A COMPARATIVE ASSESSMENT OF QUALITY PARAMETERS OF PLATELET CONCENTRATES PREPARED BY DIFFERENT METHODOLOGIES

By

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DISSERTATION SUBMITTED TO THE SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH, KOLAR, KARNATAKA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF MEDICINE
IN
PATHOLOGY

Under the Guidance of

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LIST OF ABBREVATIONS

AABP – American Association of Blood Banks

ABG – Arterial Blood Gas

ACD – Adenine Citrate Dextrose

ATP – Adenine Triphosphate

BC-PC – Buffy Coat-platelet concentrates

BCSH – British Committee for Standards in Hematology

CBC – Complete Blood Count

CCI – Corrected Count Increment

CI – Confidence Interval

CK – Cytokeratin

CPD – Citrate Phosphate Dextrose

CPDA – Citrate Phosphate Dextrose Adenine

DGHS – Directorate General Health Services

DIC – Disseminanted Intravascular Coagulation

DNA – Deoxyriboneucleic acid

FNHTR – Febrile Non-Hemolytic Transfusion Reactions

Gp – Glycoprotein

GVHD – Graft Versus Host Disease

HUS – Hemolytic Uremic Syndrome

ITP – Idiopathic Thrombocytopenic Purpura

MEP – Myeloid Erythroid Progenitor

MPV – Mean Platelet Volume

PC – Platelet Concentrate

PDW – Platelet Distribution Width

PLCR – Platelet to Large Cell Ratio

PRP-PC – Platelet rich Plasma –Platelet concentrates

PSL – Platelet Storage Lesions

RBC – Red Blood Cell

RDP – Random Donor Platelet

SAGM – Saline Adenine Glucose Mannitol

SDP – Single Donor Platelets

SOP – Standard Operating Procedures

TCP – Thrombocytopenia

TPO – Thrombopoietin

TTI – Transfusion Transmitted Infections

TTP – Thrombotic Thrombocytopenic Purpura

VWF – von Willibrand Factor

WBC – White Blood Cell

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ABSTRACT

A Comparative Assessment of Quality Parameters of Platelet Concentrates prepared by Different Methodologies

Background:

To ensure a safe and effective transfusion, the responsibility of blood banks has increased so as to produce and provide blood components which are of good quality. Platelet Count less than 1.5 lakh/cumm is thrombocytopenia and platelet transfusion is required when platelet count is less than 20,000/cumm which causes spontaneous bleeding.

There are two types of platelet concentrates available for transfusions: Random Donor Platelets [RDP] which are prepared either by Platelet Rich Plasma-Platelet Concentrate [PRP-PC] or Buffy Coat-Platelet Concentrates [BC-PC] method and Single Donor Platelet [SDP] collected by plateletpheresis with the help of an automated cell separator.

In the past decade, there was a huge demand for SDPs due to its various advantages over the RDPs. But, in developing countries the use of RDPs are still on higher side than SDP units. Both RDP and SDP platelet concentrates have their advantages and disadvantages. During storage period there are various changes which occur in the platelet morphology affecting the final yield therapeutically.

Hence, the present study was done to compare various quality parameters of PC prepared by PRP method, BC method and apheresis method so as to establish an optimum quality standard as compared to standard DGHS in India[6] in order to provide maximum therapeutic benefit to patients.

Objectives:

1. To prepare platelet concentrates by platelet rich plasma method, buffy coat method and apheresis method.
2. To assess and compare quality parameters of platelet concentrates prepared by platelet rich plasma, buffy coat and apheresis method.

Materials and Methods:

A two year study was conducted in R.L.Jalappa Blood Bank, Sri Devaraj Urs medical college, Kolar, Karnataka. A total 156 platelet concentrates prepared by PRP-PC, BC-PC and SDP units were studied to analyze the various quality parameters that include volume, pH, swirling, WBC count/bag, platelet count/bag, platelet indices [MPV, PDW, P-LCR] and ABG analysis [pH, pCO_2, $pHOC^{3-}$] to assess the morphological changes of platelets during storage and to analyze which platelet concentrate is better to be utilized clinically.

Results:

Out of 156 platelet concentrates, 52 each of PRP-PC, BC-PC and SDP were analyzed. Among the 156 platelet concentrates scored as per DGHS criteria, a score of 5 was obtained for 34.61% [18/52], 30.76% [16/52] and 11.5% [6/52] SDP, BC-PC and PRP-PC units respectively. The platelet indices [MPV, PDW, PLCR] measured for each of platelet concentrates, majority of SDP units had maintained within the normal range followed by BC-PC with a minor difference and the least was the PRP-PC. Similarly ABG analysis showed a drop in HCO^{3-} levels and an increase in pCO_2 levels in majority of PRP-PC units which in turn showed a decrease in pH.

Conclusion:

BC-PC units were comparable with SDP units in terms of scoring system as per DGHS criteria. But PRP-PC showed a lesser scoring which requires more standardization of procedure and storage. The assessment of platelet indices and ABG analysis of platelet concentrates, SDP and BC-PC showed to have a better morphology maintained than PRP-PC units. Hence, in our study, SDP was superior to BC-PC and PRP-PC but with improvement and more standardization of procedures and storage, BC-PC units can give a yield equal to that of SDP units.

Keywords: Platelet rich plasma-platelet concentrate, Buffy coat-platelet concentrates, single donor platelets, apheresis, quality parameters, platelet indices, ABG analysis.

INTRODUCTION

INTRODUCTION

Platelet count less than 1.5 lakh/cumm is defined as thrombocytopenia [TCP]. Petechiae, menorrhagia and bruising are seen when platelet count is less than 50,000/cumm, while spontaneous bleeding is seen when platelet count is less than 20,000/cumm requiring platelet transfusion[1]. Thrombocytopenia could be due to defect in platelet function [Qualitative], decreased platelet count as seen in haemato-oncology cases, infection or chemotherapy [Quantitative][1,2].

Until 1969, only whole blood was transfused, after which the transfusion of allogenic blood was introduced by separating whole blood into various components like RBCs, WBCs, platelets and plasma[3]. Since then there has been increased utility of blood components, in particular, platelet concentrates [PC] in modern medical practice to prevent and treat patients with thrombocytopenic bleeding due to various causes. There are two types of platelet concentrates available for transfusions:

- Random Donor Platelets [RDP] which are prepared either by Platelet Rich Plasma-Platelet Concentrate [PRP-PC] or Buffy Coat-Platelet Concentrates [BC-PC] method
- Single Donor Platelet [SDP] collected by plateletpheresis with the help of an automated cell separator[4]

In late 1980s, there was a decline in use of most of blood components but use of platelet component showed a rapid growth. During 1990s, there was a decline in number of platelets transfused as use of SDPs increased. As a result, the percentage of platelet transfused collected by plateletpheresis grew from 28% in 1989 to 49% in 1992[5].

In the past decade, there was a huge demand for SDPs due to its various advantages over the RDPs. But, in most of the developing countries, RDPs have not lost its importance as number of platelets in each unit by either method has doubled as a result of improvement in quality of platelet concentrate.

Though SDPs are more advantageous than RDPs, it has certain drawbacks like higher establishment charges and requires greater medical and technical skills. Apart from these, there is a risk of citrate toxicity among donors and allergic reactions among recipients. Hence, the use of BC-PC is comparatively more than SDP at various centres.

In either method of PC preparations, there is an alteration in platelet morphology and function which occurs during collection and processing to storage affecting therapeutic benefits to recipients[5]. As a result, quality parameters play an important role in assessing benefit of each PC unit.

In-vitro platelet quality is assessed by pH, swirling, volume, platelet count/bag and WBC count/bag and by various biochemical parameters. Platelet indices like mean Platelet Volume [MPV], Platelet Distribution Width [PDW] and Platelet Large Cell Ratio [PLCR] helps in assessing morphology and thus function of platelet in PC units prepared by different methods.

In view of all these, this study was done to compare various quality parameters of PC prepared by PRP method, BC method and apheresis method so as to establish an optimum quality standard as compared to standard DGHS in India[6] in order to provide maximum therapeutic benefit to patients.

AIMS & OBJECTIVES

OBJECTIVES

1. To prepare platelet concentrates by platelet rich plasma method, buffy coat method and apheresis method.

2. To assess and compare quality parameters of platelet concentrates prepared by platelet rich plasma, buffy coat and apheresis method.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Platelets are non-nucleated fragments, disc shaped and are derived from cytoplasm of megakaryocytes, the precursors in the bone marrow. These platelets circulate in the peripheral blood for a period of 7-10 days, after which non-viable and aged platelets are removed by spleen[1].

Origin of platelets:

Hewson[7] was the first to describe the small undefined particles in blood after the presentation at 'Royal Society of London' by Leewenhoeck[8]. A French histologist, Alfred Donne named it as small globular, pale corpuscles in 1842[9]. Baele, in 1850, described these as particles of germinal matter and in 1860, these were described as small corpuscles by Zimmermann[10].

In 1882, Guilio Bizzozzero was the first to state that the third morphological particle in blood were these small corpuscles and gave a precise description[11] and named these elements as 'small plates' which were later called as 'platelets'[12]. Bizzozzero described the adhesive property of platelets to damaged vasculature and their role in hemostasis[13].

Megakaryopoiesis:

Platelets are produced by proliferation and maturation of megakaryocytes in the bone marrow. The precursor cells arise from bipotential myeloid-erythroid progenitor cell [MEP] that is committed for development of the megakaryocyte. Thrombopoietin [TPO] is the major humoral factor that regulates both megakaryopoiesis and thrombopoiesis[14].

Stages of megakaryocyte development:

Megakaryocyte differentiation results due to stimulation of progenitor cell receptors by TPO and other growth factors. Megakaryoblast is earliest cell identified cytochemically by expression of Gp IIb/IIIa or platelet peroxidase. These undergo nuclear maturation first and are completed before maturation of cytoplasm begins

unlike other marrow lineages. A process where DNA content doubles called 'Endomitosis' takes place once nuclear maturation is complete[15].

As a result of repeated endomitosis, cells become polypoid within a single nuclear envelope. These polypoid cells contain DNA which range from 4N to 64N. This process of endomitosis completes at the end of stage II megakaryocyte from where cytoplasmic maturation begins [Figure 1].

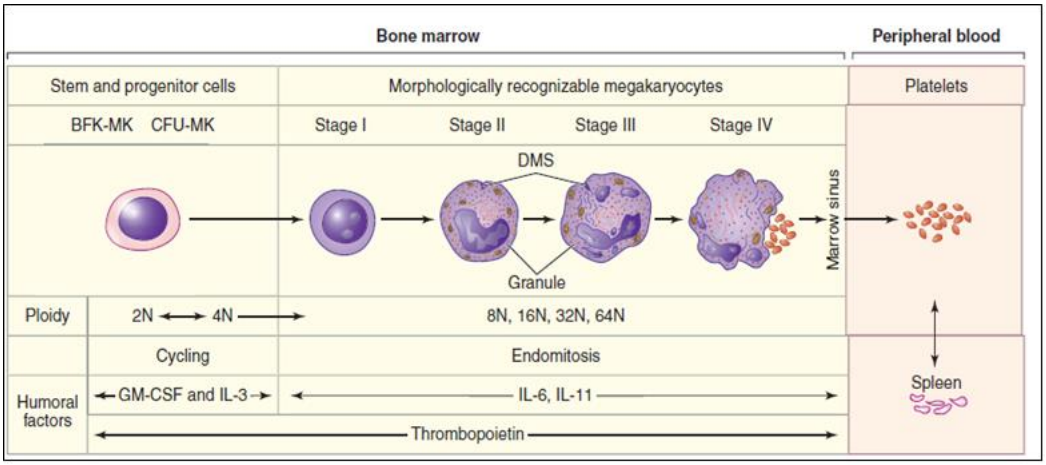

Figure 1: Stages of Megakaryopoiesis

As megakaryocyte become significantly larger, they are morphologically recognized at 8N stage. In adults, 16N stage is common ploidy stage identified. The megakaryocyte stages are divided into 4 morphologically recognizable stages which are differentiated mainly by quantity and characteristics of cytoplasm, lobulation and chromatin pattern of nucleus [Figure 2a and 2b].

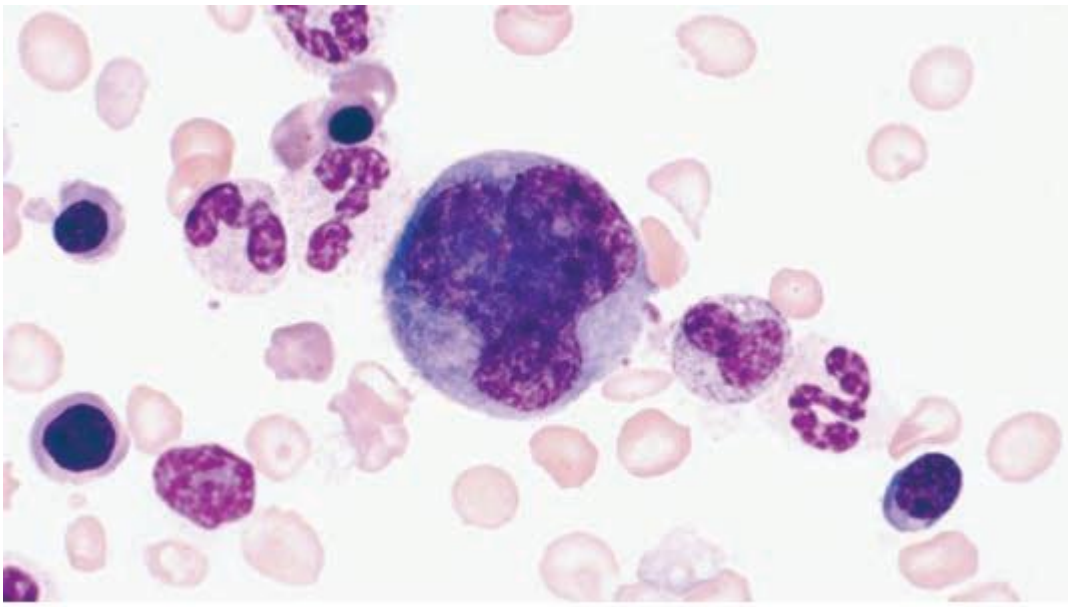

Figure 2a: Early Stage megakaryocyte

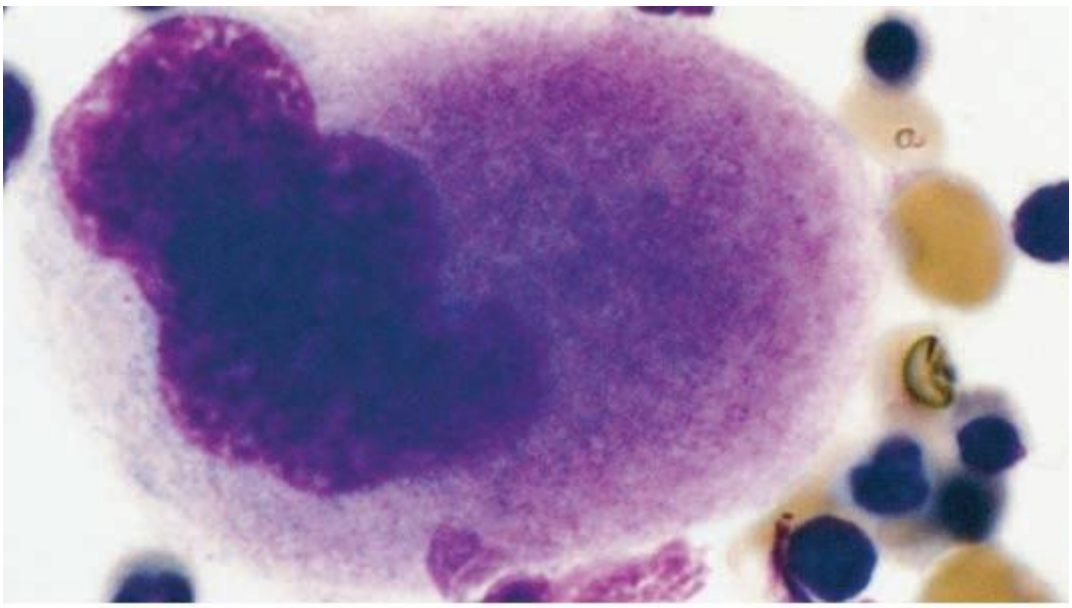

Figure 2b: Mature megakaryocyte

Thrombopoiesis:

