A STUDY ON QUALITY PARAMETERS IN VARIOUS LEUCOREDUCED RED CELL PREPARATIONS

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By

Dr. Sreethu Chand

Under the guidance of

Dr. Debasish Gupta

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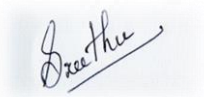
DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation titled **"A Study on Quality Parameters in Various Leucoreduced Red Cell Preparations"** is a bonafide and genuine research work carried out by me under the guidance of Dr. Debasish Gupta, Professor and Head, Department of Transfusion Medicine, Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Trivandrum and Dr. Rajbharath R., Associate Professor, Department of Transfusion Medicine, SCTIMST, Trivandrum.



Dr. Sreethu Chand

Place: Trivandrum

Date: 07-07-2021

CERTIFICATE BY THE GUIDES

This is to certify that the dissertation titled **"A Study on Quality Parameters in Various Leucoreduced Red Cell Preparations"** is a bonafide research work done by **Dr. Sreethu Chand** in partial fulfilment of the requirement for the degree of **MD Transfusion Medicine** under our guidance and supervision.

Guide: Dr. Debasish Gupta
Professor and Head
Department of Transfusion Medicine, SCTIMST

Co-Guide: Dr. Rajbharath. R
Associate Professor
Department of Transfusion Medicine, SCTIMST

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Dr. Sreethu Chand

ABBREVIATIONS

AABB	American Association of Blood Banks
ACD	Acid Citrate Dextrose
BTS	Blood Transfusion Services
CABG	Cardiopulmonary Bypass Surgery
CMV	Cytomegalovirus
CoE	Council of Europe
CPDA	Citrate Phosphate Dextrose Adenine
CPD	Citrate Phosphate Dextrose
DCA	Drug and Cosmetic Act
DGHS	Directorate General of Health Services
DNA	Deoxyribonucleic acid
EBV	Epstein-Barr virus
FC	Flow Cytometry
FFP	Fresh Frozen Plasma
FNHTR	Febrile Non-haemolytic Transfusion Reaction
GoI	Government of India
HLA	Human Leucocyte Antigen
HPA	Human Platelet Antigens
HTLV-I	Human-T-cell Lymphotropic Virus type-I
IEC	Institutional Ethics Committee
IF	Immunofluorescence
Ig	Immunoglobulin

NBTC	National Blood Transfusion Council
NC	Nageotte Chamber
NICU	Neonatal Intensive Care Unit
PC	Platelet Concentrates
PCR	Polymerase Chain Reaction
PRP	Platelet Rich Plasma
RCC	Red Cell Concentrates
RCT	Randomised Control Trial
SAGM	Saline Adenine Glucose Mannitol
SCTIMST	Sree Chitra Tirunal Institute for Medical Sciences and Technology
TAB	Top and Bottom
TAC	Technical Advisory Committee
TA-GvHD	Transfusion Associated Graft versus Host Disease
TRAP	Trial to Reduce Alloimmunization to Platelets
UK	United Kingdom
ULR	Universal Leucoreduction
UV-B	Ultraviolet-B
vCJD	Variant Creutzfeld-Jakob disease
WB	Whole Blood
WBC	White Blood Cells

CONTENTS

INTRODUCTION

The blood units collected from the blood donors contain all the different constituents of human blood. In modern transfusion medicine, the aim is to transfuse only the required component to the patient and this is achieved by whole blood (WB) processing and component separation.(1) Component preparation allows transfusing only the specific blood component to the patient. This will also allow optimal use of blood inventory. From a unit of WB, we can prepare red cell concentrates (RCC), fresh frozen plasma (FFP), platelet concentrate (PC) and cryoprecipitate depending upon the separation method applied. The common goal of component preparation is the constant preparation of RCC, FFP, and PC containing the maximum amount of therapeutic blood elements and minimum amount of unwanted residual cells. A variety of component separation methods have been developed to choose and implement the method of choice. The conventional centrifugation method is usually employed to prepare blood components from the WB collected into anticoagulant solutions. The specific gravity of red cells and granulocytes are quite similar so centrifugation is not an efficient method to separate the unwanted residual cells from RCCs. Hence the blood components can be subjected to further processing steps like leucoreduction or irradiation.

Leucoreduction is the process of reducing the concentration of white blood cells, which are the unwanted residual cells in blood components, by further processing the blood units.(2) The concept of leucocyte removal from the blood was introduced by Fleming as early as 1920 when he used a cotton wool plug in a bent glass tube having a constricted limb and forced blood through it.(3) This was the first attempt to intentionally reduce the leucocyte number in the blood by the filtration method of leucoreduction.

The need for leucoreduction arises because the viable leucocytes in the blood components and their inflammatory mediators are linked to a wide variety of acute and delayed transfusion complications.(4) The leucocytes present in the transfused blood components, with their specific allogeneic structure having the human leucocyte antigen (HLA) class I and class II on their surface are the main targets of the recipient's immune system. They can present antigens to the recipient and evoke the formation of antibodies against them resulting in HLA alloimmunisation leading to platelet refractoriness and graft rejections.(5) The state of platelet refractoriness diminishes the effect of transfused platelets. There will be no therapeutic increment of platelet count after transfusion in these patients. In these settings, as therapeutic strategies to overcome platelet refractoriness are expensive and also not so

effective, it is better to prevent alloimmunisation than treat the condition.(6) Similarly, Transfusion-associated graft versus host disease (TA-GvHD) is caused by donor leucocytes, particularly T lymphocytes in a transfused blood component. It occurs in situations where the recipient is immunosuppressed or there is HLA haploidentical match between donor and recipient. The viable lymphocytes can engraft in the recipient and attack the host tissue resulting in TA-GvHD. (7)

Leucocytes if present can also cause deleterious effects in the recipient like the Febrile Non-Haemolytic Transfusion Reaction (FNHTR).(8) FNHTR is the most common transfusion reaction encountered as reported from many hemovigilance systems. Leucocytes are one of the possible causes of febrile reaction following transfusion.(9) Hence leucoreduction of allogeneic red cells is shown to be effective in decreasing FNHTR.(10) Leucocytes also act as a carrier to transmit certain infections through blood transfusion. Cytomegalovirus (CMV) which is a double-stranded Deoxyribonucleic acid (DNA) virus that belongs to the Herpes family of viruses can be transmitted by leucocytes. CMV could be transmitted from seropositive blood donors and can cause substantial morbidity and mortality in seronegative at-risk populations like pregnant women, preterm infants, or severely immunosuppressed patients.(11) The seropositive individuals can have subclinical CMV infection with the viral DNA present in peripheral blood leucocytes. These individuals may be missed in the routine donor screening due to the subclinical nature of symptoms. But the transfusion-transmitted CMV can cause deadly infection especially in preterm infants and immunosuppressed patients. Removal of leucocytes has been shown to reduce the transfusion-transmitted CMV infection and associated morbidities.(12) Similarly viruses like Epstein-Barr virus (EBV) and human-T-cell lymphotropic virus type I (HTLV-I) can infect lymphocytes thereby getting transmitted through blood transfusion.(13) Hence leucoreduction can also help in preventing viral transmission and add more safety to blood components. It has been shown that leucoreduction filters are also capable of binding the parasite, Trypanosoma cruzi which is associated with transfusion-associated Chagas' disease.(14)

In an attempt to prevent these complications associated with leucocyte transfusion, various methods like buffy-coat reduction, filtration, washing, etc. have been developed to reduce the leucocyte count in the blood component before transfusion. In recent years many transfusion services across the globe have adopted universal leucoreduction (ULR) policy to reduce the risk associated with white blood corpuscles (WBC).(15) With the advent of various

leucoreduction techniques over the years, the transfusion services can implement the suitable method depending upon the local requirement and available finance.

It has been estimated that the average content of leucocytes in donated human WB is 10^9 per unit and their concentration continues to decrease with subsequent component processing. The number of leucocytes in a standard RCC is $5x10^8$ and after buffy-coat removal around $0.8x10^8$.(4) For RCCs that are leucocyte reduced, the Directorate General of Health Services (DGHS), Government of India (GoI), and the American Association of Blood Banks (AABB) guidelines require a residual number of $<5.0 \times 10^6$ leucocytes per unit and The Council of Europe (CoE) requires that the residual number be $<1x10^6$ per unit.(16–18) The commonly employed methods that help in reducing the leucocyte content in red cell units include centrifugation and removal of buffy-coat, filtration, washing of red cells with saline, freezing and thawing of red cells, and collection by apheresis technique. Irradiation of blood components has been shown to effectively minimise the induction of TA-GvHD in immunocompromised patients. Irradiation will eliminate the mitotic capacity of the leucocytes thereby affecting its viability and potential to cause TA-GvHD.(19) Each method has its benefits and drawbacks. In this study, the leucoreduction techniques are analysed and compared for leucoreduction efficiency as well for the changes in the quality parameters that can occur in RCCs due to the processing.

AIMS AND OBJECTIVES

The study is carried out with the following aim:

- To determine the efficacy of different leucoreduction techniques performed in a blood centre.

The study is carried out with the following objectives:

- To estimate percentage leucoreduction obtained with each method of leucoreduction
- To determine the better method of leucoreduction by comparing percentage leucoreduction and red cell recovery
- To estimate the quality parameters in the red cell concentrate leucoreduced by buffy-coat reduction, saline washing and filtration

REVIEW OF LITERATURE

Blood Component Therapy

Blood Transfusion Services (BTS) continue to evolve in the modern era. Development of a cell separator by Edwin Joseph Cohn allowed WB to be separated into its components which paved way for component therapy.(20) Development of collapsible plastic blood bags by Carl Walter, a researcher under Harvey Cushing and William Murphy in the year 1952 provided flexibility for preparing and transfusing required blood components to the patients.(21) Later interconnected plastic blood bags were developed which provided efficient use of blood from a single donor and also reduced the risk of bacterial contamination and volume overload. Now increasingly blood component therapy is practiced, where blood collected is separated into components using semi-automated or automated machines and stored in the specified temperature requirements for future use.

WB from the donors is collected in sterile plasticized polyvinyl chloride bags containing anticoagulant Citrate Phosphate Dextrose Adenine (CPDA) in the ratio of 14 ml per 100 ml WB. Blood components are prepared from the WB unit by centrifugation and separation method. WB units can be separated into RCC, FFP and PC. RCCs anticoagulated with CPDA are stored at 2-6 ºC and have a shelf life of 35 days from the day of collection or can be extended to 42 days if a red cell additive solution is used. Platelets can be prepared from WB by either of the two methods; preparation from buffy-coat or preparation from platelet-rich plasma (PRP).(22) Buffy-coat method yields more plasma and better leucocyte reduction compared to the PRP method, but at the expense of red cell loss. Murphy and Gardner have demonstrated superior platelet in vivo recovery when stored between 20 and 24 ºC. (23) Hence PCs are stored for 5 days at room temperature in continuous agitation to ensure adequate metabolic requirements. Plasma prepared by centrifugation is rapidly frozen within 8 hours of collection and stored at -18 ºC or colder for 12 months as FFP. Cryoprecipitate can be obtained from FFP by thawing at 4 ºC in a circulating water-bath.

Secondary processing of the blood components can be done to provide some added advantage to the recipients. It includes procedures like leucocyte reduction to prevent leucocyte-associated adverse transfusion reactions and irradiation which can prevent TA-GvHD.

Leucocytes in Transfused blood components:

Leucocytes are a constituent of human blood that forms the body's primary defence against bacterial pathogens. They are broadly classified into granulocytes which include neutrophils, eosinophils, and basophils, and agranulocytes that include lymphocytes and monocytes. They form the first line of host immune response with high potency and efficacy to eradicate infections. However, the role of donor leucocytes in transfused blood products is limited. Rather they are responsible for various transfusion reactions in the recipient due to their specific allogeneic structure. Leucocytes contain HLA antigens on their surface. With multiple transfusions, there is repeated exposure to leucocytes from different blood donors which can result in the development of antibodies directed against many or most HLA class I antigen types. In an animal study by Claas *et al***,** it was shown that an antibody response occurred only when platelet suspensions were contaminated with leucocytes confirming the role of leucocytes present in blood components in the development of antibodies.(24) These antibodies play an important role in some of the transfusion reactions that can occur in a recipient of allogeneic blood transfusions like FNHTR, TA-GVHD, and alloimmunisation causing platelet refractoriness. A study by Dzieczkowski *et al* characterised transfusion reactions in recipients of 12,277 transfusions and their analysis showed a reduction of alloimmunisation and FNHTR incidence after transfusion of leucocyte reduced blood components; confirming the role of donor leucocytes in the development of transfusion reactions in the recipient.(25)

Adverse reactions due to leucocytes in transfused blood components:

Leucocytes and the cytokines derived from them are associated with several adverse outcomes in transfusion therapy. (**Table No. 1**) (4)

Table No. 1: Donor Leucocyte associated adverse reactions in recipients

1. Febrile Non-haemolytic Transfusion Reactions (FNHTR)
2. HLA Alloimmunisation and Platelet Refractoriness
3. Transfusion Associated Graft versus Host Disease (TA-GVHD)
4. Transmission of infectious agents

1. Febrile Non-haemolytic Transfusion Reaction (FNHTR)

FNHTR is the development of fever and/or chills without haemolysis occurring in a recipient during or within 4 hours of completion of transfusion. The frequency of FNHTR was reported in the study by Enright *et al*, where they studied 598 leukaemia patients who received 8769 transfusions. Following transfusions, fever occurred in 4.4% of patients but rose to 22% when chills with rigors were included in the definition of FNHTR.(26)

The role of leucocytes in causing FNHTR was first recognised by Brittingham and Chaplin in the year 1957. They found an association of potent leucoagglutinins in the serum of patients who received multiple transfusions and who suffered febrile transfusion reactions. Transfusion of a fraction of blood containing more than 90% of buffy-coat to 5 patients who had a history of severe febrile reactions following blood transfusion and whose serum contained leucoagglutinins produced severe FNHTR but transfusion from the same blood unit with less than 10% of buffy-coat caused no reaction.(27) While Payne in his study on 49 patients with a history of febrile transfusion reactions, found leucoagglutinins in the serum of 32 patients, and out of the 15 patients who received repeated transfusions, 13 developed leucoagglutinins about the time of first transfusion reaction. But the blood unit containing less than 0.2×10^9 per litre caused no reaction in these patients.(28) In the year 1958, van Loghem *et al* confirmed this association of FNHTR with leucoagglutinins in their study.(29) The clinical significance of leucocyte antibodies in producing FNHTR was also studied by Brubaker in 24 patients who reported FNHTR following blood component transfusion. He identified leucocyte antibodies using immunofluorescence (IF) testing in 70 percent of his study subjects showing the association of leucocyte antibodies with the development of FNHTR.(30)

The quantity of leucocytes in the transfused allogeneic unit also influences the development of antibodies against them. In the year 1961, a detailed study on FNHTR was done by Brittingham and Chaplin on a single subject and found that transfusion of 0.4×10^9 leucocytes would not produce a reaction while the transfusion of 1.5×10^9 or more leucocytes would produce a reaction and the severity of the transfusion reaction depended on the titre of leucoagglutinins.(31) The role of leucocyte antibodies in the production of FNHTR was studied by Perkins *et al* in a study of 8 patients and found that leucocyte antibodies were the primary cause of FNHTR to blood transfusions and the least number of leucocytes that was

required to cause a transfusion reaction varied from 0.25×10^9 to more than 25×10^9 and the severity of the reaction was related to the number of incompatible leucocytes transfused.(8) These early studies indicated the role of leucocytes in febrile reactions and the importance of reducing their number before transfusing patients.

2. HLA Alloimmunisation and Platelet Refractoriness

Platelet refractoriness is defined as the failure to achieve desired platelet counts in a patient after platelet transfusions. Platelet refractoriness can occur due to both immune and non-immune causes. An immune reaction like alloimmunisation can occur due to previous pregnancies, transfusion, or organ transplantation.(32) HLA alloimmunisation can lead to refractoriness to platelet transfusions. In the majority of immunised patients, the alloantibodies are directed against HLA class I antigens as shown in the study by Schiffer *et al* in 1976, although human platelet antigens (HPA) may also be involved. (33)

It was in the 1990s, the risk of HLA alloimmunisation and the consequent risk of platelet refractoriness were first assessed. An early study by Brand and co-authors showed that leucoreduced red cell unit and random donor platelet transfusions were very unlikely to induce primary alloimmunisation against HLA antigens.(34) A prospective randomised trial was performed by Oksanen *et al*, where they provided leucocyte-reduced RCCs and PCs to adult leukaemia patients while non-leucocyte reduced RCCs and PCs to the control group and observed the development of HLA antibodies and platelet refractoriness in the control group patients.(35) A quite similar prospective randomised control study was conducted by Kooy *et al* to assess the role of leucoreduction in the development of HLA antibodies and platelet refractoriness. Refractoriness occurred in 46% and HLA antibodies were detected in 42% of the control group patients who received blood components that were not leucocyte reduced.(36)

When the number of leucocytes is not reduced in the transfused blood components; patients can develop antibodies against the HLA antigens present in the donor cells. Primary immunisation can occur as early as 10 days upto 4 weeks after transfusion, while the secondary response in an already sensitised patient can occur within 4 days and as stated by Slichter *et al*, allo-immunisation does not necessarily correlate with the number or schedule

of platelet transfusions, but increasing the number of platelet transfusions were associated with decreased post-transfusion platelet responses.(37)

There is strong evidence that leucocytes in the PC evoke antibody formation against HLA antigens rather than platelets themselves.(24) When the number of leucocytes in the transfused cell concentrate is reduced, the percentage of immunised patients decreases thereby decreasing the incidence of platelet refractoriness. This was shown in the Trial to Reduce Alloimmunisation to Platelets (TRAP) study, where leucoreduction of PCs prevented alloantibody mediated refractoriness to platelet transfusions. In the TRAP study, Ultraviolet-B (UV-B) irradiation, leucofiltration, and filtered single donor apheresis platelets were equally effective in preventing both the development of lymphocytotoxic antibodies and platelet refractoriness when it was due to alloimmunisation. Of the 530 patients with no alloantibodies at trial initiation, 45% in the control group developed lymphocytotoxic antibodies and 13% became refractory to platelet transfusions.(26)

A meta-analysis of Randomised Control Trial (RCTs) on the efficacy of leucoreduction in preventing HLA alloimmunisation and platelet refractoriness including the TRAP study data, done by Vamvakas demonstrated a significant reduction of the cumulative relative risk of HLA alloimmunisation, from the use of leucoreduced blood components.(38) While Gouttefangeas *et al* concluded that the formation of antibodies in the recipient is due to the contaminating leucocytes present in the cellular blood component, but platelets alone can induce a secondary immune response resulting in refractoriness.(39) In conclusion, there is substantial evidence that patients transfused with PC from which leucocytes were removed, were less likely to develop platelet refractoriness.(40) Hence leucoreduction plays a major role in preventing platelet alloimmune refractoriness.

3. Transfusion Associated Graft versus Host Disease (TA-GVHD)

TA-GVHD can occur in immunocompromised patients as well as in immunocompetent transfusion recipients who are heterozygous for HLA haplotype for which donor is homozygous.(41) TA-GVHD occurs when allogeneic lymphocytes expressing the HLA antigens, present in a transfused blood component engraft in the recipient, proliferate and attack the host tissues. It can cause mortality of around 90% when multiple organ systems are affected. (42)

Leucoreduction of blood components has not been shown to reduce the incidence of TA-GVHD, but leucocyte inactivation and gamma irradiation have proven efficacy.(43,44) Hence to avoid the risk of GvHD following blood transfusion, transfused blood components need to be irradiated to inactivate donor lymphocytes and 25 Gy is the dose required to inactivate the T lymphocytes.(45) Irradiation is done for at-risk target populations like patients with immune deficiencies, Hodgkin disease, and premature infants or in directed donations.

3 (a). Irradiation:

The purpose of irradiation of cellular blood components in BTS is to inactivate immunocompetent lymphocytes. Irradiated RCCs are indicated especially in immunocompromised patients, to prevent the fatal TA-GVHD. Pelszynski *et al* suggested a dose of 25 Gy for complete T cell inactivation in stored red cells as measured by a limiting dilution assay of proliferation. As this dose of radiation can affect red cell viability adversely, the shelf life of the stored RCC is reduced following the procedure.(45) Davey *et al* in 1992 studied the effect of irradiation on long-term storage of RCC and concluded that irradiation with 30 Gy damages red cells and long-term storage in the irradiated state may enhance the damage and hence irradiated RCC should not be stored for original shelf life.(46) A detailed study on storage of irradiated RCC was done by Moroff *et al* in 1999**,** which suggested that red cell viability is maintained till 28 days from the day of collection, regardless of when the cells are irradiated.(47) The AABB, recommends the irradiated RCC be stored for 28 days from the day of collection or till the original expiry whichever is earlier. While CoE guidelines recommend the irradiated RCCs to be stored only upto 14 days of irradiation or 28 days of collection whichever is earlier.(16,18) Increased efflux of potassium from the red cells was noted when subjected to irradiation. Ramirez *et al* noted an approximate doubling of potassium levels within 48 hours of storage when irradiated with 30 Gy.(48) The increased amount of potassium is of no clinical significance except in susceptible cases like massive transfusion in neonates.

