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Need to reconsider national quality standards for red cell components: Evidence from a retrospective observational analysis

Indranil Das, Dheeraj Khetan, Anupam Verma, Atul Priyadarshi & Rajendra K. Chaudhary

Department of Transfusion Medicine, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India

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***Background & objectives*: Red cell concentrates (RCCs) must comply with applicable quality control (QC) standards to achieve the desired therapeutic effect in the recipient. In this study, we assessed the effect of change in the component preparation process on the quality of RCCs and their compliance with different QC standards.**

***Methods*: A retrospective analysis of data for QC testing of RCCs over a period of 10 years, (from 2009 to 2019), was undertaken. QC testing parameters [volume, haematocrit (Hct), haemoglobin (Hb) content, white blood cell (WBC) content and percentage (%) haemolysis] were used to assess compliance with three national and three international QC standards. Linear regression analysis was done to assess the influence of donor variables.**

***Results*: Data from 5,218 RCC units was included in the analysis. A majority (>50%) of RCCs prepared did not meet the three national QC standards either for volume or for Hct. The criteria for volume, Hct and Hb content, as defined in different international standards, were fulfilled by a majority (>75%) of RCCs evaluated. RCCs prepared by the buffy coat method had overall better compliance with QC standards compared to the platelet-rich plasma (PRP) method. The method of component preparation was found to influence Hb content, WBC content and percentage haemolysis. Male gender was associated with better Hb content.**

***Interpretation & conclusions*: RCC prepared at our centre was found to have better compliance with international QC criteria compared to national standards. There is a need to reconsider the current national QC criteria for red cells with due consideration to the volume of whole blood collected and the method used for RCC preparation.**

Key words Blood component transfusions - guidelines - national - quality control - red cells - standards

Red cell concentrates (RCCs), separated from whole blood (WB), are frequently used in the treatment of anaemia due to blood loss, deficient red cell production or increased red cell destruction[1]. In India, WB is collected from healthy individuals in 450 ml or 350 ml capacity blood bags, with a maximum allowable volume variation of ±10 per cent[2]. As per the acceptable standards as laid down by Central Drugs

Standard Control Organisation (CDSCO)[3,4], one per cent of blood components are required to be evaluated routinely to ensure that they meet specific quality standards and to identify any deficiencies or errors in the preparation processes.

Some of the important quality control (QC) parameters of RCC include estimation of volume, haematocrit (Hct), haemoglobin (Hb) content and white blood cells (WBC) content. The extent of correction of anaemia in the recipient directly depends on the Hb content of RCC, which is a function of the donor Hb and the volume of WB collected[5]. Lower the WBC content of RCC, lesser is the risk of febrile non-haemolytic transfusion reaction (FNHTR), cytomegalovirus infection or Human Leukocyte Antigen (HLA) alloimmunization in the recipient[6].

The quality of RCC depends on multiple factors[7] such as separation techniques, storage conditions, biological factors related to donors and so on. Previous studies[8-10] on the quality of different blood components have mostly explored the effect of processes and storage environment, with only a few studies[11,12] available on the impact of donor factors on the same.

Both platelet-rich plasma (PRP) as well as buffy coat (BC) methods are widely used for processing WB into RCCs and other blood components[13]. For a particular volume of WB processed, RCC prepared by the PRP method (red cells, RC) usually have higher Hb and WBC content compared to RCC prepared by the BC method due to the removal of WBC-rich BC layer along with the inevitable loss of some red cells in the BC method[14]. This difference in the quality of RCCs prepared by different methods is also reflected in different QC standards[15]. RCCs prepared by the BC method (red cell concentrates in additive solution, RCC-AS), also known as leucocyte-reduced RCC, have been reported[13] to have a lower incidence of FNHTR in the recipient. Two different configurations of quadruple blood bag systems are typically used for the preparation of RCC by the BC method: top-&-top (B-TAT) and top-&-bottom (B-TAB). RCCs prepared by the B-TAT technique have been observed[16] to have a higher Hb content than those by the B-TAB technique.

At the study hospital institute, the PRP method was initially followed for component preparation, from 2010 onwards, the B-TAT method was used and more recently, from 2018 onwards, the B-TAB method is also in use. The objective of this study were to assess the impact of change in the component preparation processes at our institute on the quality of RCCs prepared over a period of 10 yr (September 2009 to August 2019) and to assess the compliance of prepared RCCs to applicable quality standards.

Material & Methods

This study was retrospective and observational, conducted at the Department of Transfusion Medicine, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, a tertiary care referral centre in northern India with due approval of the Institute Ethics Committee (IEC). At the study hospital, WB units (450 ml/350 ml) are routinely subjected to the preparation of RCCs (RCC_{450}/RCC_{350}) in accordance with departmental protocols. Data of such routine QC testing of RCCs over a period of 10 years (September 2009 to August 2019) was analyzed in this study QC criteria/standards published by three national[3,4,17] and three international[15,18,19] regulatory or accreditation agencies were used as reference to assess the QC results of RCCs prepared during the study period.

Types of red cell components: The following methods were routinely used at the study hospital for the preparation of RCCs:

RCC prepared by PRP method (Red cells, RC): 350 ml WB collected in triple bags with the CPDA (citrate-phosphate-dextrose-adenine) anticoagulant (Terumo Penpol, Thiruvananthapuram) was subjected to light spin (1,800 rpm for 10 min, at 22°C), followed by separating most of the supernatant plasma along with suspended platelets in a satellite bag, leaving behind some plasma in the primary bag to re-suspend the red cells. Prepared RC_{350} were stored at 2–6°C till issue.

