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Quality Parameters of Platelet Concentrate at Kenyatta National Hospital's Blood Transfusion Unit

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Abstract Background and objective: Platelet rich plasma-platelet concentrate (PRP-PC), were prepared and their quality parameters were assessed. **Materials and methods:** This was a prospective cross-sectional study carried out from February 2010 and May 2010 to study various quality determinants of platelets prepared at the Kenyatta National Hospital. The objectives were to assess their quality using the following parameters: swirling, volume of the platelet concentrate, platelet count, WBC count and pH. **Results:** A total of 78 platelet concentrates were analyzed. The majority 54, (69.2%) were group O RhD+, Group O RhD- was the least frequent blood group at 1.3%. A total of 77, (98.7%) of all concentrates were RhD positive. Centrifugation of whole blood was performed according to set specifications for all (100% n=78) concentrates. Separation was achieved using a separator but there was a prolonged delay of more than 1 hour before separation. Storage for all concentrates (100%, n=78) was in ambient temperature without temperature regulation. Agitation for all the concentrates (100%, n=78) was by circular motion in the horizontal plane. Only 51% (40) of all concentrates fulfilled the minimum specification for platelet count of >5.5 x 1010; the mean SD for platelet count was 6.63 ± 4.73 x 1010 with a median of 5.58 x 1010 and a range of 0.89 - 21.50 x 1010. None of the concentrates fulfilled specification for residual WBC count, (Mean + SD, Median and range of residual WBC count are 545 ± 429, 4.40 and 0.08 – 18.9(106) respectively) whereas 91% (71) and 95% (74) of the concentrates fulfilled the standards for volume and pH respectively. Forty (40) representing 51% of the coincentrates recorded a platelet count of 5.5 x 1010. The mean volume of PRP-PC was 74.6 ± 10.7 ml, with a median of 72.6 mlk and ranged from 49.8 – 97.2 ml. Their mean pH was 7.160 ± 0.426 and ranged from 6.0 – 7.8. Only 6 (7.6%) of the concentrates did not have red cell contamination. A total of 61 (78%) of the concentrates were issued on day 1, 8 (10%) on day 2 and 9 (12%) on day 3. All the concentrates were issued within three days of processing. **Conclusion:** The processes used in the preparation of platelets did not conform to the standards prescribed by NBTS resulting in only half of platelet concentrates fulfilling minimum specifications set by NBTS for platelet counts and none meeting the criteria for residual WBC count although they met the criteria for volume and pH. There is therefore need to strengthen the quality assurance program for preparation of platelet concentrates at KNH BTU and it is recommended that all platelet concentrates prepared at KNH be subjected to platelet count before issue so that only those concentrates that meet quality standards are issued.

Keywords: *platelets, quality, blood transfusion*

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1. Introduction

Since platelets were first identified in 1881, there has been considerable understanding of their function and their clinical use continues to grow [1]. Platelet transfusions are mainly used in thrombocytopenic patients most commonly as a result of myelosuppressive therapy, increased consumption as in disseminated intravascular coagulopathy (DIC), and aplastic anemia. Other less frequent indications include thrombocytopenia due to qualitative defects e.g. in Bernard Soulier syndrome or quantitative defect e.g. decreased platelet count seen in patients receiving myelosuppressive drugs like chemotherapy [2]. The cause of the thrombocytopenia should be investigated and established before platelet transfusions are administered since platelet transfusions are not always appropriate for treatment of thrombocytopenia. In some instances, for example in idiopathic/immune thrombocytopenic purpura (ITP), thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome (HUS), and heparin induced thrombocytopenia (HIT) platelet transfusions are not recommended [3,4]. There is limited data available on the clinical use of platelets in Kenya. Kenyatta National Hospital (KNH) prepared and issued approximately 450 platelet concentrates in 2008, (Unpublished KNH records), although the demand is likely to be much higher.