Release of platelets as cytoplasmic fragments from megakaryocytes into marrow sinuses is thrombopoiesis. Depending on ploidy of megakaryocyte, it is estimated to produce 1000 to 3000 platelets.

Proplatelets are released as a result of megakaryocyte membrane extensions between endothelial cells in sinuses. These proplatelets break into individual platelets and nucleus of megakaryocyte is engulfed by macrophages in marrow [Figure 3]

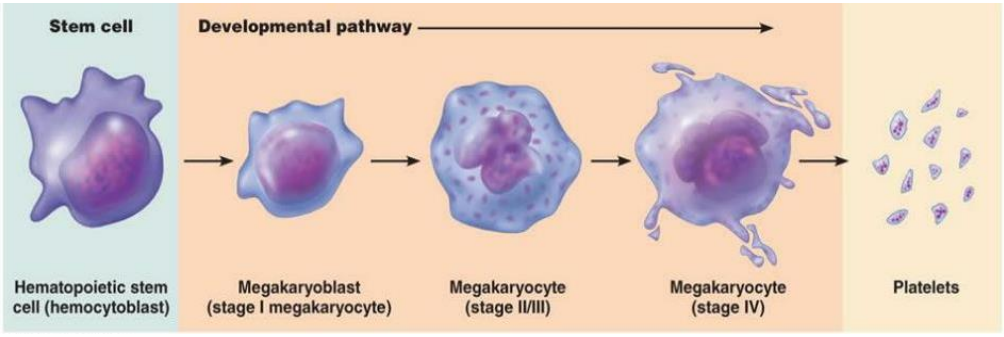

Figure 3: Thrombopoiesis

Structure of platelets:

Platelets in circulation and resting are biconvex, though they appear to be disc shaped due to effect of EDTA[16].Platelets are activated due to various morphological and biochemical changes that occur after injury to form primary haemostatic plug.

The ultrastructure of platelets is important to understand the activation process, which is divided into 4 zones: [Figure 4]

1. **Peripheral zone:**

   This zone consists of a phospholipid membrane that is covered by a surface coat exteriorly and by a thin submembranous region interiorly. The surface coat is thick and consists of glycolipids, membrane glycoproteins, proteins, mucopolysaccharides and adsorbed plasma proteins including coagulation factor V, VWF and fibrinogen. The submembranous region contains microfilaments.

   The cytoplasmic membrane contains receptors for stimuli that are involved in platelet function. Among these proteins Gp Ib, Gp IIb, Gp IIIa and Gp IX plays a major role in pathophysiology of platelets.

2. **Structural zone:**

The structural zone is composed of microtubules, which consists of protein tubulin, a submembranous network that consists of microfilaments, actin, intermediate filaments and actin binding protein.

The main functions of this zone are:

- Support and stabilize resting discoid shape
- Provide a means of change in shape when activated.

3. **Membrane systems:**

This is the fourth structural zone consisting of two membrane systems namely open canalicular system and dense tubular system.

4. **Organelle zone:**

The organelle zone lies beneath the layer of microtubes and consists of mitochondria, glycogen particles and granules dispersed in cytoplasm. These granules are of 4 types – dense bodies, α granules, lysosomal granules and peroxisomes which are storage sites for various substances necessary for regular platelet function

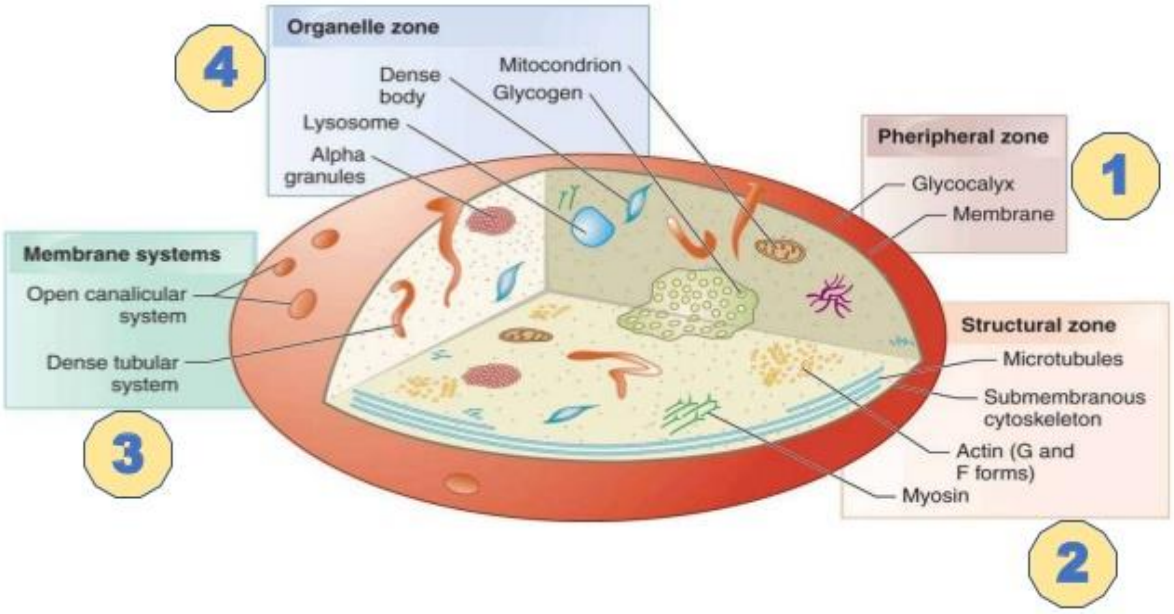

Figure 4: Structure of Platelets

Platelet function:

In 1882, the platelets role in haemostasis was established first by Bizzozzero where in his experiments on rabbits and guinea pigs' mesenteric vessels he noted platelet clumps as a part of thrombi[17].

Platelets are second most important component next to blood vessels, which play a major role in haemostatic system[1].

The role of platelets involved in haemostasis includes:

- Maintaining vascular integrity
- Formation of primary haemostatic plug
- Provides surface for fibrin
- Secondary haemostatic plug

- **Maintenance of vascular integrity:**

  On activation of platelets, granules containing cytokeratin[CK] and growth factors are released which help in stabilizing intercellular adhesion protein between endothelial cells of vessel wall[18].

- **Primary haemostatic plug:[Figure 5]**

  Activation of platelets is a response to change in normal environment as a result of injury to blood vessels. The primary haemostatic plug is result of activation of inactive platelets which require adhesion, aggregation and secretion to take place simultaneously[19].

  Platelet adhesion is the first step in formation of primary haemostatic plug where platelets get attached to subendothelial collagen.

  The collagen receptors GpIa/IIa and GpIV present on platelets help in initial interaction with VWF which slows platelets in circulation facilitating the subsequent interaction of platelets with subendothelial collagen, thus resulting in firm adhesion[20].

  GpVI binding to collagen during adhesion initiates intracellular signalling resulting in GpIIb/IIIa activation present on platelet membrane which later binds to soluble fibrinogen. As they develop high affinity, the platelets can functionally undergo platelet aggregation.

  Simultaneously platelets discharge granule contents that are a positive feedback mechanism causing activation of platelets. This in turn stimulates additional platelets and recruits platelets in multiple layers forming primary platelet plug.

- **Fibrin clot formation:**

  Fibrin is formed due to activation of coagulation cascade taking place via two pathways - extrinsic and intrinsic pathway. This activation cleaves inactive proenzyme to active thrombin which helps in converting fibrinogen into fibrin[1, 16, 21].

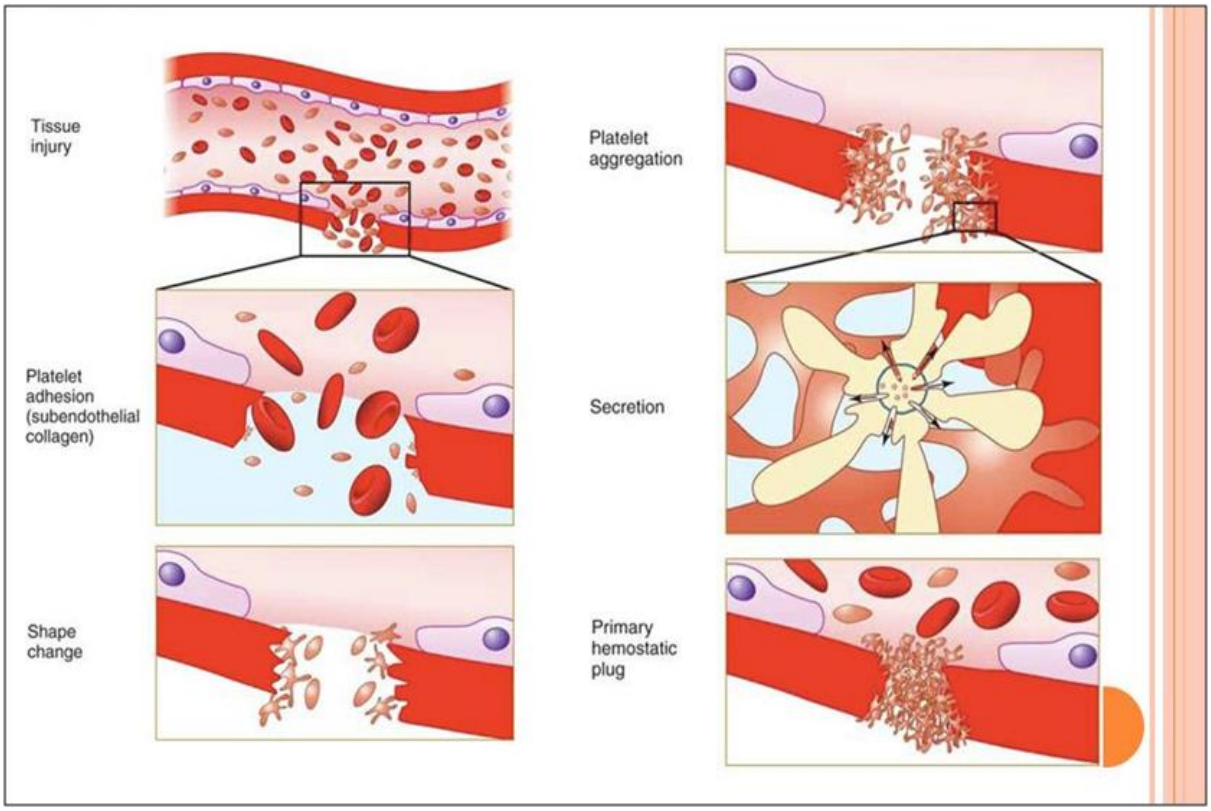

Figure 5: Schematic representation of primary hemostatic plug formation

Thrombocytopenia:

The normal reference range for platelets is 1.5 – 4.5lakh/µl and a platelet count of less than 1.5lakhs is considered as TCP[1].

Platelet count less than 1,00,000/µl does not show any clinical symptoms while platelet count less than 50,000/µl can have symptoms like petechiae, spontaneous bruising or menorrhagia. Severe TCP is considered when platelet count is less than 10,000/µl which is associated with clinical complications like spontaneous bleeding into CNS or from mucous membranes.

Causes of Thrombocytopenia:

TCP can be due to any of the following:

- Increased destruction:
  - Immune: Idiopathic Thrombocytopenia[ITP], Alloimmune TCP, drugs [heparin]
  - Non immune: Disseminated intravascular coagulation [DIC], Thrombotic thrombocytopenic purpura [TTP], Hemolytic uremic syndrome [HUS]
- Decreased production:
  - Megakatyocyte hyoplasia: Chemotherapy and Radiotherapy, Aplastic Anemia, Fanconi Anemia
  - Replacement of normal marrow: Leukemia, lymphoma, Myelodysplastic syndrome
  - Ineffective thrombopoiesis: Megaloblastic anemia
  - Inherited disorders: Wiskott-Aldrich syndrome, Bernard-Soulier, May-Hegglin anamoly
- Increased splenic sequestration
- Dilutional TCP
- Conditions with multiple mechanism of TCP

Thrombocytopenia and infection:

The most common cause of TCP is infection, accounting for nearly 50 to 75% of patients with bacterial, viral, protozoal infection[22].

Viral causes include dengue, Cytomegalovirus, Human Immunodeficiency virus, Herpes simplex virus, Parvo-B19, etc. The mechanism behind viral induced

TCP is by impairment of platelet productions due to invasion of virus into megakaryocytes, toxic effects on progenitor cells, haemophagocytosis [23, 24].

