the wash groups are significantly different from the not haemolysed not wash group (NHNW). The wash group that is very significant is the not haemolysed washed groups suspended in 0.9% saline (NHWSI). The other 3 groups not haemolysed washed suspended in SAGM (NHWSGM), haemolysed washed suspend in 0.9% saline (HWSI) and the haemolysed washed and suspended in SAGM (HWSGM) are all highly significant from the NHNW group. If there is a significant difference between the 2 groups the p-value < 0.05 and presented with a *. If there is a very significant difference between the 2 groups the p-value < 0.01 and presented in the table as **. If there is a highly significant difference between groups the p-value < 0.001 and presented as *** on the graph.

All the groups are different from the non-haemolysed not washed group. The reason for this is that when packed red blood cells are washed some of the red blood cells are lost in the red blood cells units (RBC). In RBC units containing haemolysis, there are already cells that are lysed and will be washed out. The RBC units without any haemolysis also had cells lost in the wash process. The group NHWSI had a very significant difference from the control group (NHNW) P-value <0.01. The other 3 groups NHWSGM, HWSI and HWSGM had a highly significant difference from the group NHNW. P-value < 0.01.

B.3 Red blood cell count one-week post wash

One week after the wash process it was important to see if the cells will still survive the wash process because the normal criteria for washed packed cells is 24-hour expiry date after wash. The second question in the thesis was, can the expiry date of the units be extended if SAGM a preservative is added?

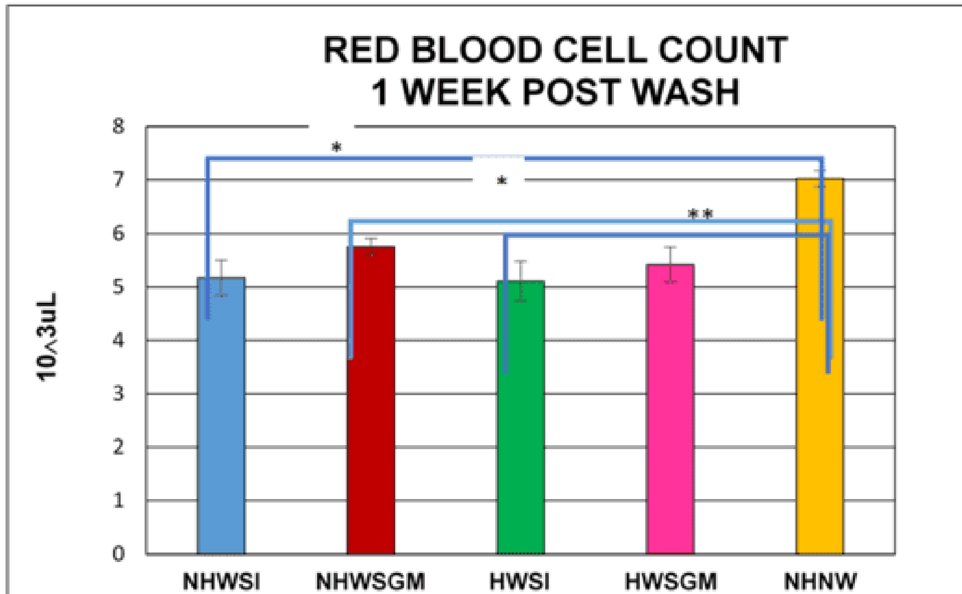


Figure B.3: This figure depicts the red blood cell count for the groups subjected to the study 1 week after washing. * P<0.05, ** P<0.01

There is a significant difference between the NHWSI group and the NHNW group that means that the p-value is < 0.5. There is also a significant difference between the NHWSGM and the NHNW group. The last group is very significant between the HWSI and the NHNW group p-value < 0.01. This means all the wash groups except the HWSGM group were not significantly different from the NHNW group.

B.4 Red blood cell count 2 weeks post wash

The red blood cell count was performed once a week for 2 weeks after the wash process to see how the cells deteriorate in storage after the wash process. It was important in the study to try and extend the expiry date. In the event where there are a lot of units that present with haemolysis in the supernatant, we need to be able to keep the units for more than 24 hours. If there is no order for this product. The aim is to save resources.

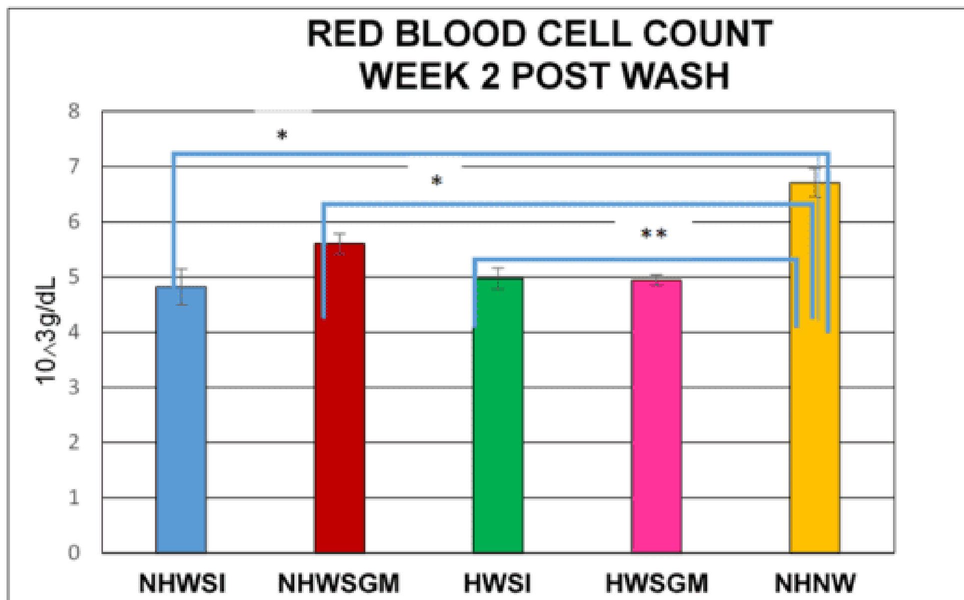


Figure B.4: This figure depicts the red blood cell count for the groups subjected to the study 2 weeks after washing. * P<0.05, ** P<0.01

Two weeks after the wash process there is a significant difference between the NHWSI and NHNW. The HWSGM and NHNW group also had a p-value < 0.05. The third group had a very significant difference between the HWSI and the NHNW group with a p-value < 0.01. The result shows that the SAGM did however preserve the red blood cells better than only with a suspension of 0.9% saline as a preservative for the cells for two weeks. The group that did not present with any haemolysis in the supernatant and the cells with haemolysis present had the same degree of degradation of cells in storage.

B.5: Red blood cell counts of the 4 groups washed and the control group, A, B, C and D

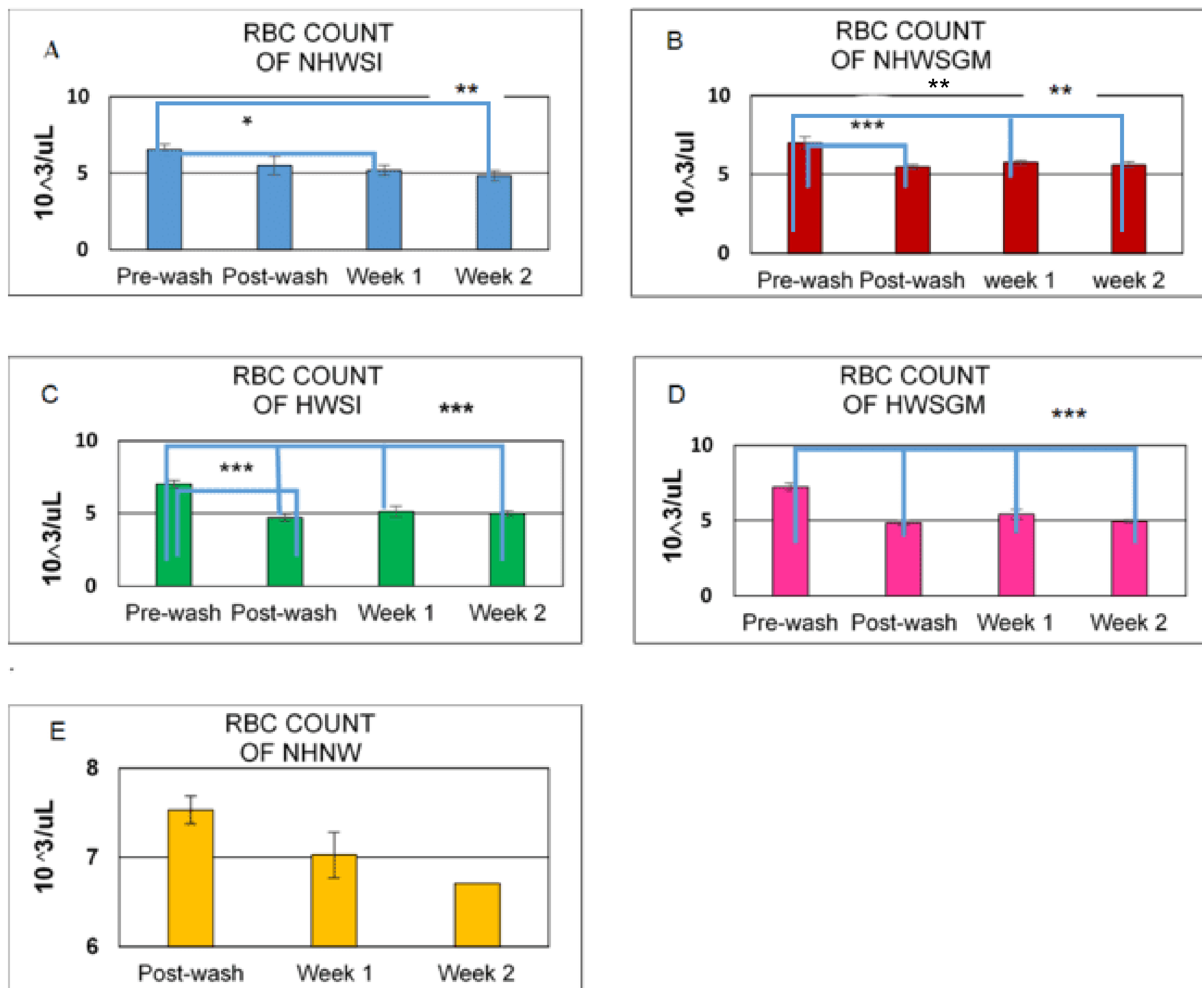


Figure B.5: Red blood cell count results of the four wash groups. The graphs for the haemoglobin results for the different groups from pre-wash till the 2nd week in storage

The red blood cell count was performed on the 4 wash groups and the control group. All the wash groups were washed with 0.9% saline. Two of the groups were suspended in saline after washing these include: haemolysed washed and suspended in saline (HWSI) and not haemolysed washed and suspended in saline (NHWSI). The other 2 wash groups suspended in the preservative saline, adenine, glucose and mannitol (SAGM) after washing with 0.9% saline was, not haemolysed washed and suspended in SAGM (NHWSGM) the other group was, haemolysed washed and suspended in SAGM (HWSGM). The control group was not washed and not haemolysed (NHNW). The control group was used to observe the washed cells degradation against the normal progression of cell death in storage. All the groups that were washed had a significant difference between the pre-wash and the post-wash. It is known in literature that red cells are lost in the wash process.

A. One week after the wash process the NHWSI had a significant difference between the pre-wash and week 1 post wash P-Value < 0.05. The pre-wash results and 2-week post-wash in storage had a very significant difference in results P-Value < 0.01.

B. The pre-wash and post-wash results of the RBC count of wash group NHWSGM had a highly significant difference P-Value < 0.001. Week 1 and week 2 post-wash had a very significant difference in results from the pre-wash P-Value < 0.01.

C. The RBC count of HWSI showed Bonferoni stats had a highly significant difference. In the results for the pre-wash and post-wash. There were also highly significant differences between the pre-wash results and post-wash week 1 and post-wash week 2 in storage. P-value < 0.001.

D. The Bonferoni stats showed a highly significant difference between post-wash in 1st week in storage and post-wash 2nd week in storage P-Value 0.001 for group haemolysed washed SAGM (HWSGM)

E. The RBC count of the NHNW control group had no significant differences in the storage period from the initial storage period till the 2nd week in storage P-Value > 0.05. The T-bar is not visible; the difference between the groups is too little to see the T-bar.

B.6: Haemoglobin test for pre-wash of the 5 study groups

The HGB was taken for all the wash groups and base groups. The result indicated there was no difference between the groups pre-wash as well as for the group that was not washed and not haemolysed.

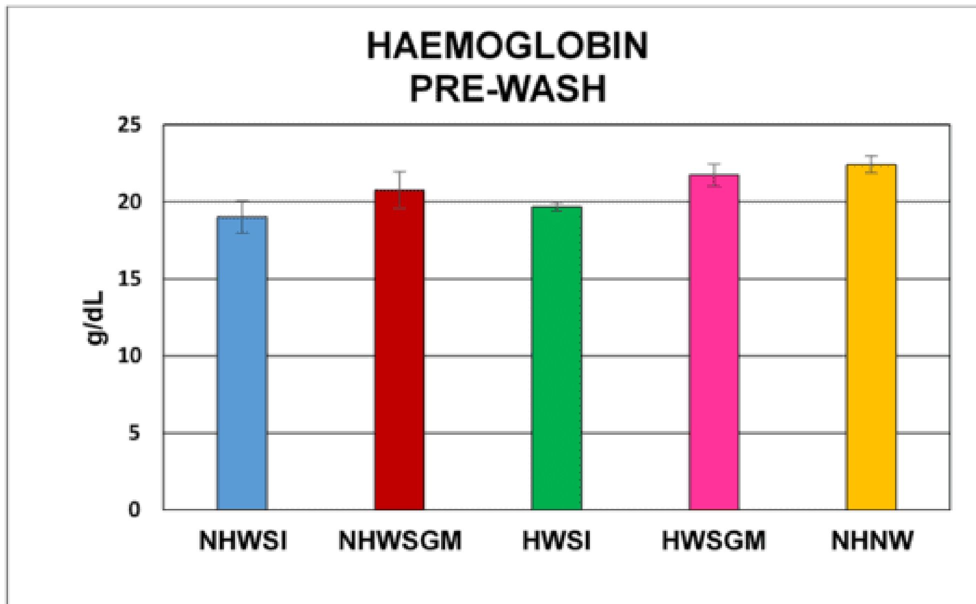


Figure B.6: This figure depicts the haemoglobin test for the groups subjected to the study, before washing

Bonferoni stats show there are no significant differences between the wash groups and the control group. All of the groups have similar haemoglobin results p-value > 0.05. The T bar for the wash group haemolysed washed and suspended in 0.9% saline is present, the differences between the groups is small that makes the T bar look absent.

B.7: Haemoglobin results for post-wash process of the 5 study groups

The HGB tests were done on the wash groups; it was expected there would be differences between the wash groups and the control group. When the cells are washed there are cells lost with every wash.

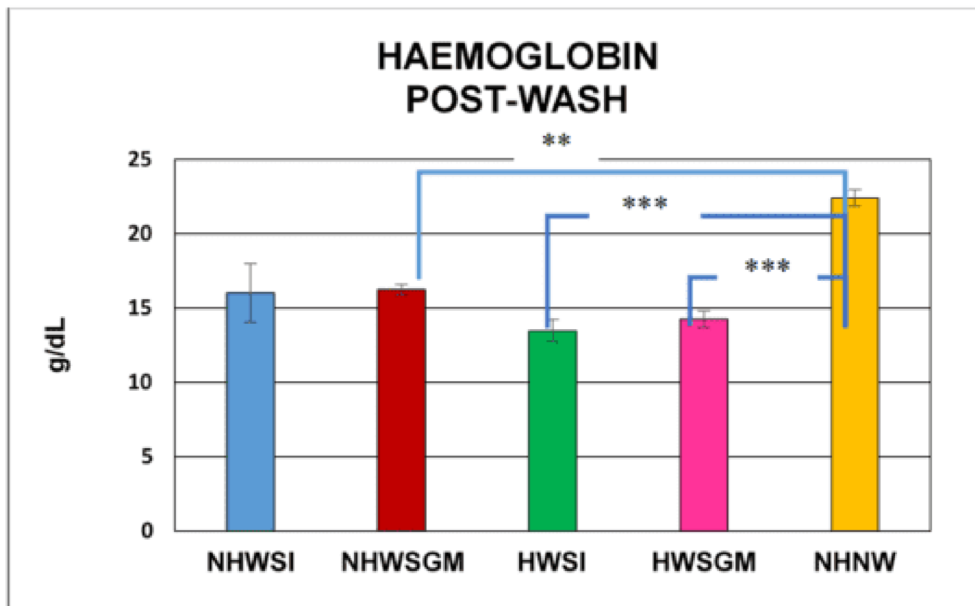


Figure B.7: This figure depicts the haemoglobin test for the groups subjected to the study directly after the wash process. ** P<0.01, ***P<0.001

Bonferoni's stats show a very significant difference between the NHWSGM and the NHNW group p-value < 0.01. The other two groups HWSI and HWSGM had a highly significant difference from the NHNW group p-value < 0.001. As expected, the HGB did drop, because of the cells that were washed out.

B.8: Haemoglobin results for the 5 groups 1-week post-wash

In the post-wash week 1, it was important to see how the cells survived in the different mediums. The haemoglobin test was done on all the wash groups and the control group.

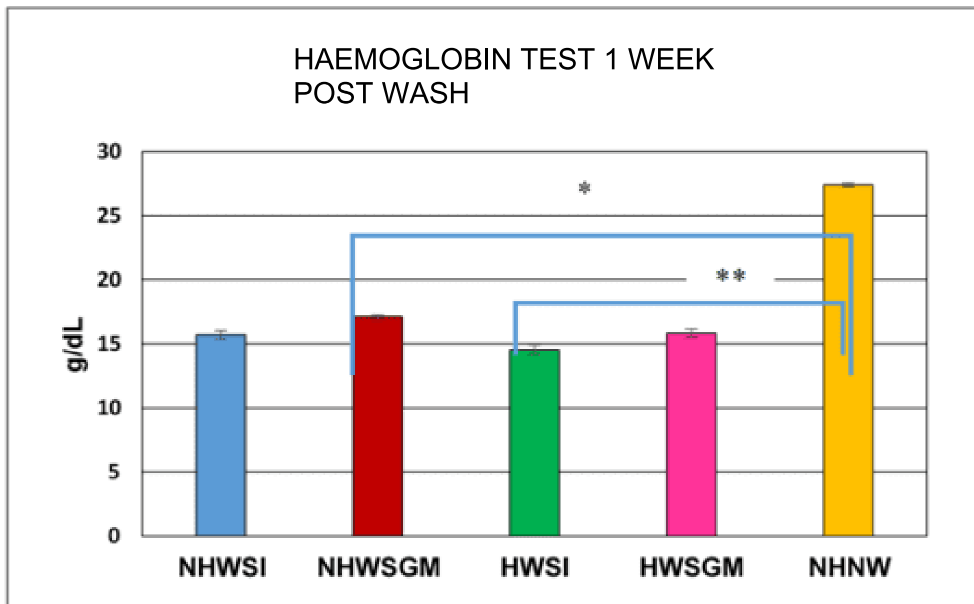


Figure B.8: This figure depicts the haemoglobin test for the groups subjected to the study 1 week after the washing. * $P<0.05$, ** $P<0.01$

Results of 1 week after wash show a significant difference between the control group (NHNW) and not haemolysed washed resuspended with SAGM p-value
< 0.05. The second group also had a very significant difference between the control group and the HWSI. These results show that the cells survived better with the SAGM than with only a substitute as 0.9% saline. The error bars on NHWSGM and NHNW are present, but the differences between the groups is so small that it is barely visible.