At KNH, the platelet concentrates are prepared from random whole blood donors and platelet concentrates are separated from platelet rich plasma derived from these donors. The buffy coat poor-platelet concentrate (BC-PC) method is rarely used mainly due to the complexity of preparing them. The single donor platelets (DPs), apharesis – PC) collected from voluntary donors with the help of an automated cell separator is not available. In North America, the Platelet-Rich-Plasma (PRP) method is preferred while in Europe, the Buffy Coat BC) method is preferred [5]. Both are not significantly different in terms of yield [6].

The recommended shelf life of platelets concentrates is 5 days stored at 22 ± 2°C with continuous agitation. It is known that platelets undergo various changes immediately after they are collected, during processing, storage and even wen being administered to the patient. This may affect the effective dosage that a patient receives [5]. There is also loss of function as high 30% that is attributed to the Platelet Storage Lesion (PSL) [6,7]. For example, the Buffy coat method produces platelets with significantly less in vitro activation and which have a lesser residual white cell count [8,9].

In the laboratory the platelet quality can be assessed by using several parameters (swirling, volume, platelet count and WBC count per bag and pH changes) [10]. Most of these parameters asses in-vitro platelet function. It's difficult to determine which in vitro platelet functions are essential for in-vivo hemostasis after transfusion [11].

The American Association of Blood Banks (AABB) recommends and sets standards for transfusion medicine in the United States. AABB has published a technical manual that details the procedures and processes to be followed in producing platelet concentrates. These include details of blood collection, spinning, separation, storage and agitation. In addition AABB has prescribed minimum requirements for platelet concentrates processed by the PRP method [12,13]. In recognizing the limited capacity of transfusion services to perform a wide array of checks, AABB has restricted the basic minimum requirements to at least include platelet count, pH, volume, red cell content, and residual WBC count in leuko-reduced products. These standards have since been adopted by the standards committee of NBTS [12]. Table 1 shows the specifications for several parameters in Kenya, United States and Europe.

In this study we have analyzed the quality of different platelet concentrates prepared at the KNH BTU as per the AABB recommended quality standards which have been adopted by the Kenyan NBTS [12,13,14].

Table 1. Platelet quality parameter specifications in different regions [12,13]

Parameter	United States	KNBTS	Europe
Platelet count ($\times 10^{10}$)	>5.5	>5.5	>6.0
Volume (mls)	To maintain		>50

2. Materials and Methods

All platelet concentrates ready for issue prepared in the period between February 2010 and May 2010 were included in the study.

2.1. Process Assessment

The processes of spinning, separation, agitation and storage of components were directly observed by the principal investigator or research assistant for all the concentrates. The data were recorded in prepared data sheets using LICKERT SCALE scoring system.

2.2. Collection of Specimen

The unique laboratory identification number, date and time of concentrate preparation and ABO/RhD group were recorded for each concentrate. Each concentrate was then assigned another identification number for the purposes of the study. The concentrates were then weighed and the volume determined. The sealed pilot tube was cut with sterile scissors and 1ml of the unit drawn into two EDTA 75×15 mm tubes. The tube was then resealed using a bag sealer and released for patient use. The samples were transported to the laboratory for analysis.

2.3. Quality Assessment of Platelets

The quality assessment of platelets preparations were tested for: Platelet concentrates volume, swirling, platelet counts per bag, WBC and RBC counts and pH changes.

2.4. Cell Counts

Platelet count, residual WBC count and contaminating RBC count were determined by Coulter® AcT™5Diff Hematology Analyzer (Beckton–Dickinser). The following formula was used to obtain the total cell count for WBC, RBC and Platelets for each concentrate.

$$\text{Cellcount / unit} = \left(\text{samplecellcount} / 1 \times \text{Volume(ml)}\right) \div 1000$$

pH was determined by an automated Bayer BG analyzer Rapid*lab* 348 model located at the intensive care unit, KNH. This instrument determines pH based on respective electrodes (Ready Sensor™ for pH reference electrode and reference cassette.

