# 1.1 Introduction

Patients who require frequent blood transfusions, such as those with certain types of anaemia like thalassemia or sickle cell disease, are at risk of iron overload due to the accumulation of excess iron from the transfused blood. This condition is also known as transfusion derived iron overload. The human body has limited mechanisms for excreting excess iron, and repeated transfusions can overload these mechanisms which then leads to buildup of iron in organs such as the heart, liver and pancreas. This can eventually lead to severe complications such as cardiac failure and liver cirrhosis.

There are multiple treatment options such as iron chelation therapy which removes excess iron from the body. Despite this, many patients suffer from iron overload, especially patients receiving chronic transfusions. This highlights the need for continued research into more effective treatment strategies.

Blood is donated from a healthy donor who has given his/her consent. The blood is then processed to form three products namely platelets plasma and red blood cells. These products are then distributed to the required hospital or doctor as requested. Red blood cell products are used when a patient is anaemic or bleeding profusely, platelets are used when a patient suffers from thrombocytopaenia and plasma can be used when a patient is suffering from hypoprotenaemia. Red blood cells have an expiry of 42 days from the date of donation and is stored at 2-6 ºC. Red blood cell (RBC) units are prepared predominantly as concentrated Packed red blood cellswhich are re-suspended in additive solutions consisting of Saline, Adenine, Glucose and Mannitol (SAGM). This allows the red cell units to be stored for up to 42 days and thereafter, due to increased cell death and haemolysis the unit is no longer viable and is discarded.

RBC haemolysis which occurs during component processing and storage, has serious clinical implications for patients receiving transfusions.. During the process of red cell haemolysis, free haemoglobin is released into the circulation. In order for the blood transfusion service to provide the best clinical outcome and reduce the adverse effects of transfusion, it is important for the quality and stored life span of red cell units to constantly be enhanced.

In an attempt to improve the longevity of stored red cells, this study aims to isolate young red cells or neocytes and to examine and compare their rate of haemolysis, biochemical changes and viability with that of conventional leucocyte reduced red cell concentrates which are pre-stored over the standard 42 day period. Neocytes, also known as young red blood cells, of 30 days old or younger, can be separated from whole blood through methods, such as, apheresis or the conventional method.

## Objectives

The objectives of this study will be to:

1) Measure the yield of neocytes collected using the conventional method compared to normally processed red blood cells.

2) To compare the plasma haemoglobin levels of neocyte rich blood at day 1 and every 14 days during storage, and to compare this to routinely prepared red cells.

3) Sodium levels between stored neocyte rich blood and routinely stored red cells

4) To compare all haematological parameters including the reticulocyte count at all stages of storage between neocyte entiched blood and routinely processed red cells.

# 1.2 Data Collection and Preprocessing

The data was collected by experimentation. Preparation of samples for testing was achieved by welding a new sample pouch to the filtered blood bag and neocytes-enriched bag every 14 days. A serum separated tube (SST) was filled with 5ml of blood. This was sent to a private pathology laboratory where it was analysed using the Architect c8000 analyzer. The instrument analysed the Sodium levels using an Ion Selective electrode. This method functions by using a membrane which is placed between the sample and the analyte’s ion. The potential difference is then analysed and a value is calculated based on the potential difference.

3 mL of blood was used in an Ethylenediaminetetraacetic acid (EDTA) for the haematological tests. The full blood count was performed using a the Sysmex XN-3000. The Sysmex XN-3000 tags the intracellular nucleic acids with a fluorescence tag. This tag is directly proportional to the content of nucleic acid. Reticulocytes cells have a much higher fluorescence tag compared to mature red blood cells and is much lower than white blood cells. There are three levels of maturation stages for reticulocytes. These include the low fluorescence ratio, medium fluorescence ratio and high fluorescence ratio.

The Sysmex XN-3000 has a RBC/PLT channel which is used to read the red blood cells and platelets. This is read by using the sheath flow direct current method. The sample is transported into the sheath flow and pushed through the detector. The function of the sheath flow is to control the route of the cells at the detector unit. The cells will then cause an electrical disturbance which is directly proportional to the volume inside the cells. This is read in the form of a pulse. The result is then transformed into graphs.

The last 2ml of the sample bag was used for the free haemoglobin test. On day 1, 14, 28 and 42 an extra 5ml of blood was collected from 30 random neocyte and LRBCPS bags. A large drop of the supernatant was pipetted out onto a slide and the plasma/low Haemoglobin (Hb) cuvette was filled. The hemocue cuvette was used as a pipette as well as a measuring cuvette. The cuvette can only be used once 20 µL of sample is drawn into the chamber by capillary action. The photometer measures two wavelengths in order to compensate for a certain degree of turbidity, and the haemoglobin level is calculated and presented. The system uses the international reference method for calculating haemoglobin.

All the results were captured in CSV format . Data wrangling was performed and all the missing results were dealt with.

# 1.3 Exploratory Data Analysis (EDA)

## 1.3.1 Descriptive statistics

Descriptive statistics (Mean, Quartiles, Min/Max and Standard Deviation) were calulated for each variable (Red blood cell count, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin Concentration, Haemoglobin, Sodium and Percentage plasma haemolysis) at each timepoint (Day 1, Day 14, Day 28 and Day 42).

## 1.3.2 Histogram

A histogram was also executed with the means for each variable. This was used to visually compare the means of the different variables.

## 1.3.3 Correlation Analysis and Heatmap

A Pearson’s Correlation with all the variables was performed. The results were then presented using a heatmap. A positive correlation presented with a colour of red and a negative correlation presented with a colour of blue. A high positive correlation is 1 while a high negative correlation is -1.

## 1.3.4 Scatterplots

This project involved generating scatterplots. If there was a strong correlation with a specific variable between Neocyte enriched and filtered blood (LRBCPS), a scatterplot was created.