RCC prepared by BC method with AS in TAT configuration [RCC-AS(TAT)]: WB collected in quadruple blood bag with CPDA and saline-adenine-glucose-mannitol (SAGM) in TAT configuration (Terumo Penpol) was given a heavy spin (3,100 rpm for 9 min at 22°C). An automated component extractor was used to first separate the plasma and then the BC from the top of the primary bag into two satellite bags, followed by the addition of SAGM into the red cell layer for preparation of RCC-AS(TAT). The empty SAGM bag and the satellite bag with BC were used for the preparation of platelet concentrates (random donor platelets, RDP). Depending on the volume of WB used, two types of RCC-AS were prepared – RCC_{350}-AS(TAT) and RCC_{450}-AS(TAT) – which were stored at 2–6°C till issue.

RCC prepared by BC method with AS in TAB configuration [RCC-AS(TAB)]: WB collected in quadruple blood bags with CPDA and SAGM in TAB configuration (Terumo Penpol) was spun at 3,800 rpm for 9 min at 22°C. The quadruple blood bag system (Terumo Penpol) was configured such that plasma was removed from the top and red cells were transferred from the bottom into a satellite bag containing SAGM. The BC remained in the primary bag with an empty satellite bag attached and was utilized for preparing RDPs. Prepared RCC_{450}-AS(TAB) were stored at 2–6°C till issue.

Therefore, during the study period, a total of four types of RCCs were prepared at the study hospital: RC_{350}, RCC_{350}-AS(TAT), RCC_{450}-AS(TAT) and RCC_{450}-AS(TAB).

Quality control testing: A representative sample in a segment from each RCC units was collected using a di-electric tube sealer to maintain a closed system. Parameters under routine QC included testing for volume (ml), Hct, Hb content (g/unit), WBC content (cells/unit) and microbial detection (aerobic culture). As per departmental protocols, QC testing of RCC was conducted within the shelf life of RCC units (*i.e.* within 35 days for RC_{350} and within 42 days for RCC_{350}-AS/ RCC_{450}-AS). Plasma Hb and percentage haemolysis were assessed for the units stored for more than one week but within the shelf life of the red cell component.

Cell counts were done using the Medonic M-Series three-part haematology analyser (Boule Medical AB, Sweden). Aerobic culture was done using the automated rapid microbial detection systems (BACT/ ALERT, bioMérieux Inc, USA). Plasma Hb estimation (g/dL) was done using microcuvettes and an analyzer of the HemoCue Plasma/Low Hb system (HemoCue AB, Sweden).

Various study parameters for individual units (including volume of RCC, Hb content per unit of RCC, and WBC content per unit of RCC) were calculated using formulae (Supplementary Material: Box).

Data collection and analysis: Records of QC testing of RCCs were taken from QC registers. Details of the type and capacity of the blood bag and the method of component preparation were collected from component preparation registers. Information on donor variables (age, gender, weight and blood group) was collected from donor registration forms and electronic database of the hospital information system (HIS).

Inclusion and exclusion criteria: Available records of all RCCs which underwent QC testing over the ten-year study period were included. Records with incomplete entries and units with missing donor data were excluded. We also excluded the units in which QC testing was done after product modifications under special circumstances, such as irradiation, saline-washing, leucofiltration, split units for paediatric patients, under-collected units, clots in blood bags and more.

Statistical analysis: Continuous data are expressed as mean with standard deviation (SD) or median with range/interquartile range (IQR). Categorical data are expressed as frequencies and percentages. Continuous data was assessed to have non-normal distribution by Shapiro-Wilk test, and therefore for further analyses, non-parametric tests were used. Mann-Whitney U test was applied for comparing of continuous QC parameters between two types of RCC as well as across gender; Kruskal Wallis test for comparison of continuous QC parameters across different donor age groups; Pearson Chi-Square test for comparison of categorical donor characteristics across two types of RCC; and Spearman's rank correlation coefficient (ρ) for Hb content and WBC content with days of storage as well as between themselves. Owing to the large sample size in this study with approximately normal distributed data (as visualized using Q-Q plots), linear regression[20-23] was used to study the association of Hb content, WBC content and percentage haemolysis with different independent factors in the study.

All analyses were done using Microsoft Excel 2010 (Washington, USA), GraphPad Prism, version 9.3.0 (California, USA) and IBM SPSS Statistics, version 26.0 (Chicago, USA) for Windows, at 95 per cent confidence limits and $P<0.05$ was considered as significant.