Volume was determined using the following formula:

$$\text{Volume} = \begin{pmatrix} \text{weightofconcentrate(g)} \\ -\text{weightofemptybag(g)} \end{pmatrix} \div 1.06$$

(Where 1.06 = specific gravity of plasma.)

2.5. Statistical Analysis

All data were expressed as mean ± SD. We performed Statistical comparison by using the student's 't'-test. A probability of $P < 0.05$ (two-sided) was used to reject null hypothesis.

3. Results

3.1. Blood Groups

A total of 78 concentrates were sampled over a four month period. The majority 54, (69.2%) were group O RhD+. Group O RhD- was the least frequent blood group at 1.3%. A total of 77, (98.7%) of all concentrates were RhD+.

Table 2. ABO and Rh blood groups distribution of the platelet concentrates

Blood	Frequency	Proportion
A+	18	23.1
B+	5	6.4
O+	54	69.2
TOTAL	78	100

3.2. Age of Platelets Concentrates

All the concentrates were issued within three days of processing. Sixty one (78%) of the concentrates were issued on day 1, 8 (10%) on day 2 and 9 (12%) on day 3.

3.3. Process Assessment

Centrifugation for all 78 concentrates was performed in a *himac*® centrifuge whose speed was not functionally and mechanically calibrated for the highest percentage platelet yield. There was a delay of more than 1 hour from the end of centrifugation to the start of separation for all 78 concentrates. A hand held Terumo® plasma extractor was used for the separation. All the 78 concentrates were agitated by a rotatory motion. The speed was fixed at four rotations per second. None of the concentrates was stored in a temperature regulated environment. Table 3 shows the scores for the processes for platelet preparation.

Table 3. Lickert scores for the processes of platelet concentrate preparation

Processes	Activity	Score	Number of	Comment.
Centrifugation	Completed	2	n=0	All 78 concentrates were centrifuged in a centrifuge which was not functionally or mechanically calibrated
	Incomplete	1	n=78 (100%)	
	Not done	0	n=0	
Separation	Separation within 60 Minutes and Use of plasma extractor)	2	n=0	There was a delay in separation after centrifugation for all the 78 concentrates
	Separation delayed (>60 minutes and use of plasma extractor)	1	n=78 (100%)	
	Plasma extract or not used	0	n=0	
Agitation/ storage with temperature regulation	Complete	2	n=0	All 78 concentrates were stored without temperature regulation
	Incomplete	1	n=78 (100%)	
	No agitation, no temperature regulation	0	n=0	

3.4. Cell Counts, Volume and pH

Forty (51%) concentrates recorded a platelet count of 5.5 x 10^{10}, residual WBC count was 545±429, see Table 4.

Table 4. The results for RBC count, pH and Volume

Parameter (n=78)	Median	Mean	Range	NBTS Standard	% meeting criteria
Platelets (1010)	6.63±4.73	5.58	0.89-21.50	5.5	51.3
WBC (106)	545 ± 429	440	8.0 -1890	<0. 83	0
RBC (109)	7.37±9.01	4.68	0.0 -64.00	Not specified	-
Volume (mls)	74.6±10.7	72.6	49.8 -97.2	60	91
pH	7.160±0.426	7.231	6.0-7.8	>6.2	95

3.5. Characteristics of the Platelet Concentrates that did not Meet the Criteria for Minimum Platelet Count, Volume and pH

The characteristic of the 38 concentrates (49%) that did not meet the NBTS requirement for platelet count were analyzed and compared with the 40 concentrates (51%) which met the minimum platelet count.

The WBC and RBC count were significantly lower in the platelets that did not meet the criteria is compared to the ones that met the criteria (p-value- <0.001) while the pH in this group was significantly higher (P- value <0.001) (Table 5).