Bacterial causing TCP are – Leptospirosis, Gram Positive and Gram Negative septicaemia, Tuberculosis, Typhoid, Mycoplasma pneumonia [24,25].

Among patients infected by malaria, 80% of them presents with TCP. The mechanism is poorly understood, but it has been postulated as immune mediated destruction, ultrastructural changes in platelets, degree of parasitemia and sequestration in spleen[26].

Indications for platelet transfusions [27, 28, 29]:

- For patients with clinically significant bleeding:
    - TCP is a major contributing factor even when platelet count is more than 10,000/µl
    - In cases of critical bleeding where massive blood transfusion is needed and may reduce the risk of mortality
    - Patients requiring surgical intervention and are actively bleeding
    - In patients who are bleeding with TCP secondary to DIC
    - Infections like dengue, malaria where there is spontaneous bleeding
- Prophylactic transfusion –
    - Severe TCP due to Chemotherapy and Stem cell transplantation
    - In critically ill patients
    - Myelodysplasia, Aplastic anaemia
    - Patients undergoing invasive procedures like cardiac bypass, intracranial surgeries and patients with history of inherited or acquired platelet function disorders
    - In patients with head injury
    - In infections when platelet counts are less than 10,000 but no active bleeding or haemostatically stable patients.

History of blood transfusion:

In 1628, William Harvey discovered blood circulation, after which first animal-to-animal transfusion was done[30]. In mid-17th century, experiments on animal-to-man transfusions were performed[31]. It was in 1818, the first man-to-man

transfusion was done[32] and discovery of ABO blood group in 1900 by Landsteiner was basis for further development in transfusion medicine[33].

Though the discovery of platelets and their role in haemostasis was known by 1870, it was W.W. Duke in 1910 who first reported that transfused platelets showed increase in platelet count thus reversing risk of bleeding.

In 1969, Dr. Scott Murphy and Frank Gardner proved that platelets could be stored at 22±2°C with maintaining the haemostatic function for up to 3 days[4, 34].Since then, there has been an improvement in platelet component preparation and storage, by development in technical aspects, additive solution, storage conditions and improvement in techniques of apheresis[35].

Platelet components:

Platelet components are derived from either whole blood or by single donor apheresis. Platelet concentrates obtained from whole blood donations can be prepared by two methods, one by pooling PRP and other method is by buffy coat both of which uses different centrifugation steps. Among these two, buffy coat method of pooling PC is being widely used in Europe[36, 37].

SDP obtained by apheresis technique was first performed by Richard Lower in 1660 on dogs, following which plasmapheresis was first done in France in 1902[38,39].

Since then, there are various experiments and research going on in the field of hemapheresis for technical improvements and its uses therapeutically as well as prophylactically in various conditions.

Though overall properties and efficacy of PC prepared by different methods are similar, most of the centres in developed countries prefer apheresis – PC as it is safe, reduces donor exposure and gives better CCI when compared to whole blood derived PC[40].

These PC prepared by any method have to be stored at 22±2°c under continuous agitation for a maximum duration of 5 days and if pathogen reduction techniques are used these PC can be stored up to a maximum period of 7 days[41].

Quality control of platelet concentrate:

Blood banks are responsible to produce and provide blood components with good quality to ensure an effective transfusion. It is difficult to examine each unit for

its efficiency, stability, safety and sterility as preparation and quality control of components differ in many aspects.

Various platelet storage changes occur during collection, processing and storage effecting therapeutic benefit to patients. Hence, quality control, which is the most important parameter in assessing benefit of each unit of PC prepared by different methods, has to be performed regularly in order to provide maximum therapeutic benefit to patients.

The quality of PC can be accessed in-vitro by certain parameters which include pH, swirling, volume, platelet count/bag, WBC count/bag and also by certain biochemical parameters as per recommendations laid by various associations like American Association of Blood Banks [AABB], British Committee for Standards in Haematology [BCSH], Directorate General Health Services [DGHS] [6, 29, 42].

The following is the brief of the quality parameters that help in assessing the quality of PC:

- **Volume of PC:**

  RDPs or SDPs are stored in donor plasma that acts as a buffering agent to maintain pH. RDPs were suspended in 40 to 70 ml of plasma when PCs were stored in first generation PVC containers. This volume range was recommended as these containers had insufficient oxygen permeability. Since the advent of second-generation containers that are highly permeable to oxygen, plasma volume required is reduced and provides less volume load to recipients[43].

- **pH:**

  pH is one of the important parameter which determines morphological changes and viability of platelets in-vivo. During storage period, pH decreases because of increased platelet glycolysis. If pH falls below 6.8 and reaches a level of 6.0 in stored PC, volume increases accelerating disc to sphere transformation of platelets affecting viability[44].

- **Swirling:**

  Assessment of swirling and respective score was given by technician who was blinded for type of PC, which was performed by visual inspection. This is a simple and non-invasive procedure which is useful technique for assessing routine quality of individual PC.

Swirling is used to detect variation in shape of platelets. The absence of swirling indicates poor post-transfusion platelet count increment[45].

- **Platelet count/bag:**

  Platelet count also plays an important role in post-transfusion platelet increment. There is a deterioration seen in platelet count and function as a result of manipulation in platelet preparations and storage[46].

- **WBC count/bag:**

  There is fall in pH, increase in lactic acid production, consumption of glucose and release of LDH during storage as a result of presence of increase in WBC count/bag in PC. During 5 day storage, the PC having high WBC count/bag there was poor platelet morphology and function was affected which could be determined by loss of platelet nucleotides, decreased ability to incorporate H-adenosine and increased excretion of β-TG[47].

  Hence, WBC count/bag should be maintained as low as possible via leuko-reduction technique, which in turn helps in improvement of platelet increment and decreases various adverse effects which result due to presence of leukocytes[48, 49].

  Platelet indices that include MPV, PDW and PLCR aids in assessing morphology of platelets in stored PC. These parameters help in determining platelet storage lesions, which will affect post-transfusion in-vivo platelet recovery, survival and hemostatic function in patients resulting in platelet refractoriness[50]. These platelet indices can be used to assess quality of PC routinely as it is less time consuming and can be performed by an automated analyser.

The availability of safe platelets is becoming a constant challenge and can be accentuated at times of emergencies. The in-vitro and in-vivo quality of PC plays an important role in transfusion therapy and hence assessing quality of each unit helps in establishing optimum quality standards in order to provide maximum benefits to recipients.

MATERIALS & METHODS

MATERIALS AND METHODS

The study included 156 voluntary blood donors and replacement blood donors coming to R.L.Jalappa blood bank from December 2017 to October 2019 were randomly selected as per donor selection criteria and the donors with platelet count between 1,50,000 to 2,50,000/cumm and enrolled for the study. The platelet concentrates prepared by three different methods was analysed for the various quality parameters, making this a laboratory based comparative study.

Donor details such as age, sex, occupation, detailed medical history that includes habits, drug history, post medical illness and physical examination of donors for general health, blood investigations including complete blood count [CBC] and screening for transfusion-transmitted infections [TTI] were done as per the proforma. The donors were counselled and explained regarding the procedure of whole blood donation and apheresis; their possible adverse effects like giddiness, pain, and bruise and citrate toxicity in case of apheresis all of which were attended by a doctor posted at blood bank. If the donor was eligible and willing for procedure, a written informed consent was obtained from them.

The criteria for donor selection were:

- Age between 18 to 60 years
- Weight more than 45Kg for RDP and more than 60Kg for SDP
- Hemoglobin greater than 12.5g/dl
- Platelet count: $150x10^3/\mu l$ to $250x10^3/\mu l$

As per the criteria, donors were selected after completion of CBC and blood grouping. The vitals of donors were checked, phlebotomy site was examined to use correct method for arm disinfection to prevent bacterial contamination of PCs.

PCs were prepared from whole blood by PRP or BC method and from apheresis technique using automated cell separator. In apheresis donors, venous access was important due to its long procedure.

The quality of 156 PCs prepared from whole blood by PRP, BC method and by apheresis method was included in this study.

The different methods of component preparation are explained in detail:

1. **Whole blood derived PC by platelet rich plasma method:**

Whole blood was collected in 450 ml triple bags [HLL lifecar triple bag] containing 63 ml of Citrate Phosphate Dextrose Adenine[CPDA] anticoagulant [Figure 6], kept at room temperature (20-24^{o}C) and PRP-PC is prepared within 6 hours of collection. Platelet rich plasma was separated from whole blood by light spin centrifugation [Cryofuge 6000i centrifuge] at 1700rpm for 12 minutes at 22^{o}C, with acceleration and deceleration curves of 5 and 4 respectively. The platelets were concentrated by heavy spin centrifugation at 3400rpm for 10minutes at 22^{o}C with acceleration and deceleration curves of 9 and 5 respectively along with subsequent discarding of supernatant plasma [Figure 7]

Figure 6: Triple Bag

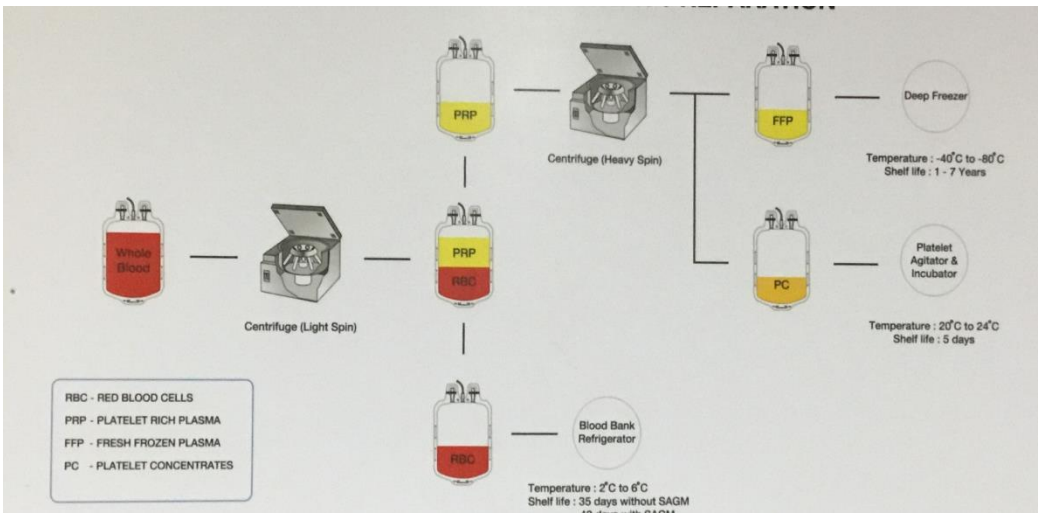

Figure 7: PRP method of Component Preparation

2. Whole blood derived PC by buffy coat method:

Whole blood was collected in 450 ml quadruple bag [HLL lifecar quadruple Bag [Figure 8], top & bottom] containing 63 ml of Citrate Phosphate Dextrose [CPD] anticoagulant, with additive solution (SAGM- Saline Adenine Glucose Mannitol). The whole blood was first subjected to hard spin centrifugation [Cryofuge 6000i centrifuge] at 3300 rpm for 9 minutes at 22°C with acceleration and deceleration curves of 9 and 4 respectively [Figure 9]. Whole blood was separated into different components [HLL Hi Care – CX component separator] according to their specific gravity [Figure 10]. Platelet poor supernatant was expressed into one satellite bag and buffy coat into another satellite bag. About 20-30 ml of plasma was returned to buffy coat. Then buffy coat was gently mixed with the plasma and again subjected to 'light spin' centrifugation at 900 rpm for 6 minutes at 22°C, with acceleration and deceleration curves of 5 and 4 respectively, along with one empty satellite bag. The supernatant platelet rich plasma was expressed into empty platelet storage bag and then the tubing was sealed [Figure11]

