

Access this article online
Quick Response Code:

Website: www.ajts.org
DOI: 10.4103/ajts.AJTS_87_19

Evaluation of quality matrix when practice changed from triple bags to quadruple (top and bottom) bags: *In vitro* analysis of blood components!

Gunjan Bhardwaj, Aseem Kumar Tiwari, Geet Aggarwal, Swati Pabbi, Jyoti Sharma, Aanchal Luthra, Anand Upadhyay

Abstract:

BACKGROUND: As a part of continuous quality initiatives, while moving from triple bags to quadruple bags, we undertook a study to compare platelet-rich plasma (PRP) and buffy-coat (BC) methods with respect to all blood components (red blood cells [RBCs], random donor platelet concentrate [RDPC], and fresh frozen plasma [FFP]) prepared by PRP and BC methods.

MATERIALS AND METHODS: It was a prospective analysis of different physical and quality parameters of RDPC, RBC, and FFP prepared out of 100 whole blood (WB) donations. Of these, 50 WB units were processed by PRP method using Triple bags and 50 WB units by BC method, using quadruple (top and bottom) bags, with an attached integral filter.

RESULTS: RBC prepared by BC method had higher hematocrit ($61.3 \pm 1.91\%$ vs. $56.03 \pm 3.37\%$; $P < 0.05$) and lower white blood cell (WBC) contamination ($6.3 \times 10^4 \pm 6.1$ vs. $5.41 \times 10^5 \pm 2.5$; $P < 0.05$) in comparison to prepared by PRP method. Higher PLT yield ($7.67 \times 10^{10} \pm 1.8$ vs. $6.47 \times 10^{10} \pm 1.5$; $P < 0.05$) and lower WBC count ($8.24 \times 10^3 \pm 1.1$ vs. $1.5 \times 10^4 \pm 2.1$; $P < 0.05$) was observed in RDPC prepared by BC method than PRP derived. CD62P expression was lower in RDPC prepared by BC method ($31.46 \pm 9.7\%$; $P < 0.05$) as compared to PRP method ($43.35 \pm 12.5\%$; $P < 0.05$). The BC method also resulted in increased plasma yield (210.56 ± 18.54 ml vs. 187.92 ± 12.93 ml; $P < 0.05$) in FFP in comparison to PRP method.

Conclusion: The blood components produced from WB by the BC method have laboratory variables suggestive of superior quality than those produced by the PRP method.

Keywords:

Buffy-coat, fresh frozen plasma, platelet-rich plasma, random donor platelet concentrate, red blood cell, white blood cell contamination

Introduction

In past few decades, there have been major strides in transfusion safety with improvements in the entire transfusion chain of events in the process of whole blood (WB) and apheresis donations, including pre- and post-donation counseling, donor recruitment, donor safety, testing

for transfusion transmitted infections, optimization and quality assurance (QA) of blood components and pre-transfusion testing.

Processing of blood donation into separate components is key link of the transfusion chain. There are two common methods of component preparation; platelet-rich plasma (PRP) method and buffy-coat (BC) method. Although both methods produce

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Bhardwaj G, Tiwari AK, Aggarwal G, Pabbi S, Sharma J, Luthra A, *et al.* Evaluation of quality matrix when practice changed from triple bags to quadruple (top and bottom) bags: *In vitro* analysis of blood components! Asian J Transfus Sci 2021;15:30-6.

Department of Transfusion
Medicine, Medanta-the
Medicity, Gurgaon,
Haryana, India

Address for correspondence:

Dr. Aseem Kumar Tiwari,
Department of Transfusion
Medicine, Medanta-the
Medicity, Sector-38,
Gurgaon - 122 001,
Haryana, India.
E-mail: draseemtiwari@
gmail.com

Submitted: 20-08-2019
Revised: 23-12-2019
Accepted: 13-09-2020
Published: 12-06-2021

a platelet (PLT) product, the products have somewhat different *in vitro* characteristics. There is noticeable quality improvement in laboratory markers of BC-produced PLTs (BC-PCs) compared to PRP-produced PLTs (PRP-PCs).^[1] This improvement is characterized by a higher proportion of discoid PLTs, lower CD62 expression, and better hypotonic shock response (HSR) at the end of storage.^[2] BC preparation of PLT is the common method of preparation of random donor platelets (RDP) in countries such as Canada, the UK, and Europe.^[3]

In India, there is a mix of processing methods; PRP and BC method of component preparation across various blood banks. Few studies comparing the PLT components prepared by these two methods have been done^[4,5] in India. However, there is paucity of data on comparison of components other than random donor platelet concentrate (RDPC), such as red blood cells (RBCs) and fresh frozen plasma (FFP). As a part of continuous quality initiatives (CQI) when we were moving from triple bags to quadruple bags, we undertook a study to compare PRP and BC methods, their compliance to published quality standards with respect to not just PLT concentrate; rather all blood components (RBC, RDPC, and FFP) prepared by PRP and BC methods.