Results

During the study period, 236,255 RCCs (RCC_{350}=89,254, RCC_{450}=147,001) were prepared, out of which 5,861 (2.48%) units were subjected to routine QC testing. Data from 643 units was excluded due to incomplete records/missing donor data (n=603) and unit modifications after preparation (n=40). Thus, data of 5,218 (RCC_{350}=2,433, RCC_{450}=2,785), 2.21 per cent of the units prepared, were included in the analysis. This accounted for 2.73 per cent and 1.89 per cent, respectively, of the total RCC_{350} and RCC_{450}

Table I. Donor characteristics of red cell concentrates (RCC) units included in the study (n=5218)

Donor characteristic	Type of red cell component					
	RCC_{350}			RCC_{450}		
	RC_{350} (PRP method) (n=297)	RCC_{350}-AS(TAT) (n=2136)	*P*-value	RCC_{450}-AS(TAT) (n=2411)	RCC_{450}-AS(TAB) (n=374)	*P*-value
Age (yr), mean±SD	29±7.9	29±8.1	0.45[a]	32±8.4	33±8.4	0.417[a]
Age groups (yr), n(%)						
18–24	89 (30)	730 (34.2)	0.349[b]	448 (18.6)	71 (19)	0.664[b]
25–44	186 (62.6)	1263 (59.1)		1714 (71.1)	259 (69.3)	
45–60	22 (7.4)	143 (6.7)		249 (10.3)	44 (11.8)	
Gender, no. of males (%)	240 (80.1)	1,929 (90.3)	<0.001[b]	2,411 (100)	374 (100)	-
Weight (kg), mean±SD	57±7	58±7.8	0.335[a]	72±9.3	72±8.5	0.349[a]
ABO blood group, n(%)						
O	94 (31.6)	695 (32.5)	0.265[b]	785 (32.6)	122 (32.6)	0.887[b]
A	78 (26.3)	455 (21.3)		554 (23)	84 (22.5)	
B	103 (34.7)	818 (38.3)		840 (34.8)	136 (36.4)	
AB	22 (7.4)	168 (7.9)		232 (9.6)	32 (8.6)	
Rh (D) positive, n(%)	282 (94.9)	2,045 (95.7)	0.532[b]	2,302 (95.5)	357 (95.5)	0.983[b]

[a] Mann-Whitney U test and [b] Pearson chi-square test

Table II. Unit characteristics of RCCs that underwent routine QC testing (n=5218)

Unit characteristics	Type of red cell component					
	RCC_{350}			RCC_{450}		
	RC_{350} (PRP method) (n=297)	RCC_{350}-AS(TAT) (n=2,136)	*P*-value[a]	RCC_{450}-AS(TAT) (n=2,411)	RCC_{450}-AS(TAB) (n=374)	*P*-value[a]
Volume of PRBC (ml), mean±SD	227±21.3	236±14.1	<0.001	299±19.4	259±40.3	<0.001
Hct (%), mean±SD	52.5±4.87	53.2±4.64	0.001	55.1±4.96	53.5±5.69	<0.001
Hb content (g), mean±SD	39.4±5.03	41.1±4.3	<0.001	54.1±6.14	45.7±7.74	<0.001
WBC/bag ($\times 10^9$), mean±SD	1.86±0.41	1.1±0.33	<0.001	1.32±0.46	0.85±0.33	<0.001
Storage age of unit (days)[$], median (IQR)	0 (0–16)	0 (0–25)	0.9	0 (0–31)	0 (0–31)	0.079

[a] Mann-Whitney U test

[$] Storage age of the RCC unit refers to the day of QC testing. PRBC, packed red blood cell; Hct, haematocrit; Hb, haemoglobin; WBC, white blood cell; IQR, interquartile range; SD, standard deviation.

units prepared during this period. According to the component processing method, there were 297 units of RC_{350}, 2,136 units of RCC_{350}-AS(TAT), 2,411 units of RCC_{450}-AS(TAT) and 374 units of RCC_{450}-AS(TAB). The donor characteristics for the different types of RCC are shown in Table I. The unit characteristics (QC parameters) are also summarized in Table II.

Quality control of RCC_{350}: The proportions of RC_{350} and RCC_{350}-AS(TAT) meeting the different reference criteria are summarized in Table III. None of the 2,433 units assessed met the volume criteria mentioned in the Drugs and Cosmetics Rules (D&C Rules)[3]. Among RC_{350}, none met the volume criteria given by the Directorate General of Health Sciences (DGHS), India[4]; only one (0.3%) unit met the Hct criteria given by both D&C Rules and DGHS. Among RCC_{350}-AS(TAT), only 632 (29.6%) and 684 (32%) units, respectively, met the volume and Hct criteria given by the National Accreditation Board for Hospitals & Healthcare Providers (NABH)[17]. The criteria for WBC content given by DGHS was met by only 49 (2.3%) units of RCC_{350}-AS(TAT).

Effect of component processing method on the quality of RCC_{350}: The proportions of units meeting the

Table III. Percentage compliance of quality control testing of RCCs to different criteria in Indian and international standards.