Table 5. Comparison of the concentrates with platelet count <5.5×10^{10} and >5.5×10^{10} per bag

PLT Count (10^{10})		Duration of storage (Days)	WBC Count (10^6)	RBC Count (10^9)	pH	Volume (ml)
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Category 1	Platelet Count >5.5 (40)	2.1± 0.5	780 ± 450	11.9 ± 10.6	6.89 ± 0.4	73.2± 11.4
Category 2	Platelet count <5.5, (38)	2.1 ± 0.4	304 ± 240	2.60 ± 2.2	7.45 ± 0.22	76.1 ± 9.9
	P-value	1.0000	<0.0001	<0.0001	<0.0001	0.235

3.6. Characteristics of the Platelets Concentrates that did not Meet Criteria for Volume

A total 7 (9%) of the concentrates were below the minimum volume of 60mls. These were compared with those with volumes exceeding 60mls.

The mean red cell content for these was 24.3 ± 20.3 x 10^9 which was significantly higher (p-value <0.001) than the mean for those with volume > 60 mls.

The mean pH 6.4 ± 0.4 was also significantly lower. There was however, no significant difference in platelet counts and residual WBC counts in these concentrates.

3.7. Characteristics of the Concentrates that did not Meet Criteria for PH

A total of four concentrates (5%) had pH below 6.2. The mean volume for these concentrates was 58.7± 11.8, which is significantly lower than in the concentrates with pH > 6.2 (p-value 0.0019). In these concentrates the mean platelet count and red cell count were significantly higher at 15.33 ± 5.3 × (10^{10}) and 34.15 ± 20.0× (10^9) (p - value <0.001).

4. Discussion

Kenyatta National Hospital (KNH) is the largest referral hospital in Kenya and runs a transfusion service that includes a donor unit, blood banking, component production, and a clinical transfusion service. KNH manages various conditions such as thrombocytopenia, bone marrow failure and in hematological and solid malignancies in adult and pediatric patients in which platelet transfusions are indicated.

This study assessed the processes and procedures used in the production of platelet concentrates at the hospital's BTU, in particular the process of centrifugation, separation, storage and agitation, and to compare these with standard procedures set by NBTS as adopted from AABB. The study also determined quality parameters of platelet concentrates which included cell counts, pH, and volume. These were compared with the standards set by Standards Committee of NBTS as adopted from AABB.

Of the 78 concentrates sampled, 69.2 % were O+ve, 23.1% were A+ve, 6.4% were B+ve and only one unit (1.3%) was O-ve. At KNH most platelet concentrates processed and issued are group O, reflecting the predominance of group O among the voluntary blood donors in Nairobi, Kenya [15].

The majority of platelet concentrates sampled were issued on day 1. According to records at the BTU, most concentrates are issued within 24 hours of processing, although the bag used allowed storage for up to 5 days. Several studies show that platelets transfused within 24-48 hours have little platelet storage lesion compared to stored platelets. At KNH, most blood components including platelets are processed and issued on demand.

All 78 platelets concentrates were centrifuged using the light spin and hard spin. Even though the centrifugation speeds was consistent for all units, functional and mechanical calibration of the centrifuge for the highest percentage platelet yields was not performed. AABB, in their technical manual, has recommended that all centrifuges used for blood product preparation should be calibrated for the lowest speed and shortest time that gives the highest platelet percent yield and the lowest residual contaminating red cells in the preparation of platelets concentrates using the PRP method from whole blood [12,13]. Lack of functional calibration of the centrifuge for speed and duration of centrifugation may affect the final products. Even though the speed used for centrifugation was 1800 rpm and 3800 rpm for the soft and hard spin respectively, there was need for calibration to ensure that these speeds resulted in the highest percent platelet yield for that particular centrifuge.

Separation was achieved using a plasma extractor. However, there were prolonged delays of more than one hour from the time of end of centrifugation to the start of separation. Delayed separation may disrupt the compartments and allow the cells to mix. This may result in higher red cell contamination in the final platelet concentrate as was observed in the study.