B.9: The haemoglobin results for the post-wash 2nd week in storage for the 5 groups

After 2 weeks post wash it is expected that the cells will break down, it is important to see if the SAGM helped preserve the cells. The haemoglobin test was done on all the wash groups and the control group.

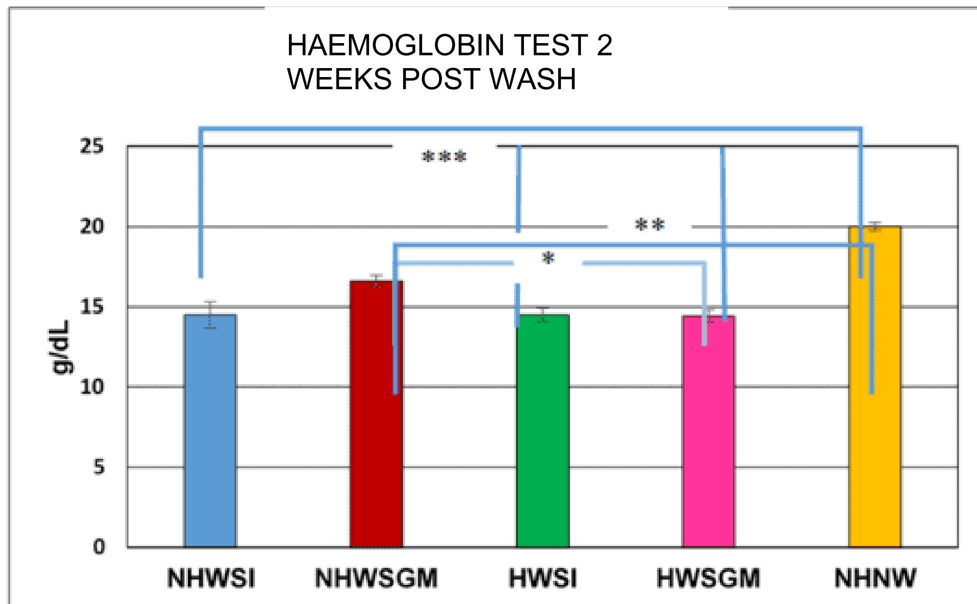


Figure B.9: This figure depicts the haemoglobin test for the groups subjected to the study 2 week after the washing. * P<0.05, ** P<0.01, *** P<0.001

After two weeks post-wash, the HGB between the NHWSGM and the HWSGM group is significantly different with p-value < 0.05. Between the NHWSGM and the NHNW group, there is a very significant difference with a p-value < 0.01. The other 3 wash groups NHWSI, HWSI, and HWSGM are highly significantly different p-value < 0.001 from the NHNW.

B.10: Haemoglobin results for the 5 groups from pre-wash till the 2nd week in storage

In this figure we can see for each group how significant the differences were from before they went through the wash process till the second week after storage.

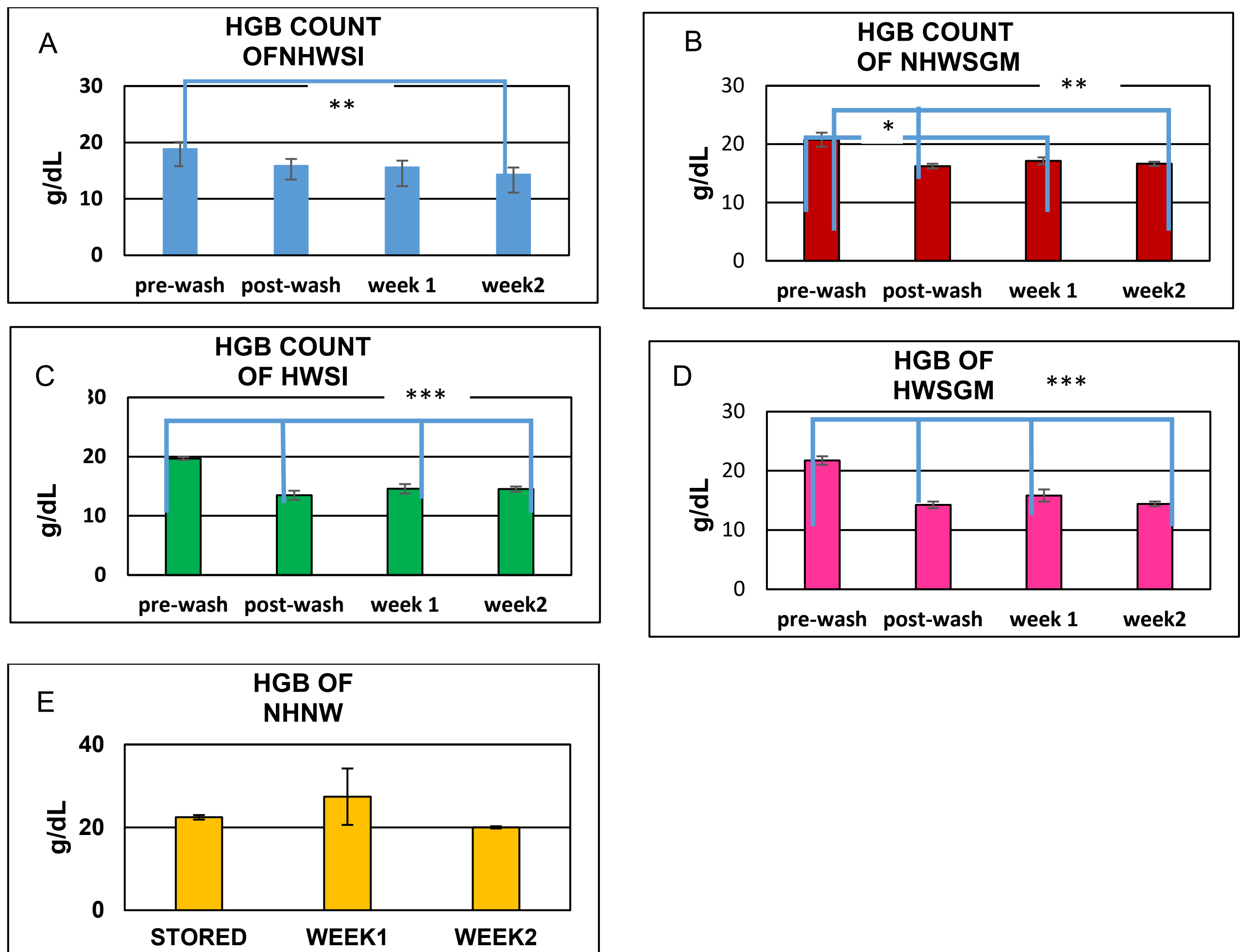


Figure B.10: Individual groups result for haemoglobin

A. Bonferoni stats show in group HGB of NHWSI there was a very significant difference between the pre-wash and the post-wash 2nd week in storage P-Value < 0.01.

B. The Bonferoni stats show in this graph HGB of NHWSGM a very significant differences in results for the haemoglobin between the pre-wash and post-wash, as well as the 2nd week in storage P-Value <0.01. There was also a significant difference between the pre-wash and the post-wash 1st week in storage P-Value <0.05.

C. There was a highly significant difference in the HBG of the HWSI between the pre-wash and the post-wash. There was also a highly significant difference between the pre-wash and 1st week and 2nd week in storage post-wash P-Value < 0.001.

D. In the Bonferoni stats there were a highly significant difference between the pre-wash and post-wash results. The results between the pre-wash, week 1 and week 2 in storage also had a highly significant difference in results P-Value < 0.001.

E. The Bonferoni stats showed for group not washed not haemolysed no significant differences between the initial storage of the cells till the 2nd week in storage P-Value

B.11 Haematocrit results for the 5 groups pre-wash

In the study we wanted to see if the red blood cells with haemolysis present would have a significant difference from the cells that did not have haemolysis. The haematocrit was done on all the RBC units these with and without haemolysis.

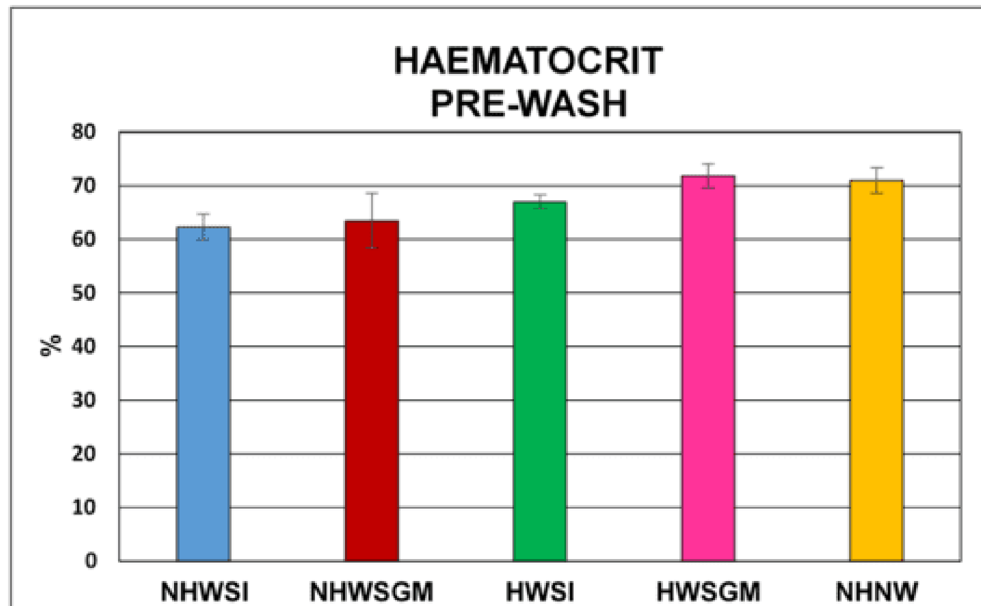


Figure B.11: Haematocrit results pre-wash

Comparing all the groups the wash groups and the control group the p-value >0.05

B.12: Haematocrit results post wash for the 5 groups

After the cells were washed it was expected that the haematocrit will be lower, because of the red cell loss in the wash process. The haematocrit was done on all the wash groups, as well as the base group.

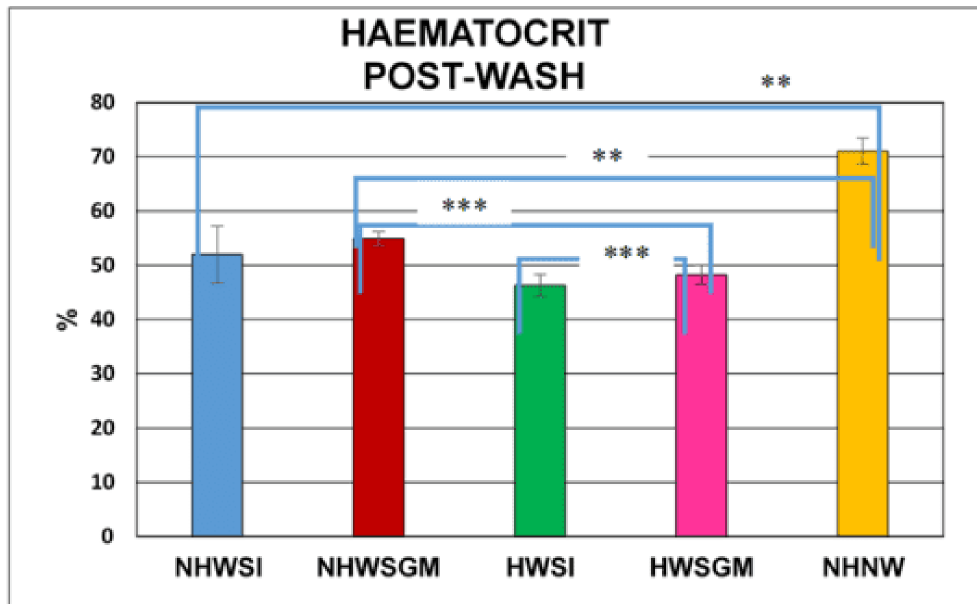


Figure B.12: This figure depicts the haemoglobin test for the groups subjected to the study directly after washing. ** P<0.01, *** P<0.001

As expected, the HCT had a very significant difference between 2 wash groups NHWSI, NHWSGM and the base group. The P value <0.01. The other 2 wash groups HWSI, HWSGM had a highly significant difference between the base group (NHNW). The P value <0.001. The RBC units that already had haemolysis present before the wash process had a higher significant difference against the base group than the control groups which had no haemolysis present before they were washed.

B.13: Haematocrit results for the 5 groups 1-week post-wash

After the wash process there were a very and a highly significant difference between the wash groups and the base group. Doing the HCT after a week of post wash it is hoped that there will not be a major difference between the wash groups.

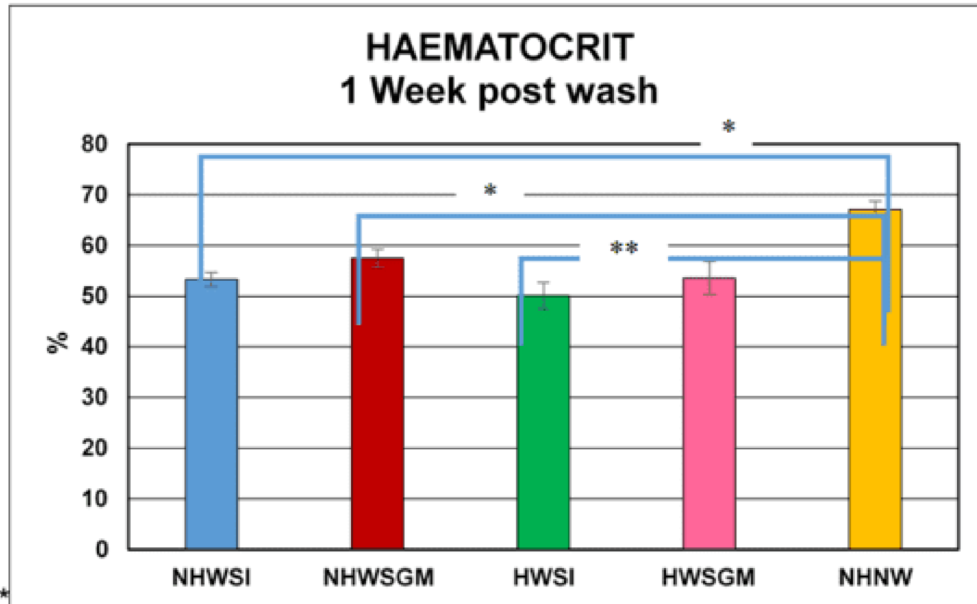


Figure B.13: This figure depicts the haematocrit test for the groups subjected to the study 2 weeks after the washing. * P<0.05, ** P<0.01, *** P<0.001

All the wash groups had a difference between the base groups. The NHWSI and NHWSGM groups both had a significant difference from the base group with a P value < 0.05. The HWSI group had a very significant difference from the base group. P value < 0.01.

B.14: Haematocrit results for the 5 groups 2 weeks post-wash

In this week it is important to see if there were a difference between the saline and the SAGM as preservative. The haematocrit was done on all the wash groups as well as the base group 2 weeks post-wash.

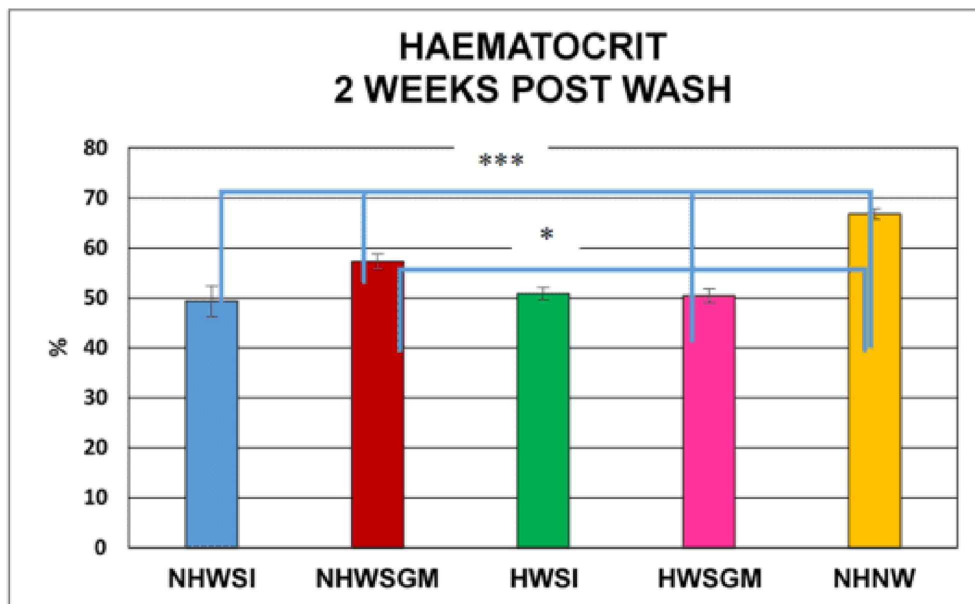


Figure B.14: This figure depicts the haematocrit test for the groups subjected to the study 2 week after washing. * P<0.05, *** P<0.001

NHWSGM has a significant difference from the base group (NHNW) P value < 0.05. NHWSI, HWSI and HWSGM had a highly significant difference from the base group. P value >0.001. Only the not haemolysed units suspended in SAGM had a less significant difference from the base group than the others. This already shows that units that were washed without haemolysis present will be able to be kept for 2 weeks if only these results are taken into consideration.

B.15: The haematocrit (HCT) was done for the individual groups from pre-wash till the 2nd week in storage

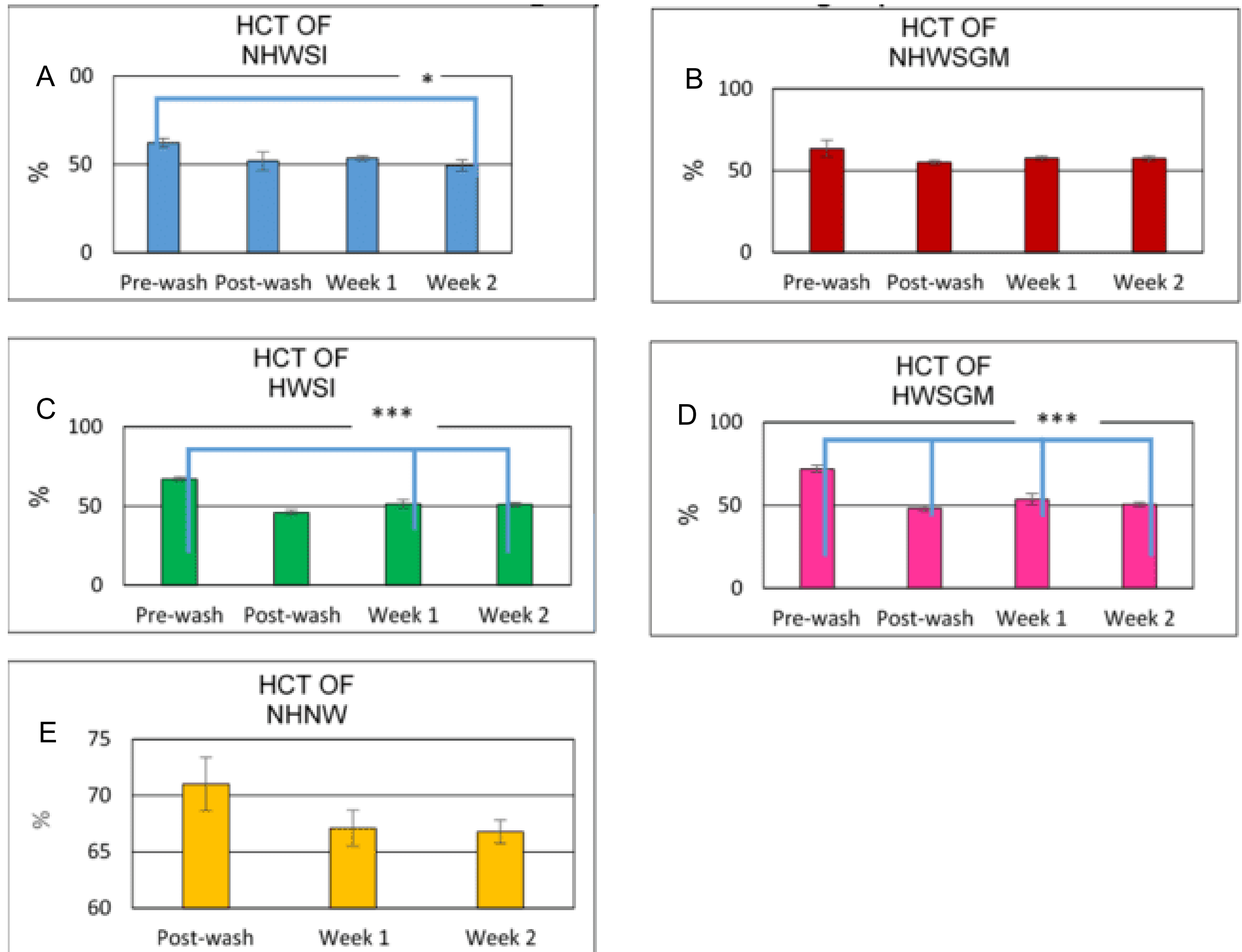


Figure B.15: The 5 different groups haematocrit result from pre-wash till 2nd week in storage

A. The Bonferoni stats of group not-haemolysed washed and suspended in 0.9% saline shows that there is a significant difference between the pre-wash group and the post-wash 2nd week in the storage group. P-Value 0.05.