4. Transmission of infections through leucocytes

Leucocytes can also be a means of transmission of viruses like CMV, EBV and HTLV type I as these viruses reside and replicate in them. CMV is a clinically significant herpes virus that solely infects humans and causes severe consequences in immunocompromised individuals and neonates with considerable morbidity and mortality.(49) CMV infection in immunocompetent individuals is often asymptomatic and therefore can be missed in seemingly healthy blood donors. A study by Kondo *et al* suggested a role for leucocytes as a reservoir for latent infection by CMV.(50) While Bowden *et al* compared the use of CMV-seronegative blood components and leucoreduced blood components in a prospective randomised trial in 502 patients and found no significant differences between the probability of CMV infection or disease in both arms.(51)

EBV is also a human herpes virus and it establishes latent infection in lymphocytes. The transmission of EBV through blood transfusion and possible seroconversion was explained in a pilot study by Fleisher in 44 children who underwent open-heart surgery and required blood transfusion.(52) While Qu *et al* in his study, described the importance of leucoreduction in eliminating the need to screen blood for EBV.(53) HTLV-I was the first human retrovirus discovered by Poiesz *et al* in 1980 in a T-cell line.(54) Infection caused by HTLV-I is usually asymptomatic; hence silent transmission can occur through blood transfusion. A retrospective serological study on 41 recipients of WB or blood components by Okochi *et al*, suggested transmission of HTLV-I through blood transfusion. They demonstrated proviral DNA in the lymphocytes of transfusion recipients.(55) A similar study by The American Red Cross HTLV I/II Collaborative Study Group on 133 recipients of prior transfusions from HTLV-I/II seropositive blood donors showed an apparent transmission rate of 12.8%.(56) Leucoreduction of blood components has been shown to provide an added advantage to anti-HTLV screening.(57)

The role of leucoreduction in preventing the transmission of infectious agents through blood transfusions is continuously being explored.(58) Studies have shown that the transmission of prion diseases like variant Creutzfeld-Jakob disease (vCJD) from blood components can only be partially reduced by buffy-coat reduction as well as by using leucoreduction filters.(59) While leucoreduction is an effective tool in preventing transmission of bacteria through blood

components in addition to the diversion pouches, has been confirmed by Andreu *et al* by analysing the bacterial infection rates before and after implementing ULR.(60) All the above information suggests the importance of leucoreduction in mitigating the adverse reactions caused by the donor leucocytes in transfused blood components.

Leucocyte reduction of Blood Components

Leucoreduction is the process by which WBCs are removed from the blood components. The average content of WBCs in donated WB has been estimated to be 10^9/unit and it has been seen to reduce with subsequent component preparation. The total number of leucocytes received by the recipient following a blood component transfusion is far more important than the percentage reduction of the initial leucocyte count. The standards have been set based on the residual leucocyte count rather than the percentage leucoreduction. Perkins *et al* estimated that RCC containing less than 0.5×10^9 leucocytes generally will not cause febrile reactions when transfused to alloimmunised patients.(8) As defined by AABB, leucoreduced RCC requires a residual leucocyte count of $< 5 \times10^6$ leucocytes per unit, which is 3 log reduction (99.9%) with a minimum of 85% red cell recovery following the procedure.(16) The same standards are set by DGHS, GoI.(17) While the CoE guidelines mandate the residual leucocyte count to be $< 1\times 10^6$ per unit.(18) (**Table No. 2**)

Table No. 2: The currently accepted standards of leucoreduction

American Association of Blood Banks (AABB)	$< 5\times10^6$ WBC/unit
The Council of Europe	$< 1\times 10^6$ WBC/ unit
Directorate General of Health Services (DGHS), India	$< 5\times10^6$ WBC/unit

Methods for preparing leucocyte reduced blood components

As leucocytes are a major reason for producing immune and non-immune transfusion reactions, there have been several attempts to remove them from the blood components before transfusion. Hence different methods were devised to achieve an adequate reduction of leucocyte content in the transfused blood units as an attempt to decrease the adverse effects caused by the donor leucocytes.

There are several acceptable options for leucoreduction, ranging from simple inexpensive methods yielding modest leucocyte removal to expensive methods that achieve exceptional leucoreduction. They also vary in their complexity and equipment required.(61,62) The currently employed leucoreduction methods for RCCs are listed in **Table No. 3**. (63)

Table No. 3: Currently Employed Leucoreduction Methods

1. Centrifugation and Buffy-coat removal
2. Saline washing of Red Cells
3. Freezing and Deglycerolisation of Red cells
4. Filtration
5. Component collection by Apheresis

Early techniques of leucoreduction involved centrifugation and buffy-coat removal and saline washing of RCC. As early as the 1920s, the concept of leucofiltration was introduced, when Fleming forced blood through a cotton wool plug in a bent glass tube having a constricted limb.(3) It closely resembled the model of modern depth filters. Other methods include freezing and deglycerolisation of red cells and apheresis procedure.(64) More efficient the leucoreduction method better is the recipient outcome following transfusion; although none of them is capable of eliminating the leucocytes from the blood components. Of all these methods, filtration and apheresis procedures have been shown to meet the current standards for leucoreduction. Other methods can achieve leucoreduction to a variable extent. **Table No. 4** lists the approximate number of residual leucocytes that are present in different cellular blood components.(17)

Table No. 4: Approximate Residual Leucocytes in Cellular Blood Components

METHOD	LEUCOREDUCTION
Fresh Whole Blood	10^9 WBCs
Buffy-coat reduced RCC	10^8 WBCs
Washed RCC	10^7 WBCs
Deglycerolised RCC	10^6-10^7 WBCs
Filtered RCC	10^6 WBCs
Apheresis RCC	10^6 WBCs

The leucoreduction procedures can be carried out either as an open system or as a closed system of leucoreduction.

Closed system: In closed methods of leucoreduction, the normal shelf life of the blood component is maintained. Here the leucoreduction is done without entering the sterile system. Sterile connecting devices and automated machines help in maintaining the closed system of leucoreduction.

Open system: When the closed system is entered, the shelf life of the blood unit is reduced to 24 hours from the time of entering the system for RCC held at 1 – 6 ºC as a measure to prevent bacterial contamination of the blood component.(16) The open system of leucocyte removal is often used in the washing of red cells or the freezing and de-glycerolisation of red cells.

Sterile connections in component preparation:

The use of special welding devices that could provide sterile connections during procedures allowed the storage of leucoreduced RCCs upto the expiration limit that is applied to the blood components prepared in closed bag systems. The development of sterile connecting devices greatly increased the use of leucoreduced blood components as it solved the issue of component sterility.(65,66)

Commonly employed leucoreduction methods include:

1. Centrifugation and Buffy-coat Removal

This is a closed system for leucoreduction. Here the cells are separated based on their differences in specific gravity (**Table No. 5**). During centrifugation of WB, components are arranged based on their specific gravity, leading to the formation of a layer of buffy-coat at the interface of plasma and red cells. The buffy-coat consists of a layer of platelets and leucocytes which are removed either by subjecting them to visually controlled expression using plasma expresser or by using semi-automated machines. The development of plastic bags with top and bottom configuration allows the use of automation for simultaneous removal of plasma and RCC from the primary bag, leaving behind the buffy-coat layer .(67)

Table No. 5: Specific gravity of blood components: (68)

COMPONENT	SPECIFIC GRAVITY (g/ml)
Plasma	1.026
Red cells	1.100
Platelets	1.058
Monocytes	1.063
Granulocytes	1.085
Lymphocytes	1.070

In this technique of centrifugation and buffy-coat removal for leucocyte reduction, the percentage leucoreduction is proportional to red cell loss**.**(69) This is the least expensive and easiest method of leucoreduction that can be done in a closed system, but it is not an efficient one. It is shown to reduce the leucocytes by 70-80%, which is less than 1 log reduction with a red cell recovery of around 80%.(17) A disadvantage of using the buffy-coat method of leucoreduction was the additional loss of red cells which has been decreased now by the use of automated separating devices. The use of automated technology has also made the buffy-coat method more reproducible. It is a predominant method of leucoreduction in developing

countries due to its lower cost and reproducibility of leucocyte removal from RCC. Also, the removed buffy-coat can be used as source material for the preparation of platelet concentrates with a very low level of leucocyte contamination.(70) A before and after study by Lieden *et al*, showed a statistically significant reduction in febrile transfusion reactions after the introduction of buffy-coat reduced RCCs. Also, reduction of leucocyte contamination to 30% and platelet content to 10% in RCC by buffy-coat reduction method prevented microaggregate formation during storage.(71)

2. Saline washing of red cells:

Washing of RCC can be done by manual methods or using automated instruments. Automatic methods are more efficient with less red cell loss during each wash cycle while manual methods are time-consuming and if done in an open system, it reduces the shelf life of RCC to 24 hours. Washed RCCs can be prepared with isotonic saline, SAGM or 5% albumin.(72) Other than leucocyte removal, washing of RCC has the advantage of removing any microaggregates formed by clumping of leucocytes and platelets during storage, as well as removing plasma proteins present.

Washed RCCs are indicated in patients who had recurrent allergic transfusion reactions and also for Immunoglobulin A (IgA) deficient persons who had formed Anti-IgA antibodies because transfusion of plasma with IgA content can result in anaphylactoid and urticarial transfusion reactions.(73) Since washing with isotonic solutions like 0.9% saline removes the plasma content in the bag along with achieving leucoreduction, washed RCC can be safely used in these susceptible patients.

3. Freezing and deglycerolisation of red cells:

An alternative way for leucoreduction is cryopreservation. In 1950, Smith reported the role of glycerol in preventing freezing injury in human red cells.(74) This paved the way to freeze red cells mixed with glycerol for a longer duration without damage. Red cells if frozen get damaged due to intracellular ice formation. Glycerol acts as a cryoprotective agent when added to red cells before freezing and when required, cells can be thawed without damage.(75) The action of glycerol as a cryoprotectant is by limiting ice formation and preventing excessive hypertonicity when cooled as it can permeate red cells during

freezing.(76) Cryoprotectants like glycerol are added to RCCs that are less than 6 days old, slowly with vigorous shaking to allow it to permeate red cells. The cells are rapidly frozen and stored at -60 ºC or lower. Two concentrations of glycerol can be used for red cell freezing, high concentration glycerol with 40% weight in volume and a low concentration glycerol with 20% weight in volume. The freezing and storage temperature depends on the concentration of glycerol used. At the time of requirement, the red cells are thawed and washed with decreasing concentrations of saline to remove glycerol content before transfusing.(17) Over 90% leucocytes were eliminated in this method with a minimum red cell loss.

Glycerolisation of the red cell unit, as well as its freezing, destroys the leucocytes present. It was found that the bulk of leucocytes were fragmented under the optimal storage conditions used for red cell freezing. Also, repeated washings required for the removal of cryoprotectants could remove most of the debris of leucocytes.(77) Even though a substantial number of recognizable lymphocytes may remain after thawing and deglycerolisation, the entire procedure would have already altered their antigenic qualities providing added advantage. The freezing experience could alter the immunological characteristics of viable cells. Cryopreservation requires highly skilled personnel, sophisticated equipment, and special storage facilities. Hence cryopreservation is rather a tedious and complicated procedure for leucoreduction.

4. Filtration

The use of leucoreduction filters have been widely accepted as a method of leucoreduction. The development of leucoreduction filters dates back to 1972 when Diepenhorst designed a leucocyte reduction filter that contained cotton wool as the filtering agent.(78) Currently, there are 4 generations of leucoreduction filters. First-generation filters were made up of nylon and 170 - 200µ screens which were capable of removing only large clots and foreign matters. Second generation filters were designed to prevent microaggregates from blood entering the bloodstream. These second-generation filters often combined technologies of screen filters and depth filters to achieve a functional pore size. One of the commonly used microaggregate filter was a 40µ filters which consisted of a woven mesh of polyester which is folded and kept in the filter (SQ40; Pall Corp, Glencove, NY). Microaggregate filters remove particles based on their size. When the red cells are stored, the platelets and leucocytes present in them will form microaggregates which were removed with these

second-generation filters. But still a large number of leucocytes that remain as individual cells will be retained, reducing the efficacy of the filter.(79)

The third-generation filters were developed that applied the barrier retention method i.e. free cells were filtered based on their size as well as leucocyte adhesion mechanism. Hence sometimes they were also referred to as adsorption filters. Leucocyte reduction of blood by using synthetic fibres was reported by Greenwalt *et al* as early as in 1962. He used tightly packed nylon wool fibres for filtration. Granulocytes were found to adhere to the fibres by phagocytosis along with a part of platelets, however mononuclear cells were almost completely eluted from the filter with the red cells. Also, optimal removal of granulocytes required temperatures higher than room temperature.(80)

It was in 1972 Diepenhorst *et al* prepared 1820 units of leucoreduced RCCs by filtration using a cotton wool leucofilter. It was shown to remove 95% or more of all types of leucocytes from WB or red cell suspensions with a red cell recovery of 96%.(78) This work led to the development of cotton wool filters like the Imugard IG-500 (Terumo Corp, Tokyo, Japan) with a fibre content of 22g. This cotton wool adhesion filter requires preliminary priming with saline, before introducing the RCC and also post-filtration the residual cells need to be flushed off with saline. A prospective randomised trial using cotton wool filtration as a method of leucoreduction in RCC and PC in 54 patients with haematological malignancy or marrow aplasia by Sniecinski *et al*, showed filtration as an effective and economical method for reducing alloimmunisation and clinical refractoriness to random donor platelets in patients receiving long term transfusion support.(81)

Nowadays the cotton wool filters have been largely replaced by filters made up of nonwoven webs of synthetic microfibres like polyester or cellulose acetate (Erypur filter, N.P.B.I., Holland), which showed much more efficacy than the cotton wool filters. Cellulose acetate filtration resulted in almost complete removal of leucocytes as well as 90% of platelets, along with a red cell recovery of 85-90%. Analysis of the Erypur filter by Rebulla *et al*, showed it as a simple, safe, and effective filtration procedure with a red cell recovery of 90 - 99% and residual leucocyte count of less than 5×10^6.(82) A comparison study on the performance of cotton wool filter (Imugard IG-500) and cellulose acetate filter (Erypur) was done by Reesink *et al*. They concluded that cellulose acetate filter has sufficient capacity to guarantee the removal of 97% of all leucocytes and 90% thrombocytes present in 500ml of fresh WB.(83) Cellselect (NPBI, The Netherlands) is also a cellulose acetate filter that could achieve more

than 99% leucoreduction with unaltered haemolysis and osmotic fragility immediately following filtration.(65)

A typical modern filter uses both barrier and adsorptive mechanism and consists of a synthetic mesh of non-woven fibres having large adsorptive surface area and minimal channeling (breaks or short circuit passageways) and bypassing (blood flowing around the adsorptive area), with high avidity of media for leucocytes, low retention volume as well as hydrophilicity. They have plastic housing and inlet and outlet ports, best designed to spread the blood over the media surface.(79) The third and fourth-generation leucofilters can efficiently remove leucocytes upto 99.99% compared to the first and second generations, which had an efficiency of only 90-96%. The filtration process is quite easy with little red cell loss.

4 (a). Filtration Processes and Mechanisms in the current generation of leucofilters

Surface or screen filtration and depth filtration are two filtration processes that are used in modern leucofilters. Screen filters have fibres arranged in multiple layers regularly (woven type). In the screen filtration method, the particles are separated at the filter surface. While in the depth filtration, leucocyte retention is not limited to the surface alone, rather at any place inside the filter by mechanical sieving or by adhesion as well as by cell-cell interaction. Adhesion of the negatively charged leucocytes to the filter material occurs by Vander Waals and electrostatic forces. Hence methacrylate polymer coating, which can increase the positive charge of the filter material, increases the efficiency of the filter. Depth filters are non-woven types, in which the fibres in filter material are compressed irregularly. They contain multiple layers that selectively retain leucocytes.

Three leucocyte retention mechanisms were recognised. One is the activation of platelets by the surface of the fibre material, followed by attachment of the granulocytes to the activated and trapped platelets and the other is the direct interaction of the granulocytes and monocytes with the fibres, most likely based on the phagocytic properties of these cells. Another proposed mechanism is the trapping of mononuclear leucocytes in the pores of the fabric based on passive sieving. This mechanism had also shown to trap some echinocytic red cells and damaged granulocytes, indicating the role of membrane flexibility of these cells in the

sieving mechanism. Because of their diapedetic property, granulocytes could squeeze through the filter pores; hence all three mechanisms are important in the filtration technique.(68) The overall effectiveness of a filtration process will depend not only on the chemical nature and number of the different fibre layers used but also on the age and number of different cell types as well as on the red cell suspension medium at the time of filtration. Also, external parameters like the environmental temperature and rate of flow during filtration were found to affect the filtration outcome. Hence each filter that is developed requires standardisation to bring out efficient filtration outcome.(84)

4 (b). Timing of Leucofiltration

Leucofiltration of blood components can be performed at three different points:

- Prestorage leucofiltration
- After storage leucofiltration at the blood centre
- Leucofiltration at the patient bedside

Studies have shown a greater advantage with prestorage leucoreduction compared to the other two methods. In an experimental animal model by Blajchman *et al*, they demonstrated significantly higher survival of allogeneic platelets and a lower rate of refractoriness to allogeneic platelets when leucoreduction was done before storage of the component than performed after storage.(85) Also study in an animal model by Bordin *et al*, on the enhancement of tumour growth associated with allogeneic blood transfusion, tumour growth was ameliorated by prestorage but not by post-storage leucoreduction.(86)

Bedside filtration carries no superior advantage. In one of the first prospective studies of bedside filtration in 123 patients, Williamson *et al* compared patients who received leucoreduced RCCs and PCs by bedside filtration and those who received non- leucoreduced blood components. They found no significant difference in the alloimmunisation rates observed between the patient groups. Also, bedside filtration did not affect the overall incidence of developing febrile transfusion reactions. They concluded that the efficacy of bedside filtration would have been hampered by intrinsic biological limitations like the possible immunogenic potential of stored blood as well as transfusion of leucocyte-derived

cytokines.(87) Leucocytes in the RCC stored at 2-6 ºC starts to disintegrate and the cytokines released can result in FNHTR. Also, the resulting white cell fragments are capable of initiating an immune response to HLA antigens. To prevent these effects it is desirable to remove leucocytes before storage. Hence it is the prestorage leucoreduction that eliminates the scope of cytokine accumulation due to leucocytes in the stored blood, thereby preventing adverse effects by them.

Prestorage leucoreduction can be performed as ULR or as selective leucoreduction for specific patient groups. ULR is the routine application of leucoreduction techniques to all units of cellular blood components before storage in a country or in a BTS. Each protocol can have its advantages and disadvantages. Universal leucoreduction protocols have been implemented by countries like France, Canada, Austria, Scotland, England, Germany, etc. as part of their blood safety program.(88)

4 (c). Leucofiltration of Buffy-coat removed red cell concentrates:

A combination method of leucoreduction is predominantly used in Canada and Western Europe which employs buffy-coat removal followed by filtration of the RCCs.(89) The use of buffy-coat reduced RCC as starting material for filtration on columns filled with cellulose acetate fibres, improved the leucocyte depletion to 99.9% as shown by Vakkila *et al*.(90)

With the development of the newer generation of leucofilters that could achieve 99.99% leucoreduction, combining buffy-coat reduction with filtration is no more advantageous. In addition, a study by Loi *et al* observed increased haemolysis during storage when buffy-coat reduction and filtration methods were combined and the combination method showed no advantage over filtration.(91)

4 (d). Counting less number of leucocytes:

After leucoreduction, counting the very low concentration of residual leucocytes in the RCC is challenging for routine quality control. The accepted level of leucocytes that could prevent alloimmunisation and CMV transmission is 5×10^6 per unit of leucoreduced RCC which is equivalent to a concentration of 3 leucocytes/ µl.(16) Electronic counting which is routinely used for blood cell counting is based on the Coulter principle. Here the leucocyte nuclei are counted after lysis of all blood cells by diluting the sample with a detergent. The lower limit

of detection using this method is about 100 leucocytes/ µl of RCC. This higher detection limit is due to the high background signal induced by red cell membranes. This lead to the development of alternative leucocyte counting methods based on either microscopic counting in large volume counting chamber like Nageotte Chamber (NC) as described by Masse *et al* or can be determined accurately by flow cytometry (FC) counting of leucocyte nuclei after specific staining as shown by Wenz *et al*.(92,93) The large volume chamber counting methods are simple but time-consuming but can be adapted as screening methods. Its efficacy is also dependent upon the skill of the operator. Trials done in 20 laboratories showed a detection limit of 0.1 leucocyte/ µl for FC and 1 leucocyte/ µl for NC technique. While in leucoreduced PCs, the leucocyte counting is more sensitive as quenching of light is negligible in these components and sample dilution is smaller. Therefore in NC, the lowest detection limit is 0.05 leucocytes/ µl of PC.

Another method that can be used to count residual leucocytes is the microtiter-plate method, which uses an epifluorescence microscope to count the number of wells that contained fluorescent nuclei. The sample is filled in 60 wells and then diluted with a staining solution. This method of leucocyte counting also revealed a lower detection limit of 0.1 leucocytes / µl and it was simpler and less strenuous to perform compared to the NC technique.(94) The use of polymerase chain reaction (PCR) to amplify leucocyte-specific DNA and quantitation of residual white cells can increase the sensitivity. However it requires validation for the reproducibility of the leucocyte-specific material during sample processing and the PCR method is rather laborious, hence making it too complicated for routine quality control.(95) Sampling error and instrument precision are hurdles in all proposed methods.

5. Component collection by Apheresis:

Collection efficiency and quality of blood components have been increased with the advent of automated blood collection instruments. RCC or different combinations of blood components can be collected from the donation by apheresis technology. The first red cell apheresis collection was performed in the year 1987. The RCC collection using apheresis technology has shown to provide an acceptable range of leucoreduction that meets the standards for leucoreduced blood components.(63,96)

Advantages of Implementing Leucoreduction Procedures:

The importance of leucoreduction has been stressed in a review on leucocyte reduction in blood component therapy by Lane *et al***,** where they suggested providing leucoreduced RCCs to patients likely to receive long-term transfusion support in order to prevent recurrent FNHTR and to prevent or delay alloimmunisation to leucocyte antigens. They also concluded that leucocyte depleted platelet transfusions may also be indicated to delay or prevent refractoriness to platelet transfusion.(97) A retrospective analysis was done by Kekre *et al* on 89 patients, who received haematopoietic stem cell transplantation. They analysed CMV infection in recipients of leucoreduced and CMV negative blood components, which showed no significant difference in both groups. Leucoreduction was equally effective as providing CMV- negative blood unit in preventing CMV transmission through blood transfusion.(98)

As stated by Perkins et al, the majority of FNHTR can be attenuated if the leucocyte count is less than 0.5×10^{9}**.**(8) These levels can be achieved by centrifugation and removal of the buffy-coat from the blood unit. Also as shown by Chaplin *et al*, red cells frozen in glycerol, thawed, and washed contain only 2% of the original leucocyte count and they are unlikely to cause FNHTR.(99) However, sensitised patients may require more rigorous methods of leucoreduction like leucofiltration. In a cohort study by Sirchia *et al* in 82 thalassemic patients receiving chronic transfusion therapy, more than one-half of the patients developed febrile reactions following transfusion with buffy-coat reduced RCC and adoption of filtration method of leucoreduction to remove additional leucocytes reduced the incidence below 5%.(100)

Time at which the leucoreduction procedure is performed also influences the incidence of transfusion reactions. Removal of leucocytes reduces febrile transfusion reactions, but this technique is most effective when performed immediately following blood collection (prestorage leucoreduction). Post storage filtration reduced the number of leucocytes from the blood component, but it failed to show a reduction in febrile transfusion reactions in the recipients as shown in the study by Williamson *et al* in 1994.(87) A similar observation was made by Wang *et al* in 2012 when a retrospective analysis was conducted on 70,015 platelet transfusions which showed a lower rate of FNHTR with pre-storage leucoreduction compared to post-storage leucoreduced PCs.(101) Hence this study confirmed that the timing of leucoreduction during storage influence the development of febrile transfusion reactions

probably because of the accumulated cytokines released from leucocytes present in the stored blood unit.