Materials and Methods

Settings

It was a prospective analytical study measuring different physical and quality parameters of RBC, RDPC, and FFP prepared from WB donated by eligible donors over a period of 2 months (June 2019 to July 2019) undertaken in the Department of Transfusion Medicine in a large tertiary care hospital in the National Capital Region (NCR).

Sample size calculation

For sample size calculation, the variable considered in two methods (PRP vs. BC) was difference in volume of PLTs made by these two different methods. The volume was calculated as the average from three different studies,^[4-6] and this was 5.8 ml considered as $(m_1 - m_2)$ in the formula.

Assumptions:

- Group 1 = PRP Group
- Group 2 = BC Group
- Parameter of Interest: RDPC Volume (ml)
- Mean value of the parameters for group 1 (m_1) = 53.6
- Mean value of the parameters for group 2 (m_2) = 59.4
- Standard deviation of the parameters for group 1 (σ_1) = 7.0
- Standard deviation of the parameters for group 2 (σ_2) = 8.9

- Difference in the parameters for two groups (m) = $(m_1 - m_2) = 5.8$.

The formula used was as follows:

$$n = (\sigma_1^2 + \sigma_2^2) (Z^\alpha + Z^\beta)^2 / (m_1 - m_2)^2$$

Where,

Z_α = Value of standard normal variate corresponding to α level of significance

Z_β = The standard normal deviate for desired power

m = Average

σ = Standard deviation

With the above assumptions, the minimum sample size for 95% confidence level and 95% power worked out 50 blood units per group.

Study samples

An analysis of blood components (RBC, RDPC and FFP) prepared out of consecutive 100 WB donations was done. Of these, 50 WB units were processed by PRP method and 50 WB units by BC method. The WB donations were collected from donors after they were administered health history questionnaire, underwent physical examination as per departmental standard operating procedures (SOP) and were found suitable for donation. Informed consent of each donor is part of the donor selection process. Few study-specific inclusions were applied; such as only male blood donors of age group between 20 and 40 years, with hemoglobin (Hb) level between 14 g/dl to 16 g/dl were included in the study. This was done to minimize the confounding effect of donor related variables (e.g., gender, age, and Hb level) on the comparative analysis of components made from PRP method and BC method.^[7]

Whole blood collection and component preparation

Four hundred and fifty milliliters of WB were collected from medically screened, apparently healthy donors in quadruple (Top and Bottom) bags (CompoFlow, Fresenius Kabi AG, Bad Homburg, Germany) with an attached integral filter and Triple bags (CompoFlex, Fresenius Kabi AG, Bad Homburg, Germany), for BC method and PRP method, respectively. Both type of bags (i.e., Top and Bottom bags and Triple bags) contained citrate, phosphate and dextrose (CPD) as anticoagulant solution in the primary collection bag (63 mL) and 100 ml of saline, adenine, glucose, and mannitol (SAGM) additive solution for RBC. The WB collection completed within 7–9 minutes from the starting time was included in the study. Blood units were given a minimum resting

period of 1 hour (h) at room temperature (20°C–24°C), before further processing and blood components. All components were prepared within 6 h of collection, using centrifuge, Cryofuge 6000i (Heraeus, Germany) and automated component separator, CompoMat G5 (Fresenius Kabi, Bad Homburg, Germany).

Sampling of units and measurements

Different physical and quality parameters were analyzed as shown in Tables 1 and 2.

For all units including WB, subjected to testing of quality parameters, sampling was done only after proper homogenization of the bag to make sure that sample in the segment represents the actual content of the bag. Samples from WB were collected 2 h from the time of collection (before separation). For RBC, samples were drawn and tested, 1 h after preparation. For RDPC and FFP, samples were drawn and tested on day-1.

Volume

Volume of WB, RBC, PLTs, and FFP was calculated from the net weight of the product divided by specific gravity (1.053 for WB; 1.09 for RBCs; 1.03 for PRP-PC and 1.06 for BC-PC; 1.02 for plasma).