Type of RCC	Parameter	Indian standards						International standards					
		NABH[17]		D&C criteria[3]		DGHS[4]		JPAC[19]		AABB[18]		EDQM[15]	
		Criteria	Compliance (%)	Criteria	Compliance (%)	Criteria	Compliance (%)	Criteria	Compliance (%)	Criteria	Compliance (%)	Criteria	Compliance (%)
RC_{350} (PRP method) (n=297)	Volume (ml)	NCD	-	150±15	0	150±15	0	NCD	-	NCD	-	NCD	-
	Hct (%)	NCD	-	65–70	0.3	65–70	0.3	NCD	-	NCD	-	NCD	-
	Hb content (g/unit)	NCD	-	NCD	-	NCD	-	NCD	-	NCD	-	NCD	-
	WBC content*	NCD	-	NCD	-	NCD	-	NCD	-	NCD	-	NCD	-
RCC_{350}-AS(TAT) (n=2,136)	Volume (ml)	245–325	29.6	150±15	0	250±25	84.4	NCD	-	NCD	-	NCD	-
	Hct (%)	55–65	32	50–60	73.3	50–60	73.3	NCD	-	NCD	-	NCD	-
	Hb content (g/unit)	NCD	-	NCD	-	NCD	-	NCD	-	NCD	-	NCD	-
	WBC content*	NCD	-	NCD	-	$<5\times10^8$	2.3	NCD	-	NCD	-	NCD	-
RCC_{450}-AS(TAT) (n=2,411)	Volume (ml)	300–400	52.6	250±25	7.2	350±35	18.9	280±60	97.3	NCD	-	NCD	-
	Hct (%)	55–65	51.1	50–60	75.7	50–60	75.7	-	-	≤80	100	50–70	89.2
	Hb content (g/unit)	NCD	-	NCD	-	NCD	-	40	96.6	NCD	-	43	93.9
	WBC content*	NCD	-	NCD	-	$<5\times10^8$	2.8	$<1\times10^6$	NA	NCD	-	$<1.2\times10^9$	37.5
RCC_{450}-AS(TAB) (n=374)	Volume (ml)	300–400	14.2	250±25	35.6	350±35	0	280±60	82.9	NCD	-	NCD	-
	Hct (%)	55–65	36.1	50–60	68.2	50–60	68.2	-	-	≤80	100	50–70	76.7
	Hb content (g/unit)	NCD	-	NCD	-	NCD	-	40	64.2	NCD	-	43	60.2
	WBC content*	NCD	-	NCD	-	$<5\times10^8$	10.7	$<1\times10^6$	NA	NCD	-	$<1.2\times10^9$	83.4

* $\times10^9$ cells/unit. NCD, no criterion defined

[19] JPAC standards are for Red cells, Leucocyte Depleted: WBC content of $<1\times10^6$ is achievable after leucofiltration, which is not routinely followed in India. Therefore, this criterion is not applicable (NA) for red cell components prepared by buffy coat removal

[18] AABB standards for red blood cells without additive solutions

[15] Council of Europe (EDQM) standards for red cells, buffy coat removed, in additive solution

criteria mentioned in the D&C Rules and DGHS were comparatively lower for RC_{350} than RCC_{350}-AS(TAT) (Table III). RC_{350}, as compared to RCC_{350}-AS(TAT),had significantly lower volume, Hct, Hb content and higher WBC content (Table II). The WBC content had a weak positive correlation with Hb content (ρ=0.1, P<0.001) and a weak negative correlation with days of storage (ρ=–0.111, P<0.001).

Quality control of RCC_{450}: The proportions of RCC_{450}-AS(TAT) and RCC_{450}-AS(TAB) meeting the different reference criteria are summarized in Table III. The

criteria for volume as per NABH standards, D&C Rules and DGHS were met overall by 1,320 (47.4%), 306 (11%) and 456 (16.4%) of the tested units respectively out of the total 2,785 units assessed. The criteria for Hct as per NABH were met overall by 1,367 (49.1%) of the tested units and as per D&C Rules/DGHS by 2,081 (74.7%) of the tested units. The criteria for WBC content given by DGHS was met by only 108 (3.9%) of the tested units.

The volume and Hb criteria given by the Joint United Kingdom Blood Transfusion and Tissue Transplantation Services Professional Advisory Committee (JPAC)[19] were met by 2,656 (95.4%) and 2,570 (92.3%) units, respectively. All the 2,785 units met the American Association of Blood Banks (AABB)[18] standards for Hct. The Council of Europe (European Directorate for the Quality of Medicines & Health Care, EDQM)[15] standards for Hct and Hb content were met by 2,437 (87.5%) and 2,490 (89.4%) units, respectively.

Effect of component processing method on the quality of RCC_{450}: The proportions of units meeting the three national and three international standards were comparatively higher for RCC_{450}-AS(TAT) than RCC_{450}-AS(TAB), except for WBC content, which met the EDQM criteria in 37.5 per cent of the former and 83.4 per cent of the latter (Table III). RCC_{450}-AS(TAT), had significantly higher volume as compared to RCC_{450}-AS(TAB), Hct, Hb content and WBC content (Table II). The WBC content had a weak positive correlation with Hb content (ρ=0.2, P<0.001) and a weak negative correlation with days of storage (ρ=–0.054, P=0.004).

The variation in QC parameters across the four different types of RCC are shown in Figure.

Effect of donor variables on Hb and WBC content of RCC: *(i)* RCC_{350}: The mean Hb content was found to be significantly lower (P<0.001) in the age group of 45–60 yr (39.6±4.25 g) as compared to 18–24 yr (41±4.46 g) and 25–44 yr (41±4.42 g) groups.

RCCs from male donors, as compared to female donors, had a significantly higher mean Hb content (41.2±4.37 g and 38.5±4.21 g, respectively, P<0.001) and a significantly lower mean WBC content ($1.18\pm0.42\times10^9$ cells/unit and $1.28\pm0.44\times10^9$ cells/unit, respectively, P<0.001). The highest WBC content ($\times10^9$ cells/unit) was observed in groups O (1.22±0.43) and A (1.21±0.45), followed by B (1.18±0.41) and AB (1.15±0.4).

Linear regression analysis of RCC_{350} showed that the significant factors influencing the Hb content were the method of component preparation and the gender of the donor. WBC content was significantly influenced by the method of component preparation, days of storage and ABO group of donors (Table IV).

