ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Quality assessment of platelet concentrates prepared from different time intervals held whole blood at Ethiopian Blood and Tissue Bank Service, Addis Ababa, Ethiopia.

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This is to certify that the thesis prepared by Muluken Kassahun, entitled 'Quality assessment of platelet concentrates prepared from different time intervals held whole blood at Ethiopian Blood and Tissue Bank Service, Addis Ababa, Ethiopia' and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology track) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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ABBREVIATIONS

AfSBT	Africa Society for blood transfusion
AABB	American Association of Blood Banking
ATP	Adenosine triphosphate
BC	Buffy coat
CRC	Concentrated red blood cell
EBTBS	Ethiopian Blood and Tissue Bank Service
ERCS	Ethiopian red cross society
FFP	Fresh frozen plasma
FMoH	Federal ministry of health
NRVBDs	Non-ruminated volunteer blood donors
PC	Platelet concentrate
PRP	Platelet rich plasma
PSLs	Platelet storage lesions
RPM	Revolution per minute
QC	Quality Control
SOPs	Standard Operating Procedures
TTIs	Transfusion transmittable infections
WB	Whole blood
WBC	White blood cell

ABSTRACT

Background: The objective of this research is to meet the ever-growing demand for blood and blood components in health facilities, especially platelet concentrate, which is extremely high. The short shelf life of platelet concentrate is another reason for the shortage. Therefore, with the need to generate a new additional method to increase the supply of platelet concentrate, this study uses platelet concentrates prepared from whole blood held at different time intervals. In addition, it included almost all quality assurance requirements to ensure its quality.

Objective: The purpose of this study was to assess quality of platelet concentrates prepared from different time intervals held whole blood at Ethiopian Blood and Tissue Bank Service, Addis Ababa.

Methods: A Cross sectional research was done on 153 samples taken from 25 female and 128 male non-ruminated volunteer blood donors and whose age was range 18 to 61 years at Ethiopian Blood and Tissue Bank Service from August 1 to October 30, 2022. Convenient sampling was used; all whole blood collected at mobile and static centers were included. The selected whole blood was held for 8, 16 and 24 hours at room temperature and processed into its component according to SOPs; then platelet concentrates were assessed for their quality parameters based on the national standards. The data collection was checked before analytical, during analytical and after analytical steps then, data was entered, checked, cleaned, and fed into Microsoft Excel software and then imported into SPSS version 26 for further statistical analysis. Mann-Whitney U test, the Wilcoxon Signed-Rank Test and Friedman's test were used for pairwise group comparison, within group pairwise comparisons and triple measure within group median comparisons, respectively. $P < 0.05$ was statistically significant.

Results: In this study, swirling was observed in all units tested. Mean of volume were within the range 50-70ml. Mean of platelet count of 8, 16 and 24hrs held prepared platelet concentrates were above $\geq 5.5 \times 10^{10}$/unit at day 1, 3 and 5. Mean of white blood cell count and pH level were in acceptable range $< 0.2 \times 10^{9}$ and 6.4-7.4 respectively. The quality parameters of the 16 and 24 hours storage held prepared platelet concentrates were comparable with those of the 8 hours. All parameters except red blood cell count met the Ethiopian Blood and Tissue Bank Service's standard requirement (75%). Statistically no significant difference were observed when compared to the platelet concentrates quality indicators of three different time interval prepared platelet concentrates, except volume and WBC count were have significant difference in comparison.

Conclusion: This study found that delaying whole blood processing for up to 16 and 24 hours had no effect on platelet concentrates quality indicators when compared to 8 hours held produced platelet concentrates.($p > 0.05$)

Key words: Whole blood; Platelet concentrate; platelet quality

1. INTRODUCTION

1.1. Background

Platelets also called thrombocytes are blood cells that originate from bone marrow megakaryocytes and which then circulate in the blood for approximately 10 days (1, 2). They possess granular cytoplasm with no nucleus and their diameter averages about 2 to 4 μm. Platelets possess mitochondria, which produce and store adenosine triphosphate (ATP), and exhibit a high sensitivity to their surrounding environment. The normal peripheral blood platelet count is 150-400x10^9/L. This count only represents two thirds of available platelets because the spleen sequesters the remainder. Platelets play an important role in blood clotting and wound healing (3-8).