Storage for all concentrates was at room temperature on the bench. There was no temperature regulation or ambient temperature measurements. Therefore the concentrates were subjected to uncontrolled environment contrary to AABB requirements for storage at 20-24°C degrees centigrade. Lower temperatures cause platelet activation, aggregation and subsequent reduced recovery after transfusion. In addition lower temperatures resulting in difficulties in re-suspension of the platelet pellet which may result in lower platelet counts [16]. Since no markers of platelet activation were performed in this study, it's not possible to ascertain what deleterious effect, if any, this storage had on these concentrates.

Only 51% of all concentrates showed platelet count of $>5.5\times10^{10}$ against NBTS requirement of >95%. The range of platelet counts was 0.89-21.50× 10^{10} showing a wide variation in the counts. Taking into account that up to 30% of the platelets fail to recover on account of platelet storage lesion [6,7] this suggests that approximately half of the platelets unit transfusions may not have the expected therapeutic effect on the recipients. The mean and standard deviation for platelet count was $6.63\pm4.73\times10^{10}$ which was well above the minimum threshold. The large SD shows a significant number of units have extremely low platelet count, since only 51% meet the criteria for minimum platelet count. This wide variation in the counts may be due to lack of standardization in the preparation procedures, particularly in centrifugation and separation. The WBC and RBC counts of these deficient platelet concentrates were lower (p-value <0.001) than those with satisfactory counts whereas the pH was significantly higher (p-value<0.001). This may be due to variability in centrifugation and separation process. Inadequate centrifugation or delay in separation after spinning may result in low platelet count.

A total of 30 (49%) of the concentrates returned platelet content of less than 5.5×10^{10} per bag. Their parameters were compared with those which returned higher counts. There was a significant difference in the WBC and RBC counts between these concentrates.

In a similar study at a tertiary hospital in Nigeria where platelet concentrates were prepared by PRP method, Fasola [17] found that only 35% of concentrates met the minimum 5.5×10^{10} platelet count threshold. The mean± SD for that study was 4.17± 3.95 as wide a range as wide as in this study. Singh RP [18] reported a mean value of 7.6± 2.97 which is well above the 5.5×10^{10} threshold in a study in which he assessed PRP, BC and apheresis platelets. These studies demonstrating such wide variations of the means reported for the same method in various regions suggesting variability in platelet production method between regions.

Low residual WBC counts in platelet productions are desirable in order to minimize side effects of residual leucocytes such as febrile reactions and to minimize the platelet storage lesion which can be enhanced by high leukocyte count [19]. All the concentrates in this study had high WBC contamination presumably because no leukoreduction procedure was performed. Therefore none of the concentrates met the criteria for residual WBC count. Even though leukoreduction was not performed, this level of contamination is considerably higher than the results reported from similar studies. Singh RP [18] reported mean WBC count of $40.5\pm4.8\times10^{6}$ for the PRP method which is far lower than levels established in this study ($545\pm429\times10^{6}$) even though no leukoreduction was performed in that study as well. These results for WBC count, like those for platelet counts suggest that the marked differences in counts are due to variability in process.

Only 6 (7.6%) of the concentrates did not have detectable red cell contamination which is considerably

higher than reported by Fasola [17] who found that 30% of concentrates in his study did not have any red cell contamination. Currently NBTS does not have guidelines for residual red cell contamination. It has been shown that as low as 0.5 mls of red cells in a platelet unit are sufficient to cause RHD and ABO alloimmunisation [20] especially if the practice of issuing platelets that are not group matched is adopted.

AABB has recommended that each transfusion center formulate a policy to be followed if they are to transfuse ABO group incompatible platelet concentrates. KNH has adopted the policy of issuing only ABO compatible platelet concentrates. However, there are instances where ABO incompatible platelets may need to be used in emergency settings or when there are no compatible platelets available. In these cases, this high level of red cell contamination of platelet concentrates may be a cause for concern for further adverse effects other than those rare effects which would arise from transfusing g r o up matched red cells. Since the separation of the platelets depends on the visual sighting of the red cell-plasma interface, it is likely that the red cell contamination is due to excessive expression of plasma beyond the interface during separation.