Universal Leucoreduction:

In Canada, prestorage universal leucoreduction (ULR) of the cell components has been performed since August 1999. In a retrospective analysis of 13902 platelet transfusions in 617 patients before and after the introduction of ULR, Seftel *el al* found that alloimmunisation significantly reduced in the post-ULR group.(102) A retrospective before and after cohort study was conducted by Hebert *et al* after the adoption of ULR in Canada, in 23 academic and community hospitals, where they found a significant reduction in in-hospital mortality after the introduction of ULR.(15) They also indicated that, when a 7% mortality rate was assumed in the control period, the decreased odds of death based on these results would translate to one life saved for every 120 patients receiving leucoreduced blood as opposed to non-leucoreduced. They found this observation consistent among all subgroups and throughout the range of blood exposures. In addition to these findings, this study also observed that ULR was associated with a decreased frequency of febrile episodes and subsequent use of antibiotics.

Following the implementation of the ULR program, a before and after study was conducted in three Canadian tertiary care neonatal intensive care units (NICUs) by Fergusson *et al*, where they evaluated clinical outcomes in premature infants admitted to NICU and requiring RCC transfusions. The study reported no significant reduction in NICU mortality or bacteraemia, but showed improvement in several clinical outcomes.(103) After the implementation of ULR in the United Kingdom (UK), a before and after study was conducted by Llewelyn *et al*. They analysed 2095 patients undergoing cardiopulmonary bypass surgery (CABG) or total hip and/or knee replacement in 11 hospitals. But this study found no impact of ULR on postoperative infection rate similar to the Canadian study.(104) In the studies by Yazer *et al*, Paglino *et al* and King *et al*, they demonstrated that switching from selective leucoreduction protocol to ULR was associated with significantly reduced rates of fevers associated with RCC and platelet transfusions.(10,105,106)

All these studies indicate that leucoreduction is associated with better clinical outcomes in a certain patient population. It is clear that reducing the leucocyte count in transfused blood components, does reduce the risk and severity of FNHTRs, the risk of CMV transmission and is also associated with reduced risk of HLA alloimmunisation and platelet refractoriness. Hence the benefits of leucoreduction are no longer debated, but the leucoreduction methods often vary according to geographic implementation.(88)

MATERIALS AND METHODS

Study Design: A comparative study on quality parameters in leucoreduced red cell concentrates prepared by methods like buffy-coat reduction, washing, and filtration. The study was done in the Department of Transfusion Medicine, Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Trivandrum, Kerala for a period of one year. The study was approved by the Technical Advisory Committee (TAC) and Institutional Ethics Committee (IEC) (IEC Regn No.ECR/189/Inst/KL/2013/RR-16).

Sample Size: The guidelines by DGHS, GoI recommend the frequency of quality control for leucoreduced red cells as 1% of all units or 4 units per month, whichever is higher.(17) This was a descriptive study on quality parameters in leucoreduced red cell concentrates prepared by methods like buffy-coat reduction, washing, and filtration. So the sample size was 1% of the prepared units or 4 units per month whichever was higher in each group (buffy-coat reduction, washing, and filtration).

In our blood centre, the average number of buffy-coat reduced red cell units per month was 100, and filtered red cell units were 38 per month. Therefore 4 units per month were included in the study. Since the study was conducted over a period of 12 months, the sample size for buffy-coat reduced and filtered red cell units for a year was 48 (4×12). The average number of washed red cell units was only 2 per month. Hence all the washed red cell units prepared over a period of 12 months were subjected to quality study. Therefore the expected sample size was 24 units (2/month×12months) in washed red cell units.

Study Method: Blood was collected with consent from voluntary non-remunerated blood donors meeting the guidelines for blood donor selection and blood donor referral by National Blood Transfusion Council (NBTC), GoI. Under sterile precautions, 350 (±10%) or 450 (±10%) ml WB was collected by single sharp phlebotomy, in conventional blood bags with anticoagulant solutions in the required ratio. Double, triple, or quadruple blood collection bags with CPDA as anticoagulant with or without an additive solution were used depending on the blood component to be prepared. During the collection procedure, an automated blood collection monitor, (Terumo Penpol Blood Collection Monitor, D601) was used with automated scales that sounded an alarm to signal phlebotomy completion and continuously mixed the WB with the anti-coagulant. The collection time was monitored with the completion alarm.

Depending on the downstream processing requirements, the immediate temperature conditioning and handling of the WB units were done. Collected WB units were kept at 2-6ºC and processed within 6-8 hours of collection to prepare RCC and FFP. The WB units intended for preparing PCs were stored at 22-24ºC and processed within 6-8 hours of collection to prepare RCC, FFP, and PC.

Special procedures on the prepared blood components, like leucoreduction were employed as per the requirement of the patient or as a request from the treating clinician. The frequently employed leucoreduction techniques in our blood centre include centrifugation and buffy-coat reduction, saline washing of red cells, and leucofiltration. When the red cell units are subjected to a leucoreduction method, it can impart changes in the quality parameters like volume and cell concentration of the units. In this study, quality parameters of leucoreduced RCCs were determined before and after a leucoreduction procedure and also the percentage leucoreduction and red cell recovery were calculated, to assess the impact and efficiency of a particular leucoreduction method that was employed. The effectiveness of a leucoreduction method was assessed and compared in this study.

Inclusion Criteria:

Leucoreduced red cell concentrates were included in the study.

Exclusion Criteria:

Leucoreduced red cell concentrates prepared by the freezing and deglycerolisation method and those obtained by apheresis donation were excluded as these procedures are not followed in the blood centre. Leucoreduced paediatric red cell units were also excluded as they have low volume.

BUFFY-COAT REDUCTION

Procedure: The WB collected in 350 or 450 ml quadruple blood collection bags (Terumo Penpol Private Limited, Puliyarakonam, Trivandrum, India) with top and bottom configuration (TAB), containing 49 or 63 ml of citrate phosphate dextrose (CPD) respectively, were used for preparing RCCs which were buffy-coat reduced (**Figure No.1**). The WB collected was kept undisturbed at an ambient temperature of 22-24 ºC for 1 hour before processing. From a unit of WB collected in a quadruple bag, buffy-coat reduced RCC suspended in an additive solution, leucoreduced PC, and plasma component were prepared. Properly balanced WB units were centrifuged in Cryofuge 6000i (Heraeus Instruments GmbH, Osterode, Germany) at 3600rpm for 9 minutes at 24ºC in an upright position with an acceleration of 5 minutes and deceleration of 9 minutes. To obtain the separation, the centrifuged unit was placed in an automated blood component extractor (T-ACE II^{+}, Automatic Component Extractor, Terumo BCT) (**Figure No.2**). The tubings were led through specific clamping devices and flow regulators on the machine. Plasma and red cells were simultaneously separated with the help of a pressure plate. The red cell was separated into the secondary bag containing additive solution – SAGM and plasma was extracted out, leaving behind the buffy-coat in the parent bag. Approximately 50g of buffy-coat was removed from a red cell unit. When the procedure comes to an end, both the top and bottom tubings were automatically clamped and the secondary bags with plasma and red cells were sealed. From the buffy-coat, leucoreduced PC was prepared through the second step centrifugation.

Storage: The buffy-coat reduced RCCs suspended in CPD-SAGM were properly labelled and stored for 42 days from the day of collection in a blood bag storage refrigerator at 2-6ºC until issue of the units.

MANUAL WASHING OF RCC

Washing of a red cell unit removes leucocytes, platelets, and plasma along with the plasma proteins. The washing procedure can be done manually or using an automated cell processor. In this study, manual washing of the red cells was performed. The RCCs were washed using 0.9 % normal saline. The manual washing was done on the day of the issue as the shelf life of RCCs washed by an open method is 24 hours post-wash.

Procedure: Under laminar flow (Laminar flow bench, Chempharm) and with strict aseptic precautions, the red cell bag was connected to 0.9 % normal saline using a bag to bottle connector, and saline was added to the bag (**Figure No. 3**). The connector tubing was clamped and the tubing of the bag was sealed distal to the needle of the connector set using a dielectric sealer (Terumo Penpol). The saline and red cells were mixed thoroughly by manual mixing. The bag was centrifuged at 3550rpm for 15 minutes at 2 – 6ºC. After proper centrifugation, the bag was placed on a manual expresser (CompoSafe Intelligent Expressor E250, Terumo Penpol) and the supernatant saline was removed into a transfer bag and discarded. The whole process was repeated three times. The red cell sediment was re-suspended in 0.9% saline, the tubings were sealed and the connector was removed. The whole process was performed under laminar flow with all aseptic precautions.

Storage and issue: RCC washed by manual technique were properly labelled and stored at 2-6ºC. The units were issued within 24 hours from the time of opening the blood bag.

FILTRATION

There are different generations of leucocyte reducing filters. The newer generations use selective absorption of leucocytes with little loss of red cells. Leucoreduction can be done at three different points; prestorage leucoreduction, after storage leucoreduction before issue, and bedside filtration, where the red cell unit is leucoreduced at the patient's side just before transfusion. In this study, after storage leucoreduction procedure was done at the blood centre just before issuing the unit to the patient. The leucoreduction filter used (Leucolab, Maco Pharma, France) was suitable for filtering RCC units suspended in CPD-SAGM (**Figure No. 4**).(107) Hence CPD-SAGM suspended buffy-coat reduced RCCs as well as CPD-SAGM suspended RCCs from which buffy-coat was not removed were used for filtration and were taken as separate categories.

Procedure: After a thorough examination of the RCC for any clot, haemolysis, or leakage, the unit was selected for leucofiltration. The RCC was mixed thoroughly to dispense any aggregates formed during storage from clogging the filters. Using a sterile connecting device (Terumo Sterile Tubing Welder TSCD), the leucoreduction filter with an attached secondary bag was connected to the red cell unit. The RCC with the attached leucocyte filter was hanged with support at room temperature (**Figure No. 5**). Nearing completion, the valve was

opened for air removal. Post-filtration, the transfer bag having the filtered RCC was sealed and removed, and examined for any visible lysis. The unit was issued on the day of filtration.

IN VITRO MEASUREMENTS:

Visual inspection of the blood unit was done to detect any white particulate matter, abnormal colour change due to bacterial contamination, haemolysis, and clots. The blood units were weighed before and after the procedure with electronic scales (CompoScale CS300, Terumo Penpol) with precision upto 1g. The weight of each blood unit was measured in grams and was converted to volume, assuming a specific gravity of 1.053 for WB**,** 1.093 for RCC, and 1.06 for RCC suspended in SAGM**.** (16,108)

The blood unit volume (ml) was calculated as follows:

[(weight of the bag+ blood components) – weight of empty bag] ÷ Specific gravity of component

Sampling: The blood units were thoroughly mixed manually and samples were taken under laminar flow with sterile precautions before and immediately after the leucoreduction procedure. The volume of the sample removed was determined to be the minimum required for the specific tests, so as not to significantly impact the total RCC volume.

Testing: The samples were tested for red cell parameters and indices using an automated haematology analyser (MEK-6420, Nihon Kohden) as described.(109) Red cell supernatants were prepared by centrifugation at 3000g for 5 min (Megafuge 1.0, Terumo Fisher Scientific). The supernatant of the centrifuged sample was used for measuring plasma haemoglobin content and extracellular potassium. Extracellular potassium was measured using a biochemistry analyser (Dimension RxL Max Integrated Chemistry System, Siemens). Plasma haemoglobin was measured from the supernatant using a plasma/low Haemoglobin system (HemoCue India). Sterility testing was performed in all the units tested using an automated microbial detection system (BacT/ALERT, Organon Teknika Corp.).

Studies have shown underestimation of WBCs when measured using a haematology analyser, in red cell units that were leucofiltered. Hence the residual WBC count post-filtration was counted using the NC.

Counting of WBC in Nageotte chamber:

The WBCs were counted in the NC, after staining with Turk's fluid. Turk's fluid is a haematological stain that destroys the membrane of WBCs, red cells, and platelets within a blood sample and stains the nuclei of WBC making them visible under the microscope. Turk's fluid was prepared using glacial acetic acid, 1% aqueous gentian violet, and distilled water. 1 ml of 1% aqueous gentian violet was mixed with 1.5 ml of glacial acetic acid and made up to 100ml with distilled water. The stain prepared was filtered before storage. With serial dilution, the sample to stain ratio that could provide optimum red cell lysis with adequate WBC staining was standardised.

Post-filtration, the sample was taken from the RCC bag. 1 part of the blood sample taken was mixed with 11 parts of Turk's fluid and mixed several times by vortexing and incubated for 10 minutes. The mixture was pipetted between the chamber and coverslip and allowed to settle for 10 to 15 minutes at room temperature beneath a moist lid to prevent evaporation of the sample mixture. All white cells detectable over one full grid (40 rectangles) were counted with a ×20 objective non-phase microscope. Counting was started immediately after removal of NC from the humidified chamber and completed before sample drying. To obtain the number of leucocytes per µl of the sample, the number of leucocytes counted was divided by the volume of one grid and then multiplied by the dilution factor of the blood sample as described below:(110)

(Number of WBCs observed per grid × dilution factor) ÷ Volume

As one grid represents 50µl and the blood sample was diluted in 1 in 11 parts, the WBC concentration per µl was obtained by the following formula:

(Number of WBCs observed per grid × 12) ÷ 50

Determining the quality parameters:

The quality parameters like volume (ml), haematocrit (%), haemoglobin content in the bag (g), absolute leucocyte (10^6) and red cell count (10^9); the extracellular potassium levels (mmol/L), plasma haemoglobin (g/dl), and percentage haemolysis were compared between the leucoreduced bag and the parent bag in each procedure. As both 350 ml and 450 ml bags were subjected to quality control, the above-mentioned pre-and post- leucoreduction parameters were analysed separately for 350 and 450 bags. In leucofiltration, as both buffy-coat reduced and RCCs without buffy-coat reduction were used, the pre-and post- values were compared separately for this group.

Quality parameters of leucoreduced red cell components were determined and compared with the quality recommendations from AABB, CoE, and DGHS, GoI. (**Table No. 6**) The percentage leucoreduction, red cell recovery, and red cell loss following each method of leucoreduction were determined and these were compared between the methods in this study.

Derived values:

Haemoglobin content in the bag (g):

Calculated using the following formula and expressed in grams:

Haemoglobin (g/dl) × Bag volume (dl)

Absolute leucocyte count (10^6):

It is the total number of leucocytes present in the blood unit and was calculated using the following formula:

Leucocyte count (cells/µL) × Bag volume (µL)

Absolute red cell count (10^9):

Represents the total number of red cells in each unit and was calculated through measurements of volume and red cell concentration before and after a leucoreduction procedure. The formula is as follows:

Red cell count (cells/µL) × Bag volume (µL)

Haemolysis (%):

The percentage haemolysis in a blood unit was calculated using the following formula:(111)

(100-Haematocrit) × Plasma haemoglobin (g/dl) ÷ Total Haemoglobin (g/dl)

Leucoreduction (%):

The percentage leucoreduction achieved shows the efficacy with which the WBCs were removed from the blood unit by each leucoreduction technique. It was measured using the following formula:(61,78)

[100 – (WBCs in leucoreduced bag ÷ WBCs in parent bag)] × 100

Red cell recovery (%):

A loss of red cell volume has to be expected during the leucoreduction procedures. Hence red cell recovery in a leucoreduction method was calculated using the following formula and was expressed as percentage:

(Red cells in leucoreduced bag ÷ Red cells in parent bag) × 100

Red cell loss (%):

The percentage loss of red cells was derived from the red cell recovery using the following formula:

Red cell loss = (100- Red cell recovery)

Statistical Analysis:

To compare pre-and post-procedure continuous variables, the Paired t-test was used. The Anova test was used to compare the three leucoreduction methods and the Post-hoc analysis was performed to do multiple comparisons.

Correlation of leucoreduction with red cell loss was analysed in the buffy-coat reduction, washing and filtration methods of leucoreduction by the Pearson correlation. In addition, correlation of haemolysis, plasma haemoglobin and potassium levels with age of the red cell units were analysed. A correlation of <0.3 was considered weak, 0.3-0.6 moderate and >0.6 was considered as a strong association.

Quantitative variables were expressed as Mean and Standard Deviation (SD). A p-value <0.05 was considered statistically significant. Data analysis was performed using SPSS version 16.0.

Table No.6: Current guidelines relating to buffy-coat reduced, washed and filtered red cell concentrates

METHOD	DGHS (17)	AABB (16)	CoE (18)
BUFFY-COAT REDUCTION	4 units a month to be tested >70% leucoreduction >70% red cell recovery	Not stated	Volume to be defined by the system used Haematocrit 0.40 -0.70 Haemoglobin minimum 43g per unit Residual leucocyte count $<1.2\times10^9$ per unit *A minimum of 90 per cent of units tested should meet the required value
WASHING	4 units a month to be tested 99% plasma removed 20% red cell recovery 85% leucoreduction	Washed Red Blood Cells shall be prepared by a method known to ensure that the red cells are washed with a volume of compatible solution that will remove almost all of the plasma	Volume to be defined by the system used Haematocrit 0.40 -0.70 Haemoglobin minimum 40g per unit Protein content <0.5g per unit *A minimum of 90 per cent of units tested should meet the required value
FILTRATION	4 units a month to be tested 99% leucoreduction 90-95% red cell recovery	$<5\times10^6$ residual leucocytes 85% red cell recovery *>95% units tested should meet the criteria	Volume to be defined by the system used Haematocrit 0.40 -0.70 Haemoglobin minimum 40g per unit Residual leucocyte count $<1\times10^6$ per unit *A minimum of 90 per cent of units tested should meet the required value

Figure No. 1: Top and Bottom blood bag system

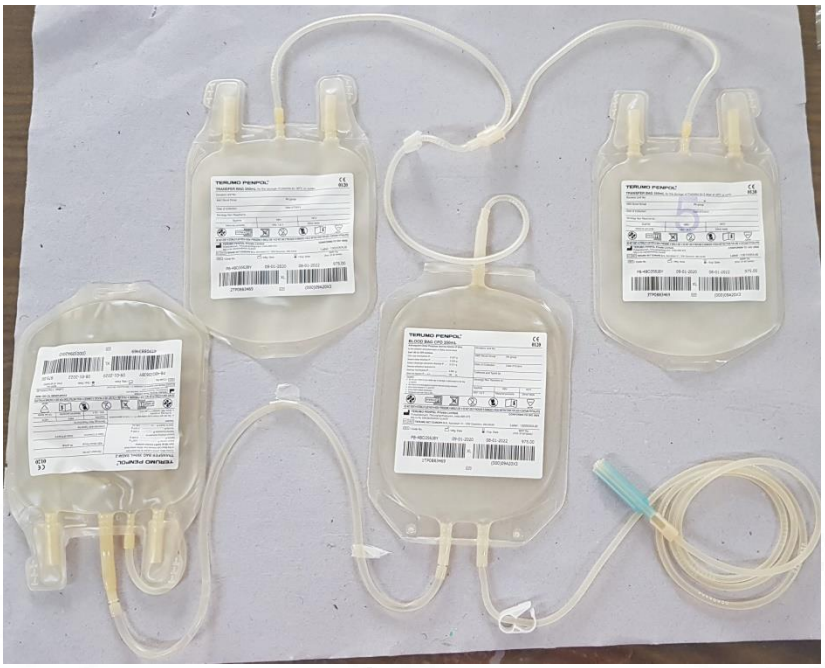


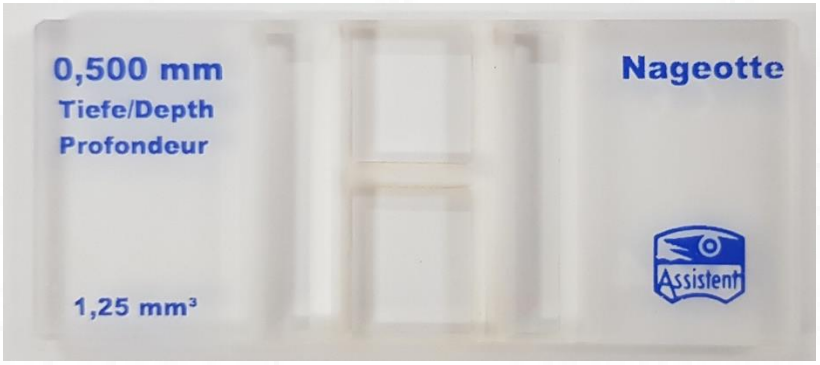
Figure No. 2: Buffy-coat reduction in automated component extractor (T-ACE II+) using the Top and Bottom system

Figure No. 3: 0.9% Normal Saline being added into RCC under Laminar flow

Figure No. 4: Hard housing Leucofilter (Leucolab, Maco Pharma)

Figure No. 5: Leucofiltration Procedure being performed

Figure No. 6: Nageotte chamber for enumeration of leucocytes



RESULTS

In this study, quality parameters were analysed and compared between three methods of leucoreduction namely buffy-coat reduction, washing and leucofiltration. Pre procedure and post- procedure quality parameters like volume, absolute red cell count, haemoglobin content, haematocrit, plasma haemoglobin, percentage haemolysis, potassium levels and absolute leucocyte count were evaluated in total of 112 blood units that underwent leucoreduction procedure. In the buffy-coat reduction and leucofiltration group 48 blood units were tested, while 16 blood units were tested in the saline washing group. RCCs processed from both 350ml and 450ml collection bags were used. In addition, leucofiltration was performed on SAGM suspended RCCs and on buffy-coat reduced SAGM suspended RCCs. The effectiveness of the evaluated methods were compared by calculating percentage leucoreduction and red cell recovery.