(ii) RCC_{450}:A significantly lower mean Hb content (P=0.006) was observed in the age group of 45–60 yr (51.9±7.27 g) as compared to the 18–24 yr (53.2±7.21 g) and 25–44 yr (52.9±6.87 g) groups.The highest WBC content ($\times10^9$ cells/unit of RCC) was observed in groups O (1.29±0.49) and A (1.29±0.48), followed by B (1.24±0.45) and AB (1.15±0.43).

Linear regression analysis of RCC_{450} showed that significant factors influencing the Hb content were the method of component preparation and the age of the donor, while the method of component preparation, days of storage and ABO group of donors were found to have a significant impact on WBC content (Table IV).

Estimation of percentage haemolysis: A total of 94 RCCs (45 RCC_{350} and 49 RCC_{450}) with more than seven days of storage were evaluated for plasma Hb, and percentage haemolysis was estimated. Both for RCC_{350} and RCC_{450}, the mean plasma Hb was 0.08±0.03 g/dl and the mean percentage haemolysis was 0.19±0.08. All these units had haemolysis within acceptable limits (<0.8%). These 45 RCC_{350} units included 8 units of RC_{350} and 37 units of RCC_{350}-AS(TAT); the 49 RCC_{450} units included 30 units of RCC_{450}-AS(TAT) and 19 units of RCC_{450}-AS(TAB). Characteristics of units subjected to estimation of plasma Hb and percentage hemolysis estimation is given in Supplementary Table.

Higher percentage haemolysis was observed in RC_{350} as compared to RCC_{350}-AS (TAT) (0.27±0.11 *vs*. 0.18±0.07, P=0.027). Lower percentage haemolysis was observed in RCC_{450}-AS(TAB) (0.16±0.07) as compared to RCC_{450}-AS(TAT) (0.20±0.09)however, this difference was not significant. Linear regression analysis showed that the percentage of haemolysis was significantly influenced by the method of component preparation (Table IV).

Sterility testing: Out of all the study units assessed, only 4 (0.08%) tested positive for microbial contamination: two units showed growth *Staphylococcus epidermidis*,

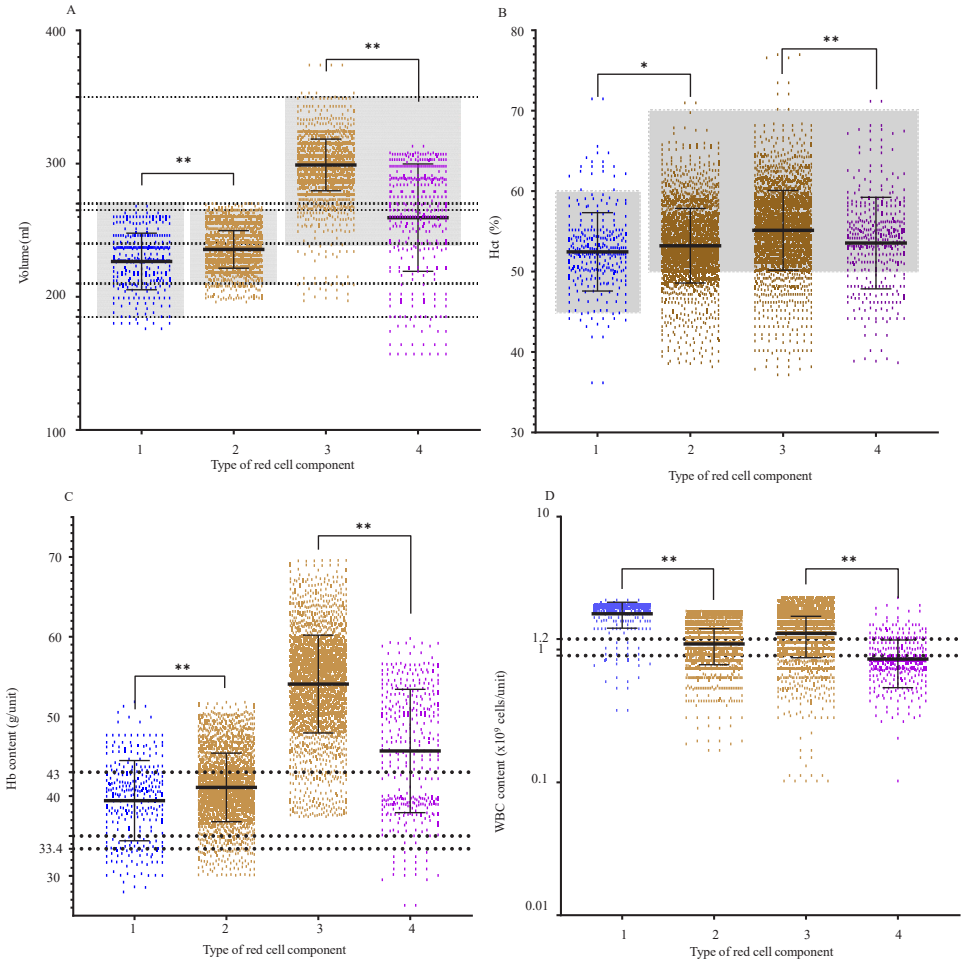

Figure. Variation in QC parameters across different types of red cell concentrates (RCC). (A) Volume of PRBC (B) Haematocrit of PRBC (C) Hb content of PRBC (D) WBC content of PRBC; 1=RC_{350}, 2=RCC_{350}-AS(TAT), 3=RCC_{450}-AS(TAT), 4=RCC_{450}-AS(TAB). Black horizontal lines and vertical error bars represent the mean and one standard deviation, respectively. Dots represent individual observations. Dotted lines and shades represent the proposed standards in Table V. Data were expressed as mean±standard deviation P^{*} <0.05, **<0.001; Mann-Whitney U test. PRBC, packed red blood cell.