The mean volume of platelet concentrates in this study was 74.6 ± 10.7 mls. A total of 71 (91%) of the concentrates were more than 60mls, therefore meeting the NBTS criteria. In addition, the 7(9%) units that did not meet criteria of minimum volume of 60mls showed statistically significant lower pH (mean 6.4±0.4, p-value <0.001) and higher residual red cell counts (mean 24.3 ± 20.3, p-value< 0.001). This study confirmed that lower volumes of PC resulted statistically significant lower pH.

In the study by Fasola [17] the mean volume was considerably low (18.52mls) whereas Singh [18] reported a mean of 62. 3 0 ±22.68 ml. Studies have shown that as little as 35-40 mls of plasma is adequate to maintain pH above 6.0, below which the platelet storage lesion is irreversible [21.] AABB has not specified the minimum volume, simply stating that the final volume should be adequate to buffer the pH to >6.2, whereas NBTS has set a minimum volume of 60 mls. This higher volume may be necessary in this setting so as to maintain an adequate buffer since neither leukoreduction nor testing for pH performed on platelet concentrates. This requirement for higher volume by NBTS may be due to lack of leukoreduction. There has been no consensus as to the final volume. Therefore, it's apparent that the 60 mls threshold set by NBTS is considerably higher than the volume required buffering the pH [21,22].

High volumes of platelet concentrate, though necessary to buffer the pH especially because leukoreduction is not done at KNH. This results in exposing recipients to high volumes of plasma, and may result in minor incompatibility reactions if platelets are not group matched, anaphylaxis due to IgA, or other allergic reactions due to plasma proteins [23]. Further studies are required to establish the minimum volume required to maintain platelet viability and pH where leukoreduction is not performed. Alternatively a shift to additive storage solutions as substitutes for plasma may be advised.

A total of 74 (95%) concentrates sampled recorded pH >6.2, in accordance with the set requirements. The pH was determined in EDTA, which has been shown to lower pH by

0.723. These results are similar to reported values. Fasola [17] reported that 100% of his concentrates recorded pH 7.25 or higher. Singh reported pH values of 6.7±0.26 (mean ± SD) with a range of 6.5-7.0. The lower pH may be due to higher mean cell counts in that study. On analysis of the concentrates that returned a pH level below 6.2, (4, 5%) there was a significant correlation between lower pH and high platelet and red cell counts (p-value <0.0001), suggesting that these two variables rather than WBC counts were responsible for the platelet storage lesion. This is despite the fact that no maximum red cell limit has been prescribed. As illustrated in the scatter plots in this study, the general effect of red cell counts, WBC counts, platelet count and volume on pH is as expected. Very high platelet count in a single concentrate, whereas desirable, appear to accelerate the storage lesion, especially if other parameters specifications such as adequate volume and WBC count are not fulfilled. This therefore emphasizes the need for stringent controls to ensure the high platelet count is not achieved at the expense of platelet viability.

In general the final effect of the various variables on the platelet storage lesion as shown in the tables (Table 2-Table 5) is as expected in a PRP platelet concentrate during storage if plasma and not additive solution is used [21]. This in effect illustrates the various interventions that may be used to prevent the deleterious effect of the platelet storage lesion.

In conclusion, the results obtained in this study indicate significant deficiencies in the quality of platelet concentrates prepared by the PRP method at KNH. In particular, the study has demonstrated the need for strict adherence to SOPs in the preparation of platelet concentrates. This study also demonstrates that pH and platelet counts can form an integral part of a rapid, simple and practical method for validation of collection, processing and storage procedures that is useful for routine quality monitoring and prerelease testing of platelet concentrates. In addition, this study shows that in transfusion units where few platelet concentrates are processed and issued, it is possible to sample all units and subject them to prerelease quality checks. Implementation of these will help in continuous quality improvement as well as entrench a standardization and harmonization program on platelet quality monitoring.

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