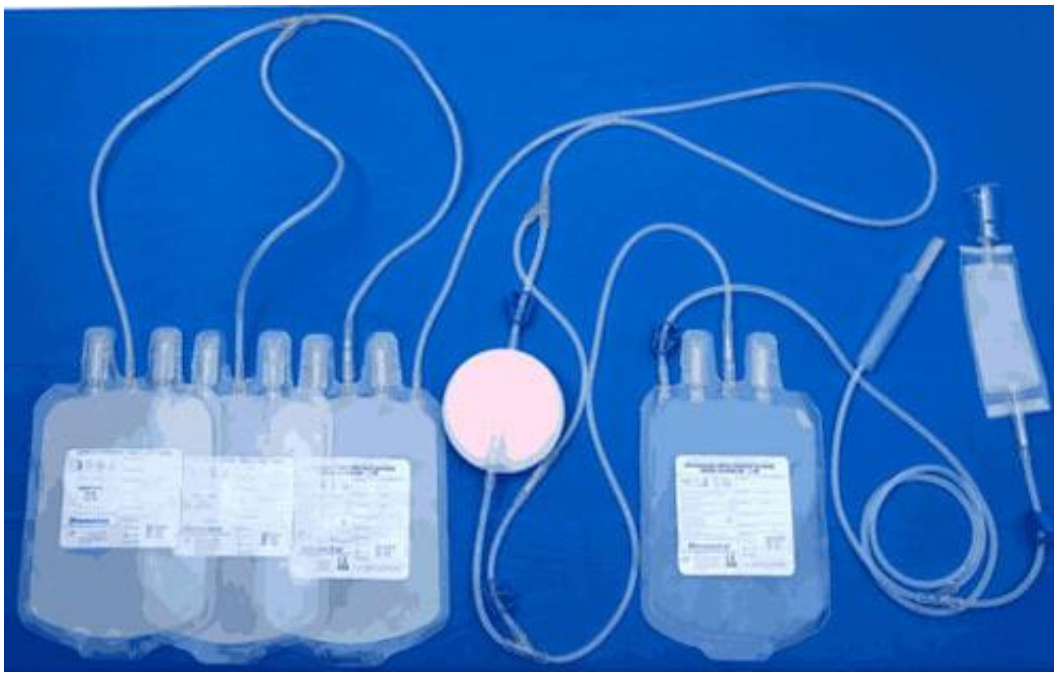

Figure 8: Quadruple Bag

Figure 9: Cryofuge 6000i

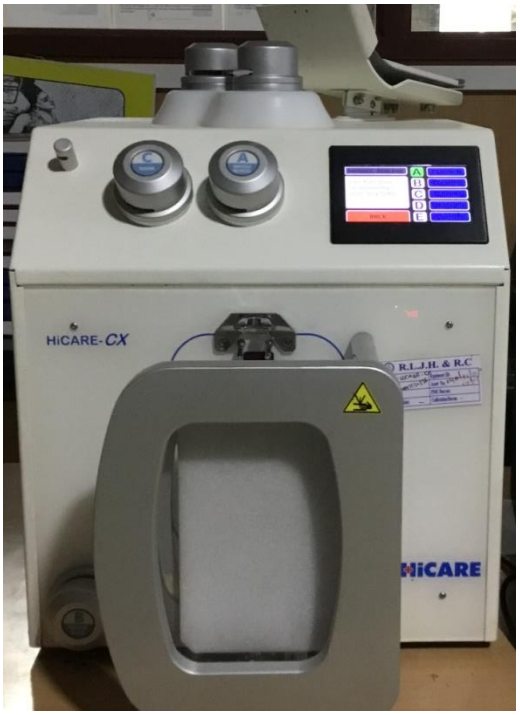

Figure 10: Component Separator

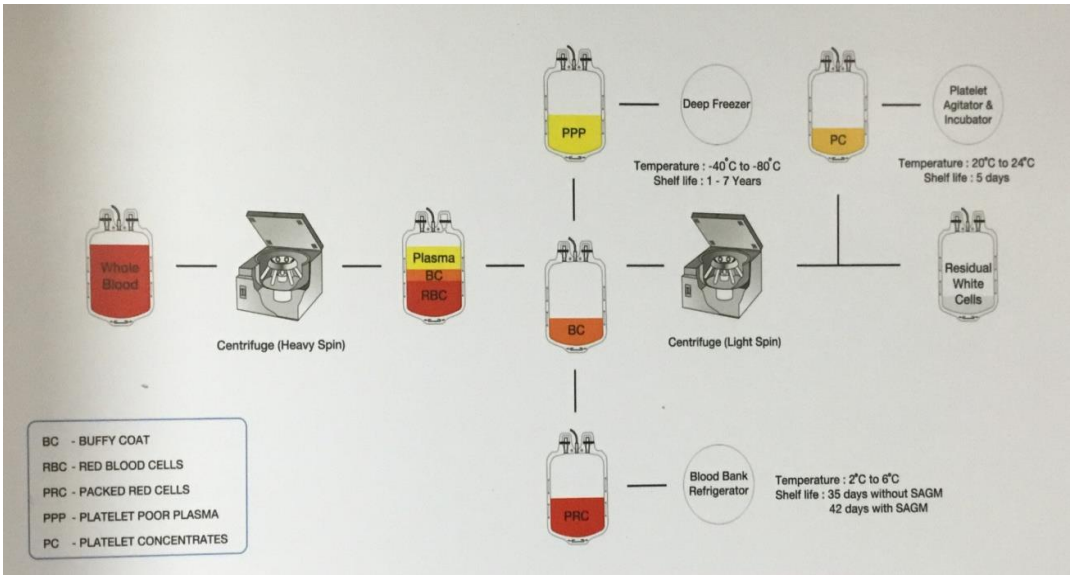

Figure 11: Buffy Coat method of component preparation

3. Whole blood derived by apheresis method:

The procedure was done in a closed system. During the procedure, the blood is anti-coagulated at the point of withdrawal in a controlled manner, and the ratio of whole blood and anticoagulant [ACD – Adenine Citrate Dextrose] was maintained at 9:1 to 11:1. The anticoagulated blood was pumped into a spinning separation container [Continuous automated cell separator]. Red cells will be packed by centrifugal force towards the outer edges of the container, and then the red cells exit the separation container. The lower density components, such as plasma, platelets, or WBCs were removed by plasma pump and entered the spinning collection container where platelets are packed by centrifugal force towards the outer edges of the container. The separated platelets remained packed in the container, while other constituents of blood were returned to the donor. At the end of the collection procedure, the platelet collection bag was shaken vigorously to detach the platelets from the wall of the bag and kept for 1 hour at room temperature to make it an even suspension. This whole procedure required 1.5-2.5 hours [Figure 12]

Figure 12: Apheresis cell separator

Each of PC prepared by the three different methods was assessed based on the following parameters:

i. Platelet concentrate volume
ii. Swirling
iii. Platelet count/bag
iv. WBC count/bag
v. pH changes
vi. Mean platelet volume [MPV]
vii. Platelet distribution width [PDW]
viii. Platelet large cell ratio [PLCR]
ix. Metabolic changes – pO_2, pCO_2, HCO^{3-}

In the present study, the quality parameters of PC were analysed as per the recommended DGHG quality norms[6] [Table 1]

Table 1: Criteria for quality assessment of PRP-PC, BC-PC and apheresis-PC (DGHS technical manual, 2nd Ed.)

Quality Parameters	PRP-PC	BC-PC	Apheresis-PC
Volume	40-70ml	70-90ml	200-300ml
Swirling	Present	Present	Present
Platelet count/bag	$>5.5 \times 10^{10}$ in 75% of unit tested	$>5.5 \times 10^{10}$ in 75% of units tested	$>3 \times 10^{11}$ in 75% of units tested
WBC count/bag	$5.5 \times 10^{7} - 5 \times 10^{8}$	$5.5 \times 10^{7} - 5 \times 10^{8}$	$< 5 \times 10^{6}$
pH	>6.0 at the end of maximum days of storage in 100% of units tested	>6.0 at the end of maximum days of storage in 100% of units tested	>6.0 at the end of maximum days of storage in 100% of units tested.

The calculation for the above quality parameters is as follows:

1. **Platelet concentrate volume:**

Volume of each unit of PC was determined by calculating with the help of specific gravity [1.03].

$$Volume\ of\ PC\ (ml) = \frac{Weight\ of\ full\ PC\ bag - Weight\ of\ empty\ PC\ bag}{Specific\ gravity\ of\ PC}$$

2. **Swirling:**

The swirling is a scoring system given by visual inspection, which was assessed against light and were scored as follows:

Score 0 – Homogenous turbid and is not changed with pressure

Score 1 – Homogenous swirling only in some part of the bag and is not clear

Score 2 – Clear homogenous swirling in all parts of the bag

Score 3 – Very clear homogenous swirling in all parts of the bag

3. **Platelet count per bag:**

The platelet count in each unit of PC was done using an automated cell counter [Sysmex XN-550].

4. **WBC count per bag:**

The WBC count per bag was performed using automated cell counter [Sysmex XN-550].

Figure 13: Automated cell analyser – Sysmex XN 550

5. **pH measurement [Figure 14]:**

The pH was measured at the end of the maximum day of storage by the use of a digital pH meter [Eutech pH Tutor].

Figure 14: pH meter

Metabolic changes:

pO_2, pCO_2, HCO^{3-} were analyzed using ABG analyzer to assess the platelet viability [Table 2][49].

Table 2

Metabolic Changes	Normal Values
pO_2	75-100mmHg
pCO_2	38-42mmHg
HCO^{3-}	22-28mEq/L

6. **Platelet indices:**

MPV, PDW, PLCR were analyzed using automated hematoanalyzer [Sysmex XN 550].

Sample size:

Sample size was estimated based on the final scores for the two methods, BC-PC and apheresis-PC as per the study done by R.S. Mallhi et.al. in the year 2015[51].

$$Sample\ Size = \frac{\left\{Z_{1-\frac{\alpha}{2}}\sqrt{2\bar{P}(1-P)} + Z_{1-\beta}\sqrt{P_1(1-P_1)+P_2(1-P_2)}\right\}^2}{(P_1-P_2)^2}$$

Where,

$$P = \frac{P_1 + P_2}{2}$$

P_1: Proportion of first group

P_2: Proportion of second group

α: Confidence Interval [95%]

$1-\beta$: Power [80%]

- A difference of 38% (as per study by R.S.Mallhi et.al.) was observed in getting an excellent result with 95% confidence interval [CI] and 80% Power.
- In the present study to obtain a difference of at least 25% of excellent result, a sample size of 52 each was obtained in each group.
- Hence, 52 samples were included in each of the three groups(PRP-PC - 52, BC-PC - 52 & apheresis platelet concentrate – 52)
- Total sample size was 52 x 3 = 156

Statistical Analysis

- Data was entered into Microsoft excel data sheet and was analysed using SPSS 22 version software
- **Statistical software:** MS Excel**,** SPSS version 22 **(**IBM SPSS Statistics, Somers NY, USA) was used to analyse data
- Categorical data was represented in the form of frequencies and proportions
- **Chi-square test or Fischer's exact test** (for 2x2 tables only) was used as test of significance for qualitative data
- Continuous data was represented as mean and standard deviation. **ANOVA** was used as test of significance to identify the mean difference between more than two quantitative variables
- **Graphical representation of data:** MS Excel and MS word was used to obtain various types of graphs
- **P value** (Probability that the result is true) of <0.05 was considered as statistically significant after assuming all the rules of statistical tests

Inclusion criteria:

- All voluntary donors and replacement donors between the age of 18-60 years
- Weight more than 45kg for RDP & more than 60kg for SDP
- Haemoglobin more than 12.5g/dl
- Platelet count - $150x10^3/\mu l$ - $250x10^3/\mu l$

Exclusion criteria:

- Platelet concentrates with RBCs or Bacterial contamination.
- Donors who are seropositive for transfusion transmitted infections
- Donors with Haemoglobin <12g/dl
- Donors with platelet count $<150x10^3/\mu l$ and $>250x10^3/\mu l$
- Platelet concentrates with inadequate volume

RESULTS

RESULTS

The quality of 156 PC were assessed, 52 each of PRP, BC and SDP which were prepared during the period of December 2017 to October 2019 at R.L.Jalappa Blood Bank, Tamaka, Kolar.

The following quality parameters are discussed below with respect to each unit prepared by PRP method, BC method and apheresis method:

- Swirling
- pH
- volume
- WBC count/bag
- Platelet count/bag

The scoring was given based on above parameters as per the DGHS recommendations[6].

- Platelet indices:
  - MPV
  - PDW
  - PLCR
- Arterial blood gas[ABG] analysis:
  - pH
  - pCO_2
  - HCO^{3-}

1. **Swirling:**

The maximum number of PCs having a **score of 3** was seen in SDP i.e. 71.2%. SDP units had better swirling score when compared to PRP-PC and BC-PC units. The difference was statistically significant [$p < 0.001$], as shown in Table 3 and Figure 15.

Table 3: Comparison of swirling score among three types

Type of PC Preparation		Swirling			Total
		Score 1	Score 2	Score 3	
PRP-PC	Number	9	26	17	52
	Percentage	17.3%	50.0%	32.7%	100.0%
BC-PC	Number	6	16	30	52
	Percentage	11.5%	30.7%	57.6%	100.0%
SDP	Number	0	15	37	52
	Percentage	0%	28.8%	71.2%	100.0%
Total	Number	15	57	84	156
	Percentage	9.6%	36.5%	53.8%	100.0%

p value <0.001, the difference was statistically significant between groups with respect to swirling score

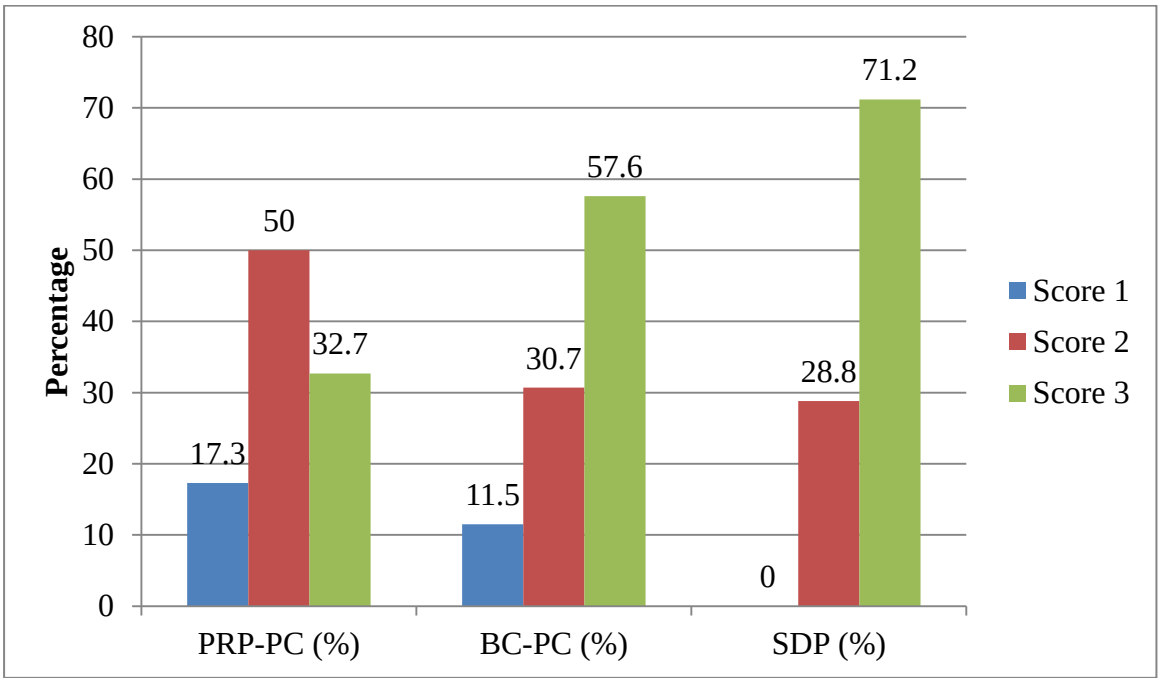

Figure 15: Graph showing comparison of swirling score among three different PCs

2. pH:

As shown in Table 4, there was no significant difference [p value 0.25] observed between three groups with respect to pH.

Table 4: Comparison of pH measured by pH meter of PCs

Type of PC Preparation		pH
PRP-PC	**Mean**	6.9329
	SD	0.37851
BC-PC	**Mean**	7.1881
	SD	0.25796
SDP	**Mean**	7.3335
	SD	0.21541

3. Volume, WBC count/bag and Platelet count/bag:

- ✓ The mean volume of PRP-PC, BC-PC and SDP units were 53.75±8.85, 73.65±6.34 and 266.83±17.57 respectively.
- ✓ 86.5% of PRP-PC (45/52 units) and 96.1% of BC-PC (50/52 units) met the desired quality criteria of volume whereas all units of SDP (100%) met the desired criteria of volume/bag. [Table 5].