Buffy-coat reduction:

Buffy-coat reduction as a method of leucoreduction was employed on the day of collection. Out of the 48 buffy-coat reduced RCCs tested, 32 units were from 450ml collections and 16 were from 350ml collections.

Leucofiltration:

Total of 48 red cell units were leucoreduced by filtration method. Leucofiltration was done on SAGM suspended RCCs (n=21) and on buffy-coat reduced SAGM suspended RCCs (n=27). Buffy-coat reduced SAGM suspended RCCs that underwent filtration included RCCs processed from 450 ml (n=14) and 350 ml (n= 13) collections.

Washing:

16 RCCs were washed and tested, which included RCCs processed from 350 ml (n= 11) and 450 ml (n=5) WB collections.

1. Age (days) of the red cell units that underwent leucoreduction by buffy-coat reduction, washing and filtration are listed in Table No. 7 and values expressed as Mean ± SD and Range

Table No. 7: Age of RCCs that underwent leucoreduction (days)

METHOD	N	MEAN	SD	RANGE
Buffy-coat reduction	48	0	0	0
Washing	16	24.06	13.04	1 - 35
Leucofiltration	48	3.65	2.39	1-10

2. Change in volume (ml) of the RCCs following buffy-coat reduction, washing and filtration are listed in Table No. 8 and values expressed as Mean ± SD.

Table No. 8: Volume change in RCCs following leucoreduction (ml)

METHOD	N	PRE-PROCEDURE		POST-PROCEDURE		p
		Mean	SD	Mean	SD	
Buffy-coat reduction						
350 ml	16	360.25	8.67	230.50	18.40	0.000
450 ml	32	457.09	17.96	291.88	16.17	0.000
Washing						
350 ml	11	239.82	26.29	236.18	35.53	0.635
450 ml	5	290.20	8.87	301.80	15.42	0.126
Leucofiltration						
Buffy-coat reduced						
350 ml	13	222.69	15.47	176.46	13.12	0.000
450 ml	14	296.57	19.88	245.43	20.29	0.000
Buffy-coat non-reduced						
350 ml	21	271.52	13.24	225.05	12.66	0.000

3. Absolute red cell count reduction following buffy-coat reduction, washing and filtration are listed in Table No. 9 and values expressed as Mean ± SD.

Table No. 9: Reduction in absolute red cell count with leucoreduction ($\times 10^9$)

METHOD	N	PRE-PROCEDURE		POST-PROCEDURE		p
		Mean	SD	Mean	SD	
Buffy-coat reduction						
350 ml	16	1703.81	58.96	1505.75	89.54	0.000
450 ml	32	2115.47	130.26	1939.53	146.25	0.000
Washing						
350 ml	11	1551.36	167.93	1422.36	160.88	0.000
450 ml	5	2091.80	124.65	1929.80	134.06	0.003
Leucofiltration						
Buffy-coat reduced						
350 ml	13	1449.62	157.49	1153.38	129.16	0.000
450 ml	14	2061.43	233.86	1679.36	213.68	0.000
Buffy-coat non-reduced						
350 ml	21	1776.33	162.56	1485.43	122.95	0.000

4. Reduction in haemoglobin content of the RCCs following buffy-coat reduction, washing and filtration are listed in Table No. 10 and values expressed as Mean ± SD.

Table No. 10: Haemoglobin content in RCCs following leucoreduction (g)

METHOD	N	PRE-PROCEDURE		POST-PROCEDURE		p
		Mean	SD	Mean	SD	
Buffy-coat reduction						
350 ml	16	47.94	2.52	45.06	4.71	0.004
450 ml	32	60.13	3.17	57.38	5.29	0.000
Washing						
350 ml	11	46.55	4.78	44.73	5.27	0.241
450 ml	5	62.80	5.40	58.80	5.40	0.022
Leucofiltration						
Buffy-coat reduced						
350 ml	13	41.77	4.44	33.23	3.68	0.000
450 ml	14	58.00	6.59	47.36	5.80	0.000
Buffy-coat non-reduced						
350 ml	21	52.14	3.93	43.86	2.92	0.000

5. A change in haematocrit observed in the RCCs following buffy-coat reduction, washing and filtration procedures are listed in Table No. 11 and values expressed as Mean ± SD.

Table No. 11: Change in Haematocrit values following leucoreduction (%)

METHOD	N	PRE-PROCEDURE		POST-PROCEDURE		p
		Mean	SD	Mean	SD	
Buffy-coat reduction						
350 ml	16	39.73	1.64	57.01	2.40	0.000
450 ml	32	39.56	1.47	57.88	2.44	0.000
Washing						
350 ml	11	58.61	5.26	59.94	11.49	0.711
450 ml	5	63.72	5.11	56.74	5.60	0.000
Leucofiltration						
Buffy-coat reduced						
350 ml	13	54.49	2.33	54.96	2.00	0.354
450 ml	14	57.52	3.50	56.81	2.69	0.311
Buffy-coat non-reduced						
350 ml	21	55.78	3.75	56.89	3.05	0.053

6. Absolute leucocyte count reduction obtained with buffy-coat reduction, washing and filtration methods are listed in Table No.12 and values expressed as Mean ± SD.

Table No. 12: Change in absolute leucocyte count in RCCs following leucoreduction ($\times 10^6$)

METHOD	N	PRE-PROCEDURE		POST-PROCEDURE		p
		Mean	SD	Mean	SD	
Buffy-coat reduction						
350 ml	16	2138.00	383.07	402.13	290.59	0.000
450 ml	32	2765.91	565.48	650.84	327.20	0.000
Washing						
350 ml	11	1545.09	676.46	303.45	336.20	
450 ml	5	1797.00	478.74	342.60	187.32	0.000
Leucofiltration						
Buffy-coat reduced						
350 ml	13	251.69	144.23	0.05	0.04	0.000
450 ml	14	573.07	344.19	0.10	0.05	0.000
Buffy-coat non-reduced						
350 ml	21	2239.62	636.81	0.13	0.05	0.000

7. Plasma haemoglobin levels changed with buffy-coat reduction, washing and filtration procedures and are listed in Table No.13. Values expressed as Mean ± SD

Table No. 13: Plasma haemoglobin levels following leucoreduction (g/dl)

METHOD	N	AGE		PRE-PROCEDURE		POST-PROCEDURE		p
		Mean	SD	Mean	SD	Mean	SD	
Buffy-coat reduction	48	0		0.03	0.01	0.01	0.01	0.000
Washing	16	24.06	13.04	0.32	0.29	0.12	0.09	0.002
Leuco-filtration	48	3.65	2.39	0.04	0.02	0.04	0.02	0.873

8. Percentage haemolysis observed in the leucoreduced RCCs are listed in Table No. 14 and values expressed as Mean ± SD

Table No. 14: Haemolysis in the leucoreduced RCCs (%)

METHOD	N	AGE		PRE-PROCEDURE		POST-PROCEDURE		p
		Mean	SD	Mean	SD	Mean	SD	
Buffy-coat reduction	48	0		0.14	0.06	0.02	0.02	0.000
Washing	16	24.06	13.04	0.52	0.31	0.25	0.17	0.000
Leucofiltration	48	3.65	2.39	0.09	0.05	0.09	0.05	0.894

9. Change in serum potassium levels were observed following the leucoreduction procedures and are listed in Table No. 15. Values are expressed as Mean ± SD

Table No. 15: Potassium levels in leucoreduced RCCs (mmol/L)

METHOD	N	AGE		PRE-PROCEDURE		POST-PROCEDURE		p
		Mean	SD	Mean	SD	Mean	SD	
Buffy-coat reduction	48	0		3.39	0.18	0.66	0.08	0.000
Washing	16	24.06	13.04	38.38	20.38	1.55	0.94	0.000
Leucofiltration	48	3.65	2.39	12.30	5.44	13.20	5.73	0.000

10. Sterility testing report of 112 red cell units that underwent leucoreduction procedures are listed in Table No. 16.

Table No. 16: Sterility testing of leucoreduced RCCs

METHOD	N	CULTURE REPORT
Buffy-coat reduction	48	Sterile
Washing	16	Sterile
Leucofiltration	48	Sterile

COMPARING THE EVALUATED METHODS OF LEUCOREDUCTION

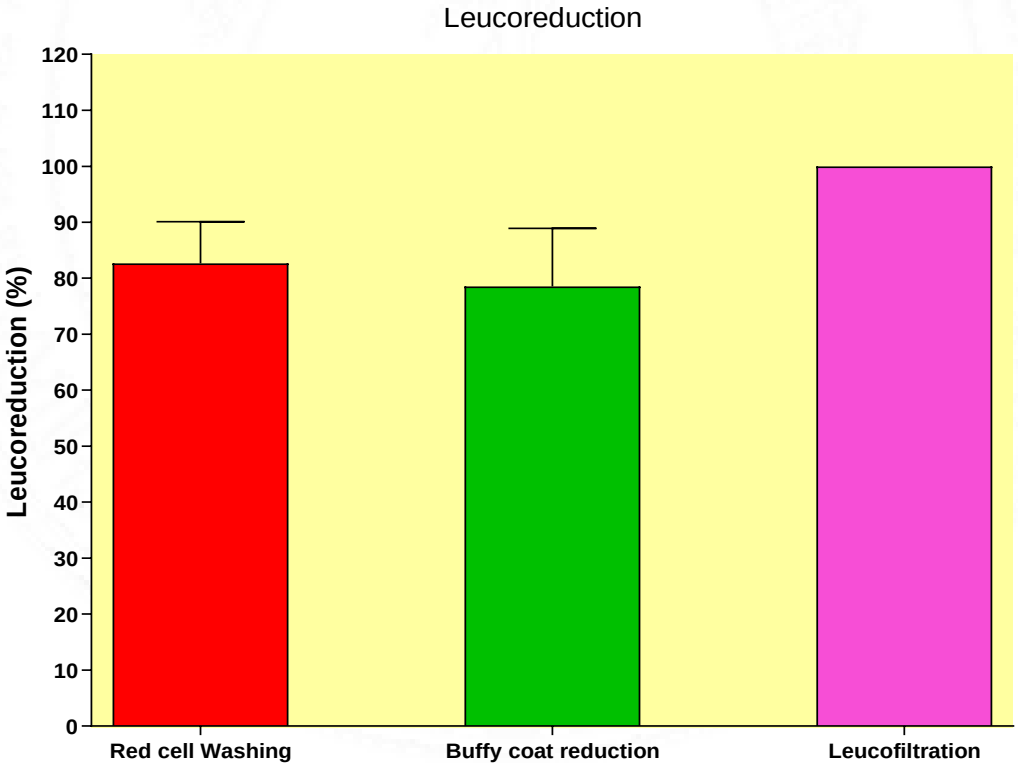
11. (a) Leucoreduction of RCCs achieved with buffy-coat reduction, washing and filtration methods were determined and are listed in Table No. 17 and Figure No. 7.

Table No. 17: Leucoreduction obtained with the three methods (%). Values expressed as Mean ± SD and Range

METHOD	N	Leucoreduction (%)		Range (%)
		Mean	SD	
Buffy-coat reduction	48	78.54	10.39	54.37 – 95.85
Red cell Washing	16	82.67	7.44	62.52 – 92.30
Leucofiltration	48	99.99	0.01	99.97 – 100.00

$p<0.001$

Figure No. 7: Leucoreduction of RCCs. Mean results obtained with washing, buffy-coat reduction and leucofiltration. The standard deviations are represented by vertical bars.

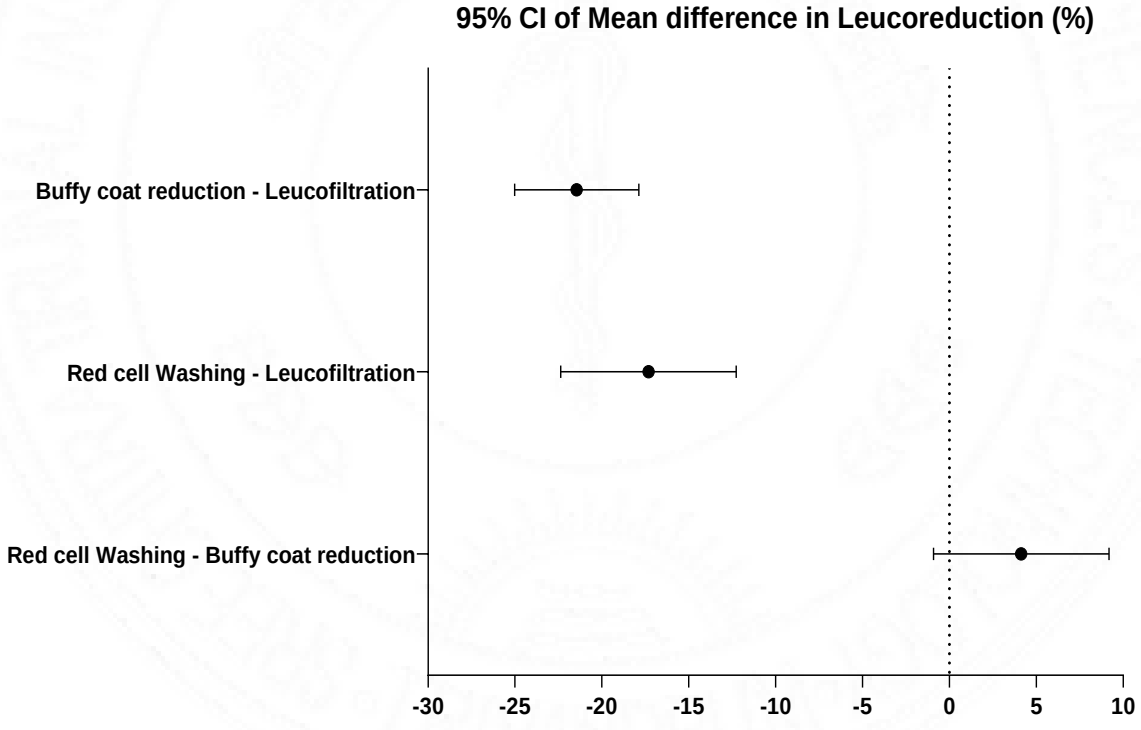


11. (b) The leucoreduction achieved by buffy-coat reduction, washing and filtration were compared between the methods and results are listed in the Table No. 18 and Figure No. 8.

Table No. 18: Comparison of leucoreduction between the methods

Multiple comparison	Mean Difference	se	95% Confidence Interval of mean difference		p
			Lower Bound	Upper Bound	
Washing Vs Buffy-coat reduction	4.14	2.13	-0.91	9.19	0.131
Washing Vs Leucofiltration	-17.31	2.13	-22.36	-12.26	0.000
Buffy-coat reduction Vs Leucofiltration	-21.45	1.50	-25.02	-17.88	0.000

Figure No. 8: Comparison of leucoreduction between the methods



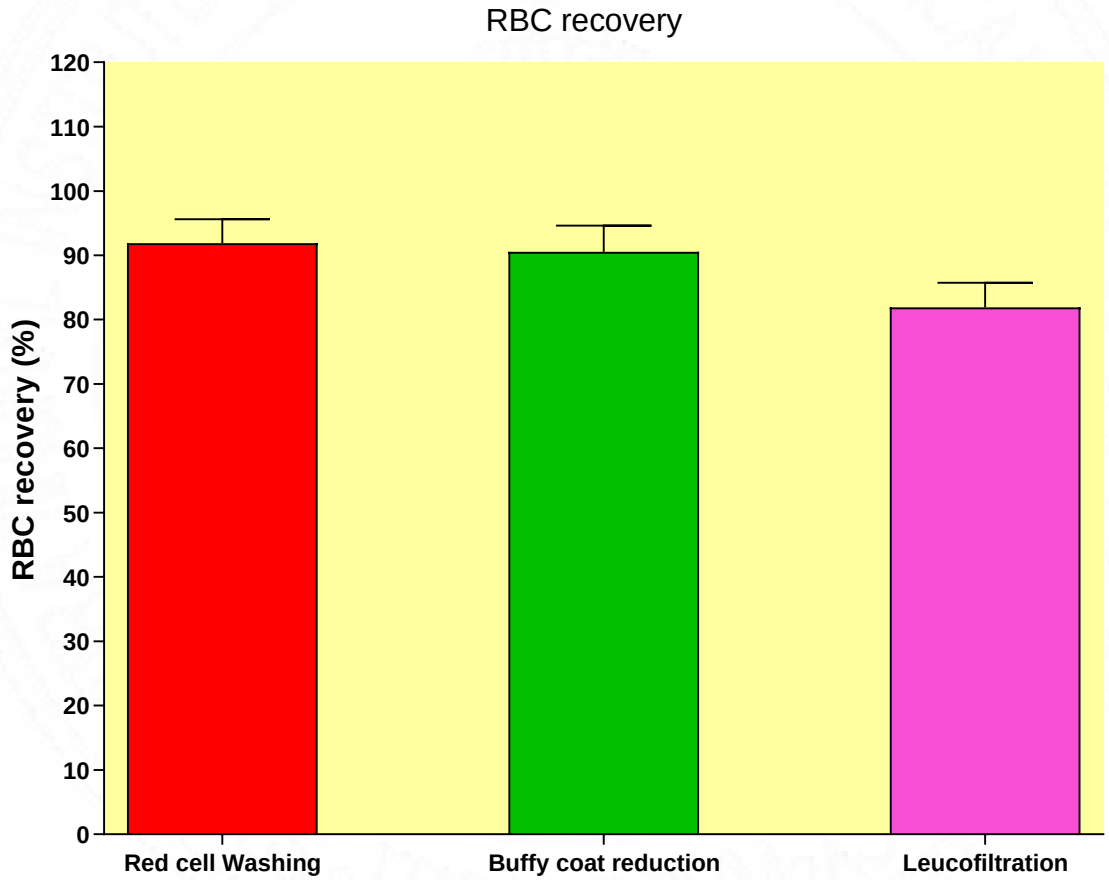
12. (a) Red Cell Recovery achieved with buffy-coat reduction, washing and filtration methods were determined and listed in Table No. 19 and Figure No. 9.

Table No.19: Red cell recovery obtained with the three leucoreduction methods (%). Values expressed as Mean ± SD and Range.

METHOD	N	RBC recovery (%)		Range
		Mean	SD	
Buffy-coat reduction	48	90.57	4.05	79.23 – 99.47
Red cell Washing	16	91.87	3.73	83.97 – 96.56
Leucofiltration	48	81.93	3.78	71.29 – 88.28

$p<0.001$

Figure No. 9: Red cell recovery in leucoreduced RCCs. Mean results obtained with washing, buffy-coat reduction and leucofiltration. The standard deviations are represented by vertical bars.

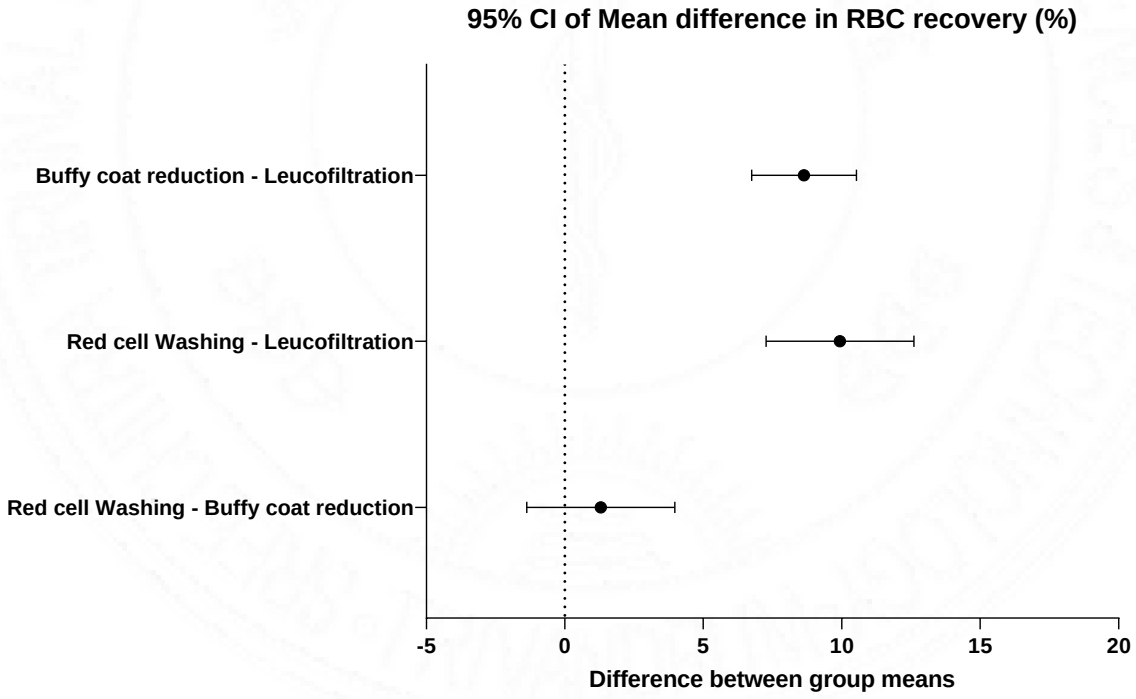


12. (b) The red cell recovery achieved by buffy-coat reduction, washing and filtration were compared between the methods and results are listed in the Table No. 20 and Figure No. 10.