Blood component production was developed in 1960, which involved the separation of whole blood (WB) to concentrated red blood cell (CRC), fresh frozen plasma (FFP) and platelet concentrate (PC) (9). PCs can be acquired using an automated technique of blood cell selection known as apheresis, or via whole blood centrifugation by randomization using the platelet-rich plasma (PRP) or buffy coat (BC) methods (4, 10-14). Both PRP and BC techniques involve manual processes such as whole blood collection, centrifugation, separation, and storage with agitation. The PRP method employs single-step heavy and two-step (light and heavy) spin centrifugation processes to extract CRC and FFP or CRC, FFP, and PC from WB (9-11).

Platelet concentrates play a crucial role as a vital constituent of whole blood, serving as indispensable support for patients undergoing intensive therapies for hematological malignancies and solid tumors (7). Platelet transfusion has exhibited a progressive increase in its employment, and has become an imperative component of cancer treatment, hematological malignancies, marrow failure, and hematopoietic stem cell transplantation worldwide (15-16).

Ethiopian Blood and Tissue Bank Service (EBTBS) has currently set the maximum storage period before component preparation to be within 8 hours at a temperature range of 20 to 24°C, in accordance with American Association of Blood Banking (AABB) and Africa Society for Blood Transfusion (AfSBT) guidelines (17-19). In Ethiopia, PC is generated from 450±45ml WB immediately after blood collection using the PRP technique and kept in plasma for 5 days (19-21). Platelets have a relatively short shelf life due to the development of platelet storage lesions (PSLs) and bacterial contamination during storage (22-24).

In addition to extending the storage time of WB and increasing production of PCs, it is imperative to assess the quality of platelets to ensure their compliance with the standards set forth by the EBTBS and international protocols. This evaluation is also necessary to determine the potential impact of PCs on the recipient (with an average transfusion of 4-6 units of PCs per patient) (7, 19, 25-27). The quality of PCs is influenced by various factors, such as the method of preparation, the type of storage bag, the temperature, time of storage, the anticoagulant used, the PCs in the bag, and the method of storage (i.e., agitation) (23,28-29).

The current quality parameters of PCs are swirling, volume, platelet count, red blood cell (RBC) count, white blood cell (WBC) count, and pH level. Thus, PC is considered quality if swirling is present, the platelet volume is within 50-70ml, platelet count should be $\geq 5.5\times10^{10}$cells/unit, RBC count should be $\leq 1.0\times10^{9}$ cell/l, WBC count should be $\leq 0.2x10^{9}$ cells/l and pH level ranged between 6.4-7.4, respectively (17-19).

As a result, the purpose of this study is to compare the quality of PCs derived from WB that has been stored for 16 and 24 hours with PCs prepared from WB that has been stored for only 8 hours. Moreover, this study also compared the PCs obtained from 16 and 24 hours stored WB against the quality requirements and specifications specified by EBTBS. Furthermore, the quality parameters of PCs such as swirling, volume, platelet count; RBC count; WBC count and pH level is assessed at EBTBS.

1. 2. Statement of the problem

Platelet transfusion is young science that plays an important role in saving lives and improving health in major medical disorders. However, it can be a source of infection for the platelet receptor. Among the infections transmitted by PC transfusions is bacterial sepsis, which the fact that the PC is stored at 20-24^{0}c temperature may be favorable to bacterial growth or may be due to a risk inherent in the order in which the blood is drawn from the donor (24, 30).

Out of the 148,800 units of blood that the EBTBS has planned to collect in 2015 E.C, 70% will be used to prepare blood products to fulfill the annual needs of PCs. According to the current standard of practice, 300 units of 450 ml whole blood must be collected every day brought to the blood product preparation room and the component preparation must be completed in 8 hour of blood collection to achieve the targeted set. Thus, adequate blood product preparation machines, manpower and a relatively prolonged time are required (19, 31).