Table IV. Significant factors influencing the Hb content, WBC content and percentage haemolysis in the study units

Parameters under study	Independent factors	RCC_{350}			RCC_{450}		
		R^2	β	*P*-value	R^2	β	*P*-value
Hb content[#]	Method of component preparation[a]	0.048	0.105	<0.001	0.17	–0.409	<0.001
	Age of donor		–0.023	0.247		–0.048	0.005
	Gender of donor[b]		–0.177	<0.001		-	-
WBC content[#]	Method of component preparation[a]	0.365	–0.587	<0.001	0.128	–0.338	<0.001
	Days of storage		–0.143	<0.001		–0.053	0.003
	ABO group of donor[c]		–0.037	0.022		–0.082	<0.001
Percentage haemolysis[##]	Method of component preparation[a]	0.144	–0.474	0.002	0.065	–0.184	0.25

[a] Method of component preparation: For $RCC_{350,}$ PRP and B-TAT methods were used, PRP method taken as base group in regression. For $RCC_{450,}$ B-TAT and B-TAB methods were used, B-TAB method taken as base group in regression.
[b] Gender of donor: Donors of RCC_{350} had both males and females; male gender was taken as base group in regression. RCC_{450} had only male donors.
[c] ABO group of donors: comprised of blood groups O, A, B and AB. Group O was taken as base group in regression.
[#] Independent variables for Hb content and WBC content: donor age, gender, weight, ABO group, method of component preparation and days of storage; [##]all these factors except ABO group of donor were considered for analysis of percentage haemolysis.

other two units showed growth of *Yersinia enterocolitica* and *Pseudomonas aeruginosa*, respectively.

Discussion

Stored blood and its components, including RCCs, are considered as drugs whose therapeutic benefit for the recipient depends on their quality as determined by QC testing. In this study, we compared our QC data to Indian as well as international standards which are widely accepted. We observed that RCCs prepared at our institute complied better with the Hct criteria as described by EDQM rather than the criteria as mentioned in the national standards (NABH, D&C Rules or DGHS) (Table III).

Hb content of ≥43 g/unit (EDQM)[15] was observed in 93.9 per cent of RCC_{450}-AS(TAT) and 60.2 per cent of RCC_{450}-AS(TAB). Currently, there are no predefined criteria for Hb content of RCC_{450}/RCC_{350} in the Indian standards. In India, for WB donation, the donor must have a minimum Hb 12.5 g/dl[3]. Therefore, theoretically, the least expected Hb content of WB collected from such donor is 56.25 g/43.75 g for 450 ml/350 ml. If 43 g/unit is taken as the minimum acceptable Hb content of RCC_{450} processed by the BC method (RCC_{450}-AS), the loss in Hb during processing is ~13.25 g (23.56%). Assuming a similar (23.56%) loss of Hb during processing of RCC_{350} by the BC method, the estimated minimum expected Hb content is ~33.4 g/unit, which was satisfactory for 94.3 per cent of our tested RCC_{350} units: 83.5 per cent for RC_{350} and 95.8 per cent for RCC_{350}-AS(TAT). A cut-off of 33.4 g/unit is above 30 g/unit[19], which is the minimum acceptable Hb content of RCC that may be used for transfusion. With no standards available for the Hb content of RCC_{350}, 33.4 g/unit may be considered in our setting for RCC_{350}-AS. Similarly, 35 g/unit is estimated as the minimum Hb content of RCC_{350} prepared by the PRP method (RC_{350}), taking a reference of 45 g/unit as the minimum requirement as per EDQM[15] criteria for red cells.

Among RCC_{350} units, RC_{350} had lower Hct and Hb content, possibly due to a significantly higher proportion of female donors with expected lower Hb as compared to RCC_{350}-AS(TAT) (Table I). Another reason could be the difference in processing (manual for PRP *vs*. semi-automated for BC method) leading to higher red cell losses. Since a significant number of WBCs from the WB is removed (near one-log reduction)[4] by the BC method, we observed lower WBC content in RCC_{350}-AS(TAT) than in RC_{350}.

Among RCC_{450}, lower Hct, Hb content and WBC content were observed in RCC_{450}-AS(TAB) than in RCC_{450}-AS(TAT), implicating higher red cell loss in B-TAB as compared to the B-TAT technique. This finding was in concordance with that of Cid *et al*[16]. We observed that units having lower WBC content also had lower Hb content, which may suggest that the method was more effective in reducing WBCs from WB also results in more red cell losses. A high demand/supply ratio of RDPs for our Hemato-Oncology patients has been observed in recent years, and therefore, the priority is to prepare RDPs from most of the WB collections. Hence, after implementation of the B-TAB technique, the component preparation method was recalibrated to maintain adequate volume and platelet yield with minimum cellular contamination of RDPs, which resulted in a lower volume of RCC_{450}, reflecting as a lower Hb content of RCC_{450}-AS(TAB).