Table No. 20: Comparison of red cell recovery between the leucoreduction methods

Multiple comparison	Mean Difference	se	95% Confidence Interval of mean difference		p
			Lower Bound	Upper Bound	
Washing Vs Buffy-coat reduction	1.30	1.12	-1.37	3.97	0.482
Washing Vs Leucofiltration	9.94	1.12	7.27	12.61	0.000
Buffy-coat reduction vs Leucofiltration	8.64	0.79	6.75	10.53	0.000

Figure No. 10: Comparison of red cell recovery between the leucoreduction methods



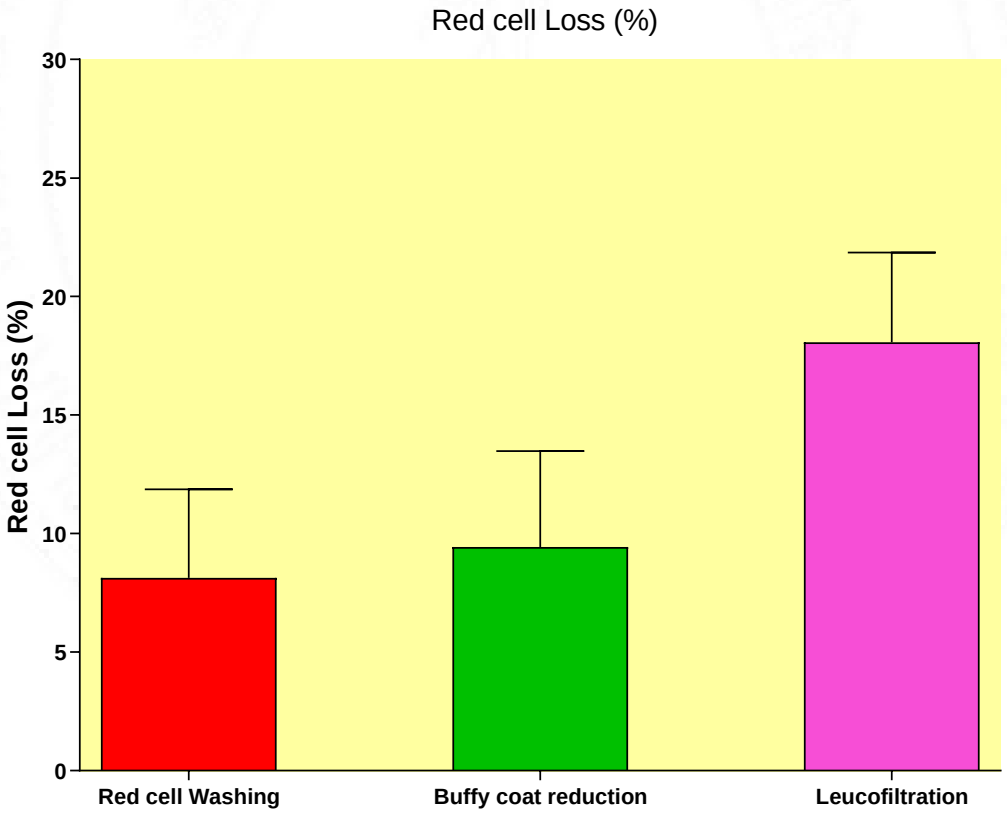
13. (a) Red Cell loss with buffy-coat reduction, washing and filtration methods were determined and are listed in Table No. 21 and Figure No. 11.

Table No. 21: Red Cell Loss with the leucoreduction methods (%).Values expressed as Mean ± SD and Range.

METHOD	N	Red cell Loss (%)		Range
		Mean	SD	
Buffy-coat reduction	48	9.43	4.05	0.53 – 20.77
Red cell Washing	16	8.13	3.73	3.44 - 16.03
Leucofiltration	48	18.07	3.78	11.72 – 28.71

$p<0.001$

Figure No. 11: Red Cell Loss with the leucoreduction methods (%).Mean results obtained with washing, buffy-coat reduction and leucofiltration. The standard deviations are represented by vertical bars.

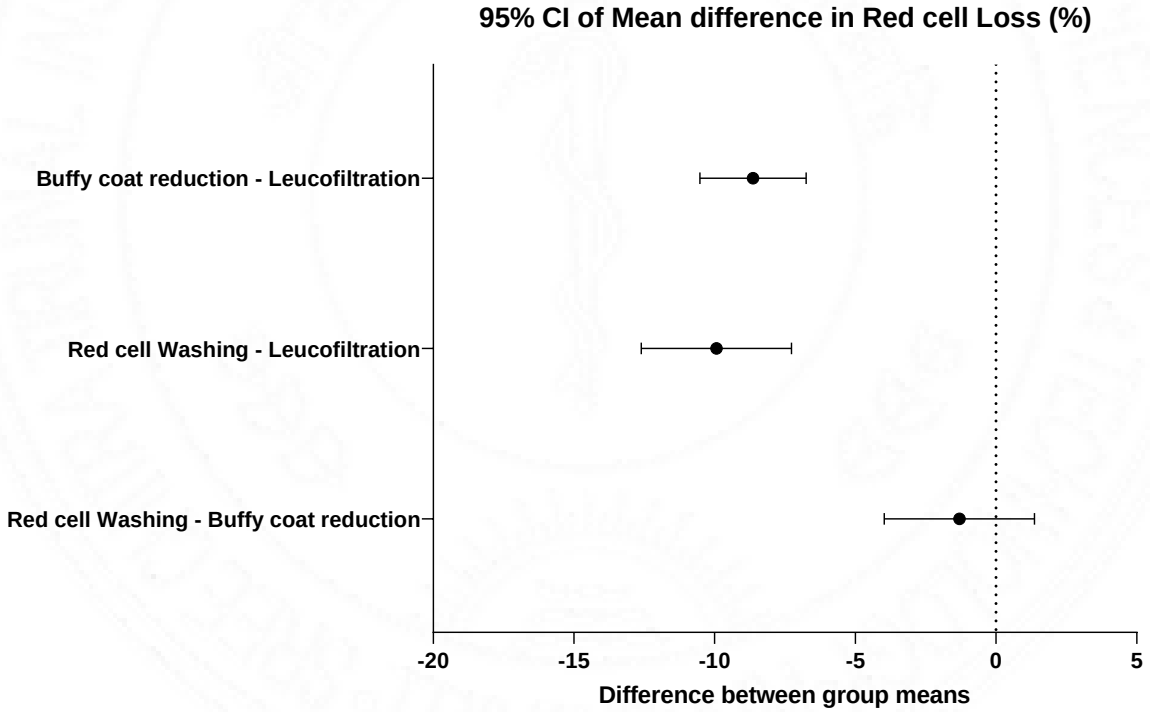


13. (b) The red cell loss with buffy-coat reduction, washing and filtration were compared between the methods and results are listed in the Table No. 22 and Figure No. 12.

Table No. 22: Comparison of red cell loss between the leucoreduction methods

Multiple comparison	Mean Difference	se	95% Confidence Interval of mean difference		p
			Lower Bound	Upper Bound	
Washing Vs Buffy-coat reduction	-1.30	1.12	-3.97	1.37	0.480
Washing Vs Leucofiltration	-9.94	1.12	-12.61	-7.27	0.000
Buffy-coat reduction vs Leucofiltration	-8.64	0.79	-10.53	-6.75	0.000

Figure No. 12: Comparison of red cell loss between the leucoreduction methods



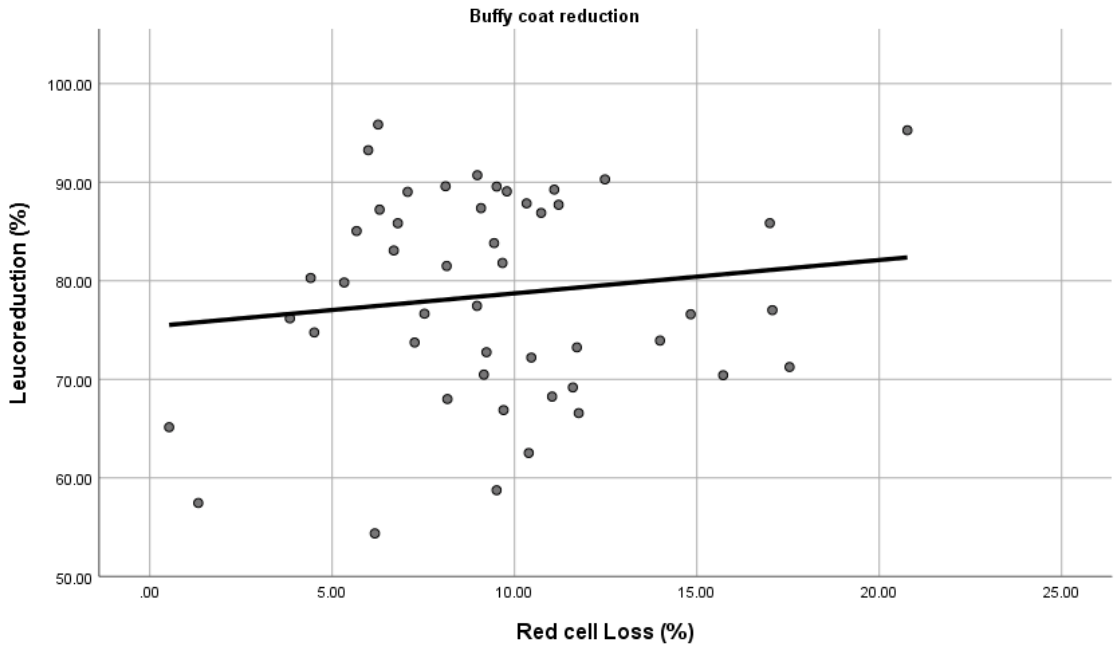
ADDITIONAL OBSERVATIONS:

14. An analysis was performed to find the correlation of leucoreduction with red cell loss in the buffy-coat reduction, washing and filtration methods of leucoreduction. The results are presented in Table No.23 and Figure No. 13

Table No. 23: Correlation of Leucoreduction (%) with Red cell Loss (%)

METHOD	Pearson Correlation r	p
Washing	-0.057	0.833
Buffy-coat reduction	0.132	0.372
Filtration	-0.181	0.219

Figure No. 13: Correlation of Leucoreduction (%) with Red cell Loss (%) in buffy-coat reduction method



15. An analysis was performed to find correlation of plasma haemoglobin levels, percentage haemolysis and potassium levels with the age of the red cell units. The correlation analysis was performed independently in RCCs suspended in CPD-SAGM (N=48) (SAGM bag) and RCCs in CPDA (N=16) (Non-SAGM bag). The results are presented in Table No. 24 - 26 and Figure No. 14- 19.

Table No. 24: Correlation of Plasma Haemoglobin levels with age of RCCs (g/dl)

GROUP	Pearson Correlation r	p
RCCs in CPDA	.630**	0.009
RCCs in CPD-SAGM	.628**	0.000

Figure No. 14: Correlation of Plasma Haemoglobin levels with age of RCCs in CPDA

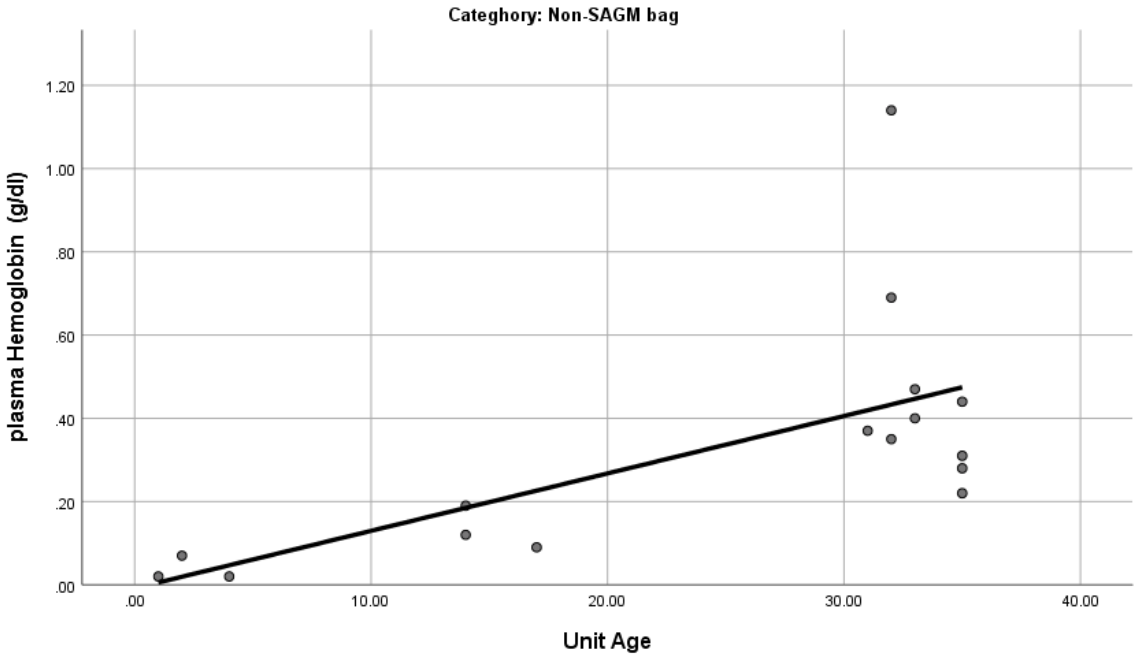


Figure No. 15: Correlation of Plasma Haemoglobin levels with age of RCCs in CPD-SAGM

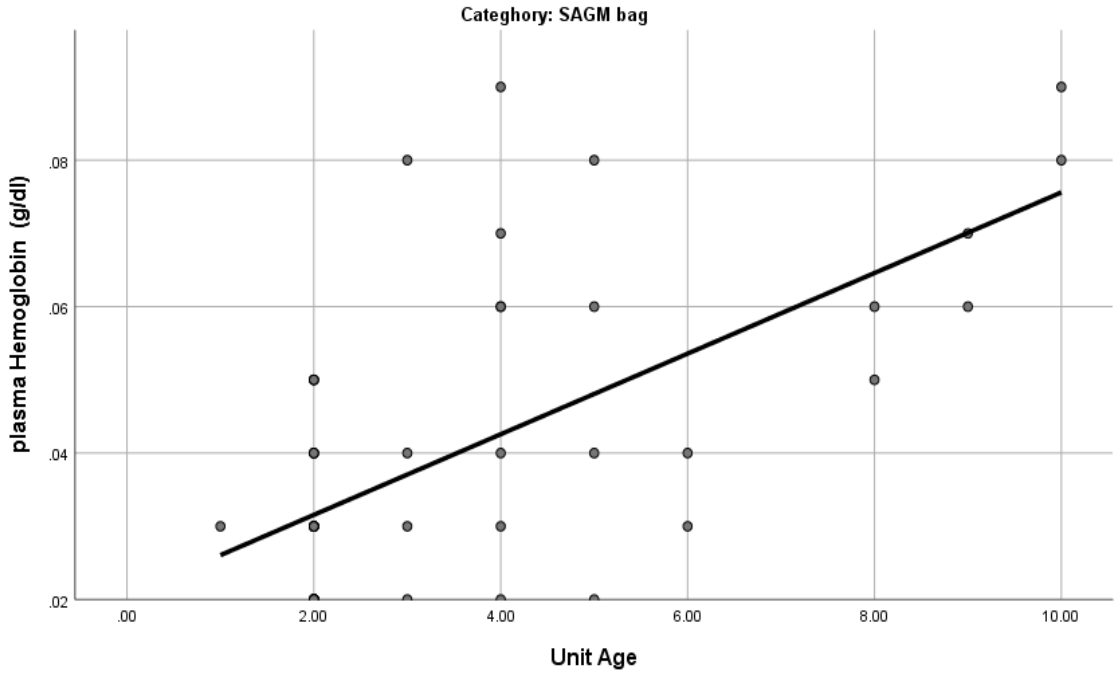


Table No. 25: Correlation of Haemolysis with age of RCCs (%)

GROUP	Pearson Correlation r	p
RCCs in CPDA	.905**	0.000
RCCs in CPD-SAGM	.678**	0.000

Figure No. 16: Correlation of Percentage Haemolysis with age of RCCs in CPDA

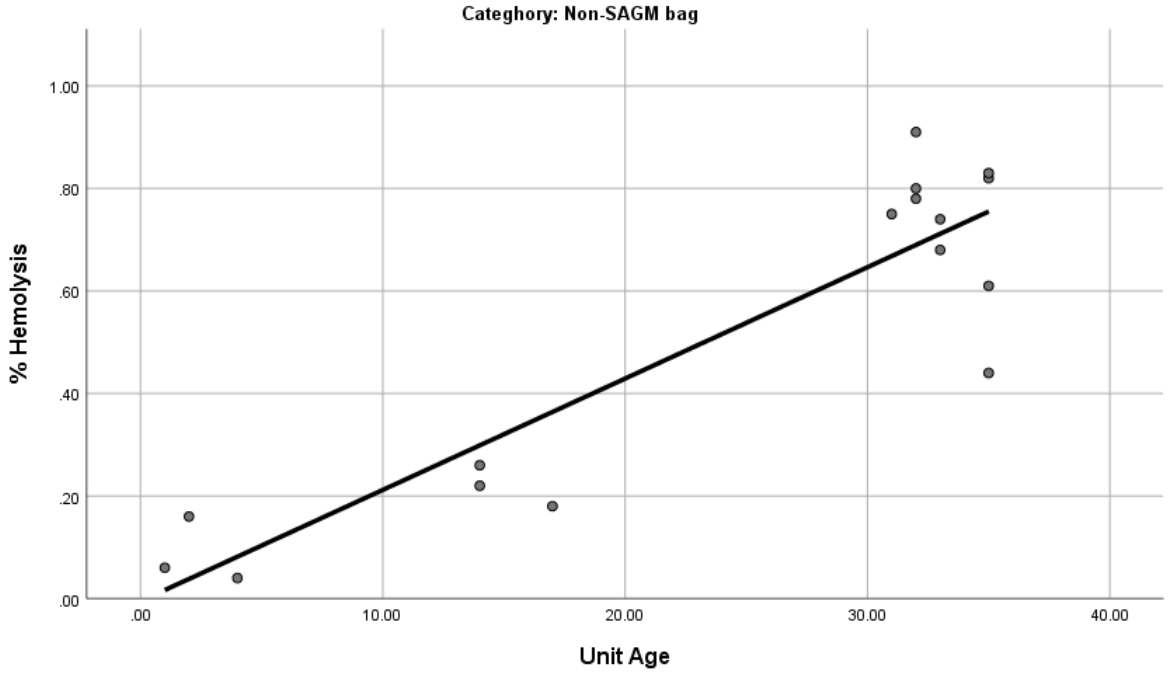


Figure No. 17: Correlation of Percentage Haemolysis with age of RCCs in CPD-SAGM

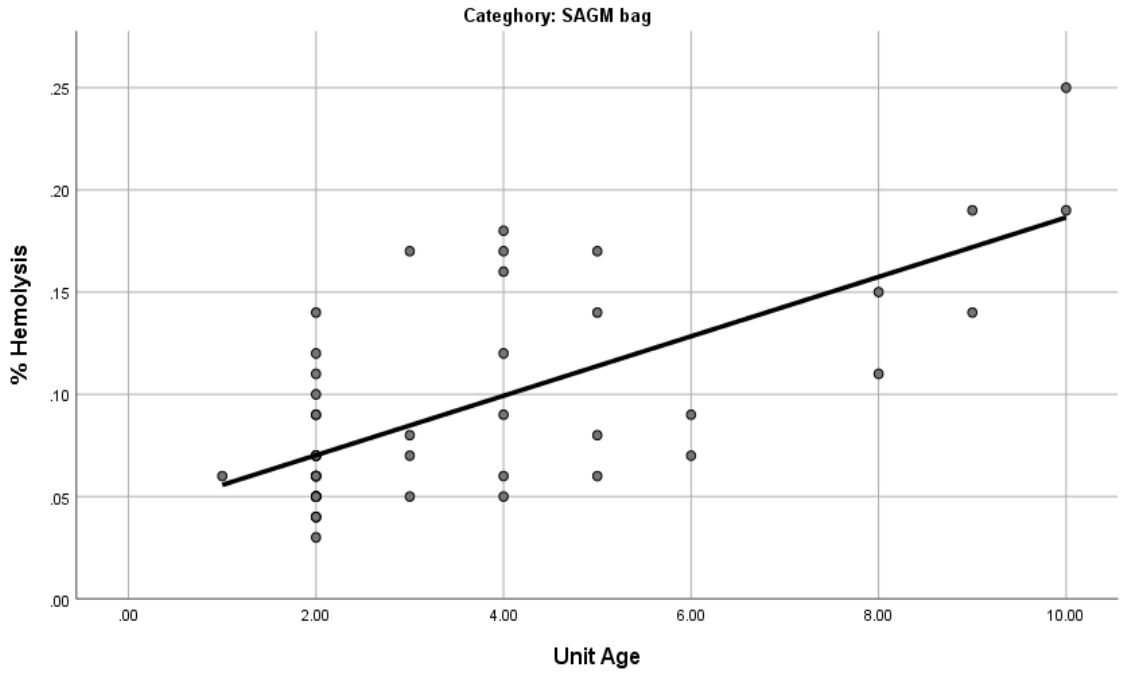


Table No. 26: Correlation of Potassium levels with age of RCCs (mmol/L)

GROUP	Pearson Correlation r	p
RCCs in CPDA	.937**	0.000
RCCs in CPD-SAGM	.750**	0.000

