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Quality analysis of red cell and platelet concentrates obtained by the automated 'Top-and-Top' blood processing system in a developing country

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Abstract

The efficacy of the widely practiced 'Top and Bottom' collection system has been established. We studied efficacy of our new 'Top-and-Top' automated blood component separation system with regards to quality analysis of red cell and platelet concentrates. At installation of machine 59 U of whole blood (WB) were used for its calibration and validation program. Optimum volume, leukocyte–platelet recovery and red cells loss in BC were adjusted as per recommended standards. WB showed mean volume of 510 ml and net hemoglobin content of 63 g/bag. BC recovered 91.7% and 62.7% of platelet and leukocytes, respectively, in a mean volume of 96 ml a hematocrit of 54% and mean platelet of 5.47×10^{10} were observed in red cell and platelet concentrates, respectively. In many aspects quality of our products could not comply with the recommended European and American standards and this requires a close insight into the series of activities associated with WB collection, separation and quality control program.

Keywords: Automated blood separation; Blood components; Quality control

1. Introduction

Blood component separation methodologies based on buffy-coat (BC) extraction have undergone a series of technical modifications since their introduction [1]. The widely practiced 'Top and Bottom' component collection system has already established its efficacy in terms of yield, product quality and ease of handling [1–4]. In addition, the system also ensures removal of >70% leukocytes thereby providing leukocyte–poor red cell or platelet concentrates that can prevent microaggregate formation, hemolysis and febrile reactions [5–7]. Recently, automated 'Top-and-Top' method has been introduced for blood component separation. However, this separation system in contrast to the 'Top and Bottom' method ensures BC collection in a dedicated BC satellite bag thereby retaining the red cell concentrate in the primary bag [8].

Majority of blood banks in India prepare blood component manually using conventional blood bags. With the introduction of automated component separators many blood banks in India have switched over to the automated 'Top-and-Top'

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method of blood component separation. However, the efficacy of the system in terms of quality of products has not been well-established. We, at our blood center in addition to the 'Top and Bottom' system (Optipress II) have also in the recent past adopted the 'Top-and-Top' quadruple bag system for component separation. In this study, we present the quality control (QC) data of the blood components obtained using this method and its compliance with the recommended guidelines.

2. Materials and methods

Quality control program for blood components, equipments and various blood bank reagents is performed routinely as a daily activity in our QC laboratory of the Department of Transfusion Medicine. We analyzed the quality of red cell and platelet concentrates prepared by the 'Top-and-Top' system (Terumo, T-ACE, version 9, France) (Fig. 1), for a period of 14 months (January 06 to February 07) and compared the quality in terms of yield and cellular contamination with the guidelines recommended by the European Council (EC), the American Association of Blood Banks (AABB) and the Drugs And Cosmetics Act (DCA), India [9–11].

2.1. Whole blood collection and processing

Whole blood (WB) 450 mL was collected from screened, voluntary, healthy donors in the 'Top-and-Top' quadruple bag system (Terumo Penpol, Trivandrum, India). This system comprises of a primary bag containing 63 mL citrate–phosphate–dextrose (CPD) as the anticoagulant, two satellite bags, one empty for the collection of plasma and another containing 100 mL of saline–adenine–glucose–mannitol (SAGM) as an additive for the red cells. The fourth bag is a dedicated bag for the collection of BC. All WB units allocated for preparation of red cell and platelet concentrates were stored at 22 ± 2 °C and separated into components within 6 h of collection as per the departmental standard operating procedure (SOP). For processing, units were first subjected to high-speed centrifugation (3100 rpm, 9 min, 22 °C) in Cryofuge 6000i centrifuges (Hereaus, Germany) and then loaded onto the T-ACE machine as per the manufacturer's instruction [8].

2.2. Installation and validation of separation system

The automated 'Top-and-Top' system of blood component separation (T-ACE, version 9), installed in our blood center by the TERUMO PENPOL Limited, Medical system group, Trivandrum, India, is functional since last 3 years. Briefly, this equipment consists of a series of optical detectors that not only monitor the interface between the plasma and red cell layers but also regulate the fluid flow rate. The machine can detect fluctuations in the BC volume during the separation process and is equipped with both clamping and sealing systems to facilitate plasma extraction at the top, red cell–SAGM collection in the primary bag and BC–platelet mixture in the dedicated BC satellite bag [8].