Table 5: Comparison of Volume, WBC count/bag and Platelet count/bag prepared by different methods

Quality Parameters	PRP-PC		BC-PC		SDP	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Volume(ml) per bag	53.75	8.85	73.65	6.34	266.83	17.57
WBC count per bag	$10.03x10^7$	$1.35x10^7$	$1.90 x10^7$	$1.05x10^7$	$1.08x10^8$	$0.14x10^8$
Platelet count per bag	$3.32 x10^{10}$	$1.60x10^{10}$	$5.52 x10^{10}$	$3.54x10^{10}$	$1.01x10^{11}$	$0.46x10^{11}$

The analysis of WBC count/bag showed the mean WBC count/bag in PRP-PC, BC-PC and apheresis-PC was 10.03±1.35 x107, 1.90±1.05 x107 and 1.08±0.14 x107 WBC/bag respectively. When compared, SDPs had least WBC contamination followed by BC-PC and PRP-PC and showed statistically a significant difference [$p<0.001$].

Platelet count/bag was analysed in all 156 PCs prepared by different methods. 5.76% of PRP-PC (3/52) units had platelet count < 55,000 cells which failed to meet required quality criteria. While 100% of the BC-PC and apheresis-PC units met the desired quality control criteria with respect to platelet count/bag.

Scoring:

Scoring was done based on the five quality parameters discussed above: swirling, pH, volume, WBC count/bag and platelet count/bag, as per DGHS criteria[6].

As shown in **Table 6**, 11.53% (6/52 units) of PRP-PC, 30.76% (16/52 units) of BC-PC and 34.6% (18/52) SDP units had a score of 5. Whereas,48.07% (25/52) of PRP-PC, 48.07% (25/52)of BC-PC and 50% (26/52) of SDP units had a score of 4 indicating that BC-PC units were in comparison with SDP units.

Table 6: Scores for the three methods of PC preparation evaluated for the quality parameters

Score	PRP-PC		BC-PC		SDP	
	No.	%	No.	%	No.	%
5	6	11.5	16	30.76	18	34.61
4	25	48.07	25	48.07	26	50
3	15	28.8	11	21.11	8	15.3
2	5	9.6	-	-	-	-
1	1	1.9	-	-	-	-

As shown in **Table 7** and **Figure 16,** maximum number of BC-PC and SDP units was within normal range. There was statistically significant difference found between PCs prepared by the three different methods [p<0.001] with respect to MPV.

Table 7: Comparison of MPV measured for each unit of PC prepared by the three methods

Method of Preparation		MPV		Total
		<8.6fl	8.6-13.5fl	
PRP-PC	Number	23	29	52
	Percentage	44.2%	55.8%	100.0%
BC-PC	Number	14	38	52
	Percentage	26.9%	73.1%	100.0%
SDP	Number	6	46	52
	Percentage	11.5%	88.5%	100.0%
Total	Number	43	113	156
	Percentage	27.6%	72.4%	100.0%

p value <0.001, there was a statistically significant difference found between groups with respect to MPV

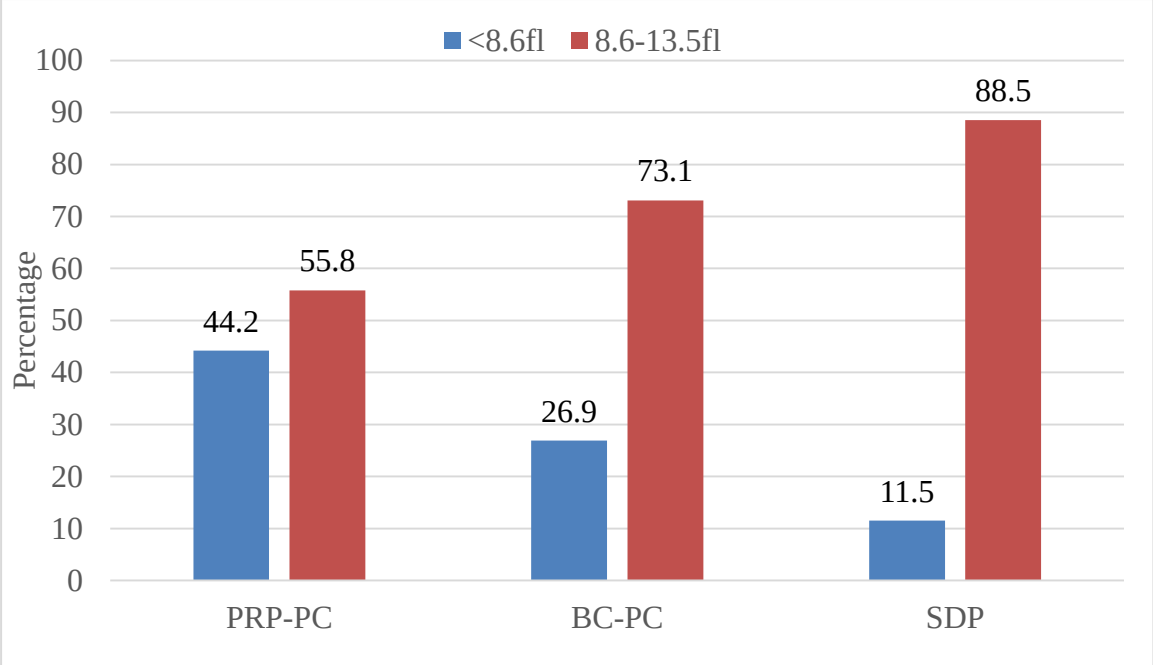

Figure 16: Graph showing comparison of MPV among the three different PCs

As shown in **Table 8** and **Figure 17,** 82.7% of SDP units were within normal range and showed a difference of 0.021, which was statistically significant.

Table 8: Comparison of PDW measured for each unit of PC prepared by the three methods

Method of Preparation		PDW		Total
		<8.2%	8.2-13.5%	
PRP-PC	Number	22	30	52
	Percentage	42.3%	57.7%	100.0%
BC-PC	Number	17	35	52
	Percentage	32.7%	67.3%	100.0%
SDP	Number	9	43	52
	Percentage	17.3%	82.7%	100.0%
Total	Number	48	108	156
	Percentage	30.8%	69.2%	100.0%

p value 0.021, there was a statistically significant difference found between 3 methods of PC preparation with respect to PDW

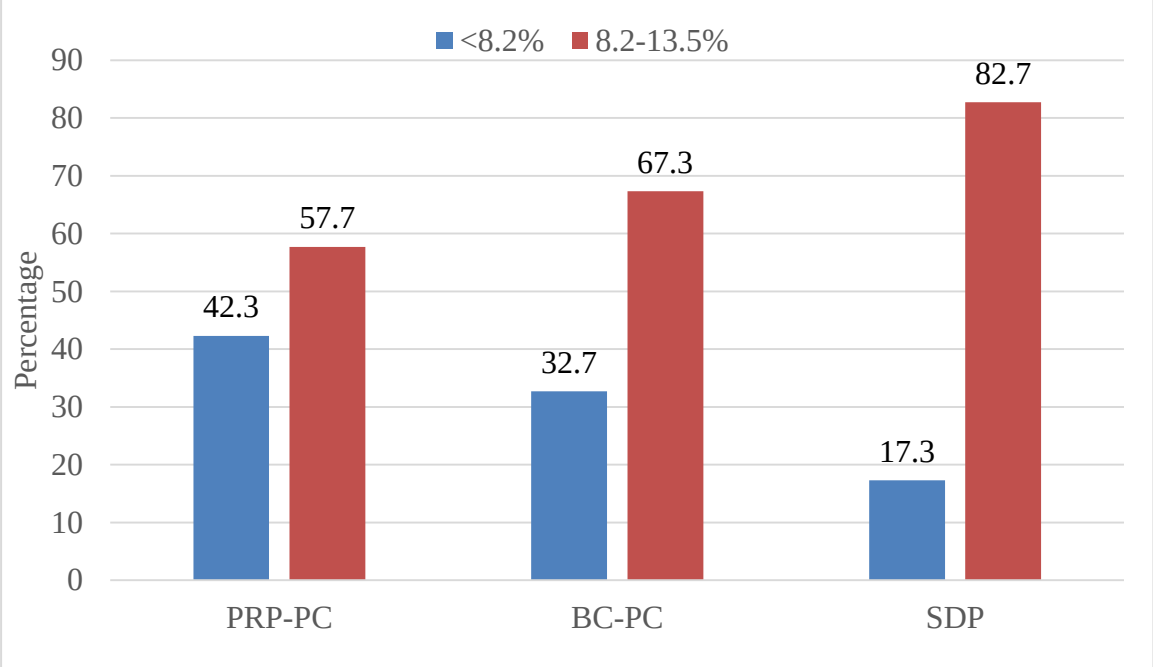

Figure 17: Graph showing comparison of PDW among the three different PCs

As shown in **Table 9** and **Figure 18,** there was no statistically significant difference between BC-PC and SDP units, while it showed significant difference between PRP-PC and SDP [p<0.023].

Table 9: Comparison of PLCR measured for each unit of PCs prepared by 3 methods

Method of Preparation		PLCR		Total
		<11.9%	11.9-66.9%	
PRP-PC	Number	19	33	52
	Percentage	36.5%	63.5%	100.0%
BC-PC	Number	12	40	52
	Percentage	23.1%	76.9%	100.0%
SDP	Number	7	45	52
	Percentage	13.5%	86.5%	100.0%
Total	Number	38	118	156
	Percentage	24.4%	75.6%	100.0%

p value 0.023, there was a statistically significant difference found between groups with respect to PLCR

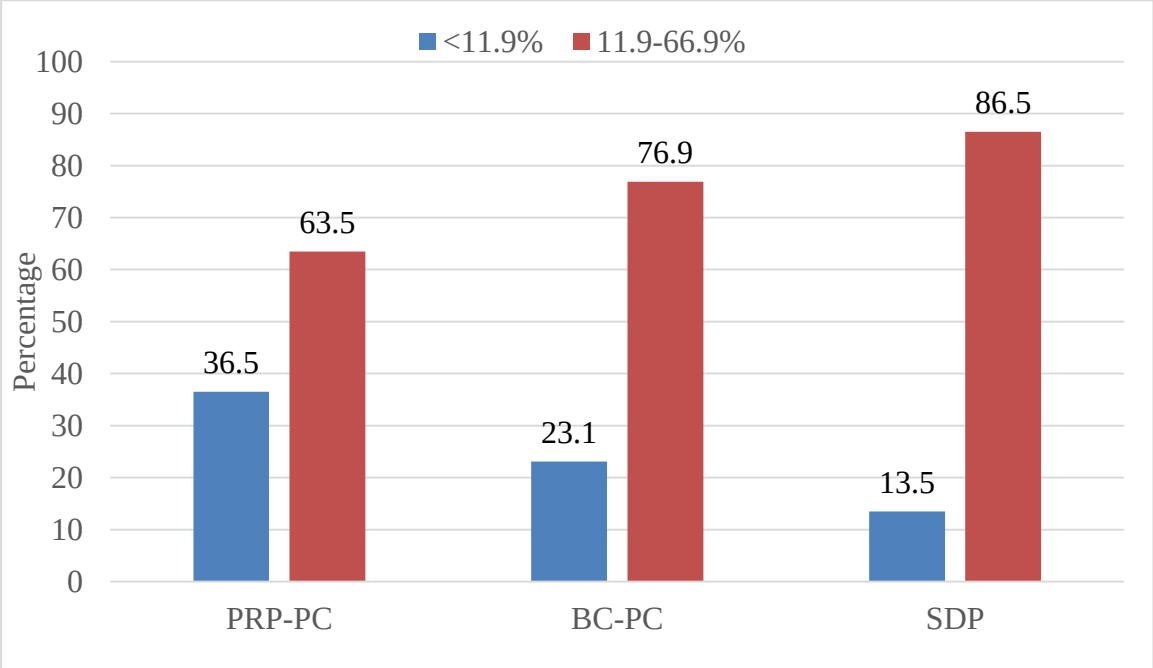

Figure 18: Graph showing Comparison of PLCR among PCs prepared by the three methods

ABG analysis:

As shown in **Table 10** and **Figure 19,** majority of PRP-PC units showed increase in pCO_2 levels i.e. 73.1%, while 50% of SDP units had low pCO_2 levels and showed to have statistically significant difference [p <0.001].

Table 10: Comparison of pCO_2 as measured using ABG analyzer

Method of Preparation		pCO_2			Total
		<35	35-45	>45	
PRP-PC	Number	1	13	38	52
	Percentage	1.9%	25.0%	73.1%	100.0%
BC-PC	Number	18	11	23	52
	Percentage	34.6%	21.2%	44.2%	100.0%
SDP	Number	26	11	15	52
	Percentage	50.0%	21.2%	28.8%	100.0%
Total	Number	45	35	76	156
	Percentage	28.8%	22.4%	48.7%	100.0%

p value <0.001, there was a statistically significant found between groups with respect to pCO_2

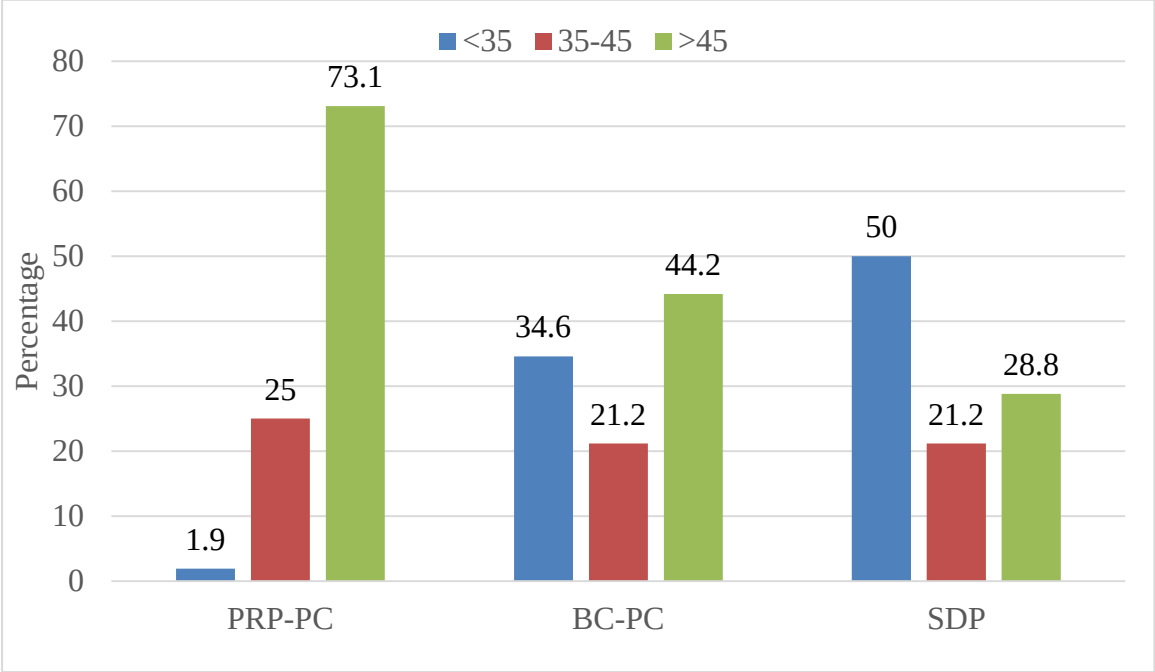

Figure 19: Graph showing comparison of pCO2 among the different methods of preparation

As shown in **Table 11** and **Figure 20,** there is a drop in the bicarbonate level in 50% of PRP-PC units. While the bicarbonate levels are maintained in BC-PC and SDP.