Volume of concentrate

$$\frac{\text{Weight of full bag} - \text{Weight of empty bag}}{\text{Specific gravity}}$$

Hematological values and cell counts

All hematological values such as Hb, hematocrit (HCT), PLT count, white blood cells (WBCs) for WB and RBC were obtained using a routinely calibrated automated hematology analyzer, Sysmex XE 2100 analyzer (Sysmex Corporation, Japan). The RBC count per bag for WB or RBC was calculated by multiplying RBC count/ μl with product volume. The Hb per bag for WB or RBC was calculated by multiplying Hb count/dl with product volume. The WBC count per bag for WB was calculated by multiplying WBC count/ μl with WB volume. The PLT count per bag for WB or PLT concentrate was calculated by multiplying PLT count/ μl with product volume.

Table 1: Whole blood parameters before separation

Parameters	PRP	BC	P value
Physical parameter, volume (ml)	452.2 \pm 10.8	458.74 \pm 10.9	0.245
Quality parameters			
RBC/bag (10^{12})	2.48 \pm 0.36	2.51 \pm 0.24	0.741
Hb/bag (g)	70.32 \pm 2.7	70.08 \pm 3.1	0.268
HCT (%)	43.8 \pm 3.48	44.3 \pm 3.3	0.142
PLTs/bag (10^{11})	0.92 \pm 0.27	0.89 \pm 0.25	0.508
WBC/bag (10^9)	3.91 \pm 0.64	3.74 \pm 0.76	0.304

CPD=Citrate phosphate dextrose, PRP=Platelet-rich plasma, BC=Buffy-coat, RBC=Red blood cell, Hb=Hemoglobin, HCT=Hematocrit, PLT=Platelet, WBC=White blood cell

Filtration time

All the blood components (RBC and PLTs) used in our center are leukoreduced pre-storage using leukocyte reducing filters. Filtration time was accessed between buffy coat method derived RBC (BC-RBC), which were filtered using integral filters and PRP method derived RBC (PRP-RBC), which were filtered using BIO- R filters (Fresenius Kabi, Bad Homburg, Germany). For PRP method "Filtration Time" included total time taken from connecting the blood bag to leukoreduction filter plus the time taken for blood bag to filter. We analyzed the filtration time for RBC only, since filtration procedure differ for PRP-RBC (where an additional filter has to be attached) and BC prepared RBCs (having integral filter).

White blood cell count and log reduction

Ten samples each of RBC and RDPC (10 each for PRP method and BC method) were analyzed for WBC contamination pre- and post-leukocyte filtration using flow-cytometry. RDPC were leukoreduced using BIO- P filters (Fresenius Kabi, Bad Homburg, Germany). WBC log-reduction was calculated by using pre- and post-filtration WBC count. WBC count was analyzed pre- and post-filtration on flow-cytometer ([BD FACS VerseTM], Becton, Dickinson and Company, BD Biosciences, San Jose, CA). WBC count per bag for RBC and RDPC was calculated by multiplying WBC count analyzed through flow cytometry by product volume.

Sterility

Randomly selected 10 samples each for PRP-PC and BC-PC and corresponding RBC units were sent for aerobic and anaerobic cultures to the microbiology department of the hospital, as per the department protocol. Sterility testing samples were drawn on day-1 postcollection and were performed as part of routine QA.

Swirling

Swirling in all the units of PRP-PCs and BC-PCs units were assessed visually against the light source and was documented as "present" or "absent."

Lactate dehydrogenase and HCO₃ levels of platelets

Fifty samples each for PRP-PCs and BC-PCs were analyzed for lactate dehydrogenase (LDH) and bicarbonate (HCO₃). The samples were sent to biochemistry laboratory of the hospital and were measured on immunoassay analyzer (Vitros immunoassay analyzer [Ortho-Clinical Diagnostics, Unites States]).

pH of platelet units

PLTs, pH was measured by a calibrated portable pH meter ([OAKTON pH 700], Oakton Instruments, IL, USA), following the departmental SOP.