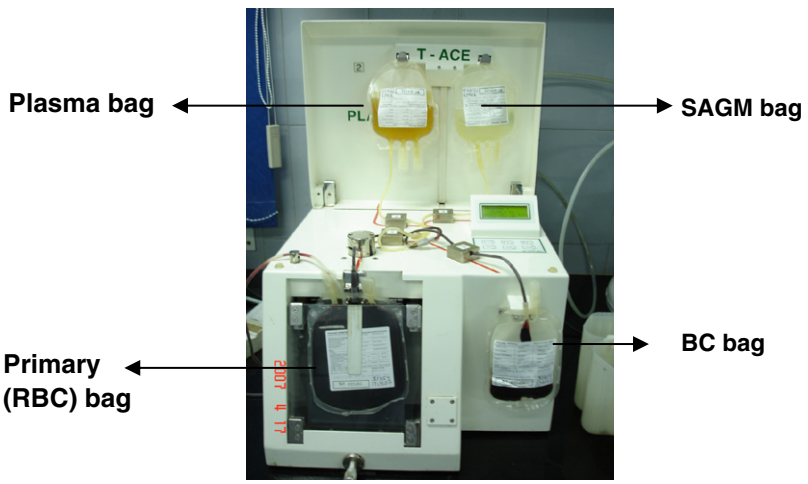

Fig. 1. T-ACE machine.

A total of 59 U of WB were used for component separation using the T-ACE machine and red cell/platelet concentrates obtained were subjected to quality analysis for calibration of the equipment and validation of results. Thus the system could be brought into routine use after a month of its installation when the entire calibration and validation programs could be fully appreciated.

2.3. Separation of blood components by T-ACE and their storage

All processed WB units were subjected to components separation using the BC method as shown in the figure below (copied from departmental SOP).

Following primary separation, red cell concentrates were refrigerated at 4 ± 2 °C, plasma stored at −40 °C and BC–platelet mixtures were subjected to low-speed centrifugation after a resting period of minimum 2 h (Fig. 2). Platelet concentrates obtained by manual extraction were stored on a flat agitator at 22 ± 2 °C (Halmer Labs Inc., USA) until their use within 5 days of separation.

2.4. Quality analysis of WB, red cell and platelet concentrates

2.4.1. Sampling of units

As per the recommended guidelines of QC testing of at least 1% of the total blood units collected [10], we randomly selected 6–8 U of respective components from their site of storage and subjected to analysis. Whole blood units were only analyzed for the calibration and validation of the T-ACE machine at the time of its installation. For all units subjected to quality testing including WB, sampling was performed only after proper homogenization of the bag to make sure that sample in the segment represents the actual content of the bag. Samples from whole blood were collected within 6 h of collection before separation. Red cell concentrates were tested within a week and platelets on the 3rd or 4th day of their preparation.

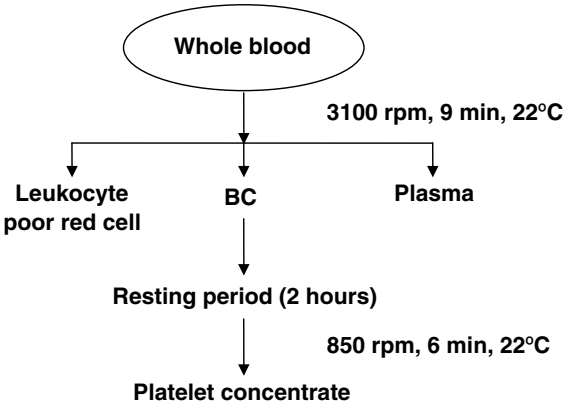

Fig. 2. Protocol for preparation of blood components by T-ACE.

2.4.2. Measurements

Unit number of all bags tested for QC was duly documented in the respective QC register before testing of the units. Volume (Vol) of WB, BC, red cell and platelet concentrates were calculated from the net weight of the product divided by specific gravity (1.053 for WB and BC, 1.09 for red cell and 1.04 for platelets). All hematological values viz. hemoglobin (Hb), platelet (Plt) count, hematocrit (Hct)%, WBC and RBC counts were obtained using a routinely calibrated automated cell counter (Micros 60, ABX diagnostics, France). The pH of platelet units were measured by a calibrated portable pH meter (Toshniwal Inst. Mfg. Pvt. Ltd., Ajmer, India) following the departmental SOP. Swirling in platelet units was assessed visually and documented as 'present' or 'absent'.

2.5. Statistical analysis

Statistical analysis was done using the SPSS statistical package (version 9, USA). Mean, standard deviation (SD) and range were the frequency descriptive statistics employed for quality analysis.

3. Results

A total of 20583 whole blood units were collected from January 2006 to February 2007 of which 99.7% were separated into red cell and 63% into platelet concentrates. Of the total red cell and platelet concentrates prepared, 255 U (1.2%) and 267 U (2.1%) were subjected for quality analysis, respectively.