Figure No. 18: Correlation of potassium levels with age of RCCs in CPDA

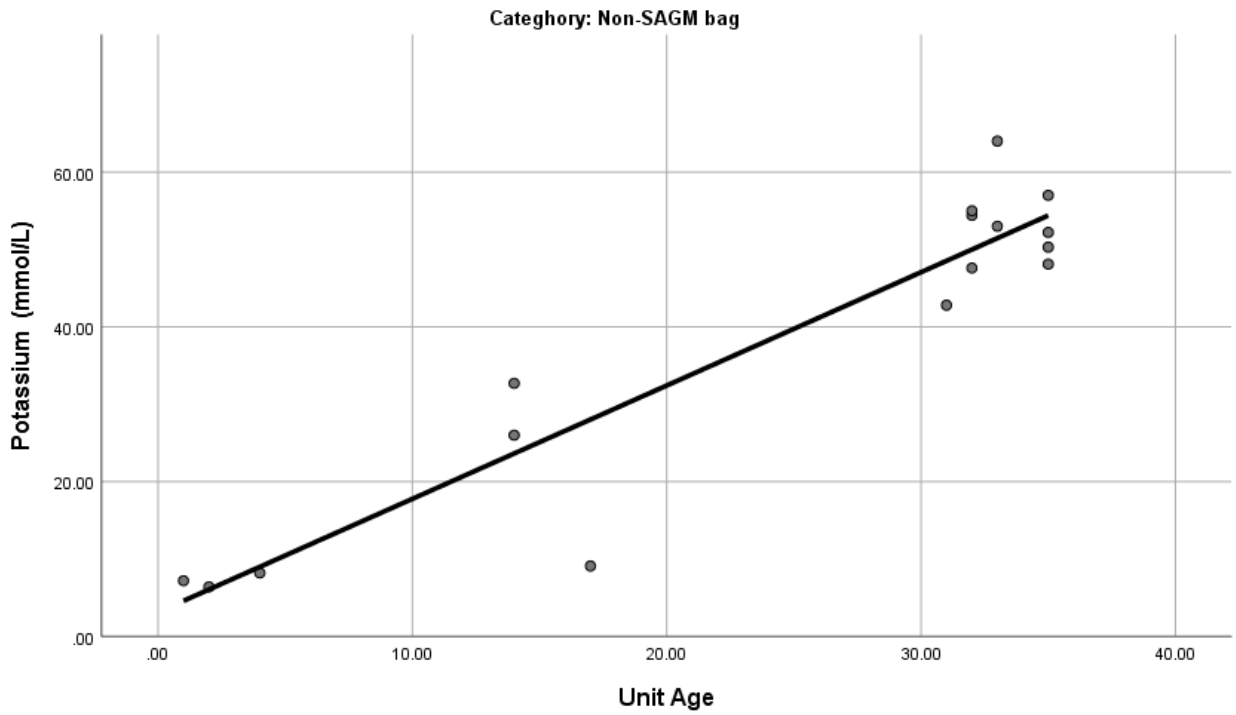
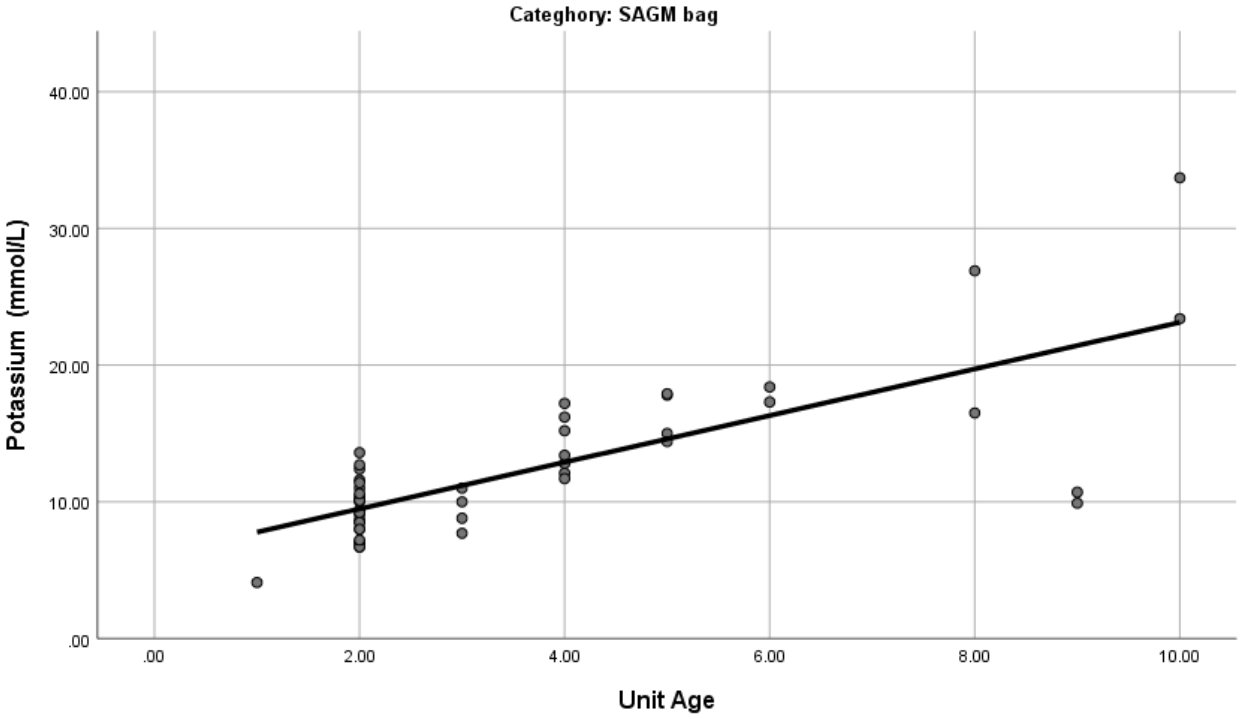


Figure No. 19: Correlation of potassium levels with age of RCCs in CPD-SAGM



DISCUSSION

Leucoreduction of blood components contributes to blood safety. It has been shown to reduce various transfusion reactions and improve clinical outcomes in transfusion recipients.(10,112) Over the years various leucoreduction techniques have been developed which differ in their method of preparation, the complexity involved, and its cost-effectiveness. Extensive analyses were carried out by many authors to understand the efficacy of each method to reduce the leucocyte content in the blood components.(113)

In this study, we performed 3 methods of leucoreduction on red cell units and analysed their efficiency to remove donor leucocytes.

BUFFY-COAT REDUCTION:

When the WB is centrifuged, cells separate based on their specific gravity, and the majority of leucocytes are present in the buffy-coat layer along with the platelets. Conventionally manual methods were used to remove this buffy-coat layer when WB was separated into components to bring down the leucocyte count in the transfused blood unit. Introduction of TAB collection bag systems allowed the separation of buffy-coat by automated and standardised methods.

Pietersz *et al* compared the manual method of buffy-coat removal with the automated system and observed significantly lower residual leucocytes in the RCCs prepared by the automated system, indicating a better buffy-coat removal compared to the manual method.(114)

In our study, buffy-coat reduction of the WB collected in the 450 ml and 350 ml TAB blood bags were performed on the day of collection using the T-ACE II+ automatic component extractor system. Post-procedure, an absolute leucocyte count of $0.4 \pm 0.29 \times 10^9$ and $0.65 \pm 0.33 \times 10^9$ was observed in the RCCs prepared from the 350 ml and 450 ml collections respectively. We achieved 78.54 ± 10.39 % leucoreduction with a red cell recovery of 90.57 ± 4.05 % using the T-ACE II+ system.

In the study by Sonker *et al*, 16 units of WB collected in 450 ml CPD-SAGM blood bags were processed into buffy-coat reduced RCCs suspended in SAGM using the T-ACE automated component extractor. The RCCs had a post-procedure volume of 301.11 ± 6.16 ml, a haemoglobin content of 53.06 ± 3.09 g, and a haematocrit of 56.51 ± 2.67 percentages. A mean absolute red cell count of $1.95 \pm .16 \times 10^{12}$ and an absolute leucocyte count of 768 ±

58.56×10^6 were present in the buffy-coat reduced SAGM suspended RCCs. Post-procedure haemolysis observed was 0.0363 ± 0.0096 %.(115) In our study with the T-ACE II+ system, using 450 ml CPD-SAGM blood bags we observed a post-procedure mean absolute red cell count of $1.94 \pm 0.15 \times 10^{12}$, haemoglobin content of 57.38± 5.29 g, haematocrit of 57.88± 2.44%, and haemolysis of 0.02± 0.02 % coinciding with the findings by Sonker *et al*. While the post-procedure leucocyte content was $650.84 \pm 327.20 \times 10^6$, less than what observed by Sonker *et al*.

Another automatic component separator for buffy-coat removal is the Optipress system from Baxter Healthcare Corporation. Quality analysis by Hurtado *et al* on 1434 units of buffy-coat reduced RCCs prepared using the Optipac-Optipress TAB system showed a post-procedure volume of 279 ± 20 ml with a mean haemoglobin content of 54.92 ± 7.16 g in the RCC unit. An absolute leucocyte count of 1.2×10^9 was obtained in 96 % of the tested units, which was in agreement with the CoE recommendations**.**(116) While we achieved $< 1.2 \times 10^9$ leucocyte counts in 97.9 % of the buffy-coat reduced RCCs tested.

Muylle *et al* performed buffy-coat reduction of WB collected in Optipac 450ml TAB bags from 72 voluntary blood donors. They used the automatic Optipress system to remove the buffy-coat and resuspend the red cells in SAGM. The buffy-coat reduced RCC showed a mean volume of 287 ± 23 ml with an absolute red cell count of $1.90 \pm 0.42 \times 10^{12}$, mean haematocrit of 60± 4% and an absolute leucocyte count of $0.50 \pm 0.77 \times 10^9$ in the buffy-coat reduced RCCs which agreed with our observation of mean absolute red cell count of $1.94 \pm 0.15 \times 10^{12}$ and absolute leucocyte count of $0.65 \pm 0.33 \times 10^9$ using the T-ACE II+ system.(117)

After buffy-coat reduction of 25 WB units using the Optipac-Optipress TAB system, Sawant *et al* obtained a haemoglobin content of 50.7 g with a haematocrit of 57.4 % in the buffy-coat reduced RCCs. The mean absolute leucocyte content was only 0.11×10^9 compared to $0.65 \pm 0.33 \times 10^9$ in our study that used the T-ACE II+ system.(111) While with the Optipac-Optipress TAB system, Hogman et al observed an absolute leucocyte count of $0.46\pm 0.25 \times 10^9$ and $0.5 \pm 0.4 \times 10^9$ in two different laboratories agreeing with our observation with the T-ACE II+ system. They also observed post-procedure haemolysis of 0.02 ± 0.007 % similar to 0.02± 0.02% observed in our study.(67)

Pasqualetti *et al* used the Compomat G4 (Fresenius-Hemocare) for automatic component separation from 450ml collection bags from 3 manufacturers (Maco Pharma, Terumo, and Fresenius). With 20 Terumo bags, they obtained haemoglobin of 62.6 ± 3.2 g and haematocrit of 52.8 ± 2.1 % in RCC unit of 340 ± 12.4 ml. Using 14 Fresenius bags, a haemoglobin content of 68.4 ± 6.5 g and haematocrit of 53.8 ± 2.6 % was obtained in RCC units of 370.4± 25.1 ml. By using 8 Macopharma blood bags with the Compomat G4 system, haemoglobin of 57.2 ± 3.9 g and haematocrit of 52.8 ± 2.2 % were seen in RCCs having a mean volume of 365.4± 18 ml.(118) In our study, by using 450ml collection bags from Terumo (n=32) with the T-ACE II+ system for automatic component separation, we obtained a haemoglobin content of 57.38 ± 5.29 g and haematocrit of 57.88 ± 2.44 % in RCCs having a volume of 291.88 ± 16.17 ml.

Buffy-coat reduction method has its advantage in preventing micro aggregate formation in the RCC during storage and if red cells are suspended in additive solution, less haemolysis will occur.(119,120) From the buffy-coat obtained using the TAB system, platelet concentrates can also be prepared. In addition, decreased incidence of FNHTR in recipients of buffy-coat poor RCCs have been reported by Lieden and Hilton.(71) A major disadvantage of the buffy-coat method is the loss of red cells especially neocytes.(121)

When the buffy layer is removed in the procedure, an expected loss of red cells is seen.(108) To check whether leucoreduction is proportional to red cell recovery, we performed correlation analysis with Pearson correlation coefficient. A weak correlation was seen in the buffy-coat reduction method, but the sample size was not found to be significant.

Quality Standards (Table No. 6):

DGHS, GoI guidelines propose quality control of leucocyte poor red cells modified by centrifugation to have white cells < 70% of original and residual red cells > 70%. CoE guidelines mandates that a minimum of 90% of the tested buffy-coat reduced RCCs suspended in additive solution to have a haemoglobin content of 43 g and haematocrit between 0.50 – 0.70. Residual leucocyte content should be $< 1.2 \times 10^9$ per unit.

Our study met both CoE and DGHS, GoI criteria for quality control of buffy-coat reduced red cells, suspended in additive solution. We obtained leucoreduction of 78.54 ± 10.39 % and red cell recovery of 90.57 ± 4.05%. All the units tested had haematocrit between 0.50 – 0.70 and > 93 % units had haemoglobin content ≥ 43g. The absolute leucocyte count was well below $< 1.2 \times 10^9$ in >95 % of the units tested.

As per the Government of India Drug and Cosmetic Act (DCA)**,** when intended to prevent febrile reactions leucocytes in the final component shall be $< 5 \times 10^8$. In our study with the buffy-coat reduction method using the T-ACE II+ system, a leucocyte count of $< 5 \times 10^8$ could only be achieved in < 46 % of the units tested. Hence even though the DGHS and CoE guidelines for buffy-coat reduced RCCs suspended in additive solution were met in our study, buffy-coat reduction as a method of leucoreduction does not seem to be an option to prevent febrile transfusion reactions as per the DCA recommendations.

WASHING:

We performed 3 cycles of manual washing using 0.9 % sodium chloride in 16 red cell units anticoagulated with CPDA. Over the years numerous methods have been developed for washing red cells and various studies have analysed and compared these procedures.

By analysing the in-vitro quality of 40 RCCs which were washed twice manually either by saline, SAGM, or 5% albumin (each cycle using 250ml wash fluid); Weisbach and colleagues observed 15.6 ± 0.1% loss of total haemoglobin which was high when compared to our study which had red cell loss of only 8.13± 3.73%, although the sample size was a limitation. They also observed that washing resulted in a reduction of free haemoglobin and potassium in all washed RCC which is agreeing with our observation.(72) A significant reduction of free haemoglobin levels from 0.32±0.29 to 0.12±0.09 g/dl and potassium levels from 38.38± 20.38 to 1.55±0.94 mmol/L was obtained with the manual washing of CPDA anticoagulated RCCs of 1-35 days old in our study. A similar observation was seen in the study by Bansal *et al*, where they washed six CPDA anticoagulated RCC units of 3 to 21 days old and observed a significant reduction in potassium levels from 36.95 ± 13.16 mEq/L to 2.15 ± 0.10 mEq/L.(122)

Toth *et al* studied quality parameters in twenty-six buffy-coat reduced SAGM suspended RCCs which were stored up to 10 days and washed twice in isotonic saline by manual technique. They observed a volume reduction from 254.2 ± 17.2 ml to 216.2 ± 18.0 ml and an associated rise in haematocrit from 62.2 ± 5.2 % to 66.1 ± 6.6 %. The volume and haematocrit in post-wash RCCs can vary with the volume of solution used for re-suspending erythrocyte sediment and a reduction in volume was observed with an increase in haematocrit.(123,124) Wenz *et al* observed no correlation between the pre and post volumes of RCCs that underwent washing procedure.(125) In our study, there was no significant change in volume with washing, and haematocrit change was seen in association to the observed volume change. The absolute leucocyte count in the washed RCCs in the study by Toth *et al* was $3.3 \pm 2.0 \times 10^8$, which was agreeing with our observation of $3.03 \pm 3.4 \times 10^8$ in the 350ml group and $3.42 \pm 1.9 \times 10^8$ in the 450ml group.

Polesky *et al* manually washed 10 RCCs thrice with 250 ml normal saline during each wash. They obtained leucoreduction of 80.3 % and a red cell recovery of 83 ± 3.5 %. The percentage leucoreduction is in line with our findings, but we obtained a higher red cell recovery of 91.87 ± 3.73 %. They also analysed the Elutramatic system, which is a semi-automatic continuous flow saline wash system, and observed only 70.8 % leucoreduction but with a red cell recovery of 87.4 ± 2.98 %.(126)

Langfelder *et al* performed triple washing of Acid Citrate Dextrose (ACD) suspended WB using normal saline by manual method and obtained a leucoreduction of 77.5 % while with triple washing of CPDA suspended RCCs, we obtained 82.67±7.44% leucoreduction. (127)

In the manual method by Proffit *et al*, they observed that all washed RCCs met the UK specification for volume (200-320ml) and haemoglobin content of ≥ 40g/unit. When the units were washed two times in 250 ml saline each and then suspended in saline, they found median haemoglobin content of 55.9 g. In our study, a mean haemoglobin content of 44.73 ± 5.27 g in the 350 ml group and 58.80 ± 5.40 g in the 450 ml group was obtained. Except for 2 units in the 350 ml group, all other washed units had a haemoglobin content more than or equal to 40 g per unit. Proffit *et al* performed sterility testing of all washed RCCs by the automated bacterial detection system and no bacterial growth was detected in any of the RCCs tested, which was the same with our study.(128)

These studies point that; manual washing techniques could remove up to 80% leucocytes with 15-20% red cell loss and these vary with the processing steps performed. Various semi-automated and automated cell processor systems were developed which varied in their ability to reduce leucocytes and obtain adequate red cell recovery.(129) Many investigators have used these systems to study leucocyte reduction and red cell recovery after washing.

Proffitt *et al* compared the manual method of washing with the ACP215 Cell processor (Haemonetics Corporation) and concluded that the overall removal of plasma proteins was better using the manual method and haemoglobin loss was lower in manually washed units than in ACP 215 washed units.(128) In the study by Smith *et al*, they washed out-dated RCCs (42-49 days old) using COBE 2991 automatic cell processor and observed a significant reduction in post-wash potassium levels to a mean of 4.63 ± 4.8 mmol/L.(130) With the manual washing of 1- 35 days (median of 32days) old RCCs in our study, a significant reduction of post-wash potassium levels to 1.55±0.94 mmol/L was obtained.

Washing red cell units have the advantage of the removal of plasma content thereby can prevent adverse reactions related to plasma proteins like allergic reactions and reducing IgA content to prevent anaphylactic reactions in IgA deficient individuals.(123) Other advantages include the removal of anticoagulants, metabolic breakdown products as well as the reduction of extracellular potassium and plasma haemoglobin levels. Even better flow characteristics have been reported by some.(124) But the washing procedure comes with its limitations like the time consumption, availability of the equipment as well as handling and expertise of the technical staff. If an open method of washing is performed, it comes with an additional disadvantage of early expiry of the washed blood components. In addition, washing techniques cannot bring down the leucocyte content in the RCCs to a level enough to prevent certain transfusion reactions. (131)

Quality Standards (Table No. 6):

According to CoE guidelines for washed red cells, a haemoglobin content of ≥ 40 g/unit and a haematocrit between 50 to 70 % is required in a minimum of 90% units tested. Haemolysis observed should be < 0.8 % of red cell mass according to CoE and < 1% according to AABB requirements. DGHS, GoI guidelines mandates at least 85% leucoreduction and 80% red cell recovery with the red cell washing procedure.

In our study, as per CoE guidelines for washed RCCs, minimum haemoglobin content was achieved only in 87% of the units tested. The 2 units which had low haemoglobin levels after washing were those processed from 350ml collections. The CoE guidelines for haematocrit in washed RCCs was met only in 81% of units tested. However, the haematocrit levels can vary with the volume of saline used for resuspending the erythrocyte sediment after the last wash. All the washed RCCs showed percentage haemolysis consistently below 0.8%.

Manual saline washing achieved a good red cell recovery, but DGHS criteria for leucoreduction was met only in < 40% of the units tested. However, leucoreduction obtained in our study was consistent with the values from previous studies on manual washing by other investigators.

LEUCOFILTRATION:

In this study, leucofiltration was performed with Leucolab Maco Pharma filter and quality parameters were analysed in 48 SAGM suspended RCCs that were ≤ 10 days old. Fresher units were selected, as leucofiltered units were issued for the paediatric population and also matched with the manufactures instructions. Following leucodepletion using new generation leucofilters, the resulting low levels of WBCs cannot be enumerated by an automated cell counter. It requires analysis by FC or microscopic methods like the NC to obtain accurate cell counts. In our study, NC was used for counting the residual leucocytes in the leucofiltered RCCs.

Masse *et al* filtered 581 buffy-coat poor SAGM suspended and 590 CPD-SAGM suspended RCCs on the fifteenth day of storage using the six commercially available leucoreduction filters. RC50 (Pall Biomedical Corp, Glen Cove, NY) and Sepacell R500N (Asahi, Tokyo, Japan) filters were used without the need for any priming or rinsing. They filtered the RCCs by gravity immediately after removing them from storage and the residual leucocyte count was determined by manual counting on NC. The median absolute leucocyte count on CPD/SAGM suspended RCCs filtered by RC50 was 1.7 (0.02-47) × 10^6 and by R500N was 1.5 (0.05- 35) × 10^6. While filtration of buffy-coat reduced RCCs showed a median leucocyte count of 0.3 (0.02-6) × 10^6 in RC50 and 0.5 (0.02-4) × 10^6 in R500N filters.(132) In our study, filtration of CPD-SAGM suspended RCCs processed from 350ml collections showed a mean absolute leucocyte count of 0.13 ± 0.05 × 10^6, and filtration of buffy-coat reduced

RCCs showed residual leucocyte count of 0.10 ± 0.05× 10^6 and 0.05 ± 0.04× 10^6 in the units processed from 450 and 350 ml collections respectively.

Williamson *et al* filtered RCCs through the Pall RC50 bedside filtration system irrespective of the day of storage and obtained a post-filtration mean leucocyte count of 4.54 (1.39- 7.78) × 10^6.(87) Though it has met the AABB criteria, it failed to meet the CoE guidelines.