Table 2: Physical and quality parameters of different blood components by platelet-rich plasma and buffy-coat method

	Physical parameters	PRP	BC	P value	Quality parameters	PRP	BC	P value
RBC	Volume (ml) with SAGM (\pm SD)	322.12 \pm 11.31	293 \pm 9.13	<0.05	RBC/bag (10^{12}) ($n=50$)	2.08 \pm 0.13	1.93 \pm 0.14	<0.05
					Hb/dl ($n=50$)	18.53 \pm 0.97	18.92 \pm 0.47	0.06
					Hb/bag (g) ($n=50$)	60.08 \pm 4.26	53.1 \pm 3.7	<0.05
	Filtration time*	23.84 \pm 2.8	19.58 \pm 2.4	<0.05	HCT (%) ($n=50$)	56.03 \pm 3.37	61.3 \pm 1.91	<0.05
RDPC	Volume (ml)	70.54 \pm 5.315	72.14 \pm 5.70	0.15	WBC/bag ($n=10$)	5.41 $\times 10^5$ \pm 2.5	6.3 $\times 10^4$ \pm 6.1	<0.05
					PLTs/bag ($\times 10^{10}$) ($n=50$)	6.47 \pm 1.5	7.67 \pm 1.8	<0.05
					WBC/bag ($n=10$)	1.5 $\times 10^4$ \pm 2.1	8.24 $\times 10^3$ \pm 1.1	<0.05
					pH ($n=50$)	6.9 \pm 0.15	6.47 \pm 0.18	0.11
					HCO ₃ ($n=50$)	14.13 \pm 3.06	13.24 \pm 2.8	0.13
					LDH ($n=50$)	154.60 \pm 35.72	159.64 \pm 40.31	0.49
					CD62P (%) ($n=20$)	43.35 \pm 12.5	31.46 \pm 9.7	<0.05
					RBC contamination (ml) ($n=50$)	0.41 \pm 0.04	0.34 \pm 0.05	0.8
FFP	Volume (ml)	187.92 \pm 12.93	210.56 \pm 18.54	<0.05	PT (s) ($n=10$)	11.550 \pm 0.87	11.4100.54	0.67
					APTT (s) ($n=10$)	25.87 \pm 1.8	27.10 \pm 1.8	0.14
					INR ($n=10$)	1.130.06	1.010.05	0.16
					Fibrinogen (mg) ($n=10$)	486.27 \pm 141.13	611.71 \pm 137.3	0.06
					F VIII (IU/ml) ($n=10$)	1.50 \pm 0.5	1.3 \pm 0.4	0.3

*In PRP method "filtration time" includes total time taken from connecting the blood bag to leukoreduction filter + time taken for blood bag to filter. PRP=Platelet-rich plasma, BC=Buffy-coat, RBC=Red blood cell, Hb=Hemoglobin, HCT=Hematocrit, PLT=Platelet, WBC=White blood cell, LDH=Lactate dehydrogenase, APTT=Activated partial thromboplastin time, PT=Prothrombin time, INR=International normalized ratio

CD62P expression

Twenty samples each of RDPC prepared by PRP method and BC method were analyzed for amount of activation by calculating their CD62P expression. PLT surface CD62P expression were measured using flow cytometry ([BDFACS Verse™], Becton, Dickinson and Company, BD Biosciences, San Jose, CA). PLTs were stained by phycoerythrin monoclonal mouse anti-human antibodies and were analyzed according to the manufacture's instruction.

Red blood cell contamination of platelets

For RBC contamination, the red cell count was multiplied with the mean corpuscular volume (MCV) of one red cell (90 fL) and PLT volume;

Prothrombin time/activated partial thromboplastin time/international normalised ratio

Ten samples each of FFP prepared by PRP (PRP-FFP) and BC (BC-FFP) were sent to hematology laboratory for analysis of prothrombin time (PT), activated partial thromboplastin time (APTT), and international normalized ratio (INR).

Fibrinogen and factor VIII

Ten samples for calculating fibrinogen level and factor VIII levels of PRP-FFP and BC-FFP were sent to hematology laboratory for analysis. The fibrinogen per bag was calculated by multiplying fibrinogen count (mg/dl) with product volume.

Statistical analysis

We used the Student's *t*-test for comparative analysis.

$P < 0.05$ was considered statistically significant. SPSS software version 23 (Statistical Package for the Social Sciences; IBM Bengaluru, India) was used for analysis.

Ethical clearance

The study was approved by the institutional ethics committee.

Results

An analysis of 100 WB units (50 each for separation by PRP and BC method, later), before separation, was done and was similar in terms of their volume, erythrocyte content, Hb, hematocrit, PLT content, and leukocyte content for both PRP method and BC methods. The mean volume of WB was 452.2 \pm 10.8 ml and 458.74 \pm 10.9 ml for PRP and BC method, respectively. The other parameters are summarized in Table 1.