We also observed a higher variation in volumes of our RCC_{450}-AS(TAB) units (SD ~40 ml) as compared to that of our RCC_{450}-AS(TAT) units (SD ~19 ml), which was contrary to that observed by Cid *et al*[16] (SD of 21 ml and 25 ml, respectively, for B-TAB and B-TAT methods). Our findings could be explained by the frequent calibration of the B-TAB method in accordance with the institutional requirements as stated above.

The current DGHS[4] criteria requires the WBC content of BC reduced RCCs to be $<5\times10^8$ cells/unit; there are no specific criteria for the WBC content of RCC_{350} in the Indian standards. The maximum estimated WBC content of RC_{350} was found to be ~3.09×10^9 cells/unit (mean+3SD) in this study. Since no RC_{450} was prepared at our centre during the study period, considering the WBC content of RC_{350} as a reference, the maximum WBC content of RCC_{450} before processing is expected to be ~3.97×10^9 cells/unit. The EDQM[15] criteria for WBC content ($<1.2\times10^9$ cells/unit) is ~30 per cent of this estimated WBC content in RCC_{450} processed by the BC method (RCC_{450}-AS). Since the BC method is known to remove ~70–80 per cent of WBCs, the WBC content found in our study units seems to be justified.

The current criteria for the volume of RCCs as defined by the Indian regulatory or accreditation agencies are not specified according to the method of preparation. However, 450 ml±10 per cent of WB collected in our settings is comparable to that laid down in both JPAC[19] (470±50 ml) and EDQM[15] (450±50 ml) standards. The JPAC criteria for the volume of red cell

Table V. Proposed quality control (QC) criteria based on our observations

Type of red cell component	QC parameters	Proposed criteria	Source of QC criteria	Overall study units passed (%)
Red cell concentrate (prepared from 350 ml whole blood by PRP method)	Volume (ml)	185–270	SPC[$]	95.3 (283/297)
	Hct (%)	45–60	SPC[$]	87.5 (260/297)
	Hb content (g/unit)	≥35	Derived[a] from EDQM criteria	77.1 (229/297)
Red cell concentrate, buffy coat reduced with additive solution (prepared from 350 ml whole blood)	Volume (ml)	210–265	SPC[$]	95.8 (2047/2136)
	Hct (%)	50–70	EDQM[##]	80 (1,708/2,136)
	Hb content (g/unit)	≥33.4	Derived[#] from EDQM criteria	95.8 (2,046/2,136)
	WBC content ($\times 10^9$ cells/unit)	<0.9	Derived[$$] from EDQM criteria	22.8 (488/2,136)
Red cell concentrate, buffy coat reduced with additive solution (prepared from 450 ml whole blood)	Volume (ml)	240–350	SPC[$]	94.7 (2,638/2,785)
	Hct (%)	50–70	EDQM criteria	87.5 (2,437/2,785)
	Hb content (g/unit)	≥43	EDQM criteria	89.4 (2,490/2,785)
	WBC content ($\times 10^9$ cells/unit)	<1.2	EDQM criteria	43.7 (1,217/2,785)

SPC[$]: defined by mean±2SD for each category rounded off to the nearest multiple of five.
[a] Derived from EDQM criteria for Hb content of red cells prepared from 450 ml WB.
[#] Calculated from EDQM criteria of ≥43 g/unit assuming 23.56 per cent loss of Hb from WB.
[##] Adopted from EDQM criteria for Hct of red cells, buffy coat reduced in additive solution, prepared from 450 ml WB.
[$$] Calculated from EDQM criteria for WBC content of red cells, buffy coat reduced in additive solution, prepared from 450 ml WB.

units was met by 97.3 per cent of RCC_{450}-AS(TAT) and 82.9 per cent of RCC_{450}-AS(TAB).

Considering the example of a 450 ml WB unit processed by the BC method, as per DGHS[4] criteria, the total volumes of all the components that can be prepared from one unit of WB (RCC: 350±35 ml, RDP: 70–90 ml and plasma: 220–300 ml) plus the volume lost during component preparation (~40–50 ml for BC discarded and the transfer tubes) comes out to be 645–825 ml, which is much higher compared to total blood volume actually available for processing (568–658 ml: WB 450±45 ml, CPDA 63 ml and SAGM 100 ml). This discrepancy in total volumes questions the practical applicability of present volume criteria for RCC in our national standards. Therefore, there is a need to reconsider the criteria for volume requirements in the Indian scenario with redefined specific ranges according to different methods of component preparation. In Table V, we propose a criterion for redefining the RCC QC standards according to the method of preparation. The criteria have been mathematically derived based on our study results as well as statistical process control (SPC) defined by mean±2SD for each category rounded off to the nearest multiple of five.