B. In the Bonferoni stats it shows no significant differences in results between the pre-wash and the post-wash, 1st week and 2nd week in storage-Value > 0.05.

C. The Bonferoni stats show a highly significant difference between the pre-wash and the post-wash group. There is also a highly significant difference between the pre-wash group and the 1st week in storage and 2nd week in storage post-wash P-Value < 0.001.

D. The Bonferoni stats shows a highly significant difference in the results for the pre-wash and the post-wash P-Value < 0.001. The pre-wash and the 1st week in storage as well as the 2nd week in storage post-wash had a highly significant difference in results for the haematocrit P-Value < 0.001.

E. The Bonferoni stats shows no significant differences for the group not haemolysed not washed the results from the beginning of storage till the 2nd week in storage. P-Value > 0.05.

B.16: The mean corpuscular volume for the pre-wash results on all the study groups

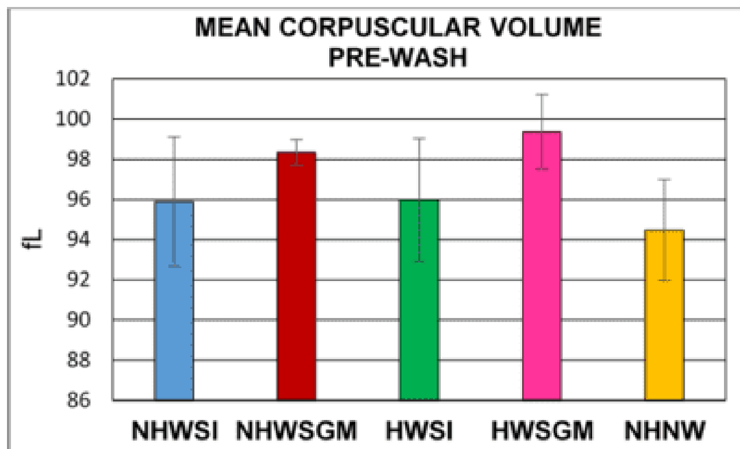


Figure B.16: Mean corpuscular volume pre-wash

The MCV show no significant differences between the wash groups and the control group p-value >0.05

B.17 The mean corpuscular volume test (MCV) was done on all wash groups and the control group post-wash

This test measures the average size of your red blood cells. After the cells have been washed the cells may miss form and may influence the MCV.

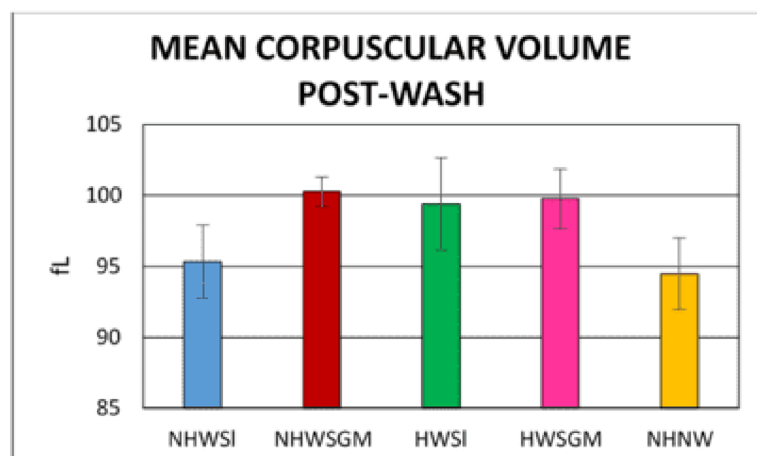


Figure B.17: Mean corpuscular volume post-wash

Post-wash indicates no significant differences between the wash groups and the control groups with a p-value >0.05.

B.18 The MCV test 1 week in storage

The mean corpuscular volume was done on all the wash groups and the base group to see if there is a difference between the base group not washed and not haemolysed. In storage, the cells go through stress and the MCV can differ after wash as they change their shapes.

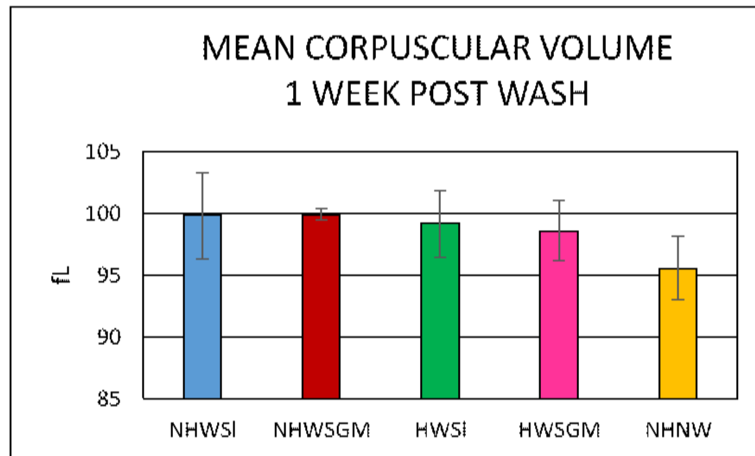


Figure B.18: Mean corpuscular volume 1-week post wash

There were no significant differences between the wash group and the control group p-value >0.05.

B.19: Mean corpuscular volume test for the 5 groups 2 weeks post-wash

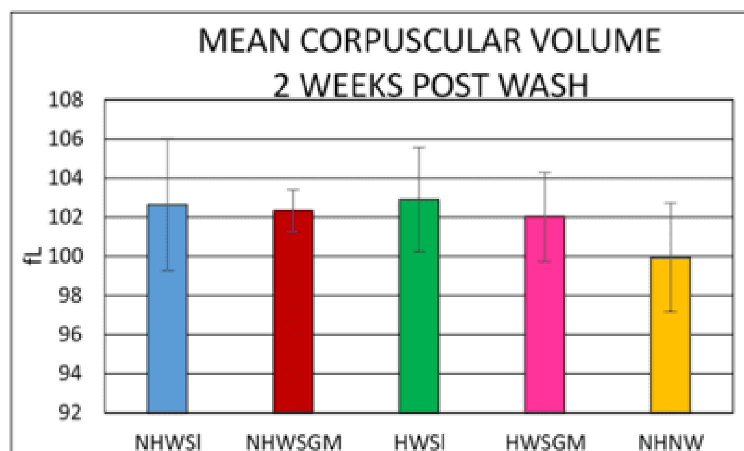


Figure B.19: The Mean corpuscular volume tests the 2nd week in storage

After the second week of storage, there is still no significant differences between the wash groups and the control group p-value >0.05

B.20: The mean corpuscular volume of all the individual groups were done form pre-wash till the second week in storage

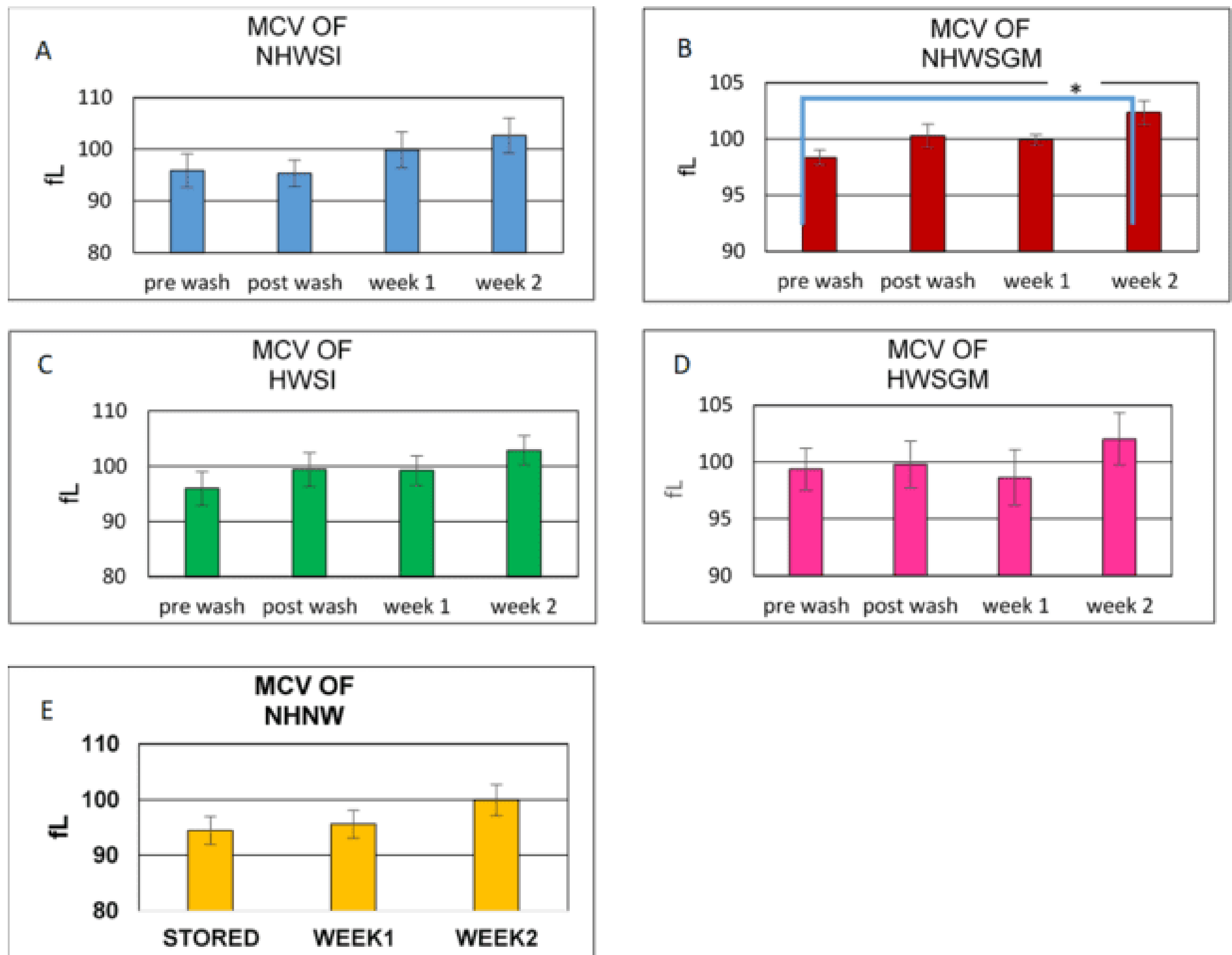


Figure B.20: The mean corpuscular volume was done on all the research groups from the pre wash till the second week in storage

A. The Bonferoni stats for group NHWSI show no significant differences in results for the pre-wash till the 2nd week in storage post-wash P-Value >0.05.

B. Bonferoni stats for wash group NHWSGM show a significant difference in results between the pre-wash and the 2nd week in storage P-Value < 0.05.

C. Bonferoni stats for HWSI show no significant differences between the pre-wash results and the post-wash results. The pre-wash and 1st week in storage as well the 2nd week in storage post-wash also had no significant differences in results.

D. Bonferoni stats for group HWSGM showed no significant differences between the pre-wash MCV tests and the post-wash tests. P-Value > 0.05. The Bonferoni results for the MCV test also showed no significant difference between the pre-wash test and the post-wash 1st week in storage as well as the 2nd week in storage results
P-Value > 0.05.

E. The Bonferoni stats for group NHNW shows no significant differences between the pre-wash and the post-wash. P-Value > 0.05. The Bonferoni stats also show no significant differences between the pre-wash and the 1st week in storage as well as the 2nd week in storage post-wash P-Value > 0.05.

B.21: The mean corpuscular haemoglobin was done on all 4 wash groups and the control group pre-wash

In the main wash we want to see post wash that the cells still have the same form and colour to produce a viable product for transfusion.

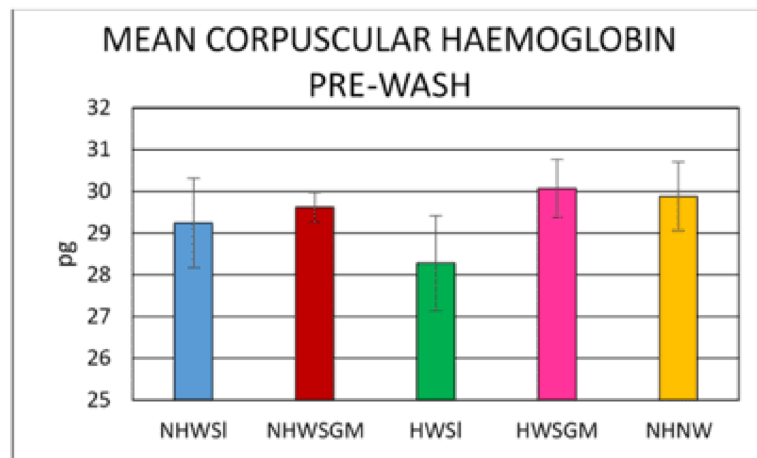


Figure B.21: Mean corpuscular haemoglobin pre-wash

The p-value >0.05 of all the groups in comparison to the control group, there were no significant differences.

The control group and all the wash groups had no significant differences from pre-wash till the second week in storage except for. The wash group haemolysed washed and suspended in SAGM from pre-wash until the week 2 in storage who had a very significant difference in results P - Value <0.01.

B.22: White blood cell count pre-wash

A comparison of white blood cell count (WBC) was done between the 5 groups before they were washed. The different groups were included not haemolysed washed suspended in 0.9% saline. Haemolysed washed and suspended in 0.9% saline, not haemolysed washed and suspended in SAGM, haemolysed washed and suspended in SAGM and the control group not haemolysed and not washed.

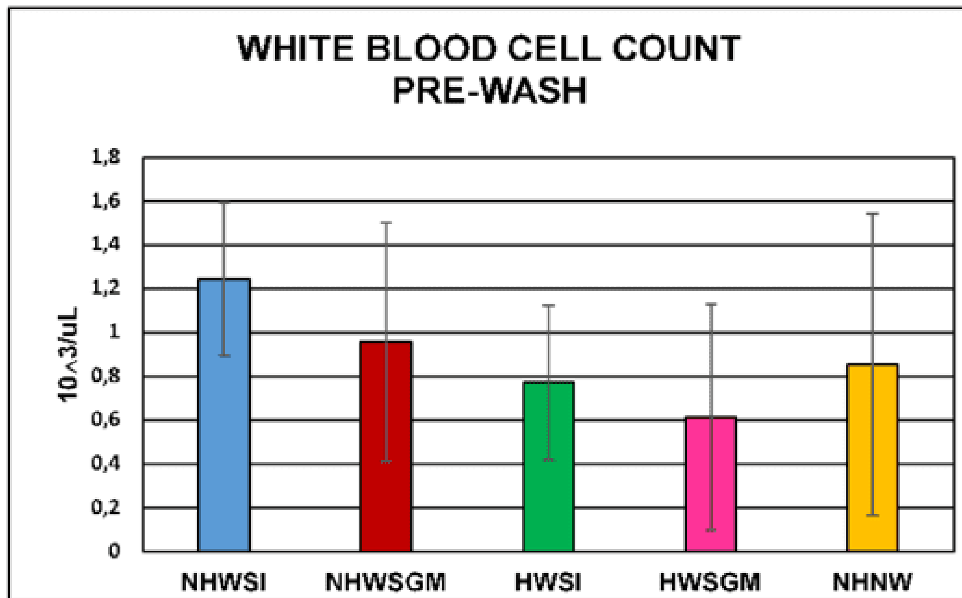


Figure B.22: This figure depicts the white blood cell count for the groups subjected to the study before washing

This graph shows there is no significant difference in the white blood cell count (WBC) between the 5 pre-wash groups. The P-Value is > 0.05.

B.23: White blood cell count post wash

White blood cell count was done on all the study groups post wash.

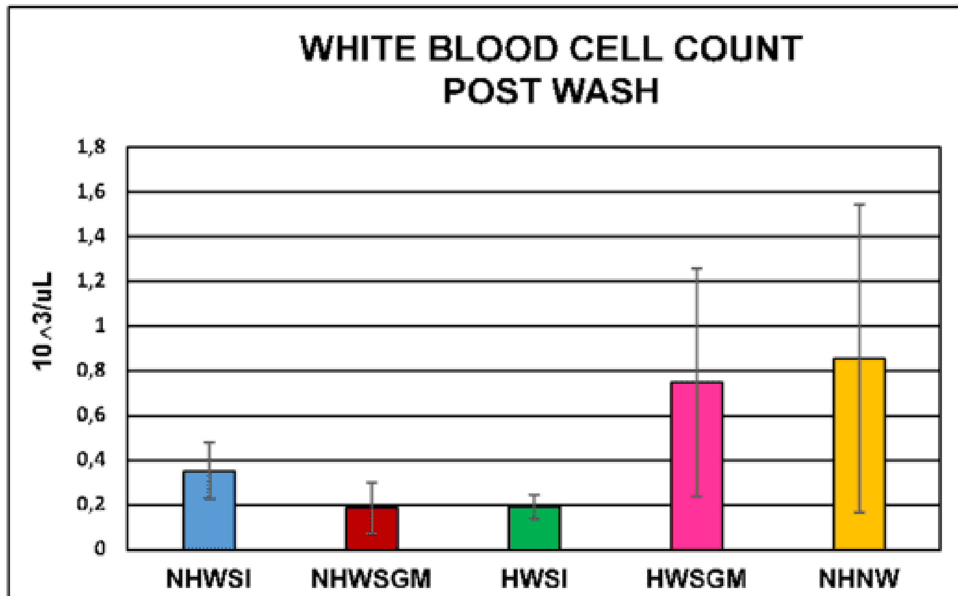


Figure B:23: This figure depicts the white blood cell count for the groups subjected to the study directly after washing

Comparing white blood cell count (WBC) between the wash groups and the base group. Bonferoni stats shows no significant differences between the base group and the wash groups with P-value >0.05.

B.24: WBC count 1-week post wash

The white blood cell count was done 1-week post wash. The WBC count was done on all the wash groups and the control group.