Analysis of physical and quality parameters of blood components

Red blood cell

The mean volume of BC-RBC was lower as compared to PRP-RBC (293 \pm 9.13 ml and 322.12 \pm 11.31 ml, respectively, $P < 0.05$). The total time taken for filtration for leukoreduction of was higher for PRP-RBC which was 23.84 min as compared to BC-RBC, which took 19.58 min.

Significant difference ($P < 0.05$) was observed between RBC count per bag, Hb per bag, and HCT per unit. The mean RBC count per bag and mean Hb per bag was more by PRP-RBC, which was 2.08 $\times 10^{12} \pm 0.13$

and 60.08 ± 4.26 g as compare to BC-RBC, which was $1.93 \times 10^{12} \pm 0.14$ and 52 ± 3.7 g, respectively. The mean Hb/dl for PRP-RBC was 18.53 ± 0.97 and for BC-RBC was 18.92 ± 0.47 and was not significant. The mean HCT per unit was significantly more for BC-RBC ($61.3 \pm 1.91\%$) than PRP-RBC ($56.03 \pm 3.37\%$). These results are summarized in Table 2.

The mean prefiltration mean-WBC count per unit for PRP-RBC was $9.1 \times 10^8 \pm 1.3$ and for BC-RBC was $6.2 \times 10^8 \pm 3.2$, respectively, and was not significant. The postfiltration mean-WBC count per unit was significant ($P < 0.05$) between PRP-RBC having count of $5.4 \times 10^5 \pm 2.5$ and BC-RBC with count of $6.7 \times 10^4 \pm 6.1$. For BC-RBC, in eight out of 10 units, a leucocyte reduction of 99.99% (4-log reduction) and in remaining two units a 99.9% (3-log reduction) reduction was observed. In PRP-RBC, in seven out of 10 units a 3-log reduction and in remaining three units a 4-log reduction was observed.

Microbiological cultures of randomly selected samples processed by both PRP method and BC method were found to be sterile.

Random donor platelet concentrate

The mean volume of PRP-PCs and BC-PCs was 70.54 ± 5.315 ml and 72.14 ± 5.70 ml respectively and was not significant.

The mean PLT count per bag was significantly higher ($P < 0.05$) for BC-PCs with mean count of $7.67 \times 10^{10} \pm 1.8$ as compare to PRP-PCs having mean count of $6.47 \times 10^{10} \pm 1.5$.

The prefiltration mean WBC count per bag for PRP-PCs and BC-PCs was $4.6 \times 10^7 \pm 1.5$ and $0.93 \times 10^7 \pm 5.7$, respectively, and was significant ($P < 0.05$). The postfiltration mean WBC count per bag for PRP-PCs and BC-PCs was $1.5 \times 10^4 \pm 2.1$ and $0.82 \times 10^4 \pm 1.1$ respectively and were significant ($P < 0.05$). For RDPC prepared each by PRP and BC method a 3-log-reduction was observed in all the 10 units analyzed.

The CD62P expression was significantly higher ($P < 0.05$) for PRP-PCs in comparison to BC-PCs, with values of $43.35 \pm 12.5\%$ and $31.46 \pm 9.7\%$, respectively.

The other quality parameters such pH, HCO_3 , LDH, and RBC contamination of PLTS which were analyzed are shown in Table 2.

Swirling was present in all the units derived by PRP and BC methods. Randomly selected RDPC units were sterile and none showed any cultivable microbial growth.

Fresh frozen plasma

The mean volume of PRP-FFP was significantly ($P < 0.05$) lesser as compared to BC-FFP (187.92 ± 12.93 ml and 210.56 ± 18.54 ml, respectively). The mean fibrinogen value for PRP-FFP units was $486.27 (\pm 141.13)$ mg and for BC-FFP units were $611.71 (\pm 137.3)$ mg.

Other quality parameters such as FVIII levels, PT, APTT, and INR were also analyzed, and no significant difference was observed. The results are depicted in Table 2.

Discussion

In India, studies^[4,5,6] carried out in the past regarding PRP method and BC method were confined to comparing the quality parameters of PLTs only. The main objective of the present study was to analyze and compare the quality of all blood components (RBC, RDPC, and FFP) made by PRP method and BC method in our setting.