Table 11: Comparison of HCO3- of PCs prepared by different methods as measured by ABG analyzer

Method of Preparation		$HCO3^-$		Total
		<22	22-26	
PRP-PC	Number	26	26	52
	Percentage	50.0%	50.0%	100.0%
BC-PC	Number	5	47	52
	Percentage	9.61%	90.38%	100.0%
SDP	Number	4	48	52
	Percentage	7.69%	92.30%	100.0%
Total	Number	35	121	156
	Percentage	22.43%	77.56%	100.0%

p value <0.001, there was a statistically significant difference found between PCs preparation methods with respect to HCO^{3-}

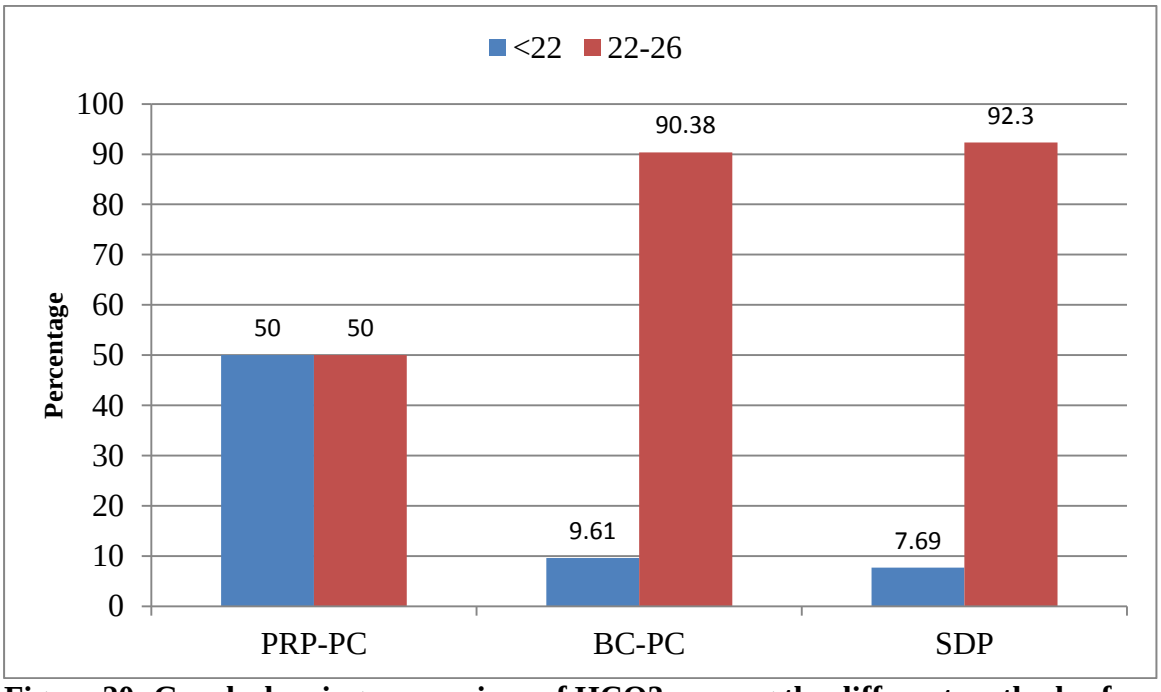

Figure 20: Graph showing comparison of HCO3- among the different methods of preparation

As shown in **Table 12**, there was statistically significant difference found between the three different methods of PC preparations [p<0.001], with PRP-PC having least viability of platelets.

Table 12: Comparison of ABG analysis among the PCs prepared by the three methods

Method of Preparation	PRP-PC		BC-PC		SDP	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
pH	6.84	.06	7.12	.29	7.18	.26
PCO2	57.78	12.70	52.25	32.61	36.78	13.10
HCO^3	7.74	2.82	13.87	3.89	20.91	3.69

DISCUSSION

DISCUSSION

Hemostasis is the main function of platelets in circulation. Decrease in platelet count i.e. TCP causing spontaneous bleeding due to various causes is most common indication for transfusion of platelet concentrates in hospitalized thrombocytopenic patients.

The functions of transfused platelets in circulation will be depending on the ex-vivo storage lesions determining platelet functionality and status of recipient. These two factors have a strong influence on platelet survival and post-transfusion recovery of platelets [52, 53].

The PCs which have been prepared and transfused immediately within 24 to 48 hours have shown good survival, preserved function and also high recovery rate which were consistent with studies done by Norol F et.al [54] and Bock M et.al [55].

Morphological changes noted in stored platelets have been defined in three categories – platelet activation, metabolic alterations and platelet senescence. As the platelets were stored for longer duration, it showed high levels of platelet activation i.e. about 30% [56, 57].

Platelet concentrates were prepared by three different methods namely PRP method, BC method and apheresis method, which causes variability in quality control of PC. Hence, quality assessment plays an important role in evaluating the in-vitro function and viability of platelets transfused and post-transfusion platelet recovery in recipients. This assessment of quality helps in providing assurance that PCs prepared are within the recommended specifications.

The various parameters used in assessing the function and viability of platelets in PCs include volume, pH, swirling, platelet count/bag and WBC count/bag. To assess the morphological changes, platelet storage lesions [PSL] occurring in platelets due to storage conditions, platelet indices that include MPV, PDW, PLCR have found to be important parameters in addition to pH, platelet count and volume[58, 59, 60]. There are other parameters that also help in assessing quality that includes measuring adenosine triphosphate [ATP], glycoprotein levels like P-selectin, GpIb and GpIIb/IIIa, but these tests are difficult to perform and are not standardised[61].

In-vivo quality of platelets transfused are assessed by 2 parameters which include corrected count increment [CCI] and percentage recovery post- transfusion at 1 hr and 24 hrs.

Among all the parameters, pH plays an important role in quality of PCs. The pH in turn is dependent on metabolic activity and gaseous exchange. During storage, HCO^{3-} in plasma acts as a buffer in maintaining pH and due to continuous metabolic activity of platelets, there is increased consumption of O_2 leading to increased production of CO_2 and a fall in the HCO^{3-}levels. This continuous metabolic activity in PCs during the storage period results in increased pCO_2 and decreased HCO^{3-} levels leading to a significant fall in pH affecting the quality of PCs[62].

The utilization of PCs prepared by different methods varies in different countries. PRP-PC and SDPs are mainly transfused in United States; while in Europe, BC-PC and SDP units are transfused regularly[63]. However, in most of the developing countries like India, BC-PC units are used for transfusion and apheresis method is being used widely to produce PCs since the last decade.

Quality assessment of platelet concentrates:

In the present study randomly selected 156 PC units were analysed for various quality parameters out of which 52 each were prepared by PRP method, BC method and apheresis method respectively. These PCs once prepared were stored at 20^{o}-24^{o}C with continuous agitation.

1. Volume:

RDP prepared from whole blood or SDPs prepared by apheresis method are suspended in donors plasma which acts as a buffering agent and maintain pH.

Out of 52, 7 PRP-PC [13.46%] and 2 out of 52[3.84%] BC-PC units did not meet the recommended quality control criteria. But all 52[100%] SDP units fulfilled the required quality criteria with respect to volume/bag.

As shown in **Table 13** the mean volume of BC-PC and SDP units were in comparison with that seen in the study done by R.S. Mallhi et.al.[51]. While in two similar studies done by R.P.Singh et.al.[2] and Talukdar B et.al.[64] showed decrease in mean volume of PCs prepared by BC-PC and aphersis methods but PCs prepared by PRP method was in comparison to present study. However, Ashish et.al.[66] showed better results with respect to volume/bag when compared to present study.

Table 13: Comparison of PCs with respect to volume/bag (ml) with various studies

Methods of Preparation	R.S.Mallhi et.al.[50]	R.P Singh et.al.[2]	Talukdar B et.al.[64]	Manish R et.al.[65]	Ashish J et.al.[66]	Present study
PRP-PC	-	62.3±22.6	59.4±10.2	53.6±7.0	65.02±6.36	53.75±8.8
BC-PC	74.33±9.61	68.8±22.95	63.7±14.3	59.3±8.9	81.50±6.5	73.65±6.34
SDP	269.13±24.1	214.05±9.91	209.8±34.6	-	294.6±8.56	266.8±17.57

2. Swirling:

Swirling is a procedure where visual inspection of PCs is performed by the observer to assess the platelet morphology. This is a simple and useful procedure thatcan be used for routine assessment of quality of PCs. There is a risk of transfusion reactions and poor CCI post transfusion if swirling is absent in PCs[67].

In the present study, a score of 5 was noted in 32.7%, 57.6% and 71.2% of PRP-PC, BC-PC and SDP units respectively, while intermediate score for swirling was seen in 50%, 30.7%, and 65% of PRP-PC, BC-PC and SDP units respectively indicating SDP units had better platelet morphology probably due to complete automation of the process and continuous agitation during apheresis procedure but BC-PC was in comparison with SDP units. The swirling scores obtained in our study was in comparison with study done by R.S.Mallhi et.al.[50] and R.P.Singh et.al.[2]

3. pH:

Maintaining pH of PC>6.0 is one of the major quality parameter that helps in assessing the morphology and function of platelets in PC. Certain conditions like volume, temperature and agitation should be optimized to maintain pH>6.0 in 100% of PC units. However, as the duration of storage of PCs increases, pH decreases. As

pH falls below 6.0, platelets change shape from disc to sphere resulting in swelling and loss of function of platelets. This results in decreased survival and affects the post-transfusion platelet count increment.

In the present study, all 156 PC units prepared by the three different methods maintained the pH>6.0 at the end of maximum storage. Various studies done on assessing quality parameters of PCs showed similar findings as was observed in the present study[2,51,64,65,66,68].

4. WBC count/bag:

PC prepared by BC method and apheresis method are effectively leukoreduced. Increase in WBC contamination of PCs affects the morphology and function of platelets by causing significant drop in pH, increase in lactic acid production and release of LDH. Also high WBC count in PC causes alloimmune reaction, febrile non-hemolytic transfusion reactions [FNHTR] and graft versus host disease [GVHD] in transfused individuals[69].

Table 14: Comparative study of WBC count/bag in PC units prepared by different methods with other studies

Methods of Preparation	R.S.Mallhi et.al.[51]	R.P.Singh et.al.[2]	Talukdar B et.al.[64]	Raturi M et.al.[65]	Ashish J et.al.[66]	Present study
PRP-PC		4.05±0.48 x10^7	3.2±2.1 x10^7	1.73±1.7 x10^7	5.99±1.44 x10^7	10.03±1.35 x10^7
BC-PC	2.92±1.2x10^7	2.08±0.39 x10^7	1.7±1.0 x10^7	1.46±1.2 x10^7	6.16±0.93 x10^7	1.90±1.05 x10^7
SDP	3.14±1.3 x10^6	4.8±0.8 x10^6	1.7±1.1 x10^6		4.87±0.75 x10^6	1.08±0.14 x10^6

In the present study, the WBC count/bag of the BC-PC and SDP units were well within the recommended quality specifications while the WBC contamination was more in PRP-PC affecting the final yield of the particular component unit.

The results obtained by Talukdar B et.al.[64] and Raturi M et.al.[65] had similar results as compared to present study with respect to WBC count/bag.

5. Platelet count/bag:

Table 15: Comparison of PCs with respect to platelet count/bag with other studies

Methods of Preparation	R.S.Mallhi et.al.[51]	R.P.Singh et.al.[2]	Talukdar B et.al.[64]	Raturi M et.al.[65]	Ahish J et.al.[66]	Present study
PRP-PC		7.6±2.97 $x10^{10}$	2.1±0.9 $x10^{10}$	5.70±1.42 $x10^{10}$	5.74±0.66 $x10^{10}$	3.32±1.6 $x10^{10}$
BC-PC	8.75±2.89$x10^{10}$	7.3±2.98 $x10^{10}$	3.1±1.1 $x10^{10}$	5.93±1.28 $x10^{10}$	6.88±0.81 $x10^{10}$	5.52±3.54 $x10^{10}$
SDP	3.92±0.74 $x10^{11}$	4.13±1.32 $x10^{11}$	2.3±0.58 $x10^{11}$		3.78±0.77 $x10^{11}$	1.01±0.46 $x10^{11}$

As per the recommended quality criteria, in the present study, all units of BC-PC and SDP units fulfilled the criteria while 5.76% of PRP-PC units failed to meet the desired criteria.

The results obtained by R.S.Mallhi et.al.[51] and Talukdar B et.al.[64] were comparable to the present study with respect to platelet count/bag. But, the results obtained by Raturi M et.al.[65] showed better platelet counts per bag as compared to present study.

Based on these five quality parameters, scoring was given with the maximum score of 5. As shown in Table 16, the present study showed similar results as observed in a similar study done by Talukdar B et.al.[64] and Trivedi MP et.al.[70].

Table 16:

Scores	Score 5 n(%)			Score 4 n(%)		
	PRP-PC	**BC-PC**	**SDP**	**PRP-PC**	**BC-PC**	**SDP**
R.S.Mallhi et.al.[51] (n=100)		26(52)	45(90)		17(34)	4(8)
Talukdar B et.al.[64] (n=105)	1(2.5)	5(12.5)	6(24)	30(75)	34(85)	17(68)
Trivedi MP et.al.[70] (n=119)	18(50)	19(32.7)	17(68)	12(33.34)	28(48.3)	6(24)
Present study (n=52)	6(11.5)	16(30.76)	18(34.61)	25(48.09)	25(48.07)	26(50)

A similar study done by R.P. Singh et.al.[2] had quoted that the results were in contrast to the present study where it was concluded that Aphersis PC units were superior followed by PRP-PC and the least was BC-PC units.