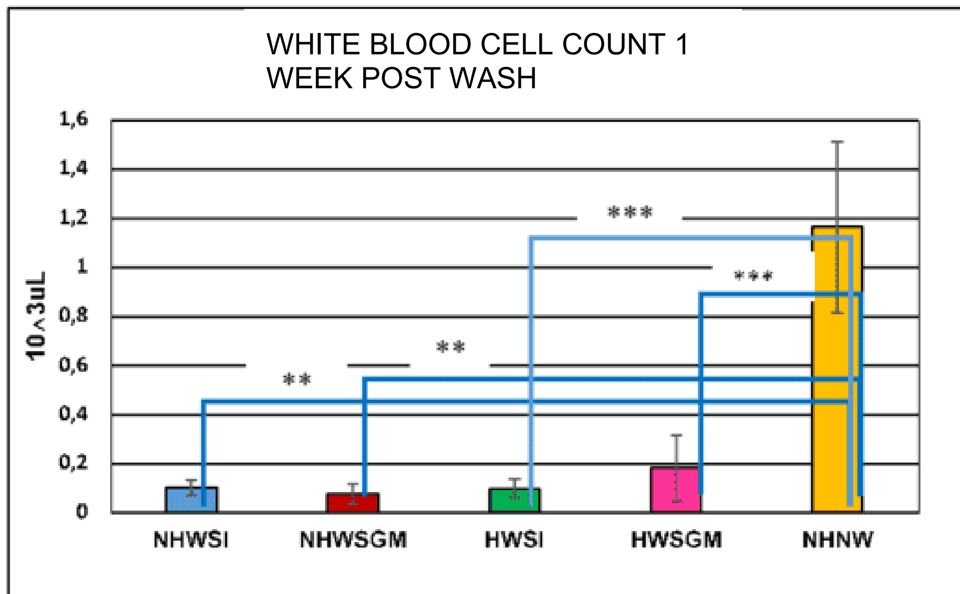


Figure B.24: This figure depicts the white blood cell count for the groups subjected to the study 1 week after washing. ** P<0.001, *** P<0.001

Comparing the WBC 1-week post wash the Bonferoni stats show a very significant difference between the NHWSI, NHWSGM and the base group (NHNW) P-Value < 0.01. There is a highly significant difference between the HWSI, HWSGM and the base group with P-Value < 0.001.

B.25: White blood cell count the 2nd week post wash in storage

Comparison between the 5 groups after 2-week in storage

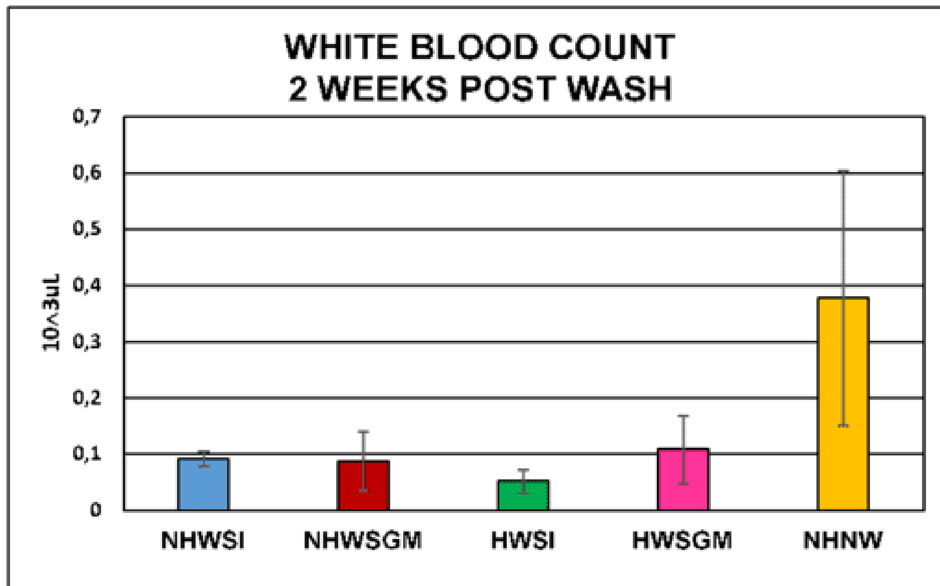


Figure B.25: This figure depicts the white blood cell count for the groups subjected to the study 2 weeks after washing

Bonferoni stats show no significant differences between the wash groups and the base group P-Value > 0.05.

B.26: The white blood cell count was done on the 4 wash groups and the control group from the pre-wash till the second week in storage.

Only the group not haemolysed washed and stored in 0.9% Saline showed significant differences between the pre-wash and the post-wash, as well in the 1st and second week in storage. The other groups had no significant differences from pre-wash till the 2nd week in storage post-wash.

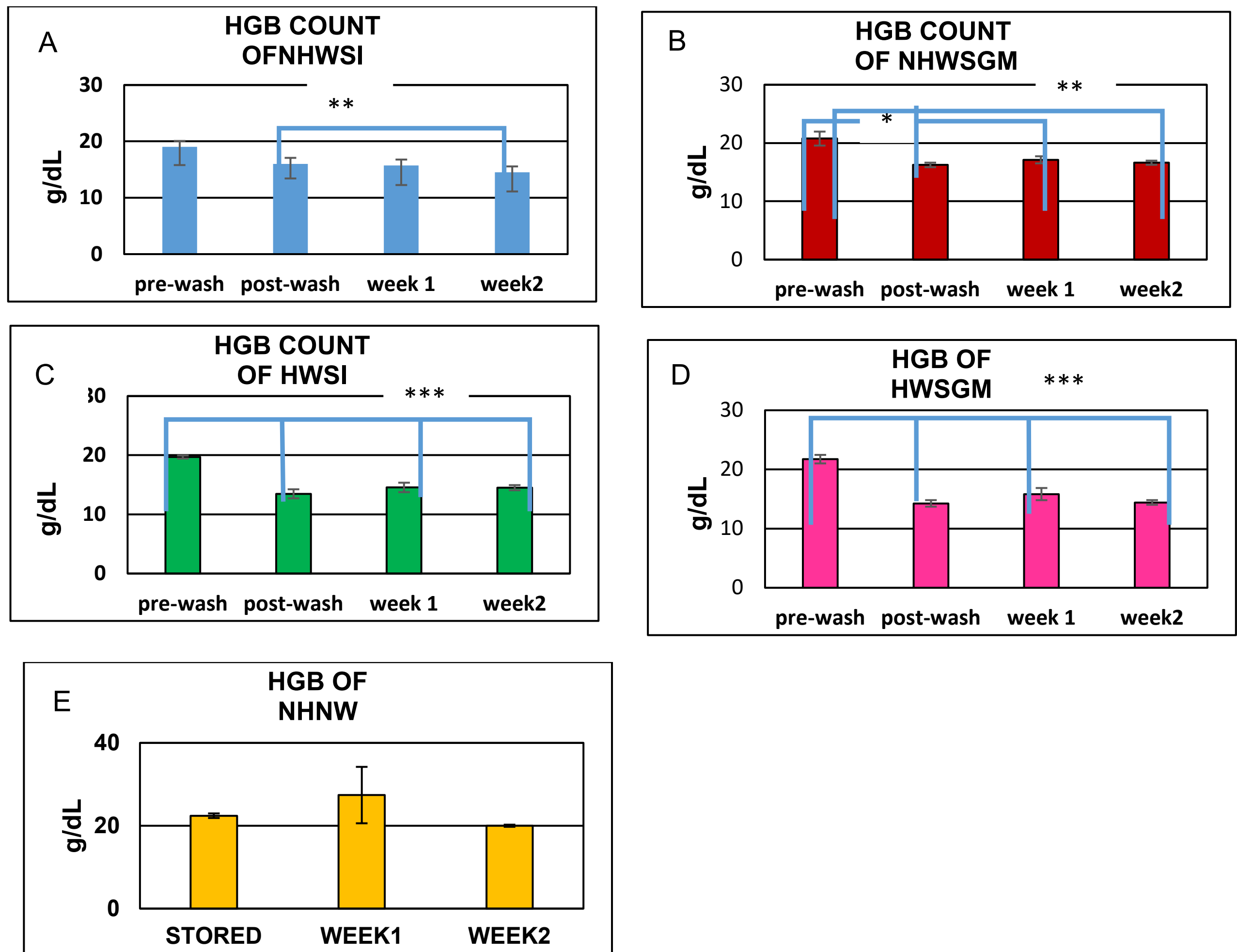


Figure B.26: HGB results for the 4 wash groups and the control group

A. Bonferoni stats show in group HGB of NHWSI that there was a very significant difference between the pre-wash and the post-wash 2nd week in storage P-Value < 0.01.
B. The Bonferoni stats show in this graph HGB of NHWSGM a very significant difference in results for the haemoglobin between the pre-wash and post-wash, as well as the 2nd week in storage P-Value <0.01. There was also a significant difference between the pre-wash and the post-wash 1st week in storage P-Value <0.05.
C. There was a highly significant difference in the HBG of the HWSI between the pre-wash and the post-wash. There was also a highly significant difference between the pre-wash and 1st week and 2nd week in storage post-wash P-Value < 0.001.

D. In the Bonferoni stats there were a highly significant difference between the pre-wash and post-wash results. The results between the pre-wash, week 1 and week 2 in storage also had a highly significant difference in results P-Value < 0.001.

E. The Bonferoni stats showed for groups not washed not haemolysed no significant differences between the initial storage of the cells till the 2nd week in storage P-Value.

B.26 HCT OF NHWSI done for all wash groups and control group

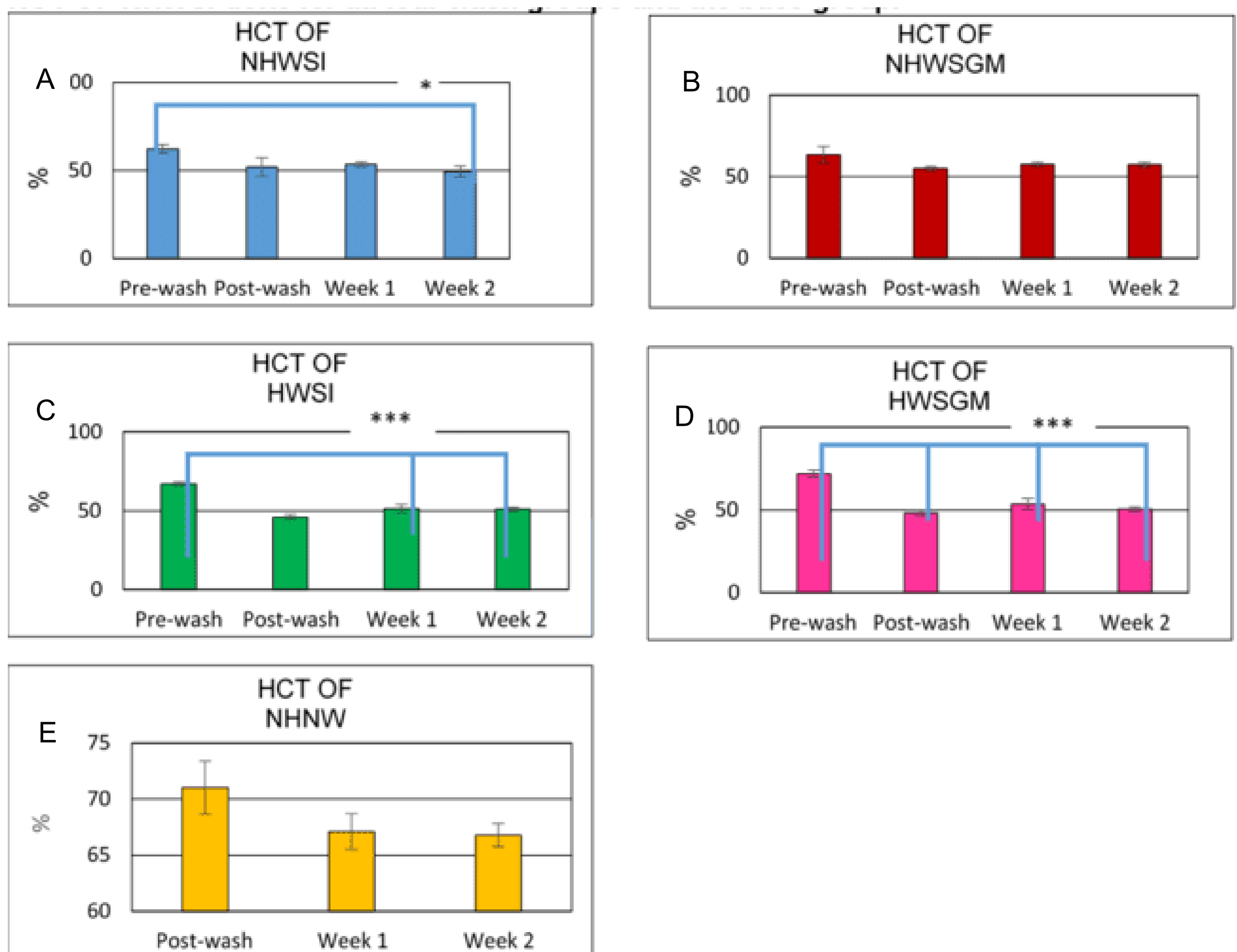


Figure B.27: The haematocrit (HCT) was done for the individual groups from pre-wash till the 2nd week in storage

A. The Bonferoni stats of group not-haemolysed washed and suspended in 0.9% saline shows that there is a significant difference between the pre-wash group and the post-wash 2nd week in the storage group. P-Value 0.05.

B. In the Bonferoni stats it shows no significant differences in results between the pre-wash and the post-wash, 1st week and 2nd week in storage-Value > 0.05.

C. The Bonferoni stats show a highly significant difference between the pre-wash and the post-wash group. There is also a highly significant difference between the pre-wash group and the 1st week in storage and 2nd week in storage post-wash P-Value < 0.001.

D. The Bonferoni stats shows a highly significant difference in the results for the pre-wash and the post-wash P-Value < 0.001. The pre-wash and the 1st week in storage as well

as the 2nd week in storage post-wash had a highly significant difference in results for the haematocrit P-Value < 0.001.

E. The Bonferoni stats shows no significant differences for the group not haemolysed not washed the results from the beginning of storage till the 2nd week in storage. P-Value > 0.05.

B.28: The mean corpuscular volume test (MCV) was done on all wash groups and the control group post-wash

This test measures the average size of your red blood cells. After the cells have been washed the cells may miss form and may influence the MCV.

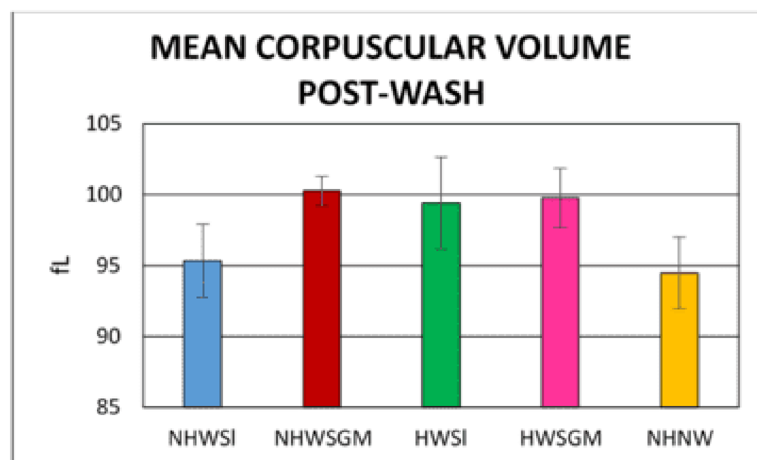


Figure B.28: Mean corpuscular volume post wash

Post-wash indicates no significant difference between the wash groups and the control group with a P value >0.05.

B.29: The MCV test 1 week in storage

The mean corpuscular volume was done on all the wash groups and the base group to see if there is a difference between the base group not washed and not haemolysed. In storage, the cells go through stress and the MCV can differ after wash as they change their shapes.

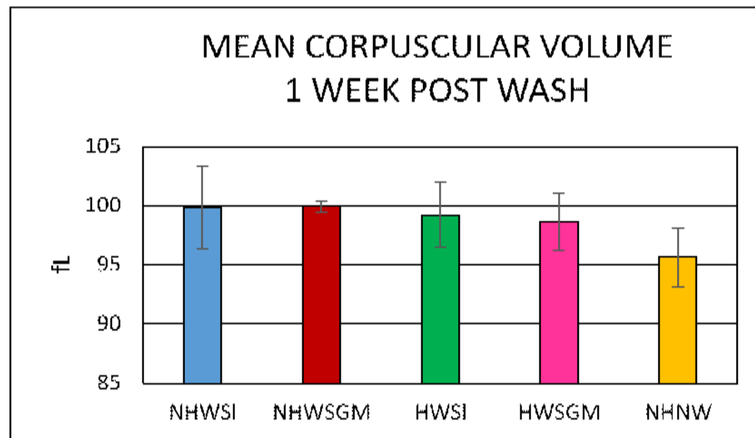


Figure B.29: Mean corpuscular volume 1-week post wash

There was no significant difference between the wash groups and the control group. P value >0.05.

B.30: The MCV 2 weeks in storage

The mean corpuscular volume was done on all the wash groups and the base group.

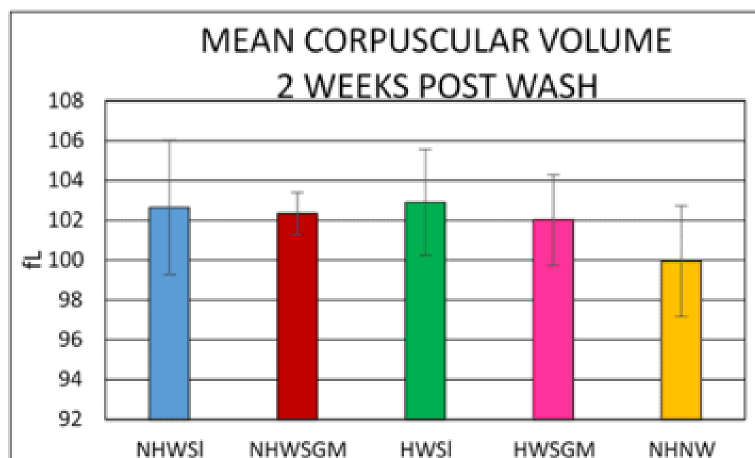


Figure B.30: Mean corpuscular volume 2nd week post wash

After the second week of storage, there is still no significance between the wash group and the control group with a P value > 0.05.

