

# Study Protocol

## **A Study Protocol to Assess the Effects of Storage on Blood Components and Packed Red Blood Cells in Blood Bank**

### **Abstract:**

**Background:** For preservation of Packed Red blood cells (PRBCs), the recommended optimum conditions includes storage at  $2-6^{\circ}$  C in specialised blood storage refrigerators. PRBCs are required for blood transfusions in some life-saving situations and can be stored for 42 days. But during this storage period, continuous degradation of RBCs occurs and oxygen carrying capacity gets reduced. These changes inadvertently affect the lifespan of stored RBCs. Therefore during this storage period, routine evaluation is recommended. This study aimed to assess the storage effects on Blood Component and internal quality control of packed red cells.

**Methodology:** This Prospective Cohort study includes analysis of 50 Packed Red blood cells bags for RBC count, Haemoglobin and RBC indices. The analysis will be done on Day 1, Day 21 and Day 42 from the date of blood collection. The study will be carried in Jawaharlal Nehru Medical College Blood Centre and Department of Pathology, JNMC, AVBRH, Sawangi Meghe, Wardha. The data collected will be analysed using appropriate statistical tools.

**Expected Results:** Haemoglobin and Red cell indices with the help of cell counter will be analysed. Effects of storage on packed red blood cells will be drawn from the analysis.

**Keywords:** Packed Red Blood Cells, Effects of storage, Blood components, Oxygen Carrying Capacity, Blood Bank

### **INTRODUCTION**

Packed Red blood cells (PRBCs) can be stored under optimum conditions at temperature of  $2-6^{\circ}$  C for up to 42 days in specialised blood storage refrigerators with temperature monitoring and power backup and are needed for various life-saving blood transfusions, but during the above-mentioned shelf life they undergo continuous degradation. The quantum of degradation of RBCs depends on a number of factors from collection, separation and. Shelf life of whole blood is 35 days while that of Packed red cells collected in triple bag is 42 days<sup>1,2</sup> Quality assurance is an important and indispensable part of any blood bank. Internal and external quality control are the backbones of all quality assurance programs. In transfusion medicine, the various protocols adopted for assurance of optimum quality (IQAC and EQAS) of blood and blood products, which aids in availability of blood and blood component of optimum quality which can deliver maximum efficacy with minimal risk of adverse effects to recipients.<sup>3</sup> Packed rbc are routinely utilised in hospitals for correction of various anaemia and other surgical and non-surgical indications. The shelf life PRC is usually around 42 days after which it is discarded<sup>2</sup>. There are a number of studies having evidence of RBCs undergoing degenerative storage

changes, but these studies assessing storage duration effects on PRBCs depict findings which are conflicting<sup>3,4,5,6</sup>. RBCs go through a variety of storage changes during the shelf life that affect health of RBC membrane and capacities of RBCs to deliver oxygen delivering, which may have an adverse effect on clinical outcomes<sup>7,8,9</sup>. These adverse variations are collectively referred to as 'storage lesions' and they include metabolic alterations (like decrease in [2,3-DPG] 2,3-diphosphoglycerate and [ATP] adenosine triphosphate), changes due to enzymatic actions (e.g. breakdown of plasma membrane, damage to proteins), effects of oxidative stress (e.g. changes in shape of cell and loss of membrane) and physiologic variations (e.g. protein restructuring, eryptosis signalling)<sup>10,11,12</sup>, because of these changes the lifespan of stored RBCs is limited during which they offer clinical benefits. It is for this reason the optimum duration of storage warrants routine evaluation and careful consideration. The main objective of this study is to assess internal quality control of packed red cells through its shelf life as an indicator of our blood centre quality and storage efficacy of Blood component packed red blood cells (PRC).

## **RATIONALE**

PRC is routinely used in hospital for various indications. Its internal quality control is done on 1 percent of bags after collection and separation before storage. This study is undertaken to evaluate the storage effects on quality of PRC during its shelf life by assessment of quality parameters on 3 different occasions during its shelf life (Day 1, Day 21 and day 42 post collection)

## **AIM AND OBJECTIVES**

### **Aim:**

To assess storage effects on Blood Component - Packed Red Blood Cells in JNMC Blood Bank, Sawangi Meghe Wardha.

### **Objectives:**

1. To test RBC count, Haemoglobin and RBC indices of Packed red blood cells at day1, day 21 and day 42 post collection in JNMC, Blood Bank
2. To compare the parameters of RBC count, Haemoglobin and red cell indices and assess the changes due to storage on 1, 21- and 42-days post collection in JNMC, Blood Centre.
3. To assess quality of blood component storage of packed rbc bags

## **MATERIALS AND METHODS:**

This is a prospective analytical type of study. The study will be carried in Jawaharlal Nehru Medical College Blood Centre and Department of Pathology, JNMC, AVBRH, Sawangi Meghe, Maharashtra, India.

**Sample size:**

Total of 50 PRC bags will be studied. Approval will be obtained from Institutional Ethics Committee before starting the study.