Quality assessment of red blood cell

In the present study, the physical and quality parameters such as volume, RBC count per bag, Hb per bag, and HCT of BC-RBC units, were similar, with the study done by Hurtado *et al.*^[8] In general, the results of the quality control analysis of RBC derived by BC method showed a high degree of compliance with the Council of Europe (CE) quality recommendations for the production and storage of blood components.^[9] The BC method are defined better by CE, since European nations predominantly prepare blood components using BC method. The DGHS does not outline any specific guidelines pertaining to BC-RBCs. The RBC units prepared by PRP method were in conformance to the DGHS^[10] and AABB guidelines,^[11] since in India and the United States, component preparation is mainly done by PRP method.

Significant difference was observed between volume, Hb per bag and HCT of RBC prepared by PRP and BC method, as shown in Table 2. PRP-RBC had higher volume and Hb per bag whereas BC-RBC had higher HCT. However, on analyzing Hg/dl, which was $18.53 (\pm 0.97)$ g/dl for PRP-RBC and $18.92 (\pm 0.47)$ g/dl for BC-RBC, no significant difference was seen. Thus, the higher Hb per bag for PRP-RBC can be attributed only to their higher volume. Hematocrit or packed cell volume is a measurement of the ratio of the volume occupied by RBCs to the total volume of the blood sample. Unit volume is the total volume of the RBC unit following processing. The administration of high volumes of transfusion products can lead to circulatory overload. In order to avoid these adverse effects but still administer enough RBC for restoring the oxygen carrying capacity of the recipient, it is important to have consistent products with a high RBC mass to volume ratio (i.e., HCT).^[12]

The volume of residual plasma in RBC does not have any clear specification or standard and is not subjected to any routine quality control. However, the residual plasma volume could be important for allergic reactions or TRALI.^[13] Case studies^[13,14] have shown that RBC-associated TRALI remains a significant cause of transfusion-related morbidity and mortality. A study carried out by Canadian Blood Services on preventive measures of TRALI, have also observed a decrease in RBC-associated TRALI cases and it was attributed to decrease in plasma content in RBC components that was a result of introduction of the buffy coat method of component production.^[15]

The mean post-leukoreduction WBC count per unit was significantly ($P < 0.05$) higher for PRP-RBC ($5.4 \times 10^5 [\pm 2.5]$) in comparison to BC-RBC ($6.7 \times 10^4 [\pm 6.1]$). This was expected since WBC separate better (move to BC layer) in BC method as compared to PRP method, where most of the WBCs remain entrenched in RBC layer. Hirosue *et al.*^[16] also reported higher WBC contamination in PRP-RBC units than in BC-RBC units.

The time taken for leukocyte filtration was significantly lesser for BC-RBC as compare to PRP-RBC. In top and bottom bags, since an in-line integral filter was already present, no extra-time and no additional workforce was required to connect RBC bags to filters, using sterile connecting device. In contrast, PRP method required both extra time and manpower and there are distinct chances of errors while labeling and then connecting the blood bags to leukocyte reduction filters.

Quality assessment of random donor platelet concentrate

The physical and quality parameters of both PRP-PCs and BC-PCs in the present study, fulfilled the quality criteria recommended by DGHS.^[10]

The volume of BC-PCs was more than PRP-PCs, but not statistically significant. The volume obtained were comparable with other Indian studies by Singh *et al.*,^[4] Raturi *et al.*,^[5] and Talukdar *et al.*,^[6] also showing higher volume for BC-PCs than PRP-PCs.

The present study results showed significantly higher ($P < 0.05$) PLT count for BC-PCs as compare to PRP-PCs with values of $7.67 (\pm 1.8) \times 10^{10}$ and $6.47 (\pm 1.5) \times 10^{10}$ respectively. The lesser PLT yield can be attributed to the fact that, the centrifugation conditions used with the PRP method results in an average of 21% of the plasma and 19% of the PLTs remaining with the infranatant red cells.^[17] Studies done by Raturi *et al.*^[5] and Taludkar *et al.*,^[6] also analyzed a comparable but not statistically significant increase in PLT count by BC method then by PRP method.