B.31: The mean corpuscular volume of all the individual groups were done form pre-wash till the second week in storage

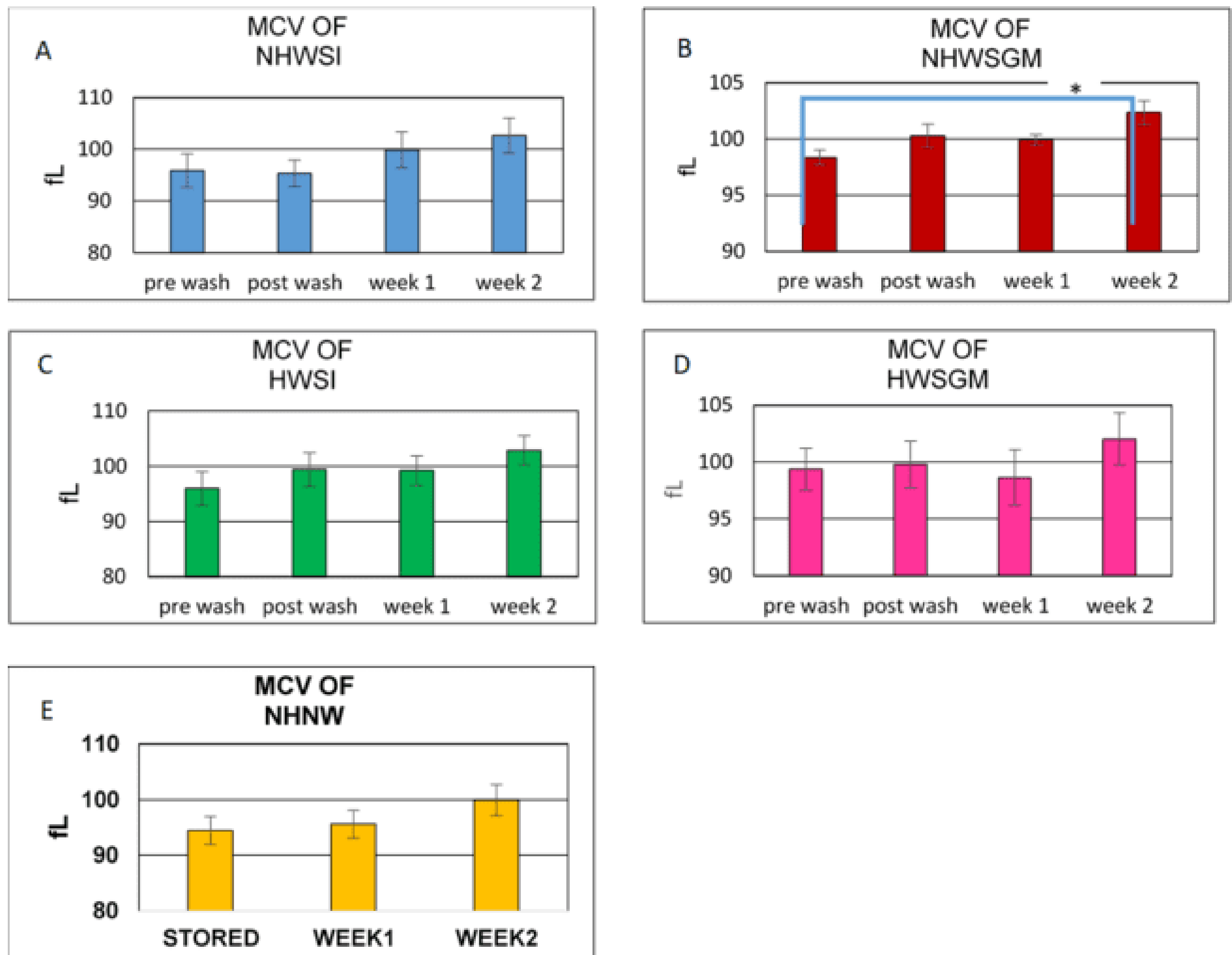


Figure B.31: The mean corpuscular volume of all the individual groups were done form pre-wash till the second week in storage

A. The Bonferoni stats for group NHWSI show no significant differences in results for the pre-wash till the 2nd week in storage post-wash P-Value >0.05.

B. Bonferoni stats for wash group NHWSGM show a significant difference in results between the pre-wash and the 2nd week in storage P-Value < 0.05.

C. Bonferoni stats for HWSI show no significant differences between the pre-wash results and the post-wash results. The pre-wash and 1st week in storage as well the 2nd week in storage post-wash also had no significant differences in results.

D. Bonferoni stats for group HWSGM showed no significant differences between the pre-wash MCV tests and the post-wash tests. P-Value > 0.05. The Bonferoni results for the MCV test also showed no significant difference between the pre-wash test and the post-wash 1st week in storage as well as the 2nd week in storage results P-Value > 0.05.

E. The Bonferoni stats for group NHNW shows no significant differences between the pre-wash and the post-wash. P-Value > 0.05. The Bonferoni stats also shows no significant

differences between the pre-wash and the 1st week in storage as well as the 2nd week in storage post-wash P-Value > 0.05.

B.32: The mean corpuscular haemoglobin was done on all 4 wash groups and the control group pre-wash

In the main wash study, it is important to see if the cells still have the same form and colour to produce a viable product for transfusion.

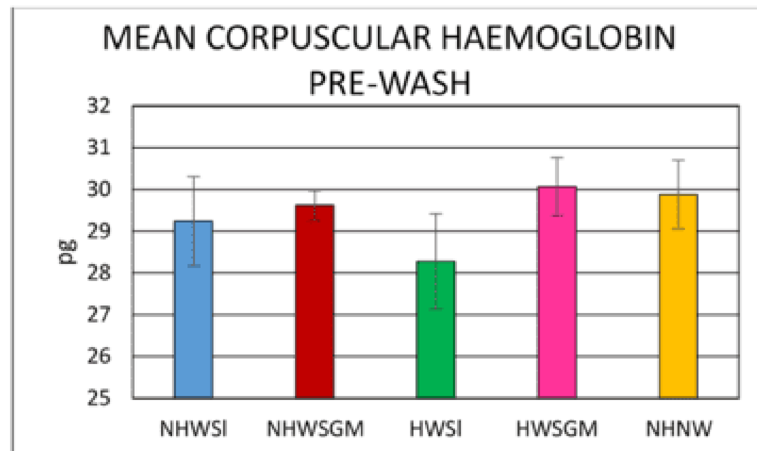


Figure B.32: Mean corpuscular haemoglobin pre-wash

The p-value > 0.05 of all the groups in comparison to the control group, shows no significant differences.

B.33: The mean corpuscular haemoglobin test (MCH) was done on all the post-wash specimens on and the control groups

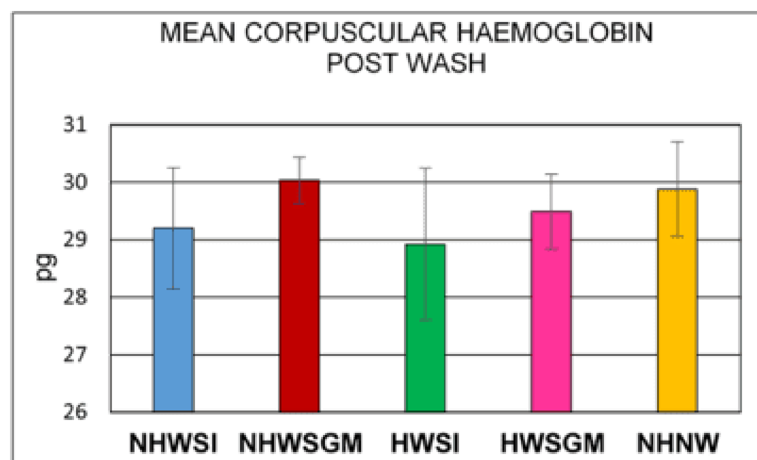


Figure B.33: Mean corpuscular haemoglobin post-wash

After the post-wash corpuscular haemoglobin shows no significant difference between the wash groups and the control group p-value >0.05.

B.34: The MCH were done on all the wash groups and the control group 1 week in storage

One week after the wash process it is important to see if the SAGM as a preservative and the saline which was suspended after washing had any significant difference on the red packed cells.

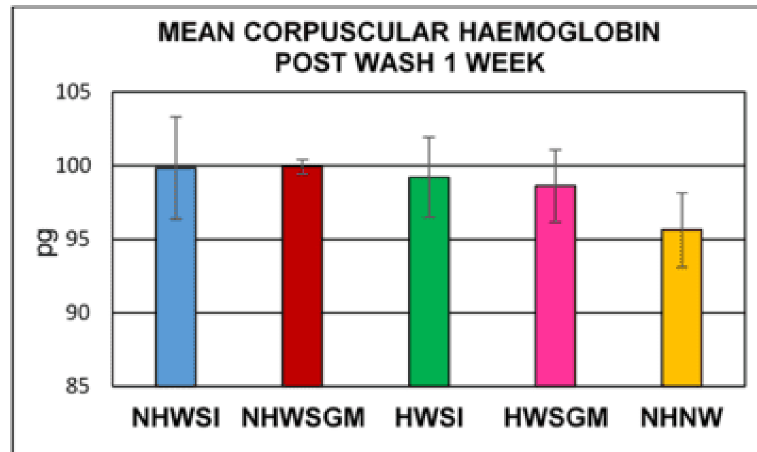


Figure B.34: Mean corpuscular haemoglobin 1-week post-wash

One-week post wash the p-value >0.05 shows no significant difference in the MCV results between the control group and the wash groups.

B.35: The MCH were done on the control group as well as the 4 wash groups 2 weeks in storage

The hope after 2 weeks is that the red blood cells will still be intact and if possible that it shows adding SAGM to the blood gives it a extended expiry time**.**

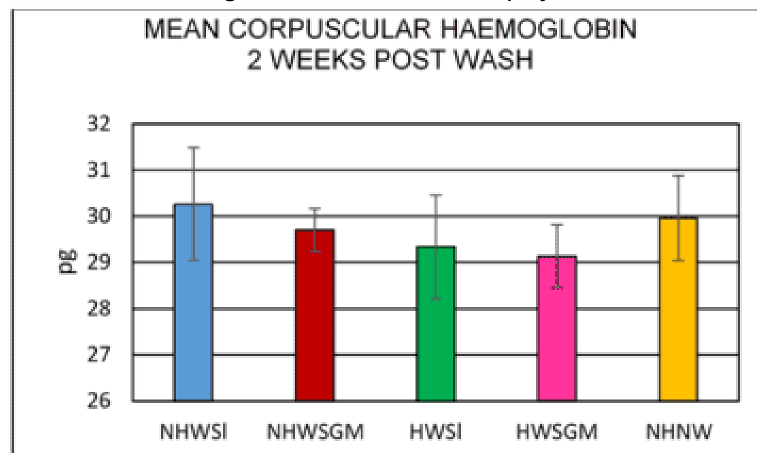


Figure B.35: Mean corpuscular haemoglobin 2nd week post-wash

After 2 weeks post wash in storage showed no significant differences between the wash groups and the control group p-value >0.05.

B.36: The mean corpuscular haemoglobin was done on each individual group for the pre-wash till the second week in storage

Mean Corpuscular Haemoglobin of all 4 wash groups and the base group.

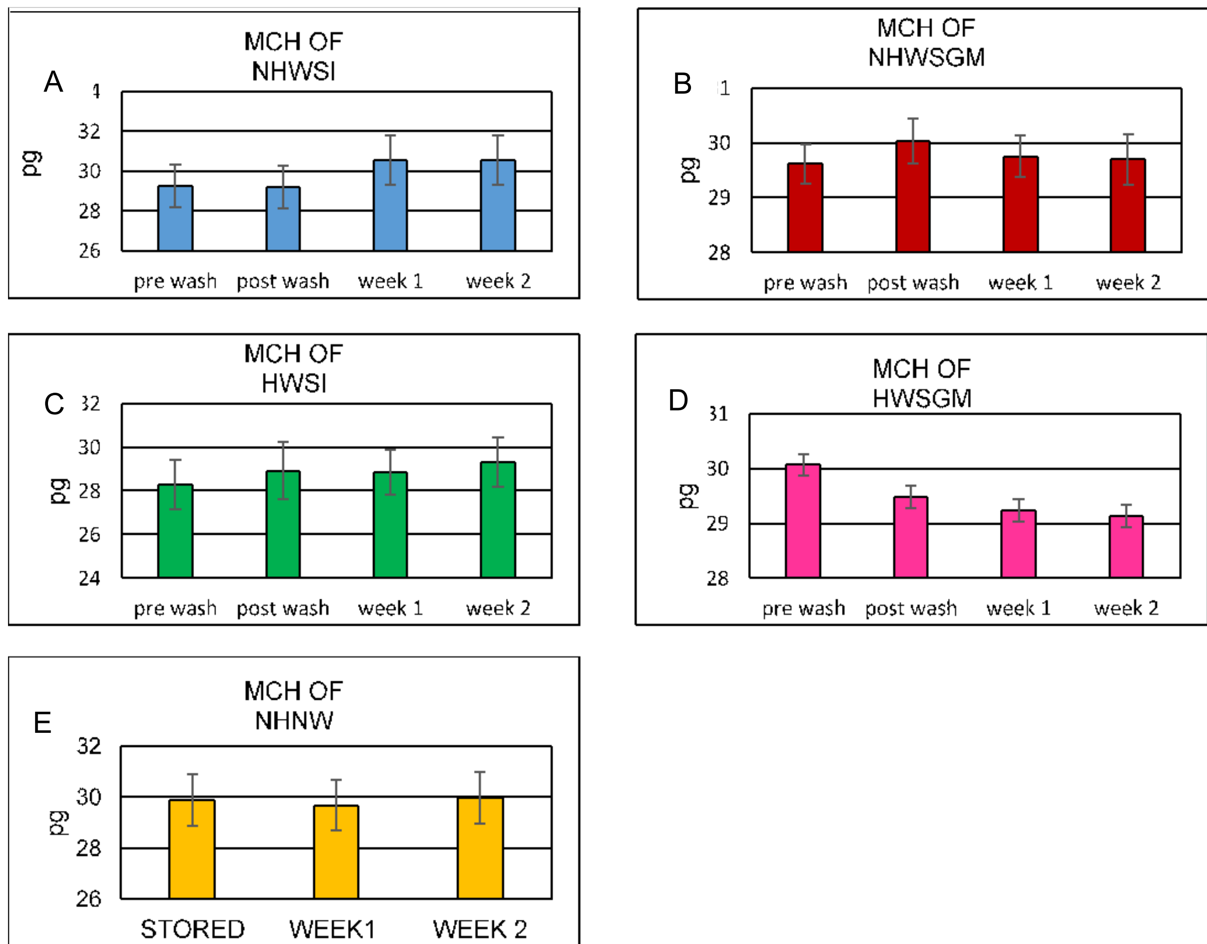


Figure B.36: The mean corpuscular haemoglobin for the 5 study groups

A. The Bonferoni stats for NHWSI show no significant differences between the pre-wash and the post-wash results. There was also no significant difference between the pre-wash results and the 1st week and 2nd week in storage post-wash. P-Value > 0.05.

B. The Bonferoni stats for NHWSG, show no significant difference between the pre-wash and the post-wash. P-Value > 0.05. There were also no significant differences between the pre-wash and the 1st week and 2nd week in storage post-wash. P-Value > 0.05.

C. Bonferoni stats for HWSI show no significant difference between the pre-wash and the post-wash P-value > 0.05. The 1st week and 2nd week in storage post-wash show no significant difference from the pre-wash. P-Value >0.05.

D. The Bonferoni start for HWSGM shows no significant differences between the initial time of storage as well as the 1st week and the 2nd week in storage P-Value > 0.05.

E. Bonferoni stats for NHNW shows no significant differences between the first and the second week in storage. P-value > 0.05.

B.37: The pre-wash for the mean corpuscular haemoglobin concentration test (MCHC) was done and compared between all the wash groups and the control group

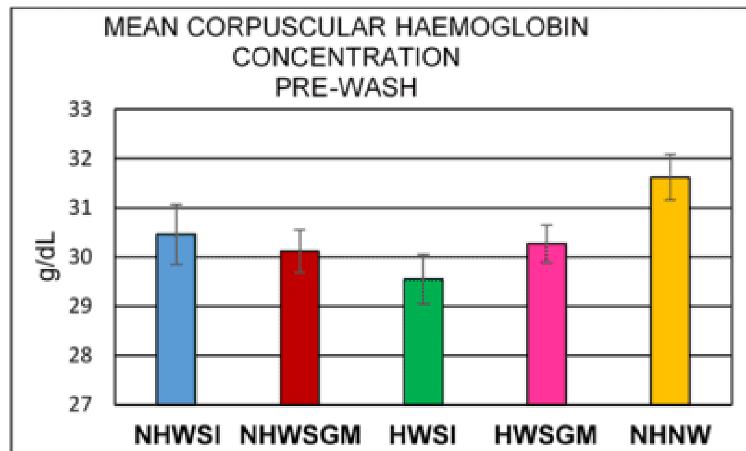


Figure B.37: Mean corpuscular haemoglobin concentration pre-wash

Before the cells were washed the p-value 0.05 showed no significant differences between the 5 groups.

B.38: The mean corpuscular haemoglobin concentration post-wash

The MCHC was done on all the wash groups as well as the control group.

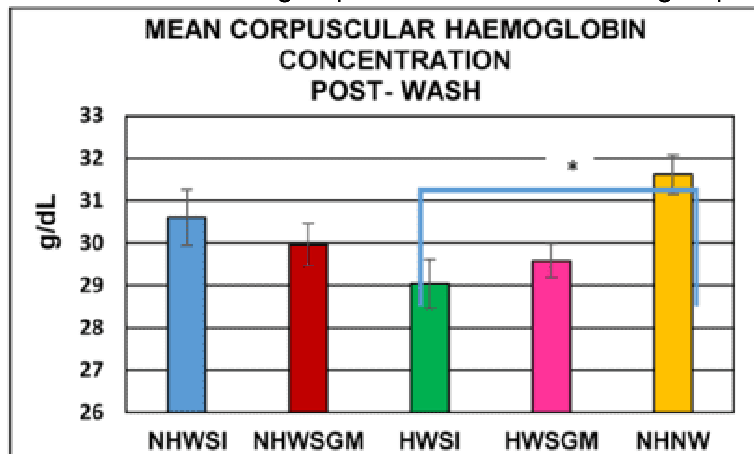


Figure B.38: Mean corpuscular haemoglobin concentration post-wash

The MCHC post-wash had only a significant difference the not haemolysed not washed and the haemolysed washed and suspended in saline group p-value >0.05. The other wash groups had no significant difference form the control group with p-value >0.05.

B.39: The mean corpuscular haemoglobin concentration (MCHC) test was done to compare the results after one week of storage between the wash groups and the control group

The MCHC was done on the wash groups and the base group 1 week after the cells were washed.

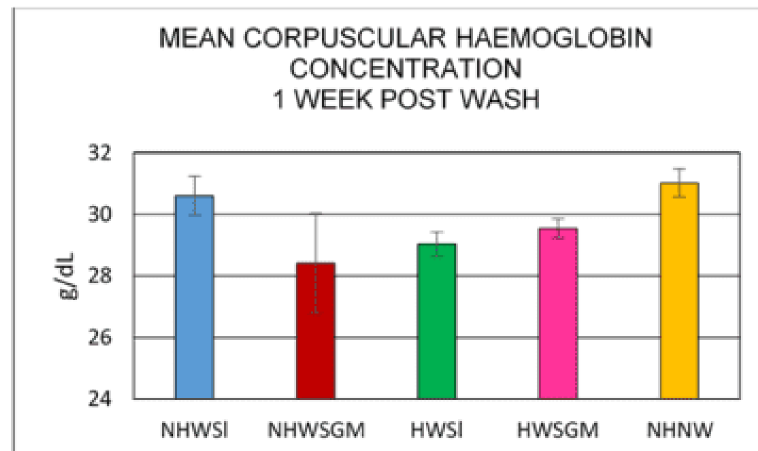


Figure B.39: Mean corpuscular haemolysis concentration 1-week post-wash

One-week post wash showed no significant difference between the control group and the wash groups p-value >0.05.

B.40: The mean corpuscular haemoglobin concentration test was done to compare the different wash groups and the control group to see if the cells had any damage in the wash process

The MCHC was done on all the wash groups as well as the control group on the 2nd week post-wash in storage.

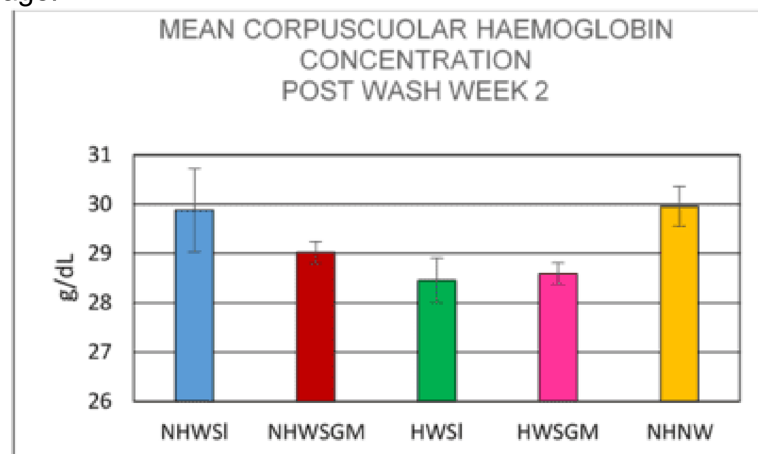


Figure B.40: Mean corpuscular haemoglobin concentration 2nd week post-wash in storage

After the second week post wash the MCHC had no significant differences between the wash group and the control group p-value >0.05.