Rebulla *et al* evaluated six commercial filters for leucocyte reduction. They filtered buffy-coat depleted SAGM suspended RCCs as well as buffy-coat non-reduced CPD anticoagulated RCCs and calculated the absolute leucocyte count using NC. They observed that all filters could achieve the target leucocyte count of <5× 10^6 cells per bag with both groups. In addition, they observed a lower post-filtration leucocyte count with buffy-coat reduced RCCs in all filters except CF1 and R500. In our study a similar observation was seen; the buffy-coat reduced RCCs after filtration showed a lower mean WBC count of 0.1 ± 0.05× 10^6 (450 ml collections) and 0.05 ± 0.04× 10^6 (350 ml collections) when compared to the filtered RCCs from which buffy-coat was not removed, which had an absolute leucocyte count of 0.13 ± 0.05 × 10^6 (350 ml collections). When they evaluated red cell recovery, the median varied from 84 to 94 percentages depending upon the filter model. Out of the six filters they evaluated, only R500 and Optima g-2 obtained a median red cell recovery below 90 percentages.(133) However, with the Leucolab filter we used, red cell recovery was only 81.93 ± 3.78 %.

Rogers *et al* filtered buffy-coat reduced SAGM suspended RCCs on the third day of collection using the third generation Sepacell R2000 (Asahi, Tokyo, Japan) filter. An absolute leucocyte count of 0.33 ± 0.08 × 10^6 was determined in the filtered RCCs using the NC manual counting method. They achieved a leucoreduction of 99.9 % with a red cell recovery of 94.47 % with the Sepacell R2000 filter. Post-filtration potassium level of 9.29 ± 1.31 mmol/L was seen in the RCC units.(134) In our study red cell recovery was only 81.93 ± 3.78 % using the 4^{th} generation leucofilter, but could achieve a leucoreduction of 99.99%. A potassium level of 13.20 ± 5.73 mmol/L was seen in the filtered RCCs.

Sonker *et al* used Imugard III-RC (Terumo Corporation, Tokyo, Japan), a pre-storage leucofiltration system to filter 16 buffy-coat reduced SAGM suspended RCCs and 16 RCCs anticoagulated with CPD; both processed from 450 ml blood bags. Post-filtration leucocyte

count was determined using NC. Buffy-coat reduced SAGM suspended RCCs after filtration had a mean volume of 251.54 ± 6.36 ml, haemoglobin content of 48.51 ± 3.62 g, haematocrit of 55.79 ± 2.59 %, an absolute red cell content of 16.75 ± 1.16 × 10^{11} and an absolute leucocyte count of 0.15 ± 0.023 × 10^{6}. This observation was similar to what we observed when 14 buffy-coat reduced SAGM suspended RCCs processed from 450 ml collection were filtered using the Leucolab filter. Post-filtration RCCs had a haemoglobin content of 47.36 ± 5.80 g, haematocrit of 56.81± 2.69 % with a mean absolute red cell count of 16.79 ± 2.13× 10^{11} and an absolute leucocyte count of 0.10 ± 0.05 × 10^{6}.

In addition, they found a significant decrease in post-filtration absolute leucocyte count in buffy-coat reduced SAGM suspended RCCs when compared to the RCCs anticoagulated with CPD. However, both the groups fulfilled the criteria of < 1 × 10^{6} as per the CoE and < 5 × 10^{6} as per the AABB guidelines. They observed a 4 log reduction (99.99%) in leucocyte count with less than 15 % red cell loss by the Imugard III-RC filtration system.(115) Even though we obtained 99.99% leucoreduction, a red cell loss of 18.07 ± 3.78 % was incurred with the Leucolab system.

Kijkornphan *et al* used the Imugard III-RC (4B) leucofiltration set for bedside use to filter 20 CPD anticoagulated RCCs on day one. They achieved a 99.99 percentage leucoreduction with a mean absolute leucocyte count of 0.134 ± 0.063 × 10^{6} and a red cell loss of 15 percentages in the filtered RCCs with no additive solution.(135)

In 2005, Musuraca and colleagues performed the quality control of RCCs filtered by the commercially available leucofilters in Europe and compared them. They analysed the laboratory filters Leucolab LCG 2 (Maco Pharma Italia, Rho, MI, Italy) and Imugard III RC (Terumo Italia, Rome, Italy) and the in-line filters Leucored RC/PL SK (Grifols Italia, Ghezzano, PI, Italy), RCT 434CL (Pall Italia, Milan, Italy), R7546 (Baxter Fenwal, Rome, Italy), T3945 (Fresenius HemoCare, Midolla, MO, Italy), BB*WGQ45613 (Terumo) and LPT6265LR (MacoPharma). Comparing the parameters such as yield of red cells and haemoglobin content, there were no substantial differences in the performance characteristics of these filters. Among the 8 filters that were investigated, red cell yield was lowest for Leucolab (222 ± 16.4 g) and highest for Baxter (251 ± 9.2 g), and the total haemoglobin content varied from 44.7 g for Leucolab to 52.4g for Baxter. All the filters produced a post-filtration leucocyte count below 1 × 10^{6}. RCCs filtered by the Leucolab Macopharma filter showed a mean absolute leucocyte count of 0.14 ± 0.23× 10^{6}.(136) Among the 8 filters

studied by Musuraca et al, the Leucolab filter which was also used in our study showed the lowest red cell yield. This, along with the red cell loss due to sampling could explain the lower red cell recovery encountered in our study using the Leucolab filter, when compared to the studies from investigators on other available leucoreduction filters.

Over the years the development of new filtration technologies has improved the efficiency of leucoreduction. The earlier Danulon fibre column filter could achieve only 88% leucocreduction, whereas the newer generation filters like Imugard III or Leucolab could reduce leucocyte count by 99.99%.(127,135,136)

Quality Standards (Table No. 6):

According to AABB standards for leucofiltered red cells, absolute leucocyte count should be $< 5 \times 10^6$ with a red cell recovery of 85% in > 95% units. While CoE mandates the absolute leucocyte count to be $< 1 \times 10^6$ with minimum haemoglobin content of 40g/unit and haematocrit of 50-70% in > 90% units tested. As per DGHS, GoI guidelines, a minimum of 99% leucoreduction with 90-95 % red cell recovery is to be obtained in leucofiltered red cell units.

In our study, all the filtered RCCs achieved absolute leucocyte count $< 1 \times 10^6$ accounting for leucoreduction of 99.99%, which met the AABB, CoE, and DGHS recommendations for leucocyte count in filtered RCCs. Haematocrit values were between 50-70 % in all the units tested. As with the haemoglobin content in the leucofiltered RCCs, 13 units that were buffy-coat reduced before filtration, and a single unit from the 350 ml buffy-coat non-reduced filtered group had < 40 g of haemoglobin thereby not satisfying the CoE criteria for minimum haemoglobin. Only less than 50% of the buffy-coat reduced RCCs that underwent filtration had haemoglobin content ≥ 40 g. This signifies the red cell loss that can happen when buffy-coat reduction and leucofiltration methods are combined. In line with the observation by Michael-Muller-Steinhardt, pre-procedure haemoglobin content in 350 bags was lower compared to the 450 bags, and buffy-coat reduction before filtration incurred additional red cell losses. (137)

The mean red cell recovery after filtration using the Leucolab filtration system was 81.93 ± 3.78 %. Only < 25 % of the filtered RCCs was found to achieve 85% red cell recovery thereby not satisfying the AABB or DGHS criteria. In the filtration method of

leucoreduction, loss of red cells is inherent with the procedure which represents the dead space volume in the filter set used as well as in this study sampling loss should also be considered.

As with the observed quality parameters following leucofiltration in this study, even though the mean absolute leucocyte count was lower in the buffy-coat reduced RCCs that were filtered compared to the filtered RCCs from which buffy-coat was not removed, all of them met the AABB, CoE, and DGHS criteria for residual leucocyte count after filtration. Considering the haemoglobin loss when both buffy-coat reduction and filtration were performed on a red cell unit, we conclude that going for a single effective method of leucoreduction is as good as or even better than combining buffy-coat reduction and filtration as far as new generation leucofilters are considered because additional red cell loss during buffy-coat separation can be avoided in the leucofiltered RCCs. In addition, Loi *et al* in their study observed that combining buffy-coat reduction and filtration resulted in increased haemolysis when compared to filtration and the combination method had no advantage over filtration.(91)

Comparison studies:

When the methods of leucoreduction are compared in terms of their ability to reduce leucocyte content, filtration provides the best results. Lichtiger *et al* compared red cell washing using the IBM 2991 cell processor with leucofiltration using the lmugard filter and observed that both leukocyte removal and red cell recovery were superior with the filtration procedure. Hence they concluded leucofiltration as a practical, effective, and cost-saving alternative to the 2991 wash.(138) A similar observation was made by Rock *et al* when they compared washing using IBM 2991 with filtration using Imugard filter.(139)

Grunnet and Rasmussed compared leukocyte depletion and red cell recovery in five leucoreduction methods: saline washing in a cell separator, spin and wash in glass bottles, Imugard filter, inverted spin, and dextran sedimentation. When both leukocyte removal and red cell recovery were considered together, they found the Imugard filter to be superior. (113)

With technology development, leucofiltration has emerged as a simple inexpensive method to provide the best possible leucoreduction. In addition, leucofiltration has the definite

advantage of reducing the incidence of transfusion reactions mediated by donor leucocytes in the transfused blood components.(140)

An analysis with red cell unit age:

When plasma haemoglobin, percentage haemolysis, and potassium levels were compared with age of RCCs using the Pearson correlation coefficient, we observed a strong correlation of these parameters with unit age. A similar relation of unit age with percentage haemolysis was observed in the study by Muller-Steihardt *et al*. Their analysis showed that haemolysis rates increased with the storage time of RCCs. (137)

Shastry *et al* compared non-leucoreduced CPD-SGAM suspended and non-leucoreduced CPDA anticoagulated RCCs for plasma haemoglobin, percentage haemolysis, and potassium levels with storage age and observed the levels to be remarkably low in the CPD-SAGM suspended RCCs.(141) In our analysis of these parameters with the age of the unit using Pearson coefficient, the correlation was found to be stronger with the CPDA anticoagulated RCCs than with the CPD-SAGM suspended RCCs, indicating better red cell viability with the use of additive solution.

Leukocytes are a part of blood component therapy and are responsible for a variety of adverse effects such as FNHTR, HLA- alloimmunisation, and platelet refractoriness, as well as transmission of viruses like the CMV. Leucoreduction technologies are constantly evolving to provide the standard of care treatment. With the development of fourth-generation leucofilters, a 99.99% reduction in leucocyte count could be achieved in the transfused blood components. Hence filtration has evolved as the most efficient method for leucoreduction and is capable of preventing a majority of adverse transfusion reactions caused by leucocytes present in the transfused blood components.

CONCLUSION

Buffy-coat reduction, saline washing and filtration methods were able to achieve leucoreduction in the red cell units to a variable extent, while the efficiency to reduce leucocyte content was found to be more with filtration. The current generation leucofilter is shown to achieve 99.99% leucoreduction, bringing down the leucocyte content in the filtered red cell units to $< 1 \times 10^6$ cells.

In a resource-poor country like India, selective leucoreduction of blood components using the new generation filters is a viable option to potentially reduce the frequency of donor leucocyte associated adverse events such as febrile non-hemolytic transfusion reactions, HLA- alloimmunisation and platelet refractoriness, as well as transmission of viruses like the cytomegalovirus in the recipients and improve the outcome, particularly of the multitransfused patient population.

LIMITATIONS

The study has few limitations:

- Only three methods of leucoreduction were compared in this study
- For counting the residual leucocytes in the filtered red cell units, Nageotte Chamber was used which is associated with subjective variation and underestimation of leucocytes
- The day of the leucoreduction procedure was not comparable between the groups

SUMMARY

This is a comparative study on quality parameters in leucoreduced red cell concentrates prepared by methods like buffy-coat reduction, saline washing, and filtration. The study was performed in the Department of Transfusion Medicine, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum, Kerala for a period of one year.

The study aimed to determine the efficacy of different leucoreduction techniques performed in a blood centre. This study analysed the quality parameters in 112 red cell concentrates prepared by three methods of leucoreduction and more specifically compared the efficacy in reducing leucocyte load in the transfused blood component. 48 red cell units underwent buffy-coat reduction using an automated component extractor and 16 underwent manual saline washing. Filtration was performed using a fourth-generation leucofilter in 27 buffy-coat reduced red cell units and in 21 red cell units from which buffy-coat was not removed. RCCs processed from both 350ml and 450ml collection bags were used in the study. Quality parameters were measured before and after each leucoreduction procedure and the percentage leucoreduction and red cell recovery obtained were compared.

In this study, we obtained 99.99 ± 0.01% leucoreduction with filtration, 82.67 ± 7.44% with manual saline washing, and 78.54 ± 10.39% with buffy-coat reduction method. Though all three methods were found to satisfy the quality recommendations, the leucofilter we used could achieve 4 log reductions, with a mean residual leucocyte count $< 1 \times 10^6$ per bag which could potentially reduce the frequency of leucocyte associated adverse events in the transfusion recipients and improve patient outcome.

There is an inherent red cell loss that is associated with each leucoreduction procedure. In this study, a red cell loss of 8.13±3.73 % with manual saline washing, 9.43±4.05% with buffy-coat removal, and 18.07±3.78 % with filtration was observed.

An interesting finding in our study was that only less than 50 % of the buffy-coat reduced red cell units that underwent filtration met the European standards for minimum haemoglobin content. This led us to the conclusion that performing filtration using the available new generation leucofilters is more beneficial than combining buffy-coat reduction and filtration procedure in a red cell unit.

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ANNEXURE

Technical Advisory Committee (Clinical Studies)

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES & TECHNOLOGY
THIRUVANANTHAPURAM – 695011, INDIA

TAC Registration No: SCT-/S/2019/920 **Date:24.06.2019**

Project title: A STUDY ON QUALITY PARAMETERS IN VARIOUS LEUCOREDUCED RED CELL PREPARATIONS.

Principal Investigator:	
Dr. Sreethu Chand, Junior Resident, Department of Transfusion Medicine, SCTIMST	Degree: MBBS
Co-Principal Investigator(s)	
Dr. Debasish Gupta, Professor and Head, The Department of Transfusion Medicine, SCTIMST	Degree: MD
Dr. R. Raj Bharath, Associate Professor, Department of Transfusion Medicine, SCTIMST	Degree: MD

Members who participated in the TAC meeting on 01/06/2019
Dr. Rupa Sreedhar (Chairperson)
Dr. Sankara Sarma P
Dr. Prasantakumar Dash
Dr. Sylaja. P.N
Dr. Ashalatha
Dr. Krishna Kumar K
Dr. Sanjay G
Dr. Bijulal S
Dr. Syam K
Dr. Jayadevan ER
Dr. K. Shivakumar (Member Secretary)

Dr. Jayadevan ER, Dr. Sylaja. P.N, Dr. Bijulal S, Dr. Ashalatha, Dr. Rupa Sreedhar, Dr. Prasantakumar Dash and Dr. Sanjay G stayed away from the proceedings when the projects in which they are involved as investigator were discussed (#921, 925, 929, 934, 937, 938, 942, 943, 945, 948).

Risk Classification of the project (Minimum/ Moderate/ High): Minimum

Requirement of DSMB: No

Recommended members of DSMB: Not applicable

Recommendations of TAC:
Recommended for consideration of IEC in the light of the responses received from the investigator

The PI may note that there can be no additions / alterations in the documents approved by TAC when they are submitted to the IEC.

Signature of the Member Secretary, TAC (Clinical Studies)

Note for IEC
Copy of the investigator's responses to questions/suggestions from TAC is attached (Appendix-1).

श्री चित्रा तिरुनाल आयुर्विज्ञान और प्रौद्योगिकी संस्थान, त्रिवेन्द्रम
तिरुवनन्तपुरम - ६९५०११, केरल, इंडिया
SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY, TRIVANDRUM
Thiruvananthapuram - 695 011, Kerala, India
(An Institute of National Importance under Govt. of India)
Grams : Chitramet, Phone : +91-471-2443152, Fax : +91-471-2550728 / 2446433, E-mail : sct@sctimst.ac.in, Website : www.sctimst.ac.in

Institutional Ethics Committee

(IEC Regn No. ECR/189/Inst/KL/2013/RR-16)

SCT/IEC/ 1392/JULY-2019 07.08.2019

Dr. Sreethu Chand
Junior Resident
Department of Transfusion Medicine
SCTIMST, Thiruvananthapuram

Dear Dr Sreethu Chand,

The Institutional Ethics Committee reviewed and discussed your application to conduct the study entitled "A STUDY ON QUALITY PARAMETERS IN VARIOUS LEUCOREDUCED RED CELL PREPARATIONS (IEC/1392)" on 26th July, 2019.

The following documents were reviewed:

1. *Covering Letter addressed to the Chairman, IEC, SCTIMST dated 25.06.2019 with checklist*
2. *TAC Approval Letter*
3. *IEC Application Form*
4. *Project Proposal*
5. *Proforma*
6. *Application for waiver of Informed Consent Form*
7. *CV of Principal Investigator and Co-Principal Investigators*

The following members of the Ethics Committee were present at the meeting held on 26th July, 2019 at Noshir H Wadia Conference Hall, AMCHSS, SCTIMST

SL. No.	Member Name	Highest Degree	Gender	Scientific /Non Scientific	Affiliation with Institution(s)
1.	Dr. Harikrishnan S	MD, DM (Cardiology) DNB (Cardiology)	Male	Clinician	Yes
2.	Dr. Kala Kesavan. P	MBBS, MD	Female	Basic Medical Scientist	No
3.	Smt. Sathi Nair	MA (English Literature)	Female	Lay Person	No
4.	Dr. Christina George	MD Psychiatry	Female	Clinician	No
5.	Dr. Mala Ramanathan	PhD	Female	Social Scientist (Member Secretary)	Yes

IEC Decision

The IEC approved the conduct of the study in the present form.

Remarks:
The Institutional Ethics Committee expects to be informed about the progress of the study, any SAE occurring in the course of the study, any changes in the protocol and patient information/informed consent and asks to be provided a copy of the final report.

There was no member of the study team who participated in voting / decision making process. The ethics committee is organized and operated according to the requirements of Good Clinical Practice and the requirements of the Indian Council of Medical Research (ICMR).

Sincerely,

Mala Ramanathan
Member Secretary, IEC

PLAGIARISM CERTIFICATE

Curiginal

Entire Document

1 INTRODUCTION The blood units collected from the blood donors contain all the different constituents of human blood. In modern transfusion medicine, the aim is to transfuse only the required component to the patient and this is achieved by whole blood (WB) processing and component separation.(1) Component preparation allows transfusing only the specific blood component to the patient. This will also allow optimal use of blood inventory. From a unit of WB, we can prepare red cell concentrates (RCC), fresh frozen plasma (FFP), platelet concentrate (PC) and cryoprecipitate depending upon the separation method applied. The common goal of component preparation is the constant preparation of RCC, FFP, and PC containing the maximum amount of therapeutic blood elements and minimum amount of unwanted residual cells. A variety of component separation methods have been developed to choose and implement the method of choice. The conventional centrifugation method is usually employed to prepare blood components from the WB collected into anticoagulant solutions. The specific gravity of red cells and granulocytes are quite similar so centrifugation is not an efficient method to separate the unwanted residual cells from RCCs. Hence the blood components can be subjected to further processing steps like leucoreduction or irradiation. Leucoreduction is the

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PROFORMA

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY

DEPARTMENT OF TRANSFUSION MEDICINE

QUALITY CONTROL OF LEUCOREDUCED RED CELL PREPARATIONS

PRODUCT/ BAG: **SEQ NO:**

UNIT NO: **DATE OF COLLECTION:**

DATE OF PROCEDURE:

PROCEDURE PERFORMED:

QUALITY PARAMETERS	PRE-PROCEDURE	POST-PROCEDURE
VOLUME:		
APPEARANCE		
HAEMOGLOBIN		
HAEMATOCRIT		
RED CELL COUNT		
WBC COUNT		
PLASMA HAEMOGLOBIN		
K+ LEVELS		
CULTURE		

% LEUCOREDUCTION:

% RED CELL RECOVERY:

%HAEMOLYSIS:

1= Pre-procedure value; 2= Post procedure value 1. Red cell Washing	BAG type	Unit Age	volume1 (ml)	volume2 (ml)	Absolute leucocyte count 1 (10*6)	Absolute leucocyte count 2 (10*6)	Leucoreduction (%)	Absolute Red cell count- 1 (10*9)	Absolute Red cell count- 2 (10*9)	RBC recovery (%)	Red cell Loss (%)
1	350	32	203	187	1422	112	92.12	1424	1344	94.38	5.62
2	350	32	211	207	1374	207	84.95	1547	1416	91.55	8.45
3	350	31	256	225	820	135	83.53	1732	1672	96.56	3.44
4	350	2	247	215	3384	1269	62.52	1638	1484	90.58	9.42
5	350	14	271	236	1842	425	76.93	1901	1619	85.18	14.82
6	350	33	239	250	1648	250	84.84	1617	1549	95.79	4.21
7	350	35	260	256	1091	179	83.57	1393	1306	93.81	6.19
8	350	35	261	301	1565	120	92.3	1557	1415	90.88	9.12
9	350	35	208	230	1059	92	91.33	1477	1327	89.89	10.11
10	350	1	269	289	1533	347	77.37	1479	1422	96.15	3.85
11	350	4	213	202	1258	202	83.92	1300	1092	83.97	16.03
12	450	33	293	281	1376	169	87.75	2108	1890	89.67	10.33
13	450	14	299	316	2603	663	74.53	2298	2137	93.00	7.00
14	450	35	276	290	1658	290	82.51	2036	1958	96.14	3.86
15	450	32	295	313	1532	282	81.62	1974	1771	89.72	10.28
16	450	17	288	309	1816	309	82.97	2043	1893	92.62	7.38