The unit characteristics of QC of red cell components which have an impact on the therapeutic outcome of the recipient are Hb content[5] and WBC content[6], where the former is directly related to the correction of anaemia and the latter is responsible for certain adverse effects of transfusion. Strict compliance with the NABH[17] criteria of volume and Hct for RCC_{450} would result in modifications of the component preparation methods in such a manner that a lot of red cells are lost during processing, or there could be a possibility of preparing diluted red cell units with larger volumes, part of which may get wasted or such units may pose a risk of volume overload to the patient. Considering D&C Rules[3] criteria of minimum volume and Hct for both RCC_{450} and RCC_{350} would result in RCC units with low Hb content not beneficial to the patient, while maximum limits of these QC characteristics would result in red cell loss during processing. Considering the DGHS[4] standards for RCC_{450}-AS with minimum volume of 315 ml with Hct 50 means that the Hb content in the RCC should be minimum 52.5 g, which is practically impossible to achieve from a donor with Hb 12.5 g/dl, and 450 ml volume of WB collected, which would result in only 56.25 g of Hb collected from the donor, followed by further red cell losses during processing; maximum volume of 385 ml is a large volume for transfusion. BC method is a cost-effective method of component preparation with some benefit of WBC removal in the Indian scenario, but only a low proportion of RCC units prepared by the BC method in this study had compliance with the current DGHS criteria (Table III). Thus, meeting the present reference national criteria for QC of red cell components is not only difficult to achieve by the blood centres across the country, but also RCC units produced in compliance

with the existing national criteria may often result in transfusion of many units with such characteristics, which may not benefit or may produce adverse outcome in the recipient. Our proposed QC criteria are aimed at achieving an acceptable proportion of quality with more emphasis on Hb content and WBC content for the therapeutic benefit of patients.

Apart from the method of component preparation, the other significant factors influencing the Hb content were the age and gender of the donor, while the WBC content was influenced by days of storage and the ABO group of donors.

RCC_{350} prepared from blood donated by female donors was observed to have lower Hb content and Hct compared to male donors. A similar finding was reported by Jordan *et al*[11]. It is commonly known that females in the general population have lower Hb due to dietary deficiency of iron and menstrual blood losses. Males have higher Hb due to higher levels of the hormone testosterone that stimulates the red cell production[24].

Our observation of decreasing WBC content of RCCs with increasing duration of storage may be attributed to the senescence, loss of membrane integrity and subsequent lysis of WBC during storage[25]. Higher WBC content was observed in units of female donors. Gwak *et al*[26] in a study on postoperative patients, found females to have higher neutrophils. However, with no differential leucocyte count data being documented in routine QC at our centre, we are not able to generalise the effect of gender on the WBC content of RCC. We observed higher WBC content in O and A group RCCs. This finding correlated with a study on 2,864 patients with acute coronary syndromes by Johansson *et al*[27].

Similar to Sawant *et al*[28], we observed higher haemolysis in RCCs prepared by the PRP method as compared to the BC method. RCC_{450}-AS(TAB) had the minimum percentage of haemolysis, suggesting the B-TAB method to have lesser physical stress on the RBC membrane as compared to the B-TAT technique. RCCs of female donors were previously[11] observed to have lesser haemolysis than that of male donors, possibly due to the antioxidant effect of oestrogen in females[29] and the propensity of testosterone in males to induce instability in RBC membrane[30]. However, such a finding could not be ascertained in this study.

This study had certain limitations. Throughout the study period, the pre-donation Hb of the donors was checked by either quantitative or qualitative methods, resulting in non-uniformity in records of this parameter, which could not be considered for analysis. During the study period, no RC_{450} (RCC prepared from 450 ml WB collection by the PRP method) units were prepared at our centre and hence were not available for analysis. Also, RCCs were prepared using the blood bag system from a single manufacturer; therefore, the possibility of better compliance with QC standards with the use of blood bag system from another manufacturer(s) cannot be ruled out.

Overall, this study shows that the quality of RCCs is significantly influenced by the processing method and certain donor factors which may be considered in upgrading our current quality standards. Evidence generated from this retrospective study alone, however, is not sufficient for redefining the QC criteria of red cell components. A multi-centric study with a larger sample size is required to confirm these observations.

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For correspondence: Dr Dheeraj Khetan, Department of Transfusion Medicine, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow 226 014, Uttar Pradesh, India
e-mail: dheerajkhetan@gmail.com

Supplementary Material: Box. Formulae used for calculation of various study parameters for individual RCC units

- Volume of RCC (ml)
  = [weight of RCC (g) – weight of empty bag[#] (g)]/specific gravity[$]
  [#] weight of empty bag is 35 g for RCC_{350} and 45 g for RCC_{450};
  [$] specific gravity is taken as 1.09.
- Hb content (g/unit)
  = [Hb (g/dL)×Volume of RCC (ml)]/100
- WBC content (cells/unit)
  = [WBC count (/mm^3)×Volume of RCC (ml)] × 1000

  RCC units within shelf-life with a storage period of more than seven days were randomly selected from the inventory for estimation of percentage haemolysis, in addition to routine QC parameters.
- Percentage haemolysis (%)
  = [(100–Hct)×plasma Hb (g/dL)]/Hb concentration (g/dL)

Note: RCC units within shelf-life with a storage period of more than seven days were randomly selected from the inventory for estimation of percentage haemolysis, in addition to routine QC parameters.
RCC, red cell concentrate; Hb, haemoglobin; WBC, white blood cell; QC, quality control

Supplementary Table. Unit characteristics of RCCs subjected to percentage haemolysis testing (n=94)

Unit characteristics	Type of red cell component			
	RCC_{350}		RCC_{450}	
	RC_{350} (PRP method) (n=08)	RCC_{350}-AS(TAT) (n=37)	RCC_{450}-AS(TAT) (n=30)	RCC_{450}-AS(TAB) (n=19)
Plasma Hb (g/dl), mean±SD	0.09±0.04	0.07±0.03	0.08±0.03	0.06±0.02
Storage age of unit (days), median (Range)	12 (9–16)	10 (9–25)	10 (9–31)	19 (10–31)