B.41: The mean corpuscular haemoglobin concentration was done on each individual wash groups and the control group and compared from the pre-wash till the 2nd week in storage

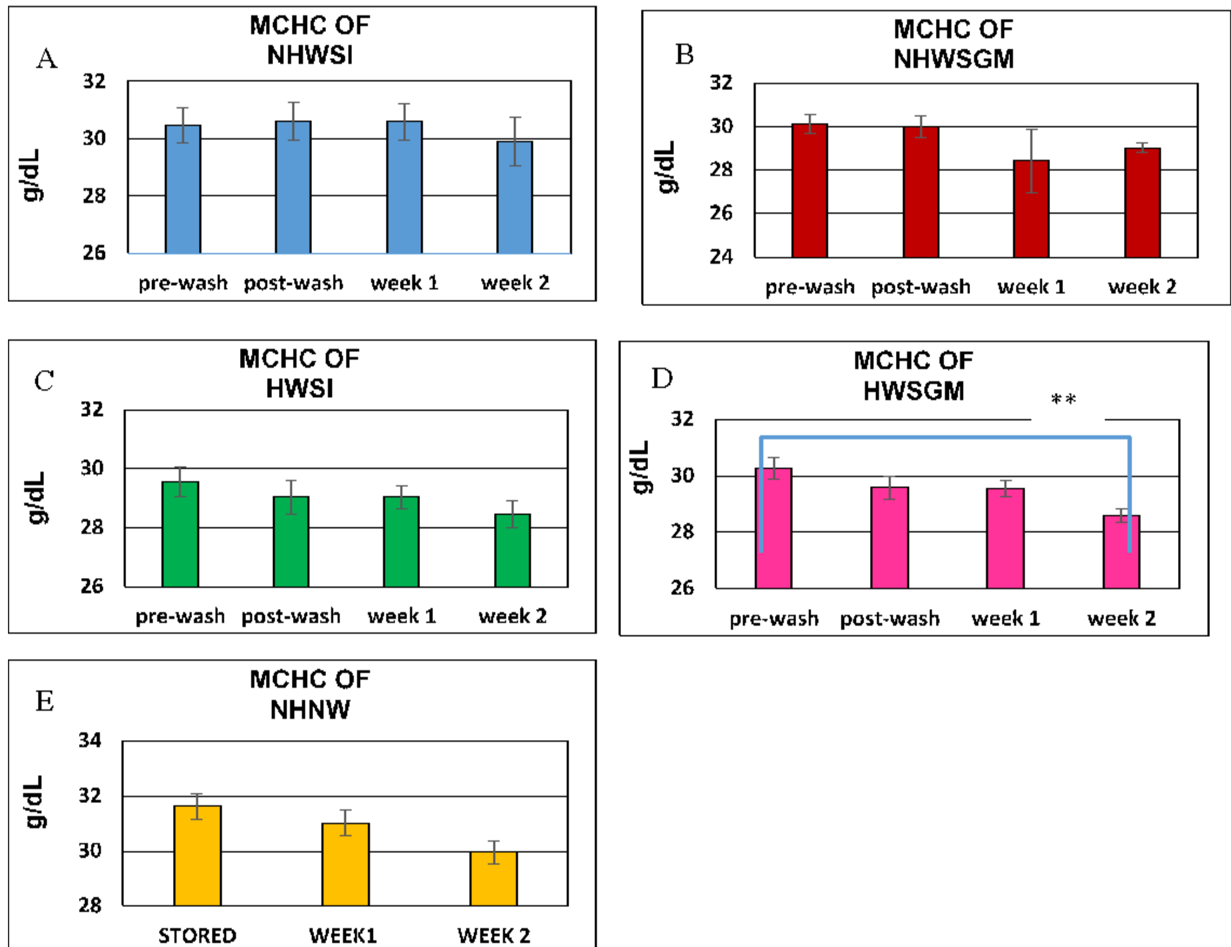


Figure B.41: The MCHC for the individual groups from pre-wash till 2nd week in storage

A. Bonferoni stats of NHWSI show no significant differences between the pre-wash and post-wash results P > 0.05. There are also no significant differences between the pre-wash and the 1st and 2nd week in storage post-wash P-Value > 0.05.

B. The Bonferoni stats shows no significant differences between the pre-wash and the post-wash. P-Value > 0.05. The pre-wash MCHC results showed no significant differences between the 1st and 2nd week in storage post-wash P-Value > 0.05.

C. MCHC OF HWSI. The pre-wash and post-wash showed no significant differences in results P-Value > 0.05. The pre-wash results and week 1 and week 2 in storage had no significant differences in the MCHC results P-Value > 0.05.

D. The pre-wash and the post-wash MCHC HWSGM showed no significant difference in results P-Value > 0.05. The pre-wash and week 1 in storage had no significant differences in the

results for the MCHC P-Value >0.05. The pre-wash and week 2 in storage had a very significant difference in results P - Value <0.01.

E. The Bonferoni stats for group NHNW shows no significant differences for the MCHC between the initial week when the cells were stored and 1st and 2nd week in storage P-Value > 0.05.

B.42: The red blood cell distribution width coefficient deviation was done on the pre-wash for all the groups. The red cell distribution width measures the size and the volume of the red blood cells. The red blood cells are normally all the same size. If the distribution width is high, it means that there is a big difference between the sizes of the red blood cells from small to large.

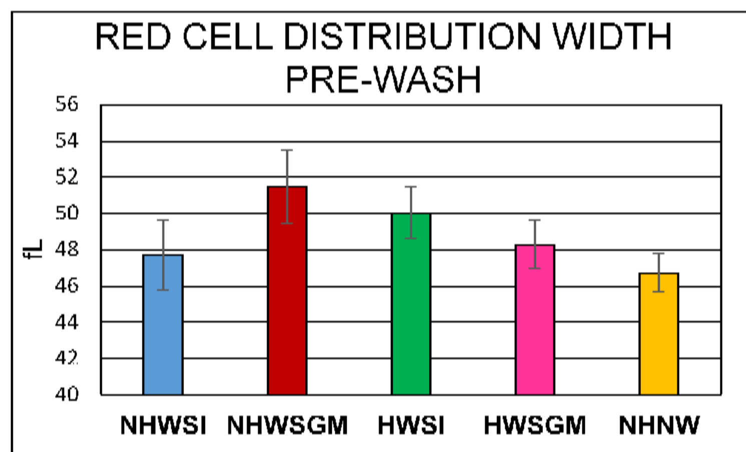


Figure B.42: Red cell distribution width pre-wash

The Bonferoni stats shows no significant differences from the pre-wash between the groups p->0.05.

B.43: Red cell distribution width post-wash

The red cell distribution with (RDW-SD) was done on all the units post wash these with and without haemolysis, as well as the base group.

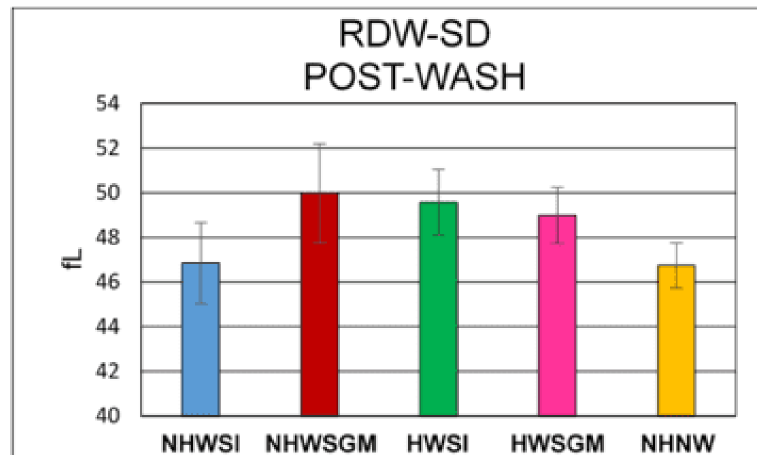


Figure B.43: Red cell distribution width post-wash

The Bonferoni stats shows no significant difference between the wash groups and the control group p-value >0.05.

B.44: Red cell distribution width 1 week in storage

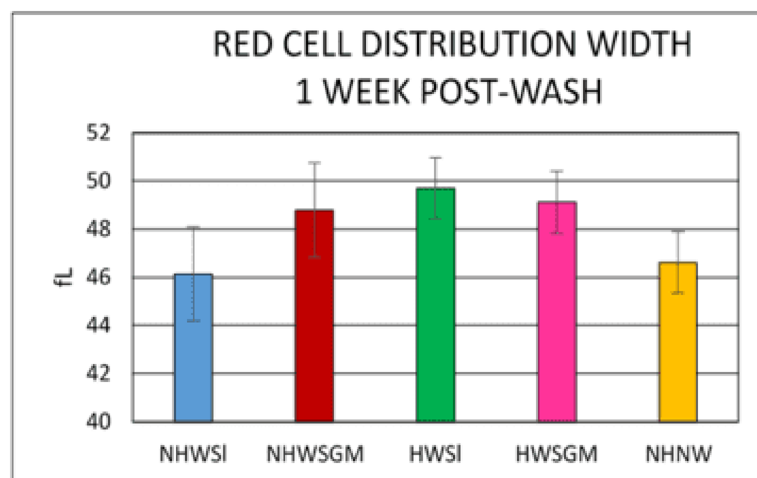


Figure B.44: Red cell distribution width 1st week post-wash

The Bonferoni stats shows no significant difference between the wash groups and the control group p-value >0.05.

B.45: Red cell distribution width was done on all the wash groups and the control group

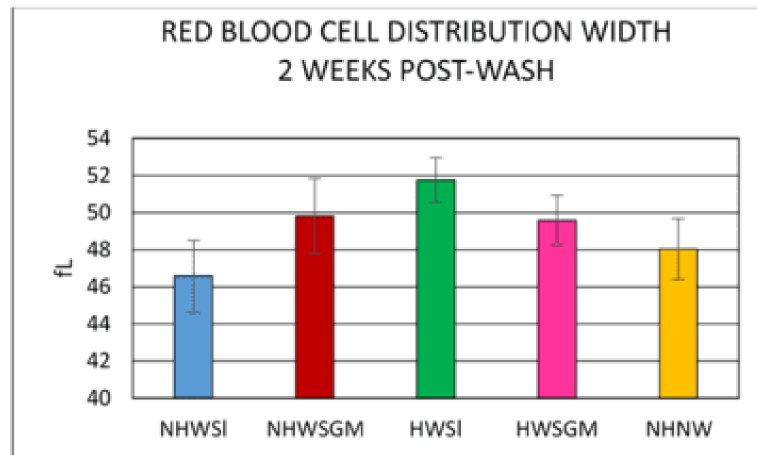


Figure B.45: Red cell distribution width 2nd week post-wash

The Bonferoni stats shows no significant difference between the wash groups and the control group p-value>0.05.

B.46: The red cell distribution width standard deviation was done on individual groups from the pre-wash till the second week in storage

Red cell distribution with 4 the four wash groups and the base group.

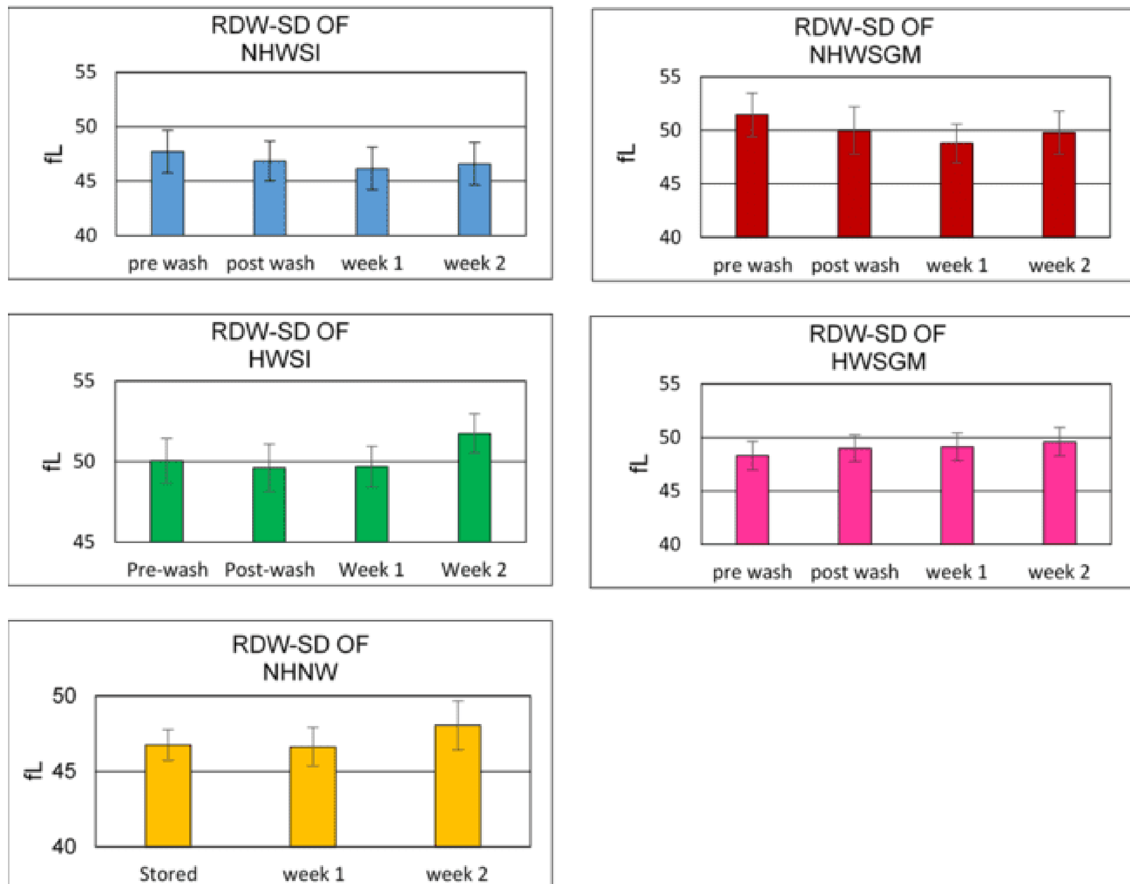


Figure B.46: Shows the red cell distribution width of all the research groups from the pre-wash till the second week post wash

A. The Bonferoni stats for NHWSI show no significant differences between the
pre-wash and the post-wash results. There were also no significant differences between the pre-wash and the 1st week and 2nd week in storage. P-Value > 0.05.
B. The Bonferoni stats for NHWSGM show no significant difference between the pre-wash and post-wash P-Value > 0.05. There were also no significant differences
between the pre-wash and the 1st week and 2nd week in storage post-wash. P-Value > 0.05.
C. The Bonferoni stats for HWSI shows no significant differences between the pre-wash and the post-wash group P-Value > 0.05. There are also no significant differences between the pre-wash and the 1st and 2nd week in storage post-wash.
D. The Bonferoni stats for HWSGM shows no significant difference between the pre-wash and the post-wash p-Value > 0.05. There were no significant differences between the pre-wash and the 1st week and 2nd week in storage post-wash P-Value > 0.05.
E. The RDW-SD were done on the NHNW group from the time it was stored. The RDW-SD was repeated on the 1st and 2nd week in storage. The Bonferoni stats showed no significant differences between the initial storage and the 1st and 2nd week in storage p-value >0.05.

B.47: Red cell distribution width coefficient of variation was done on the 5 study groups before the wash process

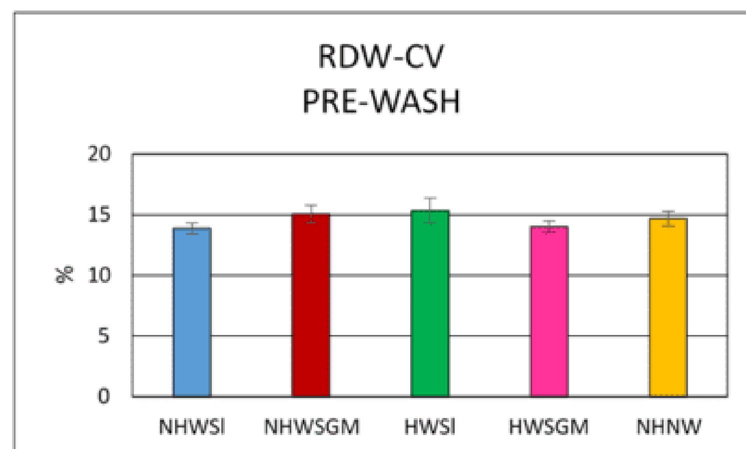


Figure B.47: Red cell distribution width coefficient of variation

RDW-CV Pre-wash. The red blood cell distribution coefficient of variation was done on the pre-wash for all the groups except the control group. The Bonferoni stats shows no significant differences between the wash groups and the control group p-value >0.05.

B.48: The red blood cell distribution width in percentage was done post-wash on all the wash groups and the control group

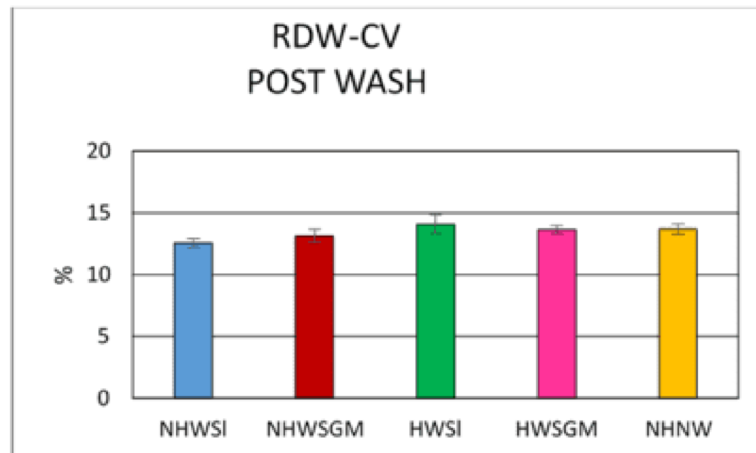


Figure B.48: Red cell distribution width coefficient of variance post wash

The Bonferoni stats shows no significant differences for the wash groups and the control group in storage post-wash p-value >0.05.

B.49: Red blood cell distribution width coefficient of variation test was done on all the wash groups and the control group 1-week post-wash in storage

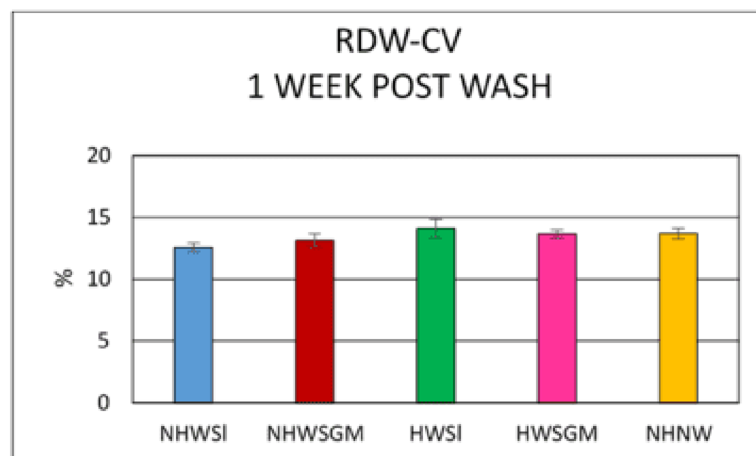


Figure B.49: Red cell distribution width in percentage 1 post-wash

The Bonferoni stats shows no significant differences for the 5 group 1-week in storage post-wash p-value >0.05.