Haemoglobin content in bag -1 (g)	Haemoglobin content in bag -2 (g)	Haematocrit-1 (%)	Haematocrit-2 (%)	Plasma Haemoglobin - 1 (g/dl)	Plasma Haemoglobin - 2 (g/dl)	Haemolysis -1 (%)	Haemolysis-2 (%)	Potassium - 1 (mmol/L)	Potassium - 2 (mmol/L)	Culture
43	42	62.5	67.1	0.69	0.32	0.91	0.46	55	1.86	Sterile
46	41	64.7	58.2	1.14	0.23	0.80	0.48	54.4	1.23	Sterile
51	49	60.1	63.9	0.37	0.12	0.75	0.20	42.8	0.6	Sterile
48	41	56.6	89.4	0.07	0.06	0.16	0.03	6.4	0.3	Sterile
57	47	61.6	58.2	0.12	0.04	0.22	0.08	26	3.1	Sterile
51	54	63.9	62.6	0.4	0.19	0.68	0.33	53	1.73	Sterile
43	47	51.4	54.5	0.28	0.14	0.82	0.35	57	1.64	Sterile
45	49	54.1	51.3	0.31	0.11	0.83	0.33	52.2	0.9	Sterile
41	37	60.2	48.1	0.22	0.13	0.44	0.42	50.3	1.2	Sterile
44	47	48.6	49.1	0.02	0.02	0.06	0.06	7.2	0.4	Sterile
43	38	61	56.9	0.02	0.02	0.04	0.05	8.2	2.8	Sterile
64	56	66	60	0.47	0.08	0.74	0.16	64	0.5	Sterile
71	67	68.1	60.6	0.19	0.04	0.26	0.07	32.7	3.4	Sterile
63	61	68	61.7	0.44	0.2	0.61	0.37	48.1	1.6	Sterile
57	53	56.8	48.7	0.35	0.16	0.78	0.49	47.6	2.1	Sterile
59	57	59.9	53.1	0.09	0.03	0.18	0.08	9.1	1.40	Sterile

1= Pre-procedure value; 2= Post procedure value 2. Buffy coat reduction	BAG type	Unit Age	volume1 (ml)	volume2 (ml)	Absolute leucocyte count 1 (10*6)	Absolute leucocyte count 2 (10*6)	Leucoreduction (%)	Absolute Red cell count- 1 (10*9)	Absolute Red cell count- 2 (10*9)	RBC recovery (%)	Red cell Loss (%)
1	450	0	462	292	3237	730	77.45	2104	1916	91.03	8.97
2	450	0	472	306	3068	520	83.07	2322	2167	93.31	6.69
3	450	0	464	275	2786	824	70.42	2122	1789	84.28	15.72
4	450	0	450	274	2341	548	76.6	2296	1955	85.17	14.83
5	450	0	504	289	1664	202	87.85	2184	1958	89.67	10.33
6	450	0	464	306	3111	1284	58.75	2192	1983	90.49	9.51
7	450	0	443	292	2173	641	70.48	2173	1974	90.84	9.16
8	450	0	470	286	2256	1030	54.37	2106	1976	93.83	6.17
9	450	0	448	273	3227	928	71.25	2264	1867	82.46	17.54
10	450	0	456	283	2051	651	68.25	2183	1943	88.97	11.03
11	450	0	486	321	2674	932	65.14	2256	2244	99.47	0.53
12	450	0	425	279	3616	1198	66.88	1817	1640	90.3	9.70
13	450	0	455	277	3412	913	73.24	1983	1751	88.29	11.71
14	450	0	437	288	2097	230	89.02	2023	1880	92.93	7.07
15	450	0	445	294	2450	618	74.76	2093	1999	95.49	4.51
16	450	0	481	336	4237	1178	72.21	2258	2022	89.54	10.46
17	450	0	453	273	2220	273	87.71	1989	1766	88.79	11.21
18	450	0	448	287	3317	1062	68	1959	1799	91.84	8.16
19	450	0	462	276	2312	303	86.89	1915	1709	89.27	10.73
20	450	0	446	278	2009	527	73.74	2053	1904	92.74	7.26
21	450	0	442	276	2650	276	89.59	1956	1798	91.89	8.11
22	450	0	406	290	2676	637	76.18	2040	1961	96.16	3.84
23	450	0	463	291	3012	203	93.25	2155	2026	94.01	5.99
24	450	0	456	296	2644	533	79.83	2047	1938	94.67	5.33

Haemoglobin content in bag -1 (g)	Haemoglobin content in bag -2 (g)	Haematocrit-1 (%)	Haematocrit-2 (%)	Plasma Haemoglobin - 1 (g/dl)	Plasma Haemoglobin - 2 (g/dl)	Haemolysis -1 (%)	Haemolysis-2 (%)	Potassium - 1 (mmol/L)	Potassium - 2 (mmol/L)	Culture
60	56	39	56.8	0.01	0	0.05	0.00	3.6	0.7	Sterile
65	62	41.6	60.8	0.03	0.01	0.13	0.02	3.2	0.7	Sterile
60	52	39.3	56.1	0.03	0.01	0.14	0.02	3.12	0.6	Sterile
63	54	41.9	58.8	0.04	0.01	0.16	0.02	3.4	0.7	Sterile
64	59	36.9	58.1	0.04	0.01	0.20	0.02	3.32	0.7	Sterile
64	59	41.5	57.7	0.05	0.03	0.21	0.07	2.9	0.7	Sterile
56	53	39.5	55.1	0.02	0	0.10	0.00	3.3	0.5	Sterile
59	57	38.9	60.6	0.02	0.01	0.10	0.02	3.5	0.7	Sterile
60	50	41	55.7	0.03	0.02	0.13	0.05	3.2	0.8	Sterile
57	52	37.9	55.5	0.02	0.01	0.10	0.02	3.5	0.8	Sterile
63	65	39.9	60.3	0.02	0.01	0.09	0.02	3.16	0.6	Sterile
55	52	39.5	54.7	0.04	0.02	0.19	0.05	3.12	0.7	Sterile
57	51	37.9	55.0	0.03	0.01	0.15	0.02	3.6	0.7	Sterile
61	57	40.7	58.4	0.02	0.01	0.09	0.02	3.4	0.8	Sterile
59	61	39.3	60.7	0.02	0.01	0.09	0.02	3.17	0.6	Sterile
66	68	40.8	59.5	0.03	0.01	0.13	0.02	3.14	0.7	Sterile
57	53	37.3	55.1	0.04	0.01	0.20	0.02	3.1	0.6	Sterile
58	55	39.1	56.1	0.02	0.01	0.09	0.02	3.4	0.6	Sterile
58	54	38	57.4	0.02	0.01	0.10	0.02	3.6	0.7	Sterile
57	53	38.1	57.3	0.05	0.02	0.24	0.04	3.45	0.6	Sterile
57	54	38.4	57.7	0.03	0.01	0.14	0.02	3.6	0.7	Sterile
55	56	40.9	57.8	0.04	0.02	0.18	0.04	3.4	0.7	Sterile
61	60	40	59.4	0.04	0.01	0.18	0.02	3.5	0.6	Sterile
61	60	39.4	57.7	0.09	0.03	0.41	0.06	3.42	0.7	Sterile

1= Pre-procedure value; 2= Post procedure value	BAG type	Unit Age	volume1 (ml)	volume2 (ml)	Absolute leucocyte count 1 (10*6)	Absolute leucocyte count 2 (10*6)	Leucoreduction (%)	Absolute Red cell count- 1 (10*9)	Absolute Red cell count- 2 (10*9)	RBC recovery (%)	Red cell Loss (%)
25	450	0	451	301	2481	451	81.81	2102	1899	90.33	9.67
26	450	0	469	291	3753	349	90.71	2130	1939	91.02	8.98
27	450	0	462	288	2590	604	76.66	2026	1877	92.65	7.35
28	450	0	458	335	3067	1305	57.45	2302	2272	98.67	1.33
29	450	0	450	292	2926	437	85.05	2260	2132	94.33	5.67
30	450	0	457	287	2695	344	87.22	1932	1810	93.7	6.30
31	450	0	481	309	3178	588	81.5	2297	2110	91.86	8.14
32	450	0	461	299	2579	508	80.29	2156	2061	95.59	4.41
33	350	0	358	231	2148	231	89.25	1701	1512	88.91	11.09
34	350	0	371	237	1819	190	89.56	1734	1569	90.49	9.51
35	350	0	368	233	2903	791	72.75	1602	1454	90.77	9.23
36	350	0	349	224	2132	269	87.37	1719	1563	90.92	9.08
37	350	0	347	217	2010	195	90.29	1650	1444	87.52	12.48
38	350	0	357	235	2321	328	85.85	1789	1485	83	17.00
39	350	0	352	216	1409	324	77.02	1635	1356	82.93	17.07
40	350	0	364	225	2073	541	73.93	1720	1480	86.01	13.99
41	350	0	351	224	2038	628	69.18	1662	1469	88.4	11.60
42	350	0	365	204	2152	102	95.27	1692	1341	79.23	20.77
43	350	0	354	228	1877	205	89.07	1668	1505	90.21	9.79
44	350	0	356	222	2849	1068	62.53	1638	1468	89.61	10.39
45	350	0	374	230	2544	851	66.57	1785	1575	88.24	11.76
46	350	0	366	233	2157	349	83.82	1729	1566	90.56	9.44
47	350	0	373	291	2053	291	85.84	1803	1680	93.2	6.80
48	350	0	359	238	1723	71	95.85	1734	1625	93.74	6.26

Haemoglobin content in bag -1 (g)	Haemoglobin content in bag -2 (g)	Haematocrit-1 (%)	Haematocrit-2 (%)	Plasma Haemoglobin - 1 (g/dl)	Plasma Haemoglobin - 2 (g/dl)	Haemolysis -1 (%)	Haemolysis-2 (%)	Potassium - 1 (mmol/L)	Potassium - 2 (mmol/L)	Culture
61	56	40.7	55.0	0.04	0.01	0.18	0.02	3.3	0.8	Sterile
61	56	39.4	57.5	0.03	0.01	0.14	0.02	3.48	0.6	Sterile
58	56	38	57.1	0.04	0.01	0.20	0.02	3.53	0.7	Sterile
65	74	43.1	66.7	0.02	0	0.08	0.00	3.4	0.5	Sterile
59	58	40	58.6	0.03	0.01	0.14	0.02	3.5	0.6	Sterile
58	56	37.4	56.4	0.04	0.03	0.20	0.07	3.6	0.8	Sterile
65	64	40.2	58.1	0.02	0.00	0.09	0.00	3.6	0.7	Sterile
64	63	40.2	60.4	0.03	0.01	0.13	0.02	3.6	0.6	Sterile
51	47	41.9	59.0	0.02	0.02	0.08	0.04	3.12	0.7	Sterile
52	49	40.9	58.7	0.02	0.01	0.09	0.02	3.5	0.6	Sterile
48	45	38	54.6	0.04	0.01	0.19	0.02	3.16	0.6	Sterile
48	45	40.50	57.9	0.01	0.01	0.04	0.02	3.47	0.7	Sterile
44	43	37.60	57.6	0.03	0.02	0.15	0.04	3.50	0.60	Sterile
46	47	38.20	59.0	0.01	0.01	0.05	0.02	3.71	0.80	Sterile
44	38	37.9	51.3	0.04	0.01	0.20	0.03	3.4	0.6	Sterile
47	42	38.9	54.6	0.02	0.01	0.09	0.02	3.5	0.6	Sterile
47	43	41.3	57.5	0.03	0.01	0.13	0.02	3.44	0.7	Sterile
47	38	39	55.9	0.02	0.01	0.10	0.02	3.3	0.5	Sterile
49	46	41.5	58.5	0.04	0.01	0.17	0.02	3.3	0.7	Sterile
45	41	37.9	54.5	0.04	0.01	0.20	0.02	3.34	0.7	Sterile
48	43	38.9	56.3	0.03	0.02	0.14	0.05	3.6	0.7	Sterile
49	47	41.1	59.1	0.02	0	0.09	0.00	3.5	0.6	Sterile
50	57	39.5	56.8	0.02	0.01	0.09	0.02	3.46	0.6	Sterile
52	50	42.5	60.9	0.02	0.01	0.08	0.02	3.14	0.7	Sterile

1= Pre-procedure value; 2= Post procedure value	BAG type	Unit Age	volume1 (ml)	volume2 (ml)	Absolute leucocyte count 1 (10*6)	Absolute leucocyte count 2 (10*6)	Leucoreduction (%)	Absolute Red cell count- 1 (10*9)	Absolute Red cell count- 2 (10*9)	RBC recovery (%)	Red cell Loss (%)
3.(a). Leucofiltration in Buffy coat reduced RCCs											
1	450	1	313	264	438	0.13	99.97	2204	1858	84.30	15.70
2	450	4	310	240	838	0.12	99.99	2228	1698	76.22	23.78
3	450	6	279	225	306	0.05	99.98	1646	1322	80.33	19.67
4	450	9	252	199	76	0.00	100.00	1534	1157	75.39	24.61
5	450	2	319	269	701	0.07	99.99	2202	1844	83.72	16.28
6	450	4	284	236	199	0.06	99.97	1909	1559	81.66	18.34
7	450	2	292	244	729	0.12	99.98	1945	1703	87.54	12.46
8	450	9	287	234	947	0.11	99.99	2097	1666	79.43	20.57
9	450	5	309	257	711	0.19	99.97	2233	1799	80.55	19.45
10	450	4	329	279	230	0.13	99.94	2326	1953	83.98	16.02
11	450	4	308	260	247	0.05	99.98	2088	1782	85.36	14.64
12	450	4	296	252	1096	0.12	99.99	2083	1792	86.02	13.98
13	450	2	294	241	412	0.12	99.97	2293	1635	71.29	28.71
14	450	2	280	236	1093	0.17	99.98	2072	1743	84.11	15.89
15	350	2	232	184	556	0.04	99.99	1518	1283	84.53	15.47
16	350	2	233	182	163	0.04	99.97	1627	1274	78.31	21.69
17	350	8	201	166	40	0.00	100.00	1240	993	80.11	19.89
18	350	5	234	186	234	0.05	99.98	1507	1201	79.72	20.28
19	350	5	248	197	198	0.05	99.98	1632	1309	80.23	19.77
20	350	5	207	156	454	0.15	99.97	1312	1008	76.87	23.13
21	350	4	240	194	288	0.09	99.97	1612	1299	80.57	19.43
22	350	10	227	178	341	0.09	99.97	1542	1193	77.38	22.62
23	350	4	198	153	277	0.07	99.97	1224	926	75.61	24.39
24	350	10	207	168	228	0.04	99.98	1189	1045	87.87	12.13
25	350	8	225	175	135	0.00	100.00	1484	1131	76.18	23.82
26	350	2	224	178	292	0.09	99.97	1427	1101	77.18	22.82
27	350	6	219	177	66	0.00	100.00	1531	1231	80.42	19.58

Haemoglobin content in bag -1 (g)	Haemoglobin content in bag -2 (g)	Haematocrit-1 (%)	Haematocrit-2 (%)	Plasma Haemoglobin - 1 (g/dl)	Plasma Haemoglobin - 2 (g/dl)	Haemolysis -1 (%)	Haemolysis-2 (%)	Potassium - 1 (mmol/L)	Potassium - 2 (mmol/L)	Culture
64	53	60.1	60.1	0.03	0.01	0.06	0.02	4.1	4.3	Sterile
64	49	59.6	58.6	0.06	0.04	0.12	0.08	16.2	15.5	Sterile
55	44	57.1	56.8	0.04	0.04	0.09	0.09	18.4	19.3	Sterile
45	34	52.2	50	0.07	0.08	0.19	0.23	9.9	10.4	Sterile
64	54	59.2	59.1	0.03	0.02	0.06	0.04	10	10.3	Sterile
56	45	57.5	57	0.03	0.04	0.06	0.09	17.2	17.4	Sterile
54	47	54.6	57.7	0.03	0.03	0.07	0.07	11	11.5	Sterile
53	44	55.6	54.5	0.06	0.08	0.14	0.19	10.7	12	Sterile
62	50	59.4	58.1	0.04	0.03	0.08	0.06	17.9	18.8	Sterile
66	57	59.2	58.7	0.09	0.05	0.18	0.10	12.8	12.7	Sterile
60	52	56.7	58.1	0.04	0.04	0.09	0.08	12.1	13.1	Sterile
51	44	52.3	53.1	0.06	0.03	0.17	0.08	13.4	14.3	Sterile
66	47	65.8	57.6	0.03	0.04	0.05	0.09	11.6	12.7	Sterile
52	43	56	56	0.02	0.02	0.05	0.05	11.4	12.3	Sterile
39	33	50.8	54.3	0.02	0.02	0.06	0.05	9.4	9.6	Sterile
47	37	57.8	58	0.03	0.02	0.06	0.04	12.4	12.8	Sterile
40	32	56.1	54.7	0.05	0.05	0.11	0.12	16.5	21.5	Sterile
45	35	55.3	55.7	0.06	0.03	0.14	0.07	15	18.8	Sterile
50	40	56.5	57	0.08	0.07	0.17	0.15	17.8	21.1	Sterile
36	27	51.4	52.4	0.02	0.03	0.06	0.08	14.4	16.1	Sterile
46	38	56.8	57.5	0.07	0.08	0.16	0.17	11.7	13.4	Sterile
43	33	55.8	55.4	0.08	0.09	0.19	0.21	23.4	24.1	Sterile
36	28	53.1	51.9	0.02	0.03	0.05	0.08	15.2	16.1	Sterile
36	31	51	55.6	0.09	0.09	0.25	0.21	33.7	34.5	Sterile
42	32	53.7	52.4	0.06	0.06	0.15	0.16	26.9	27.8	Sterile
41	32	54.1	53.4	0.02	0.02	0.05	0.05	8	8.5	Sterile
42	34	55.9	56.2	0.03	0.09	0.07	0.21	17.3	18.2	Sterile

1= Pre-procedure value; 2= Post procedure value 3.(b). Leucofiltration in RCCs not Buffycoat reduced	BAG type	Unit Age	volume1 (ml)	volume2 (ml)	Absolute leucocyte count 1 (10*6)	Absolute leucocyte count 2 (10*6)	Leucoreduction (%)	Absolute Red cell count- 1 (10*9)	Absolute Red cell count- 2 (10*9)	RBC recovery (%)	Red cell Loss (%)
28	350	2	253	206	2279	0.10	100.00	1644	1347	81.93	18.07
29	350	2	287	237	3443	0.06	100.00	1667	1334	80.03	19.97
30	350	2	247	203	2344	0.15	99.99	1559	1308	83.89	16.11
31	350	2	266	227	1785	0.11	99.99	1766	1506	85.26	14.74
32	350	2	278	235	1832	0.17	99.99	1579	1335	84.51	15.49
33	350	2	290	246	1304	0.12	99.99	1779	1507	84.70	15.30
34	350	2	268	221	2521	0.16	99.99	1805	1502	83.21	16.79
35	350	2	298	249	2981	0.24	99.99	1574	1390	88.28	11.72
36	350	2	280	235	1963	0.11	99.99	2243	1724	76.87	23.13
37	350	2	257	210	2185	0.15	99.99	1848	1539	83.30	16.70
38	350	2	273	221	1228	0.11	99.99	1741	1401	80.44	19.56
39	350	2	275	228	2281	0.06	100.00	1885	1610	85.41	14.59
40	350	2	263	213	814	0.00	100.00	1739	1438	82.73	17.27
41	350	3	260	215	2156	0.16	99.99	1790	1515	84.66	15.34
42	350	3	255	211	2807	0.15	99.99	1656	1434	86.61	13.39
43	350	2	267	223	2299	0.16	99.99	1700	1432	84.22	15.78
44	350	2	275	227	3050	0.16	99.99	2006	1712	85.37	14.63
45	350	2	290	240	2984	0.12	100.00	1898	1602	84.42	15.58
46	350	2	278	231	2359	0.11	100.00	1643	1367	83.16	16.84
47	350	3	270	227	2404	0.16	99.99	1861	1567	84.21	15.79
48	350	3	272	221	2013	0.11	99.99	1920	1624	84.56	15.44

Haemoglobin content in bag -1 (g)	Haemoglobin content in bag -2 (g)	Haematocrit-1 (%)	Haematocrit-2 (%)	Plasma Haemoglobin - 1 (g/dl)	Plasma Haemoglobin - 2 (g/dl)	Haemolysis -1 (%)	Haemolysis-2 (%)	Potassium - 1 (mmol/L)	Potassium - 2 (mmol/L)	Culture
50	41	56.6	57.8	0.02	0.02	0.04	0.04	12.7	13	Sterile
53	43	54	52.5	0.02	0.02	0.05	0.05	9.1	9.9	Sterile
46	38	54.2	55.6	0.05	0.02	0.12	0.05	10.2	10.7	Sterile
57	49	61.5	61.7	0.04	0.03	0.07	0.05	10.4	12.9	Sterile
48	41	50.8	51.3	0.03	0.03	0.09	0.08	6.7	7	Sterile
52	44	52.3	52.6	0.02	0.02	0.05	0.05	7	7	Sterile
56	46	59.4	60.4	0.05	0.05	0.10	0.09	8.5	8.8	Sterile
46	46	46.2	54.9	0.04	0.04	0.14	0.10	8.7	9.3	Sterile
61	47	62.8	57.6	0.02	0.02	0.03	0.04	7.1	7.9	Sterile
51	41	57.9	58.9	0.04	0.04	0.09	0.08	13.6	14.2	Sterile
55	42	55.4	55.1	0.02	0.05	0.04	0.12	10.1	10.9	Sterile
54	47	57.8	60.1	0.02	0.02	0.04	0.04	9.2	9.6	Sterile
49	41	54.5	55.9	0.02	0.02	0.05	0.05	8.5	8.6	Sterile
53	44	59.2	60.9	0.04	0.04	0.08	0.08	10	10.6	Sterile
47	41	53.2	57.1	0.02	0.08	0.05	0.18	11	11.5	Sterile
51	44	56	56.9	0.03	0.03	0.07	0.07	6.7	6.9	Sterile
52	45	56.9	58.9	0.05	0.04	0.11	0.08	8	8.7	Sterile
57	49	57.1	58.2	0.03	0.02	0.07	0.04	7.2	7.4	Sterile
49	42	51.8	51.9	0.02	0.03	0.06	0.08	10.6	10.5	Sterile
53	44	56.2	56.5	0.03	0.03	0.07	0.07	8.8	9.3	Sterile
55	46	57.6	59.9	0.08	0.09	0.17	0.17	7.7	12	Sterile