Platelet Indices:

Platelet storage lesions are multifactorial and it is a complex process caused by various chemical, metabolic changes, stress and mechanical trauma[71]. This affects the final product of PC resulting in decreased quality and abnormal function of platelets, which can be assessed by measuring MPV, PDW and PLCR.

These platelet indices help in assessing the platelet morphology thatis dependent on platelet activation during storage. As there is increase in platelet activation, MPV, PDW and PLCR increase affecting the post-transfusion platelet count increment, survival and function of platelets in recipients[72].

In the present study, platelet indices MPV, PDW and PLCR had maintained within normal range in maximum number of SDP units followed by BC-PC and the least being PRP-PC units indicating the morphology and function was well maintained in SDP and BC-PC units.

The results obtained by Das et.al.[68] was in comparison to present study with respect to platelet indices in PRP-PC and BC-PC units.

ABG analysis:

When first generation platelet storage containers were used, there was a decrease in permeability of gases. This resulted in high metabolic activity of platelets causing increased accumulation of CO_2 and decrease in O_2 affecting the pH of platelets in PC units.

As the duration of PC storage increased, the continued metabolic activity caused an increase in the mean pO_2 and decrease in mean HCO^{3-} levels resulting in a significant fall in pH affecting the function, survival and morphology of platelets[73].

In the present study, ABG analysis was performed on all 156 PC units. The pCO_2levels were increased in PRP-PC and BC-PC units but was maintained in SDP units while HCO^{3-} levels showed a decrease in PRP-PC units thus affecting the final pH. As the HCO^{3-} in plasma present in PC units acts as a buffer in maintaining pH, in the present study though the pH was maintained above 6.0 there was a fall in pH to less than 6.7 in PRP-PC units. A decrease in pH to less than 6.7 affects the

morphology of the platelets which in turn affects the quality of final platelet concentrate.

As there is paucity in literature with respect to ABG analysis of PC, comparison of these parameters could not be done with other studies.

CONCLUSION

CONCLUSION

In the present study, due to complete automation procedure and continuous centrifugation of apheresis method, the PCs prepared by this method showed slightly higher score of 5 for the quality parameters that is volume, pH, swirling, WBC count/bag and platelet count/bag when compared to BC-PC. Though a few BC-PC units were not fulfilling the desired quality parameters, maximum number of units were in par with the SDP units. This minor lack in quality between BC-PC units and SDP units could be because of the variations in the preparation methods, as complete automation was not involved in platelet preparation by BC method. Thus suggesting if we formulate and follow strict standard operating procedures [SOP] this lack in quality can be reduced.

On the other hand, PC units prepared by PRP method showed a major variation in achieving a score of 5 in the quality parameters as compared to SDP and BC-PC units. However, pH of all 156 PC units was maintained above 6.0, which were within the recommended criteria.

The other parameters included in the present study that is platelet indices [MPV, PDW, PLCR], which help in understanding platelet morphology and thus assessing the PSLs, were well maintained within the normal range in majority of SDP and BC-PC units.

The additional parameter, ABG analysis [pH, pCO_2, HCO^{3-}] that was analysed in the present study showed that maximum number of SDP units had maintained pCO_2 and HCO^{3-} levels followed by BC-PC and thus pH were maintained.

Our study suggested that apheresis PCs were superior to BC-PC and PRP-PC, but with incorporating a standard SOP for BC method of preparation, the minor deficiency between SDP and BC-PCs can be reduced and achieve a quality equal to apheresis-PCs.

However, further large-scale studies are required to attain more standardization of procedures to attain better quality of PC units. Further studies are needed to analyse PSL, extend duration of storage of PCs and to achieve a better post-transfusion yield, with respect to platelet indices, ABG analysis, estimation of lactate and clinical correlation.

SUMMARY

SUMMARY

1. This was a prospective study on "A Comparative Assessment of Quality Parameters of Platelet Concentrates prepared by different Methodologies" conducted from 2017 to 2019. A total of 156 platelet concentrates prepared by PRP, BC and apheresis methods with 52 in each method of preparation.
2. The quality parameters that include volume, pH, swirling, WBC count/bag and platelet count/bag were analyzed for each of the platelet concentrate and was scored as per DGHS criteria
3. The platelet indices that are MPV, PDW and P-LCR along with ABG analysis was analyzed for each of platelet concentrate to assess the morphology of platelets during process of collection and storage
4. Among the 3 types of platelet concentrates, 37/52 SDP units [71.2%] had a score of 3 for swirling while 30/52 BC-PC [57.6%] and 17/52 PRP-PC units [32.7%] had a score of 3 for swirling
5. The intermediate scoring was seen in 28.8%, 30.7% and 50% of SDP, BC-PC and PRP-PC units respectively.
6. Therefore there was a better swirling seen in SDP as compared to BC-PC and PRP-PC probably due to complete automation and continuous agitation during collecting process of apheresis.
7. With respect to pH, all the 156 platelet concentrate units had shown a pH of more than 6.0 as measured by pH meter
8. The mean volume of platelet concentrates collected by PRP-PC, BC-PC and SDP was 53.75±8.85, 73.65±6.34 and 266.83±17.57ml respectively
9. Out of 156 platelet concentrate units prepared, 45/52 [86.5%] PRP-PC units, 50/52 [96.1%] and 100% of SDP units had met the recommended desired quality criteria with respect to volume/bag
10. The mean WBC count/bag was 10.03 $x10^7$±1.35 $x10^7$, 1.90 $x10^7$±1.05 $x10^7$ and 1.08 $x10^7$±0.14 $x10^7$ for PRP-PC, BC-PC and SDP units respectively
11. SDP units had the least WBC contamination followed by BC-PC and the PRP-PC units had highest WBC contamination. This showed a statistically significant difference with p value of <0.001

12. The mean platelet count/bag was 3.32 $x10^{10}$±1.60 $x10^{10}$, 5.52 $x10^{10}$±3.54 $x10^{10}$ and 1.01 $x10^{11}$ for PRP-PC, BC-PC and SDP units respectively.
13. Among the 156 platelet concentrate units, 5.76% of PRP-PC units failed to meet the required quality criteria for platelet count/bag.
14. But 100% of BC-PC and SDP units had met the desired quality criteria with respect to platelet count/bag
15. 11.53% (6/52 units) of PRP-PC, 30.76% (16/52 units) of BC-PC and 34.6% (18/52) SDP units had a score of 5. Whereas,48.07% (25/52) of PRP-PC, 57.6% (30/52)of BC-PC and 50% (26/52) of SDP units had a score of 4 indicating that BC-PC units were in comparison with SDP units.
16. The 5 parameters were scored as per recommended by DGHS. Among the total 156 platelet concentrates, 11.53% (6/52 units) of PRP-PC, 30.76%(16/52 units) of BC-PC and 34.6% (18/52) SDP units had a score of 5.
17. While,48.07% (25/52) of PRP-PC, 48.07% (25/52)of BC-PC and 50% (26/52) of SDP units had a score of 4 indicating that BC-PC units were in comparison with SDP units.
18. With respect to MPV, 46/52 [88.5%], 38/52 [73.1%] of SDP and BC-PC units respectively showed MPV within normal range
19. Among the 52 PRP-PC units, 55.8% showed MPV value within normal range while 44.2% of units had decreased MPV. This showed a statistically significant difference between platelet concentrate units
20. When PDW was measured for all the 156 platelet concentrate units, 43/52 [82.7%], 35/52 [67.3%] and 30/52 [57.7%] of SDP, BC-PC and PRP-PC units were within the normal range. There was a statistically significant difference of p value <0.021
21. 45/52 [86.5%], 40/52 [76.9%] and 33/52 [63.5%] of SDP, BC-PC and PRP-PC units showed PLCR within the normal range
22. The platelet indices which were measured for each of platelet concentrate units were within normal range in maximum number of SDP and BC-PC units
23. This showed that SDP units had maintained a better platelet morphology and thus the pH as compared to BC-PC and PRP-PC units
24. The mean of pCO_2 as measured by ABG was 57.78±12.70, 52.25±32.61 and 36.78±13.10 for PRP-PC, BC-PC and SDP units respectively

25. The mean of HCO^{3-} as measured by ABG for PRP-PC, BC-PC and SDP was 7.74±2.82, 13.87±3.89 and 20.91±3.69 respectively
26. The ABG analysis of all 156 platelet concentrate units showed to have decrease in pH in PRP-PC units
27. This fall in pH was due to continuous metabolic activity of platelets which was mainly due to increase in pCO_2 and a decrease in HCO^{3-} levels which acts as a buffer for maintaining the pH

Hence, in the present study, the quality of SDP was superior to BC-PC and PRP-PC units and also showed a better morphology of platelets during storage period. As there is a minor deficiency in quality of BC-PC units, more standardization of collecting procedure and storage is required which makes BC-PC units in par with the SDP units. But further large scale studies are required for more standardization of the procedure.

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ANNEXURES

ANNEXURE – I

PATIENT PROFORMA

Name and Address: Bag No. -

Age/Sex:

Clinical Examination:

1. Weight:
2. Pulse:
3. BP:
4. Temperature:
5. Haemoglobin:
6. Platelet count:
7. HIV
8. HBsAg
9. HCV
10. VDRL
11. MP

Examination of quality parameters:

a) Volume:

b) Swirling:

c) WBC count/bag:

d) Platelet count/bag:

e) pH:

f) ABG Analysis – pO2:
pCO2:
HCO^{3-} :

g) MPV:

h) PDW:

i) PLCR:

ANNEXURE – II

INFORMED CONSENT FORM

I _____, have read or have been read to me the donor information sheet and understand the purpose of the study, the procedure that will be used, the risk and benefits associated with my involvement in the study and the nature of information that will be collected and disclosed during the study.

I have had the opportunity to ask my questions regarding various aspects of the study and my questions are answered to my satisfaction.

I, the undersigned, agree to participate in this study and authorize the collection and disclosure of my personal information for the dissertation.

Name and signature/ Thumb impression (Subject) Date: Place:

Name and signature/thumb impression (Witness/Parent/Guardian/Husband) Date: Place:

ANNEXURE – III

DONOR INFORMATION SHEET

Study Title: A Comparative Assessment of Quality Parameters of Platelet Concentrates Prepared by Different Methodologies

Study Site: Blood Bank, R.L Jalappa Hospital And Research Centre, Tamaka, Kolar.

The aim of the study is:

1. To prepare platelet concentrates by platelet rich plasma [PRP], buffy coat [BC] and apheresis methods
2. To compare the quality parameters of platelet concentrates prepared by platelet rich plasma [PRP], buffy coat [BC] and apheresis methods

This study is approved by the institutional ethical committee. The information collected will be used only for dissertation and publication. There is no compulsion to agree to participate. You are requested to sign/provide thumb impression only if you voluntarily agree to participate in the study.

All information collected from you will be kept confidential and will not be disclosed to any outsider. Your identity will not be revealed. You will have to pay for the basic investigation and you will not receive any monetary benefits to participate in this research.

This informed document is intended to give you a general background of study. Please read the following information carefully and discuss with your family members. You can ask your queries related to study at any time during the study. If you are willing to participate in the study you will be asked to sign an inform consent form by which you are acknowledging that you wish to participate in the study and entire procedure will be explained to you by the study doctor. You are free to withdraw your consent to participate in the study any time without explanation and this will not change your future care.

For any further clarification you can contact the primary investigator:
Dr. Varsha Shree. R
Mobile no: 9886841958
E-mail id: varshashree@gmail.com

ANNEXURE – IV

KEY TO MASTER CHART

WBC – White blood cell

MPV – Mean Platelet Volume

PDW – Platelet Distribution Width

P-LCR – Platelet to Large Cell Ratio

MASTER CHART

Sl. No.	Donor bag no.	Volume(ml)	pH	Swirling	WBC count [cells/cumm]	Platelet count [x10³/µl]	MPV [fL]	PDW [fL]	PLCR [%]	ABG Analaysis			Score
										pH	pCO2	HCO^{3-}	
1	1783	75	7.03	3	530	558	8.9	8.3	15	7.27	40.7	18	4
2	1784	65	7.17	2	70	219	9.6	9.5	20.2	7.2	54.9	20.4	4
3	1785	60	6.41	2	40	387	8.6	7.7	12.3	7.18	63.2	22.4	4
4	1786	75	6.94	1	230	245	7.8	6.6	6.5	7.25	55	21.2	4
5	1787	65	6.94	2	170	339	9	8.4	15	7.11	73.2	22.3	4
6	1788	55	7.01	3	840	362	8.4	7.3	11.9	7.18	63.2	22.4	5
7	1789	50	7.09	2	540	274	7.3	5.7	4	7.17	63.2	22.4	4
8	1790	50	6.13	2	150	342	8.1	7.1	9.1	7.25	40.7	18	3
9	1792	65	6.93	3	250	531	8.9	8.1	14.2	7.22	41.3	23	5
10	1795	50	6.07	2	450	454	9.4	9.2	18.7	7.3	63.2	22.4	4
11	1796	60	6.21	2	600	256	8.7	7.8	13.2	7.2	40.7	17	4
12	1805	50	6.77	1	120	555	7.9	6.4	7.9	7.14	42.3	20.2	4
13	1806	70	7.11	3	50	812	9.2	8.9	15.8	7.27	40.8	18	5
14	1807	65	7.77	3	280	239	8.1	7.5	7.9	7.17	63.5	22.8	5
15	1808	60	7.64	1	320	420	9.8	10.2	22.4	7.18	63.2	23.1	3
16	1809	50	6.86	2	240	348	7.8	6.4	8.2	7.2	54.6	20.4	4
17	521	40	6.92	1	300	14	7.4	6.2	4.4	7.27	40.7	18	1
18	3632	50	7.22	2	520	528	8.2	8.4	11.9	7.17	63.2	22.4	4
19	3636	60	7.41	3	210	525	9.8	11	24.8	7.2	54.9	20.4	5
20	3638	60	7.2	2	280	418	8.9	9.8	17.2	7.18	63.2	22.4	3
21	3642	50	7.1	3	3230	232	10.6	12.7	29.9	7.14	71.1	23.1	5
22	3757	50	7.2	2	1890	535	8.7	9	14.6	7.12	64.4	20.2	4
23	3758	50	7.23	3	1750	520	8.8	9	15.5	7.15	64.5	21.3	5
24	3771	50	7.2	2	770	498	9.7	10.4	23.3	7.25	49.1	20.6	3
25	3811	40	6.8	1	740	120	11.3	13.3	34.9	7.11	73.2	22.3	3
26	3812	50	7.3	3	740	285	9	9	16.1	7.14	67.8	22	5
27	3813	50	7.1	2	940	450	9.3	9.9	20.4	7.13	73.5	23.5	4
28	3959	50	7.2	3	820	185	8.6	8.4	15.1	7.18	57.3	20.3	4
29	3960	40	6.23	1	1480	40	8.7	8.7	13.3	7.23	48.2	19.7	3
30	5416	65	7	2	3260	80	8.8	8.5	15	7.07	71.8	19.6	4
31	5417	50	7	2	1630	285	8.8	9	15.2	7.23	73.5	22.8	4
32	5418	50	7.2	2	2190	233	10.2	10.7	25.1	7.13	73.5	23.5	4
33	5419	65	6.95	3	8290	249	8.8	8.9	14.7	7.21	31	12	5
34	5420	50	6.92	2	130	187	8.5	8.8	14.1	7.12	35.1	18	3
35	5422	60	6.8	2	2690	185	10.5	12.3	28.3	7.22	70.1	23.1	4
36	5425	50	7.3	2	60	245	9.8	13.2	14.1	7.1	40.1	20.2	4
37	5430	40	7.25	1	760	35	9.2	8.3	14.3	7.2	71.2	24.1	3
38	5431	60	6.92	3	100	210	10.1	14.2	23.1	7.12	54.1	22.3	4
39	5432	50	7	2	660	322	9.5	12.1	28.1	7.21	71.3	23.1	3
40	5433	60	6.82	1	120	420	10.3	13.3	15.5	7	40.2	18.2	4
41	5434	60	6.72	2	2060	225	9.1	12.2	22.2	7.21	71.3	25.2	3