Leukocytes found in blood components are responsible for most transfusion reactions and exert adverse effects on the blood component quality and stability (storage lesion) and are also responsible for transfusion associated virus transmission (CMV).^[18] The WBC count obtained in BC-PCs was lower in comparison to PRP-PCs in the present study in both prefiltration and postfiltration stages. Since the preleukoreduction WBC counts were significantly lower in BC-PC as compared to PRP-PC, this difference was carried forward in the post-leukoreduction stage, as well. The filtration was constant in both the groups. Post-leukoreduction WBC in the present study was in concordance with studies conducted by Fijnheer *et al.*,^[19] Singh *et al.*^[4] and Raturi *et al.*,^[5] since they also reported higher post-leukoreduction residual WBC count per unit in PRP-PCs than in BC-PCs.

CD62P expression measures PLT secretion and it indicates the level of activation of the PLTs. An increase in CD62P expression associated with PLT storage lesions as well and it can be used as a sensitive quality marker.^[20] In our study, the expression of CD62P, was significantly higher for PRP-PC in comparison to BC-PC, as analyzed on Day one. A study by Ali,^[21] displayed, during storage for up to 5 days' PRP-PCs units displayed a significant increase in the CD62P, as compared with BCs units ($P < 0.05$).

Recommendations for erythrocyte contamination as proposed by DGHS standards^[10] is supposed to be < 0.5 mL in each bag, in order to prevent RBC-associated alloimmunization. The PLT units prepared in the present study by both PRP and BC method had RBC values well below the desired limits.

A simpler technique to determine the quality of PLTs in the laboratory is by using the "Swirling test." The discoid morphology of PLTs correlates with the *in vivo* survival and can be demonstrated by the swirling phenomenon.^[5] In the present study, all freshly prepared PRP-PCs and BC-PCs, exhibited swirling, indicating their intact discoid morphology.

Biochemical tests such as decrease in pH, LDH accumulation, and changes in bicarbonate levels can be employed to assess PLT viability. These *in vitro* tests are more economical and rapid. All the PRP-PCs and BC-PCs were found within the acceptable limit ($\text{pH} > 6.0$ units) as per DGHS standards. No significant difference was observed between HCO_3 and LDH levels between the PLTs prepared by these two different methods. Although studies^[22] have shown difference in biochemical changes between PRP-PCs and BC-PCs, with storage, in our study, we analyzed the samples only on day of component preparation.

Quality assessment of fresh frozen plasma

The different physical and quality parameters of FFP, prepared by both PRP and BC method, met the DGHS guidelines.^[10]

The volume of BC-FFP was significantly higher ($P < 0.05$) in comparison to PRP-FFP. The lesser volume of PRP-FFP is due to the centrifugation conditions used with the PRP method resulting in an average of 21% of the plasma remaining with RBCs. The fibrinogen level per bag was considerably more for BC-FFP as compared to PRP-FFP, though not statistically significant. This can be attributed to the higher volume obtained in BC plasma bags.

No significant difference was observed between other quality parameters such as Factor VIII, PT, APTT, and INR. A study by Sheffield *et al.*,^[23] analyzing the coagulation variables in plasma during the transition period in the Canadian Blood Services, when they switched from PRP method to BC method of component preparation, observed that there was no reduction in frozen plasma (FP) quality made by the BC method (FP-BC) in the characteristics they tested such as fibrinogen, factor (F) V, ABO-matched FVIII, and antithrombin levels in comparison FP made by the PRP method (FP-PRP). They also concluded that, FP-BC supported global clotting, as measured by prothrombin time or activated partial thromboplastin time.

Conclusion

The blood components produced from WB by the BC method have laboratory variables suggestive of a superior quality than those produced by the PRP method, such as better HCT of RBCs, higher PLT count, and lesser WBC contamination in both RBC and PLTs. The BC method gives an increased plasma yield in FFP. The kinetics of CD62P expression are influenced by the method used to prepare the PLTs, with activation in PRP-PCs exceeding BC-PCs.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