Table 1 shows the quality of WB used for calibration of the T-ACE machine at its installation. All samples showed a normal distribution in terms of unit volume and hematological values. Mean volume of units including CPD was calculated to be 510 mL with net Hb content of 63 g/U.

Quality analysis of BC was performed as a part of our calibration program to measure the cellular contents (Table 2). Mean volume of the BC layer

Table 1
Volume and hematological values of whole blood (WB) units ($N = 59$)

Parameters	Vol (ml) with CPD	Hct (%)	Hb (g/bag)	RBC ($\times 10^{12}$)	Plt ($\times 10^{11}$)	WBC ($\times$109)
Mean ± SD	510 ± 8.1	43.7 ± 3.2	63 ± 4.4	1.99 ± 0.31	0.97 ± 0.3	3.4 ± 0.7
Range	491–522	38–52.5	56.7–74.2	1.57–2.39	0.5–1.7	2–4.9

Table 2
Volume and hematological values of BC units ($N = 59$)

Parameter	Vol (ml)	Plt ($\times 10^{11}$)	Plt recovery (%)	WBC ($\times 10^{9}$)	WBC recovery (%)	RBC ($\times 10^{12}$)	RBC loss (%)
Mean ± SD	96.4 ± 6.1	0.89 ± 0.2	91.7 ± 7.6	2.52 ± 0.5	62.7 ± 13.3	0.65 ± 0.13	19 ± 6.47
Range	79–105	0.58–1.2	59.2–98.4	1.33–4.1	49.3–77.4	0.4–0.8	13.8–34.1

measured 96 mL (range 79–105 mL). The mean platelet and WBC recovery in BC from whole blood was calculated to be 91.7% and 62.7%, respectively, with a mean red cell loss of 19%.

Tables 3 and 4 present the properties of red cell and platelet concentrates measured in the routine QC program. A mean red cell volume of 285 mL was observed with a net Hct and Hb values of 54% and 52.5 g/U, respectively. On analyzing the quality of platelets we observed that all units were within the normal pH range with a mean volume of 54 mL containing a net 5.4×10^{10} platelets per unit.

4. Discussion

Automation in blood component preparation has contributed significantly to the modern good laboratory practice in blood banking [12]. Moreover the inclusion of QC programs has greatly revolutionized the current good manufacturing practice in Transfusion medicine [1]. However existence of inadequate resources and poor laboratory settings in this subcontinent highlighted the practice of component preparation as a very new concept even today. In addition, total lack of knowledge in quality programs has greatly hindered the development of good manufacturing practices.

An analysis of the quality of components prepared in our laboratory will not only help us to improve our program and assure product reproducibility, but may also help and stimulate the other developing laboratories to adopt the programs in the near future to ensure a global establishment of good transfusion practices.

With regards to the cost effectiveness, availability of leukocyte–poor blood product and regular

Table 3
Quality of red cell concentrates ($N = 255$)

Parameters	Vol (ml) with SAGM	Hct (%)	Hb (g/bag)	RBC ($\times 10^{12}$)	RBC recovery (%)	WBC ($\times 10^{9}$)
Mean ± SD	285 ± 24.3	54 ± 4.2	52.5 ± 5.7	1.77 ± 0.37	77.7 ± 7.89	1.3 ± 0.63
Range	198–350	41–69	38–59.2	1.04–2.07	66.7–86	0.3–3.4
Within CE criteria (%)	NA	85.1	92.9	NA	NA	42
Within AABB criteria (%)	NA	100	NA	NA	NA	Not applicable

NA, not available.

Table 4
Quality of platelet concentrates ($N = 267$)

Parameters	Vol (ml)	pH	Plt ($\times 10^{10}$)	Plt recovery (%)	WBC ($\times 10^{9}$)
Mean ± SD	54 ± 7.6	7.04 ± 0.2	5.4 ± 2.9	55.7 ± 13.4	3.4 ± 2.8
Range	34–67	6.8–7.4	1.5–15	35–71	0.5–17
Within CE criteria (%)	65	100	37.5	NA	76.7
Within AABB criteria (%)	NA	100	43.5	NA	Not applicable
Within DCA criteria (%)	NA	100	71	NA	NA

NA, not available.

manufacturer's support in terms of consumable accessibility and machine maintenance, we adopted the 'Top-and-Top' quadruple bag system in our laboratory and planned to use the system as a routine method of component separation after its standardization. A total of 59 WB units (Table 1) were employed for calibration and validation study of the machine. The final set program was to extract at least 70% of leukocytes, >95% of platelets and as low as 10% red cells in a BC volume of 100 mL, so as to obtain leukocyte–poor products of an optimum yield. With this programmed settings we separated 20,583 U of whole blood in 14 months and subjected >1% each of red cell and platelet concentrates for quality analysis. We also analyzed the quality of our products as per the quality guidelines recommended by the CE, the AABB and the DCA, India (Table 5) [9–11].