B.50: The red blood cell distribution width coefficient of variance (RDW-CV) was done on all 4 wash groups and the control group 2 weeks post-wash in storage

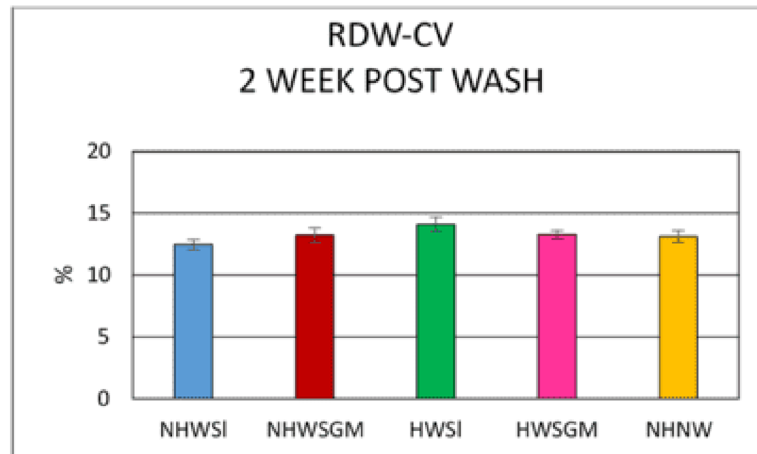


Figure B.50: Red cell distribution width coefficient of variation for the 2nd week in storage post-wash

The Bonferoni stats shows no significant differences between the wash groups and the control group for the RDW-CV p-value >0.05.

B.51: The RDW-CV was done on all the individual groups, the 4 wash groups and the control group

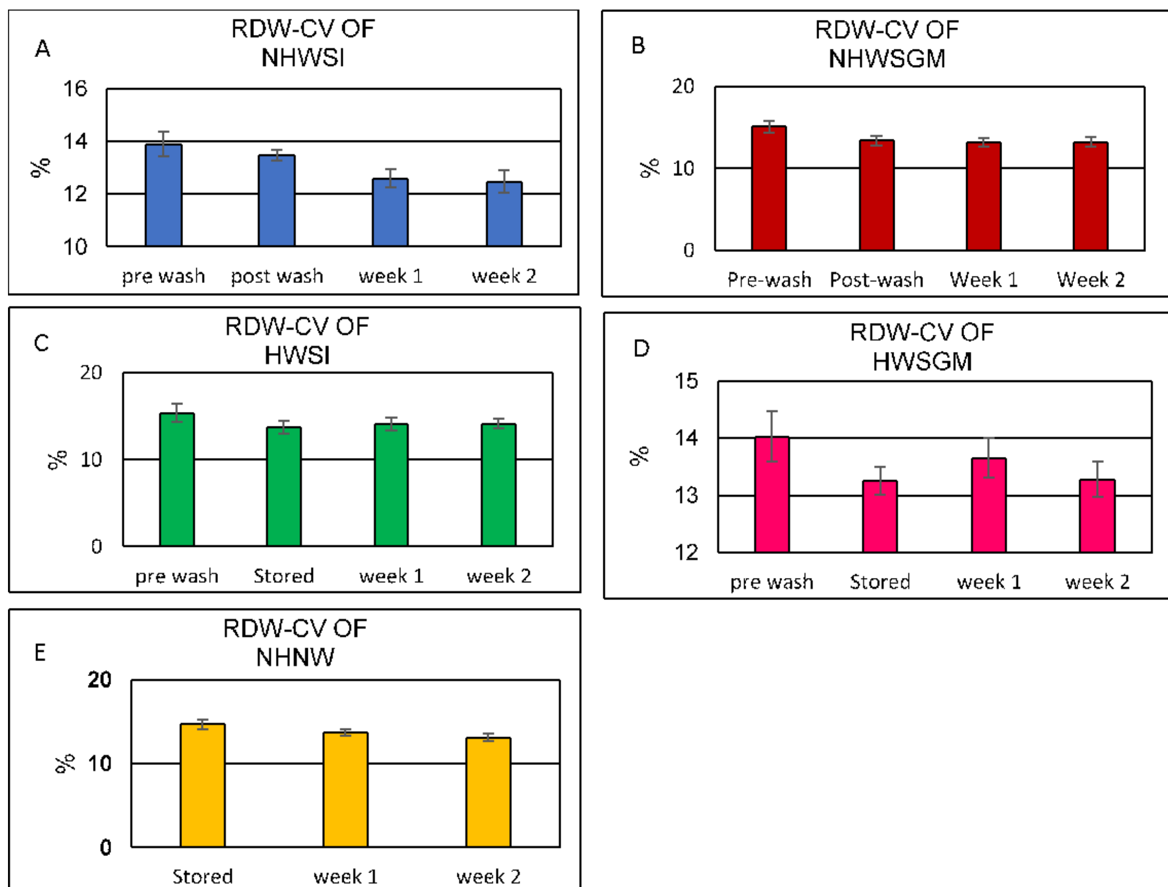


Figure B.51: Red cell distribution width coefficient of variance for all the groups from pre-wash till 2nd week in storage

A. The Bonferoni stats for group NHWSI shows no significant difference between the pre-wash and the post-wash. There were also no significant differences between the pre-wash and the 1st week and 2nd week in storage for the RDW-CV.

B. RDW-CV OF NHWSGM**.** The Bonferoni stats shows no significant difference between the pre-wash and the post-wash. The pre-wash and the 1st week and the 2nd week in storage post-wash also had no significant difference P-Value > 0.05.

C. The Bonferoni stats for HWSI shows no significant difference between the pre-wash and post-wash. There were also no significant differences between the pre-wash and the 1st week and 2nd week in storage P-Value >0.05.

D. The Bonferoni stats for HWSGM shows no significant difference between the pre-wash and the post-wash. There were also no significant differences between the pre-wash and the 1st week and 2nd week in storage post-wash P-Value

E. The Bonferoni stats for NHNW show no significant differences between the initial storage of the cells as well as the 1st and 2nd week in storage. P-value > 0.05.

B.52: The white blood cell count was done on the 5 study groups before they were washed

The comparison of white blood cell count (WBC) between the 5 groups before they were washed. The different groups were not-haemolysed washed and substituted with 0.9% Saline. The other group were not-haemolysed washed and substituted with SAGM (saline, adenine, glucose and mannitol). The third group was haemolysed, washed and substituted with 0.9% Saline. The fourth group is haemolysed washed and substituted with SAGM. The last group were the base group not haemolysed, not washed.

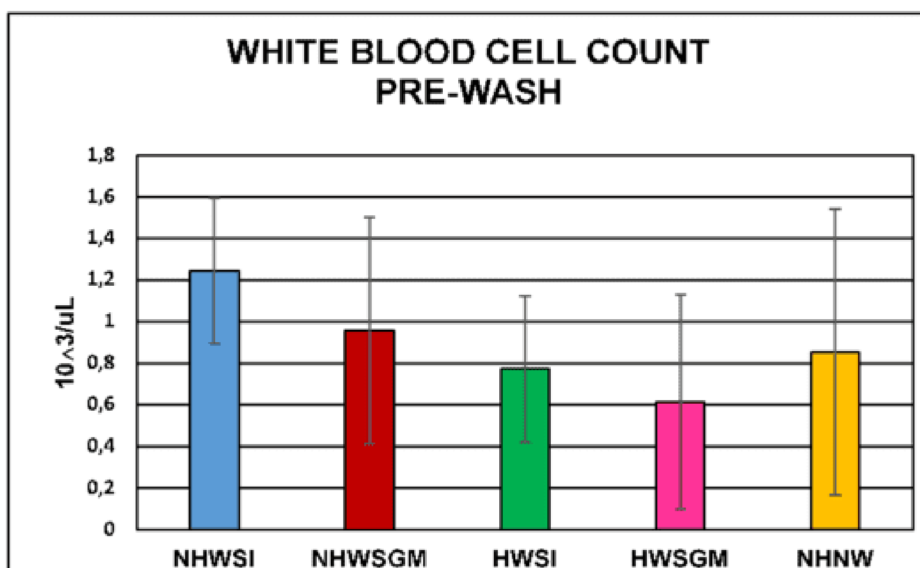


Figure B.52: This figure depicts the white blood cell count for the groups subjected to the study before washing

This graph shows there is no significant differences in the white blood cell count (WBC) between the 5 pre-wash groups p-value >0.05.

B.53: White blood cell count post-wash on the 5 study groups

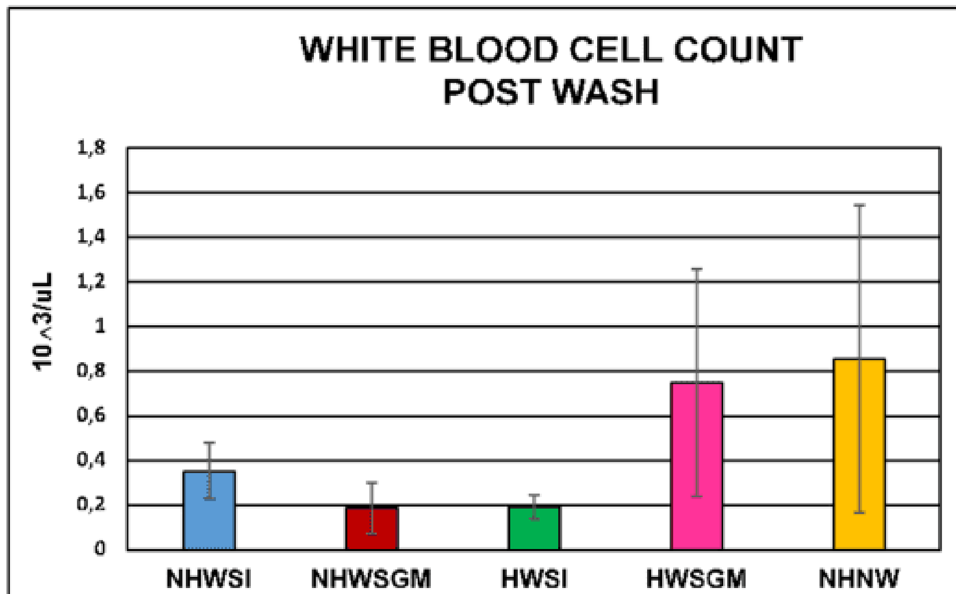


Figure B.53: This figure depicts the white blood cell count for the groups subjected to the study directly after washing

Comparing white blood cell count (WBC) between the wash groups and the base group. Bonferoni stats shows no significant differences between the base group and the wash groups with P-value >0.05.

B.54: WBC count 1-week post-wash on all the study groups

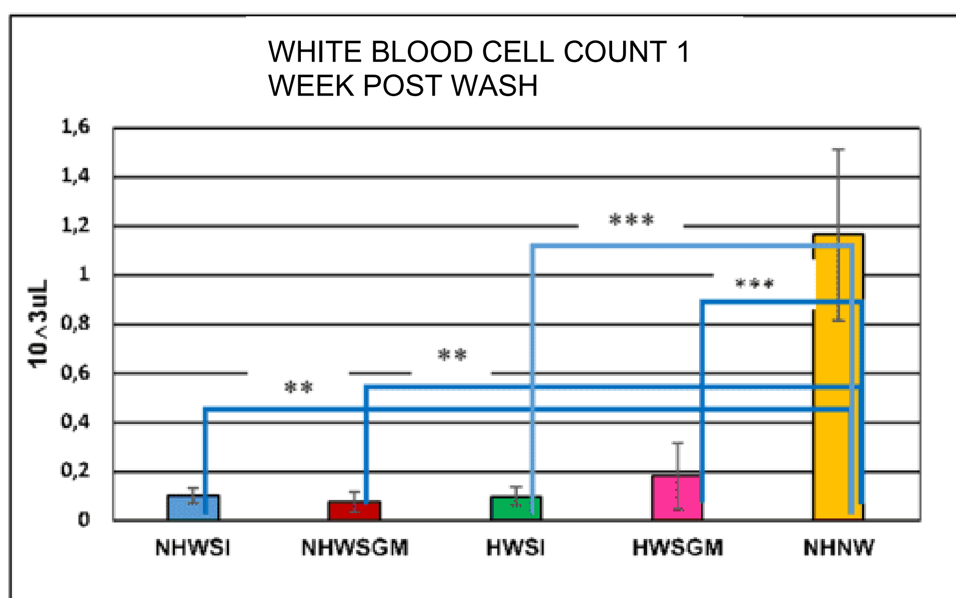


Figure B.54: This figure depicts the white blood cell count for the groups subjected to the study 1 week after washing. ** P<0.001, *** P<0.001

Comparing the WBC 1-week post wash the Bonferoni stats show a very significant difference between the NHWSI, NHWSGM and the base group (NHNW) P-Value < 0.01. There is a highly significant difference between the HWSI, HWSGM and the base group with P-Value < 0.001.

B.55: WBC count for the 5 study groups 2 weeks post-wash

Comparison between the 5 groups after it has been stored for 2 weeks I a controlled environment. After the wash process, in the groups that were not washed and stored for 2 weeks.

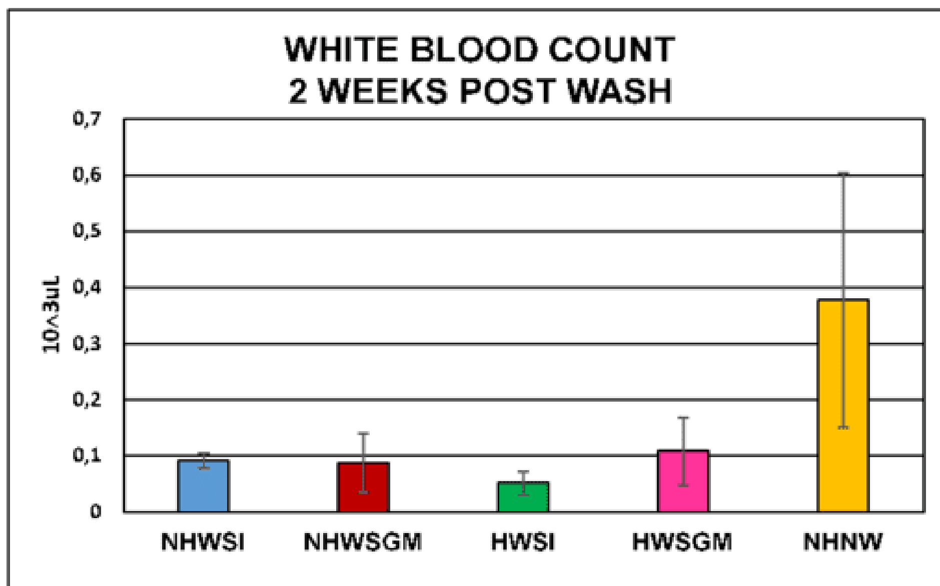


Figure B.55: This figure depicts the white blood cell count for the groups subjected to the study 2 weeks after washing

Bonferoni stats show no significant differences between the wash groups and the base group P-Value > 0.05.

B.56: White blood cell count for all 4 wash groups

White blood Cell Count of the four wash groups and the base group.

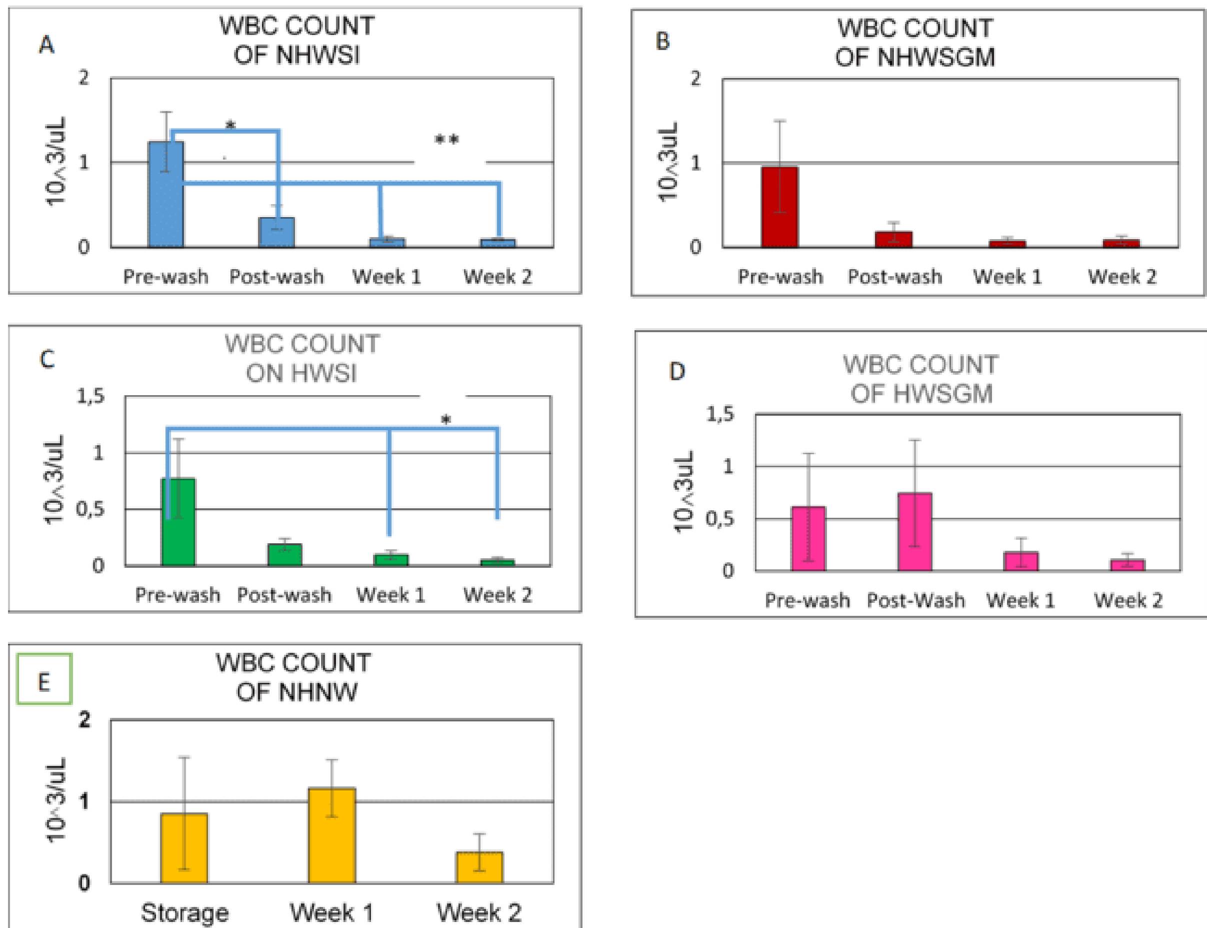


Figure B.56: The white blood cell count was done on all the research groups from the pre-wash till the 2nd week in storage

A. The Bonferoni stats for NHWSI shows a significant difference between the pre-wash and the post-wash WBC result was P-Value < 0.05. There is a very significant difference in the WBC between the pre-wash and weeks 1 and 2 in storage post-wash P-Value < 0.01.
B. The Bonferoni stats of NHWSGM shows that the WBC count for the pre-wash and the post-wash had no significant difference P-Value > 0.05. The pre-wash and week 1 and week 2 in storage also had no significant differences in the WBC count post-wash P-Value > 0.05.
C. WBC COUNT OF HWSI: The Bonferoni stats shows no significant differences between the pre-wash and post-wash WBC count results P-Value > 0.05. The Bonferoni stats shows a significant difference between the pre-wash WBC count and the 1st week and 2nd week in storage P- Value < 0.05.
D. The Bonferoni stats of HWSGM shows no significant differences between the WBC of the pre-wash and the post-wash results P-Value > 0.05. The Bonferoni stats shows no significant differences for the WBC between the pre-wash and the 1st week and 2nd week in storage post-wash P > 0.05.