Sl. No.	Donor bag no.	Volume(ml)	pH	Swirling	WBC count [cells/cumm]	Platelet count [x10³/µl]	MPV [fL]	PDW [fL]	PLCR [%]	ABG Analaysis			Score
42	5435	50	6.1	3	240	325	11.2	14.2	24.2	7.17	63.3	22.4	4
43	5436	50	7.21	2	240	450	10.2	10.7	25.1	7.13	42.2	18.2	4
44	5437	60	6.82	3	2090	520	9.8	12.1	34.5	7.14	64.5	12	5
45	5438	50	6.91	3	1710	488	9.7	10.4	23.3	7.11	73.2	22.3	4
46	5439	40	7.2	2	30	185	8.8	9	15.5	7.15	42.2	21.2	3
47	5440	50	6.25	3	2200	450	9.3	9.9	20.4	7	58.2	23.4	4
48	5441	50	6.9	2	2020	470	8.4	8.4	10.1	7.23	63.4	22.1	4
49	5442	40	7.23	1	2060	285	8.1	8	10.4	7.3	71.4	24	4
50	5443	50	6.8	2	40	335	8	8.2	11.2	7.22	70.1	22.8	4
51	5444	40	6.2	2	660	220	8.5	8.2	11	7.12	40.4	14	3
52	5445	50	6.82	3	370	185	8.6	8.3	11.8	7.18	57.4	22.4	3

Sl. No.	Donor bag no.	Volume	pH	Swirling	WBC count	Platelet count	Score	MPV	PDW	PLCR	ABG Anlaysis		
											pH	pCO2	HCO^{3-}
1	1301	70	7.2	3	410	1109	5	9.7	9.8	20.9	7.43	21.4	13.9
2	1302	70	7.14	3	460	145	5	9.5	10.5	20.7	7.41	23.3	14.4
3	1306	70	7	2	250	146	4	8.5	7.7	11.7	7.37	31.2	17.6
4	1409	65	6.9	2	130	57	4	8.2	7.2	10.5	7.49	21.7	16.5
5	1410	80	7.1	3	640	360	5	10	10.5	23.7	7.49	18.7	14
6	1411	70	7	2	280	266	4	8.2	6.9	9.9	7.34	25.8	13.7
7	1412	80	6.7	3	200	285	5	8.3	7.1	10.3	7.44	20.1	15.4
8	1532	70	7.35	3	770	412	5	8.5	7.7	11.8	7.49	22.4	16.7
9	1533	70	7.08	3	160	208	5	8.9	8.5	14.1	7.46	24.2	17.1
10	1534	70	7.39	2	370	449	3	8.8	8.3	13.7	6.76	76.3	10.3
11	1535	70	7.15	3	310	179	5	10.6	11.2	28.5	7.4	21.4	11.5
12	1536	70	7.4	3	420	130	5	9.7	9.4	20.9	7.42	18.6	11.9
13	1537	70	7.18	3	120	302	5	8.6	7.7	12.7	7.52	16.6	13.3
14	3426	70	7.14	3	90	330	5	8.3	7.2	10.2	7.02	52.1	14.3
15	3427	70	7.14	2	750	521	3	8	6.8	8.8	6.97	66.1	14.5
16	3428	70	6.9	2	170	343	4	8.1	6.7	9.4	7.11	33.5	10.4
17	3525	75	7.14	2	30	404	4	9.1	9.1	15.9	7.23	32.3	13.1
18	3529	70	7.11	3	340	434	5	10	10	23.1	7.22	36	14.1
19	3530	70	7.35	2	430	623	4	9.7	9.8	20	7.17	42.2	14.7
20	3531	70	7.45	2	30	407	4	8.8	7.8	13.1	7.18	36.2	13
21	3834	70	7.14	2	510	1035	4	10	9.7	22.4	6.84	91.8	14.9
22	3836	70	7.35	2	460	1058	4	9.8	9.6	21	7.26	40.7	17.6
23	3841	70	7.45	1	320	1090	4	9.9	9.8	21.5	7.23	54.4	21.8
24	4056	75	7.49	3	20	445	4	8.3	7.4	10.4	7.22	35.3	13.8
25	4057	75	7.5	3	780	966	5	9.6	9.6	20.1	7.2	40.1	15
26	4059	85	7.8	2	380	445	4	8.4	7.5	10.9	7.15	50.6	16.9
27	4071	85	7.66	2	260	336	4	8.9	8.4	14.5	7.28	44.6	20.3
28	4072	70	7.35	3	50	346	5	8.2	7.2	9.7	7.24	35.9	14.7
29	4155	50	7.74	2	310	815	4	9	9.2	16.9	6.83	99	15.4
30	4157	70	7.23	2	280	1521	4	10.4	11.3	27.3	6	123.1	2.9
31	4161	70	6.98	2	360	687	4	9.1	8.6	16.2	6.72	132.2	16
32	4164	80	7.22	1	470	968	3	9.8	10.5	22.9	6.42	118	10.2
33	5014	80	7.35	1	220	240	3	9.4	10.3	20.8	6.81	80.6	12
34	5096	75	7.14	3	580	406	4	9.2	8.8	17.2	7.05	59	15.7
35	5097	80	7.35	2	280	706	4	11.1	12.3	32.5	7.07	38.8	10.7
36	5099	80	7.11	3	370	356	4	8.8	8.2	14.2	7.08	49.7	14.1
37	5115	85	7.14	2	320	1672	4	10.4	11.2	26.5	6.58	91.4	8
38	5116	75	6.98	3	40	191	4	9.7	10.2	21.6	7.12	49.5	15.4
39	5117	70	7.02	2	50	625	3	8.6	7.8	12.3	6.94	78.5	16.1
40	5125	70	6.75	3	220	193	4	9.6	9.6	20.3	7.07	60.6	16.6
41	5128	70	7.14	2	40	663	4	10	10.2	23.6	6.85	84	13.8
42	5154	85	7.11	3	30	769	3	9.6	9.4	19.5	7.49	21.7	16.5
43	5155	80	7	2	260	780	4	9.6	9.4	19.5	6.94	49.5	10.1

Sl. No.	Donor bag no.	Volume	pH	Swirling	WBC count	Platelet count	Score	MPV	PDW	PLCR	ABG Anlaysis		
44	5177	80	7.14	1	340	749	3	9.3	9.3	17.6	7.14	55	17.9
45	5178	75	7.14	2	20	1025	3	10.4	11	26.3	7.04	146.9	2.8
46	5180	80	7.14	3	40	106	4	9.8	9.7	22.5	7.34	25.8	13.7
47	5200	75	7.14	2	20	455	4	9.8	10	22.1	7.02	49.8	12.1
48	5201	80	7.35	1	280	460	3	9.8	10.3	22	6.98	120.2	3.1
49	5427	70	7.35	1	380	663	3	10	10.2	23.6	7.23	30.7	12.5
50	5429	75	7.45	3	450	438	3	10.7	12	29.2	7.13	47.2	15
51	5275	85	7	2	570	625	4	8.6	7.8	12.3	7.25	30.2	12.9
52	5276	70	6.25	3	50	778	5	9.7	10	22.2	7	42.2	22.4

Sl. No.	Donor bag no.	Volume	pH	Swirling	WBC count	Platelet count	Score	MPV	PDW	PLCR	ABG Anlaysis		
											pH	pCO2	HCO^{3-}
1	12	260	7.41	3	2	1030	5	9.5	10.2	18.6	6.93	47.2	9.3
2	13	270	7.3	3	0	1102	5	8.8	9.3	14.3	6.91	42.6	8.1
3	14	280	7.39	3	0	1654	5	9	9.5	17.4	6.36	29.6	7.6
4	18	260	7.41	3	0	1647	5	9.3	9.4	18.6	6.93	47.2	9.3
5	19	260	7.39	3	2	1035	5	10.5	11.2	27.1	6.91	42.6	8.1
6	20	260	7.63	3	0	1013	5	12	15.2	39.1	6.63	23.4	2.3
7	24	320	7.35	3	10	3169	4	10.1	11.2	25.3	7.1	26.7	2.5
8	25	250	7.45	3	2	1436	5	9.9	10	21.5	6.36	29.6	7.6
9	26	290	7.45	3	1	1439	5	10.3	11.1	26	7.02	31.7	7.7
10	27	295	7	3	2	1445	5	10.4	10.9	26.3	6.42	46.5	2.9
11	28	295	7.45	2	28	922	3	8.1	6.7	9.6	7.08	14.1	4
12	29	230	7.48	3	20	876	4	11.4	13.5	35.1	7.08	25.6	7.2
13	32	270	7.14	2	6	462	3	11.6	13.8	36.7	7.07	71.8	19.6
14	36	260	7.2	3	13	897	4	9.5	9.2	19.1	7.48	20.9	15.3
15	37	260	7.1	3	7	1161	4	11	12.3	31.6	7.09	72.5	20.8
16	38	250	7.45	2	9	688	3	8.8	8.2	14.6	6.9	47.1	9.3
17	39	260	7.35	3	20	1231	4	9.2	8.9	17.4	6.92	42	8.1
18	46	250	7.24	3	13	1021	4	8.9	8.5	15.3	6.91	42.2	8.2
19	48	260	7.41	2	3	433	5	8.6	7.7	12.3	6.93	47.2	9.3
20	49	260	7.63	2	2	974	5	9.5	9.4	29.6	6.63	23.4	2.3
21	50	280	7.45	3	5	781	5	9.3	8.7	17.7	6.36	29.6	7.6
22	52	250	7.41	3	20	1776	4	9.4	9.5	18.4	6.93	47.2	9.2
23	54	290	7.45	2	16	484	3	10.1	10.4	24.2	7.02	31.7	7.7
24	55	260	7.4	3	48	936	4	9.9	10.3	22.8	6.36	29.6	7.6
25	56	295	7.3	2	85	630	3	9.4	9	18.6	7.08	14.1	4
26	57	260	7.41	3	15	1731	4	9.6	9.8	20.4	6.93	47.2	9.3
27	59	260	7.63	3	38	1029	4	9	8.5	15.9	6.68	23.4	8.1
28	60	270	7.2	3	10	751	4	8.7	7.8	13.8	6.91	42.6	8.1
29	61	280	7.45	3	5	795	5	8.3	7.6	10.4	6.4	29.6	7.8
30	62	240	7.42	2	0	348	5	8.2	7.3	8.9	7.08	14.1	4
31	63	290	7.2	3	10	822	4	8	6.8	8.4	7.02	31.7	7.7
32	64	270	7.39	2	13	774	3	8.9	8.7	15.6	6.91	42.6	8.1
33	65	260	7.42	3	3	825	5	9.3	9.1	17.7	6.93	47.8	9.8
34	66	260	7.45	3	6	1229	4	9	8.6	15.7	7.08	14.1	5.2
35	67	240	6.8	2	9	413	4	9.3	8.9	16.6	6.63	23.4	2.3
36	68	260	7	3	6	690	4	9.6	9.9	21.2	6.83	47.2	8.1

Sl. No.	Donor bag no.	Volume	pH	Swirling	WBC count	Platelet count	Score	MPV	PDW	PLCR	ABG Anlaysis		
37	69	260	6.83	3	6	690	4	9.6	9.9	21.2	6.36	14.1	7.7
38	70	260	7.39	3	1	700	5	8.1	6.9	8.6	6.91	42.6	8.1
39	71	260	7.42	2	13	861	4	9.6	9.6	20.6	6.82	47.5	9.3
40	73	260	7.41	3	8	1343	4	10.2	11	25.2	6.93	47.2	9.3
41	74	270	7.39	3	2	758	5	8.4	7.5	11.7	6.91	42.6	8.1
42	76	250	7.39	3	11	714	4	10	10.6	24.1	6.91	42.6	8.1
43	78	260	7.25	2	6	503	4	9.4	9.4	18.6	6.63	42.6	2.3
44	79	290	7.41	3	19	1252	4	9.2	9.1	1252	6.93	47.2	9.3
45	80	260	7.41	3	11	1363	4	9.3	9.3	18.1	6.93	47.2	9.3
46	83	280	7.39	3	18	1045	4	8.3	7.1	10.4	6.4	23.4	2.3
47	84	260	7.39	3	8	1084	4	9.3	8.7	17.8	6.91	42.6	8.1
48	86	280	7.2	2	10	1122	4	10.2	10.4	20.4	7.02	42.4	9.4
49	87	260	7.4	2	15	861	4	10.2	11	25.2	6.93	31.6	9.3
50	88	260	7.63	3	4	1093	4	8.7	8.4	14	6.82	40.2	5.2
51	89	250	6.42	3	1	452	5	10.1	10	23.7	6.28	42	10.4
52	90	310	7.4	3	1	1524	5	9.4	9	19.9	7.1	26.7	2.2