On analyzing the quality of BC (Fig. 2), we observed a platelet recovery of 91.7% (mean), WBC removal of mean 62.7% and mean red cell loss of 19%. These deviations from the final set program may be attributed to some unrecognizable fault in the working protocol or fault in the technical operation of the machine despite regular servicing and calibration of system by the manufacturer. Hurtado et al. [1] observed mean platelet recovery and WBC removal of 92% and 74%, respectively, and a red cell loss of 13–15% while analyzing the quality of their components separated by the 'Top and Bottom' (Optipress II) system. A WBC removal of 88% was observed by Pietersz et al. [4] by the 'Top and Bottom' system (Biotrans, Dreieich, FRG). Though the platelet recovery and red cell loss in our study are comparable with the previous studies but we obtained a low WBC recovery in our BC units and this deserves a review of departmental SOP and manufacturer instruction.

Table 5
A comparison of the AABB, EC and DCA (India) guidelines

RED CELLS	AABB	EC	DCA, India
Hct (%)	<75	50–70	No established guidelines
Hb (g/U)	NA	>43	
WBC/unit	$\leqslant 5 \times 106$ (P-L)	$<1.2 \times 10^9$	
PLATELETS			
Volume	NA	>50	NA
Yield/unit	5.5×10^{10}	6×10^{10} (75%)	$3.5/4.5 \times 10^{10}$ (350/450 mL WB)
pH	>6.2	6.5–7.4	$\geqslant 6$
WBC/unit	$\leqslant 5 \times 10^6$ (P-L)	$\leqslant 0.05 \times 10^9$	NA

NA, not available; P-L, post-leuko-filtration.

We could not retrieve any guidelines set by the AABB regarding the leukocyte and Hb contents of red cell units derived by BC method. In this context, leukocyte depletion in 42% red cell units and Hb content in 92.9% units were as per the recommended European guidelines (Table 3). Though Hct value as low as 41% was observed in our units however 85.1% bags have met the CE criteria. We obtained poor leukocyte-depletion in the red cell concentrates and this is attributed to the retention of leukocyte in the primary bag during separation of BC units. The AABB recommends Hct of <75% in all red cell tested and all our units could fulfill the criteria. We observed a mean Hct and Hb of 54.6% and 51.9 g in our units. Pietersz et al. [4] analyzed the quality of 156 units of red cell concentrates and observed a mean Hct of 58%. Similarly Hurtado et al. [1] observed a mean Hct and Hb of 60.87% and 54.9 g in a quality control study on 672 U. All these findings are comparable and show compliance with the recommended guidelines. The DCA, India has no established guidelines with regards to the quality of red cell concentrates [11].

We observed a mean platelet yield of 5.47×10^{10} in the platelet concentrates tested, with 76.7% of the units showing leukocyte-depletion as per the EC standards (Table 4). Hurtado et al. [1] and Pietersz et al. [4] observed mean platelet yield of 6.4×10^{10} in their studies. The pH of all our units (range 6.8–7.4) was within the recommended guidelines. Adequate Swirling was present in all the platelet concentrates. As far as platelet yield is concerned, Hurtado et al. [1] found 59.7% of their tested units fulfilling the EC criteria. In the present study only 37.5%, 43.5% and 71% of the units showed compliance with the EC, AABB and DCA guidelines, respectively. This poor result can be attributed to the lower normal platelet count in our donor population. We observed 67% of our healthy donors having a platelet count of $\geqslant 250 \times 10^3/\mu l$ [13].

We conclude that the quality of blood components is dependent on multiple factors of which the efficiency of the separation machine is a critical part. Strict adherence to the departmental SOP and machine manufacturer's guidelines also play important roles. Moreover platelet yield or hematocrit/hemoglobin content of a unit depend on the blood counts of the donor and low normal counts have been observed among most donors in this region [13]. This study provides data that WB processing

in an automated separation machine gives optimum QC results, nevertheless improvement is desired to comply with the international guidelines. The AABB describes leukocyte-depletion in leukocyte-filtered units which is a rarely practiced method in developing countries, however as high as 60% of our red cell units could not meet the EC criteria of leukocyte-depletion by BC method. This requires a close review of our laboratory practices and expert calibration of the T-ACE machine. To our observation, as far as the quality of blood component is concerned, it will be prudent for all developing countries to follow the guidelines recommended by the European Council. In addition more multi-centric studies are required in India which will enable the DCA (India) to establish adequate guidelines on the quality of red cell and platelet concentrates that in turn will definitely improve the national laboratory and clinical practices in transfusion medicine.

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