E. WBC COUNT OF NHNW**:** The Bonferoni stats shows no significant differences between the pre-wash and post-wash WBC count for the control group P > 0.05. The pre-wash WBC count and the 1st week and 2nd week in storage shows no significant differences in results P > 0.05.

B.57: Platelet count was done for the pre-wash on all the wash groups and the control group

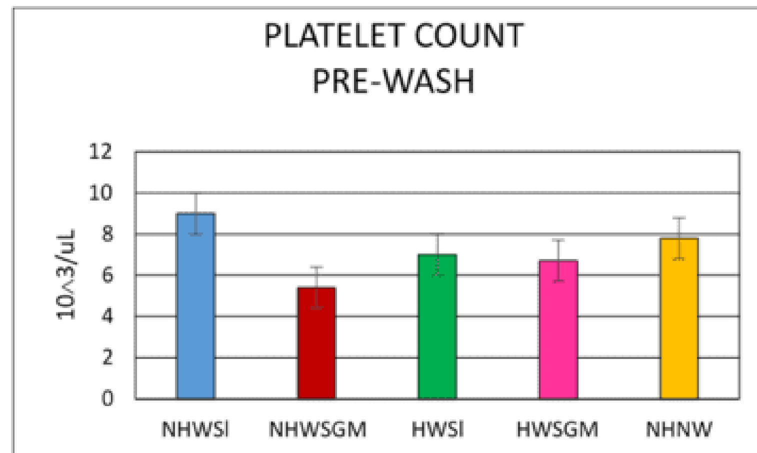


Figure B.57: Platelet count done pre-wash

Bonferoni stats shows no significant differences between the 5 groups p-value >0.05.

B.58: Platelet count was done post-wash on all the wash groups and the control group

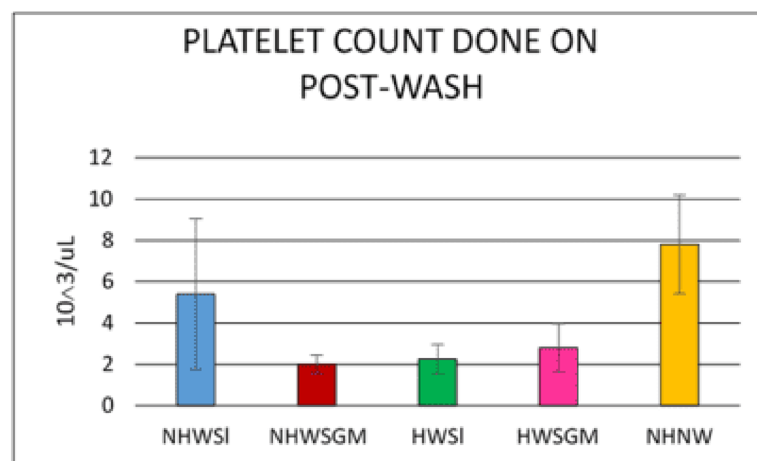


Figure B.58: Platelet count done post-wash

The Bonferoni stats show no significant differences in platelet count between the wash groups and the control group results p-value >0.05.

B.59: The platelet count was done 1 week in storage post-wash on all the wash groups and the control group

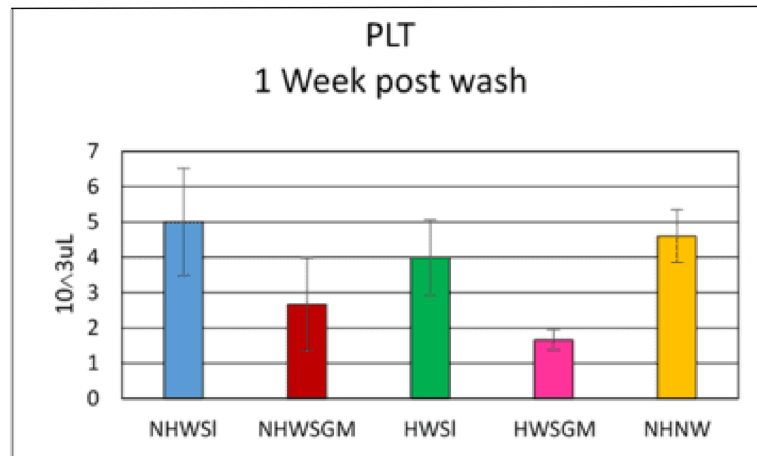


Figure B.59: Platelet count done 1-week post-wash

The Bonferoni stats shows no significant differences between the groups for the platelet count 1 week in storage post-wash p-value > 0.05.

B.60: Platelet count 2nd week post-wash

The platelet count was done 2 weeks after the units was washed. The platelet count was done on all the wash groups and the control group.

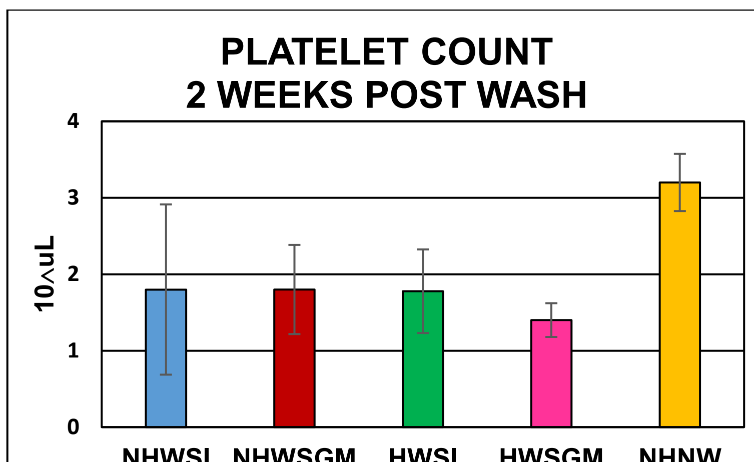


Figure B.60: Platelet count done 2nd week post wash

The platelet count was performed on all the groups washed and the control group, 2 weeks after the wash process. The Bonferoni stats shows no significant differences between the groups and the platelet count results p-value >0.05.

B.61: The platelet count was done on each individual group from the pre-wash till the 2nd week storage

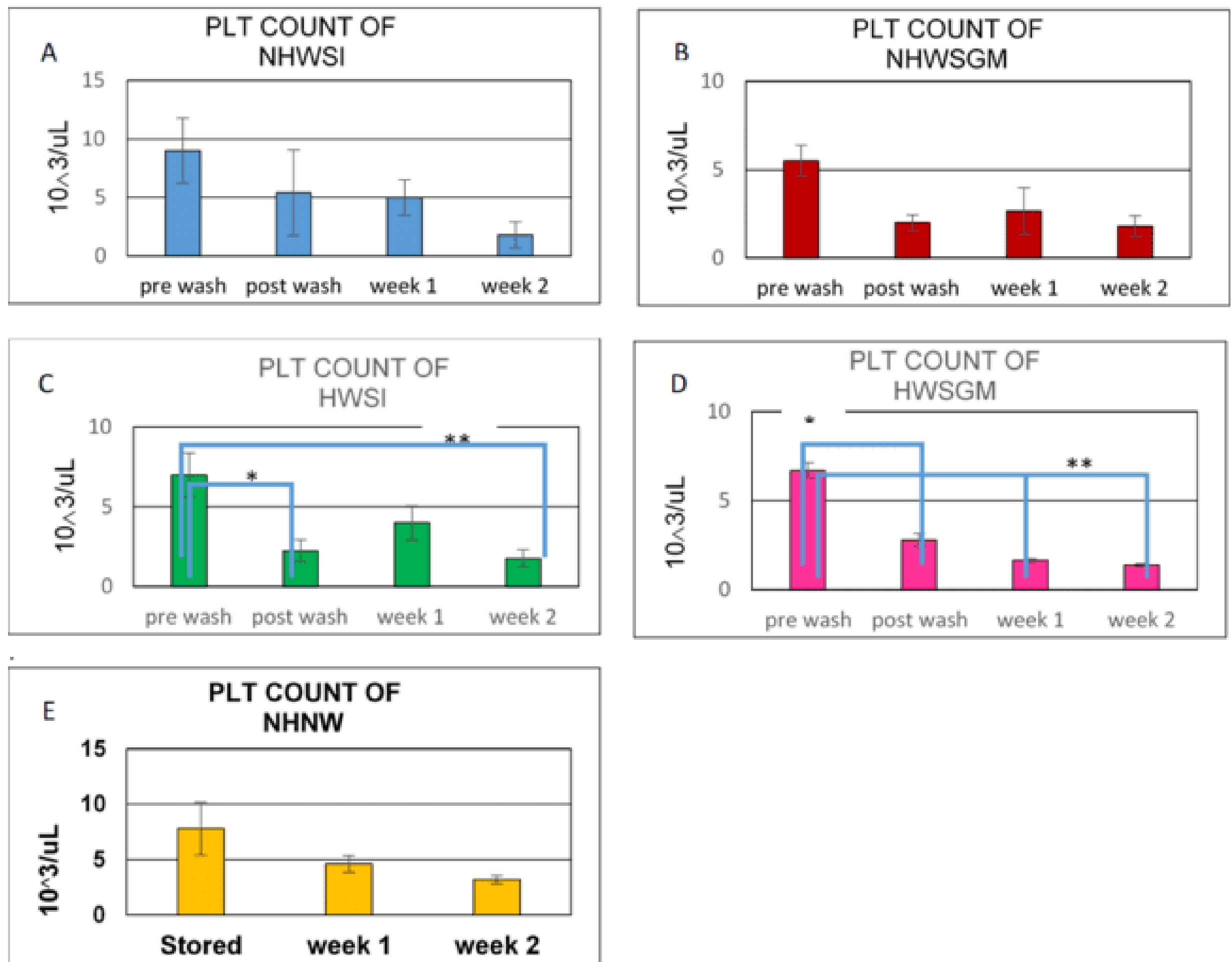


Figure B.61: The platelet count for all the individual groups from pre-wash till the 2nd week in storage

A. The Bonferoni stats for NHWSI show no significant differences between the pre-wash and the post-wash platelet count $P > 0.05$. The platelet counts also did not show any significant differences between the pre-wash and the 1st and 2nd week in storage post-wash P-Value > 0.05.

B. The platelet count shows for NHWSGM no significant differences between the pre-wash and the post-wash results P-Value > 0.05. The platelet count showed no significant differences in results between the pre-wash and the 1st week and second week in storage post-wash P-Value > 0.05.

C. The Bonferoni stats in HWSI shows a significant difference between the pre-wash and the post-wash platelet count P-Value < 0.05. Bonferoni stats also showed a very significant difference between the pre-wash platelet count and the 2nd week in storage post-w3.
ash P-Value < 0.01.

D. The Bonferoni stats shows a significant difference between pre-wash and post-wash platelet count results $P < 0.05$. The Bonferoni results for the platelet count show a very

significant difference between the pre-wash and the 1st week and 2nd week in storage post-wash results P-Value < 0.01.

E. The Bonferoni stats shows there were no significant differences in the platelet count results for the initial storing of the cells as well also no significant differences for the 1st week and the 2nd week of storage P > 0.05.

APPENDIX C - ETHICS APPROVAL LETTER

HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE (HW-REC)
Registration Number NHREC: REC- 230408-014

P.O. Box 1906 • Bellville 7535 South Africa Symphony
Road Bellville 7535
Tel: +27 21 959 6917
Email: simonsy@cput.ac.za

5 December 2019
REC Approval Reference No:
CPUT/HW-REC 2019/H29

Dear Ms Charlotte Veronica Roelfofse

Re: APPLICATION TO THE HW-REC FOR ETHICS CLEARANCE

Approval was granted by the Health and Wellness Sciences-REC to Ms Charlotte Veronica Roelofse for ethical clearance on 5 December 2019. This approval is for research activities related to student research in the Department of Informatics of this Institution.

TITLE: **Salvage of packed red cell units containing haemolysis by a cell wash process in the Western Cape**

Supervisor : **Dr DJ Bester**
Comment

Approval will not extend beyond 6 December 2020. An extension should be applied for 6 weeks before this expiry date should data collection and use/analysis of data, information and/or samples for this study continue beyond this date.

The investigator(s) should understand the ethical conditions under which they are authorized to carry out this study and they should be compliant to these conditions. It is required that the investigator(s) complete an **annual progress report** that should be submitted to the HWS-REC in December of that particular year, for the HWS-REC to be kept informed of the progress and of any problems you may have encountered.

Kind Regards

Dr. Navindhra Naidoo
Chairperson – Research Ethics Committee
Faculty of Health and Wellness Sciences

APPENDIX D – WCBS DONOR QUESTIONNAIRE

www.wcbs.org.za | info@wcbs.org.za
Head Office
3 Oude Molen Road, Ndabeni 7405 • P.O. Box 79, Howard Place 7450
t: 021 507 6300 / f: 021 531 0322

Western Cape Blood Service
Do something remarkable

CONFIDENTIAL DONOR QUESTIONNAIRE

DONOR LABEL | DATE STAMP | SERIAL NUMBER

Section 1 | Personal Details

First-time donors: **Complete all sections in full.**
Repeat donors: **Only complete this section if your personal information has changed.**

Please circle the relevant answers where applicable e.g. YES NO

SURNAME:

FIRST NAME:

TITLE: Prof | Dr | Mr | Mrs | Ms

SEX AT BIRTH: (This is required for your haemoglobin reference range) Male | Female

DATE OF BIRTH: D D M M Y Y Y Y AGE:

RSA ID NUMBER:

HOME / POSTAL ADDRESS:

FOREIGN PASSPORT NUMBER:

TELEPHONE NUMBER (HOME):

TELEPHONE NUMBER (WORK):

POSTAL CODE:

CELL PHONE NUMBER:

EMAIL ADDRESS:

LANGUAGE: English | Afrikaans

ETHNIC GROUP: Asian | Black | Coloured | White

PREFERRED PLACE OF DONATION:

I consent to receive notifications and reminders from WCBS. Yes | No

If yes, please select by which method *(you may select more than one)*: SMS | Phone call | Email

I understand that all calls received from WCBS will be recorded for quality purposes.
I understand that although I indicated my preferred method(s) of communication above, WCBS will contact me via telephone after my first donation.
I understand that I can withdraw my consent at any time by contacting WCBS.
I understand that I will receive the WCBS blood donor newsletter if I select email.

I hereby declare that I would like to enrol as a blood donor. | DONOR SIGNATURE: | DATE:

Your 1 donation could save up to 3 lives. Thank you for donating blood today!

For office use only:

STATS CODE: | PANEL CODE:

RECEPTIONIST SIGNATURE:

DEFERRALS:

GIFT RECEIVED:

DONOR SIGNATURE:

Attach the malaria tag to blood pack until (date):

DONOR CODE:

CLN12 (10 Jul 24)

Section 2 | Health Questionnaire

DATE STAMP

SERIAL NUMBER

Please circle the relevant answers e.g. (YES) NO

Q1.	**Will you be involved in any of the following activities?**		
	Driving a public or heavy-duty vehicle, working on scaffolding or using power tools in the next 24 hours?	YES	NO
	Sky diving, deep-sea diving, flying an aeroplane or mountaineering in the next 3 days?	YES	NO
	Participating in a major sporting event (e.g. full marathon or cycling race over 100 km) in the next 7 days?	YES	NO
	Having a surgical procedure in the next 6 weeks?	YES	NO
Q2.	**In the past 3 days:**		
	Have you taken any painkillers, anti-inflammatories or aspirin (including Ecotrin)?	YES	NO
Q3.	**In the past 7 days:**		
	Have you had a cold, flu, sore throat, fever, infection, open wound or allergies?	YES	NO
	Have you been to the dentist?	YES	NO
	Have you had acupuncture, Botox or dry-needling?	YES	NO
Q4.	**In the past 30 days:**		
	Have you had diarrhoea or vomiting that lasted more than 24 hours?	YES	NO
	Have you had an immunisation or vaccination?	YES	NO
Q5.	**In the past 3 months:**		
	Have you taken any medication (including traditional medication) by mouth or injection?	YES	NO
	Have you been admitted to hospital or had a surgical procedure performed in a doctor's room?	YES	NO
Q6.	**In the past year:**		
	Have you taken part in a drug trial, vaccine trial, or clinical research?	YES	NO
Q7.	**In the past 2 years:**		
	Have you used any medication for the treatment of acne, epilepsy, hair-thinning, prostate problems, rheumatoid arthritis or anticoagulation (blood-thinning)?	YES	NO
Q8.	**Have you ever had:**		
	Heart (e.g. stents), lung or circulatory problems (e.g. clots) or a bleeding disorder?	YES	NO
	Convulsions (fits), epilepsy or strokes?	YES	NO
	Cancer, skin cancer (melanoma, basal cell carcinoma, squamous cell carcinoma) or leukaemia?	YES	NO
	Diabetes, asthma, tuberculosis (TB) or kidney disease?	YES	NO
	Any other serious illnesses, severe allergic reactions, tropical diseases or used medication not mentioned above?	YES	NO
Q9.	**Has your doctor advised you to donate blood to treat a medical condition such as high iron, 'thick blood', polycythaemia or haemochromatosis?**	YES	NO
Q10.	**Hepatitis:**		
	Have you had yellow jaundice, hepatitis, liver disease or tested positive for hepatitis after 1 year of age?	YES	NO
	In the past 3 months, have you been in sexual contact or lived with anyone who has hepatitis (jaundice)?	YES	NO
Q11.	**Travel history:**		
	Have you or your sexual partner travelled outside South Africa **in the last 3 months?**	YES	NO
Q12.	**Malaria:**		
	Have you had malaria **in the past 3 years?**	YES	NO
	Have you been in a malaria area **in the past 3 months?**	YES	NO
	Did you grow up in a malaria area or country (including Zimbabwe, Botswana or Swaziland)?	YES	NO
	If "yes", have you been in **any** malaria area **in the past 3 years?**	YES	NO
Q13.	**For women only:**		
	Are you pregnant or undergoing fertility treatment?	YES	NO
	In the past 3 months have you had a baby, miscarriage or abortion?	YES	NO
	Are you breastfeeding?	YES	NO

STAFF SECTION

CLN12 (10 Jul 24)

FOR OFFICE USE ONLY
(to be completed by clinic staff members)

DONOR LABEL	DATE STAMP	SERIAL NUMBER

PRE-DONATION OBSERVATIONS

Hb: g/dL	Sign:	BP:	Pulse:	Regular	Irregular	Sign:

DONATION PROCEDURE

Donor set-up by: (sign)	HemoFlow Machine No.:
Samples taken by: (sign)	Phlebotomist No. 1: (sign)
Needle removed by: (sign)	Phlebotomist No. 2: (re-needling) (sign)

IRON REPLACEMENT

Iron replacement tablets taken by the donor	Yes	No	Batch No.:	Expiry date:

Dispensed by Professional Nurse:
(name & signature)

DONOR ADVERSE EVENTS (please circle answer)

Faint:	Immediate (before leaving the donor clinic)	Delayed (after leaving the donor clinic)	Mild	Moderate	Severe*

* If marked "Severe", complete all the following information:

Sweating:	Yes	No	Loss of consciousness:	Yes	No	Vomiting:	Yes	No	BP:	Pulse:

Medication administered:	Yes	No	IV Therapy:	Yes	No
Type: Lot No.: Expiry date:			Type: Lot No.: Expiry date:		

Haematoma:	Mild	Moderate	Severe	Accident:	Immediate (before leaving the donor clinic)	Delayed (after leaving the donor clinic)
Delayed bleed: (returns after having left the clinic)				Citrate reaction:		

DETAILS / COMMENTS

QUESTIONNAIRE CHECK AT END OF THE CLINIC

Checked by: Signature:

CLN12 (10 Jul 24)