- Nasiri S. Conversion from platelet-rich plasma platelet production to buffy coat platelet component production: Benefits and limitations. *Iran J Blood Cancer* 2014;6:188-207.
- Levin E, Culibrk B, Gyöngyössy-Issa MI, Weiss S, Scammell K, LeFresne W, *et al.* Implementation of buffy coat platelet component production: Comparison to platelet-rich plasma platelet production. *Transfusion* 2008;48:2331-7.
- Philip J, Samantha K, Chatterjee T, Biswas AK, Mallhi RS. Evaluation of random donor platelets produced from buffy coat stored for 24 h at ambient temperature: should this be implemented in India? *Indian J Hematol Blood Transfus* 2015;31:264-8.
- Singh RP, Marwaha N, Malhotra P, Dash S. Quality assessment of platelet concentrates prepared by platelet rich plasma-platelet concentrate, buffy coat poor-platelet concentrate (BC-PC) and apheresis-PC methods. *Asian J Transfus Sci* 2009;3:86-94.
- Raturi M, Shastry S, Raj P. Cumulative quality assessment for whole blood-derived platelets: A compliance review. *Glob J Transfus Med AATM* 2017;2:38-43.
- Talukdar B, Chakraborty S, Hazra R, Biswas K, Bhattacharya P. Quality assessment of platelet concentrates: A comparative study using three different methods. *Int J Biomed Res* 2017;8:194-9.
- Sparrow RL. Red blood cell components: Time to revisit the sources of variability. *Blood Transfus* 2017;15:116-25.
- Hurtado C, Bonanad S, Soler Mf, Mirabet V, Blasco I, Planelles Mf, *et al.* Quality analysis of blood components obtained by automated buffy-coat layer removal with a top & bottom system (Optipress ®II). *Haematologica* 2000;85:390-5.
- Council of Europe. Guide to the Preparation, Use and Quality Assurance of Blood Components. EDQM, 18th ed.; 2015. Available from: <https://www.avis.it/userfiles/file/News/EDQM%20Guide%2018th%20edition.pdf>. [Last accessed on 2019 Aug 18].
- Saran RK, editor. quality assurance in blood Transfusion. In: *Transfusion Medicine Technical Manual*. 2nd ed. New Delhi: DGHS, Ministry of Health and Family Welfare; 2003. p. 352-55.
- Kakaiya R, Julleis J. Whole blood collection and component processing at blood collection centres. In: Robock JD, editor. *AABB Technical Manual*. 17th ed. Bethesda: AABB; 2011. p. 217-21.
- Acker JP, Marks DC, Sheffield WP. Quality assessment of established and emerging blood components for transfusion. *J Blood Transfus* 2016;2016:486028.
- Weber LL, Roberts LD, Sweeney JD. Residual plasma in red blood cells and transfusion-related acute lung injury. *Transfusion* 2014;54:2425-30.
- Pérez-Caballero-Macarrón C, Moreno-Jiménez G, Coca-Pérez A, Vázquez-Martínez JL, Tenorio-Núñez M, Stanescu S, *et al.* Transfusion related acute lung injury in paediatric patients. Case report and review of the literature. *Acta Pediatr Mex* 2018;38:386-93.
- Lin Y, Saw CL, Hannach B, Goldman M. Transfusion-related acute lung injury prevention measures and their impact at Canadian Blood Services. *Transfusion* 2012;52:567-74.
- Hirosue A, Yamamoto K, Shiraki H, Kiyokawa H, Maeda Y, Yoshinari M. Preparation of white-cell-poor blood components using a quadruple bag system. *Transfusion* 1988;28:261-4.
- Heaton WA, Rebullia P, Pappalettera M, Dzik WH. A comparative analysis of different methods for routine blood component preparation. *Transfus Med Rev* 1997;11:116-29.
- Vuk T, Strauss Patko M, Gulan-Harcet J, Očić T, Šarlija D, Jukić I. Quality control of leucocyte-depleted platelet concentrates obtained by buffy-coat method. *Transfus Med* 2013;23:338-43.
- Fijnheer R, Pietersz RN, de Korte D, Gouwerok CW, Dekker WJ, Reesink HW, *et al.* Platelet activation during preparation of platelet concentrates: A comparison of the platelet-rich plasma and the buffy coat methods. *Transfusion* 1990;30:634-8.
- Levin E, Jenkins C, Culibrk B, Gyöngyössy-Issa MI, Serrano K, Devine DV. Development of a quality monitoring program for platelet components: A report of the first four years' experience at Canadian Blood Services. *Transfusion* 2012;52:810-8.
- Ali SF. Platelet activation in stored platelet concentrates: Comparison of two methods preparation. *J Hematol* 2012;1:15-9.
- Jain A, Marwaha N, Sharma RR, Kaur J, Thakur M, Dhawan HK. Serial changes in morphology and biochemical markers in platelet preparations with storage. *Asian J Transfus Sci* 2015;9:41-7.
- Sheffield WP, Bhakta V, Jenkins C, Devine DV. Conversion to the buffy coat method and quality of frozen plasma derived from whole blood donations in Canada. *Transfusion* 2010;50:1043-9.