**Inclusion criteria:**

PRC bags prepared from triple bags at JNMC Blood Bag

**Exclusion criteria:**

- PRC Bags with low volume
- HIV, HBsAg, HCV, Syphilis positive PRC bags

**Materials:**

- 1) Triple Bags
- 2) Refrigerated Centrifuge
- 3) Digital Weighing scales
- 4) Laminar airflow
- 5) Tube sealer
- 6) Plasma Expressor
- 7) PRC storage fridge
- 8) Cell counter

**Methods:**

A total of 50 bags of PRC prepared from Triple Bags at JNMC Blood bank, Sawangi Meghe, Wardha will be enrolled in the study and analysed for RBC count. The potential donor of blood will be selected after taking proper history and medical examination as per the SOP of the blood centre according to the statutory guidelines of donor selection. Informed consent in patient's vernacular language will be taken. A suitable vein will be selected for donation based on the basis of location and other parameters, the site of venepuncture will be prepared by cleaning the area by betadine and spirit, starting from centre to periphery. The phlebotomy will be done by experienced medical officer with minimal trauma. Pilot sample will be collected at the time of collection from primary blood bag and sent to transfusion transmitted infections testing laboratory within the blood bag. The collected bag will be then sent to component laboratory for separation

The following steps will be followed for separation of blood components for the triple within 6 hours of collection:

1. Triple are balanced using standard weights and centrifuged for 8 mins at light spin (1900) rpm at 22-24-degree Celsius (Using refrigerated cold centrifuge by thermo scientific 40553401) and PRP is expressed from the primary bag leading to formation of PRBC (packed red blood cells) bag and PRP (Platelet rich plasma) bag
2. Preparation of PRBC (packed red blood cells) bag is complete and it is cut off and stored at 2-6-degree Celsius (in walk in cooler - bluestar 93691309 or remi BDI4571)
3. PRP (Platelet rich plasma) bag is further processed by hard centrifugation at 3800 rpm for 7 min at 22-24 degree Celsius (Using refrigerated cold centrifuge Remi VCAM-595) and expressed (using plasma expessor – Baxter T-29219) leading to formation of platelet and plasma bags

The platelet concentrates bags will be stored in platelet incubator (Terumo Penpol PI200) over platelet agitator (PA300) as per protocol for up to 5 days. The platelet poor plasma stored as fresh frozen plasma (FFP) by freezing it in deep freezers at or below -40°C up to a period of one year (in Deep freezers- Remi BD4564 or Bluestar - dw - hl398s).

The pilot sample from the blood unit will be tested for transfusion transmitted infections (TTI). HIV, HbSAg, HCV, Syphilis will be screened by elisa using automated ELISA analyser, screening for malaria will be done by microscopic examination of Leishman stained slides. If the blood unit comes positive for any infection the bags will be discarded as per protocol (SOP).

Haemoglobin and Red cell indices with help of cell counter will be analysed on Day 1, Day 21 and Day 42 post collection. The data collected will be tabulated and compared by various statistical tools.

## **OBSERVATION**

The values of RBC count Haemoglobin and RBC indices will be analysed on Day 1, Day 21 and Day 42 post collection, the results obtained will be tabulated and analysed using appropriate statistical tools. Conclusion regarding the effects and quality assessment of storage of packed red cell bags will be drawn accordingly.

## **DISCUSSION**

Blood Centres have to take triad of responsibilities - primarily provision of sufficient blood and blood products for those needing transfusion and essentially to ensure maximum possible safety from adverse drug reactions and transfusion transmitted infections while providing maximum efficacy of the products by maintaining optimum quality. Various quality control measures are taken to ensure optimum quality of products quality. The quality of products can be affected by a number of factors during collection, preparation, storage, testing and administration. The quality, adequacy and safety of blood and blood components have been improving significantly with time, owing to technological advances in all aspects of the manufacturing processes and practices.

Developing countries sometimes face obstacles in ensuring safest blood transfusions, due to cost effectiveness and paucity of availability of technological advances. A balance has to be made considering these factors. Quality control in Blood Centre services involve all aspects of flow of events from blood collection from donor to administration to recipients (including blood collection, Transfusion transmitted infections (TTI) screening, preparation of component, storage of blood and blood components, transportation, and transfusion to recipients.

Internal quality control and proficiency testing are aspects of quality systems concerned. A number of studies have been done to assess the quality control of blood and blood components at various blood centres. Studies for assessment of quality of blood and blood products have been done at all levels of like collection, blood grouping, TTD testing, separation and storage. We have planned to study the storage effect on packed RBCs which is the most important and most used blood component. A few studies have also been done on similar subject. Some of them have been discussed below in brief.

In a cross-sectional study of Sultan et al on 100 units of blood component PRBCs were studied for hematocrit (HCT). The PRBCs had a mean HCT of was  $69.5 \pm 7.24$ , and in 98% units. The values of HCT were under permissible limits and ( $<80\%$  of HCT) met the standard <sup>4</sup>

In the study of T. Brune et al 15 components were prepared using the hollow fibre filter device U-shaped. Hemoglobin was estimated in these sample at the time of collection and 42 days after collection. The findings of haemoglobin values were compared with 15 bags prepared by centrifugation. The findings were found to be within the acceptable limits as per the guidelines of the council of Europe.<sup>13</sup>

Zhensong Xu et al studied the storage effects or prbcs on stiffness of RBCs with the help of polydimethylsiloxane (PDMS) device and found an increase in stiffness of RBCs on storage.<sup>14</sup>

R. N. Makroo et al did a study of evaluation of red blood cells hemolysis on processing and storage on 7, 14, 21, 28, 35 and 42 days after collection of blood on 40 bags of PRC stored in SAGM and found that the RBCs had an increase in hemolysis with storage, but haemolysis did not exceed the limits permissible as per guidelines even at its highest level at any time.<sup>15</sup>

The findings of our study will be discussed in relation to the findings of study done by others and incoherence's will be analysed and attempt to find the reason for the same will be made, also the quality control of storage will be assessed.<sup>17-21</sup>

## CONCLUSION

The tabulated findings of effects of storage on RBC count, Haemoglobin and RBC indices at 7, 21 and 42 days in Packed red blood cell bags, post collection will be analysed with the help of suitable statistical tools and conclusion. Conclusions will be drawn regarding the quantum of degradation of PRBC (Packed red blood cells) on storage as well as assessment of quality control of storage of PRBC (Packed red blood cells) will be done.

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