Moreover, in Ethiopia, mobile or onsite blood donation campaigns initiated to fulfill the temporary demands are common practices since the awareness of the population about regular blood donation and the numbers of regular donors are limited. During blood donation campaigns, increased number of blood is collected within a short period; in which completing the component production process within 8 hours of collection by the available staff and machineries is difficult. Hence, switching from 450 ml to 350 ml blood collection which will be transfused as a whole blood rather than components is common during campaigns. Whole blood therapy has its limitations in economical use of blood, its effectiveness in case of specific component or factors deficiencies as well as increased transfusion reaction (32-34).

This particular standard, 8 hours maximum holding time of WB, has imposed limitations on blood banks with regards to their production of various blood components, particularly PC, which has a short shelf life and is in high demand by health facilities. Consequently, to meet the demand for blood components, extending the shelf life to WB might be helpful to solve the problem. Studies found no significant difference in the quality characteristics of PCs derived using the PRP Method within 8 hours and 24 hours (15, 20, 29, 35) and emphasized the importance of ensuring PC availability in healthcare facilities without a scarcity.

Hence, the purpose of this study was to assess the quality of PCs prepared at different time intervals and to consider the difference between 8 hours and 16 and 24 hours, and to show the differences and closeness with EBTBS standards at EBTBS in Addis Ababa, Ethiopia.

1. 3. Significance of the study

This study on the quality of platelet concentrates will allow policy makers to use the results of the study to develop new procedures for the preparation of blood products; this could mean increasing platelet concentrate preparation from the current 8 hours to 24 hours.

Another is that if this research is implemented, it will lead to a significant improvement in blood bank services in the supply of platelet concentrate which are highly demanded by health facility and soon expired type of blood products.

In addition, this study will be useful for other researchers, especially those involved in blood banking.

2. LITERATURE REVIEW

The quality of blood and blood components can only be assured if all procedures related to blood collection, component preparation, testing, storage, and transports are constantly controlled and monitored (9,36). Additionally, in accordance with blood bank regulations, at least 1% of blood and blood components must be analyzed for quality control, with 75% of the tested components needing to meet specified standards for quality (17-19). In this review of the literature, the study on platelet concentrates prepared from whole blood using the platelet rich plasma approach was examined. According to the set standards, swirling, volume, platelet count, RBC and WBC count, pH level, and other quality criteria were tested.

In 2011, Coelho *et al*. conducted a cross-sectional investigation on 80 platelet samples in Brazil to examine platelet aggregation and biochemical characteristics of PCs using quality control tests required by their current criteria, revealed that occurrence of swirling was in all evaluated platelet, the average platelet concentrate volume was 60ml, the mean of platelet concentrate count at day 1, 3, and 5 was 5.45×10^{10}/unit, 5.8×10^{10}/unit and 5.76×10^{10}/unit respectively, the mean of WBC contamination was 0.1/unit and the mean of pH level was 7.42, 7.72, and 7.73 at day 1, 3, and 5 respectively. Even meeting the required specifications, platelet concentrates showed low aggregation rates. They propose that a functional evaluation assessment be included in the quality control of platelet concentrates to ensure a more effective response to platelet concentrate transfusion (4).

Shabani *et al*. conducted a prospective, controlled study on 64 platelet samples in Malaysia in 2014 to examine the quality of PCs prepared freshly and after WB overnight storage via PRP PC manufacturing methods, showed that the mean of volume of PC freshly prepared and overnight storage was 67.18 ±1.58ml and 66.82 ±1.67ml respectively, the mean of platelet counts freshly prepared were $11.16 \pm 19.66 \times 10^{10}$/unit, $9.64 \pm 18.70 \times 10^{10}$/unit and $9.24 \pm 18.74 \times 10^{10}$/unit at day 1,3 and 5 respectively and overnight storage were $9.92 \pm 21.88 \times 10^{10}$/unit, $9.80 \pm 21.97 \times 10^{10}$/unit and $9.04 \pm 20.38 \times 10^{10}$ /unit at day1,3 and 5 respectively, the mean of RBC count of fresh platelet was $0.01 \pm 0.01 \times 10^{6}$/µl, $0.01 \pm 0.01 \times 10^{6}$/µl and $0.02 \pm 0.01 \times 10^{6}$/ µl at day 1,3 and 5 respectively and overnight platelet was $0.02 \pm 0.01 \times 10^{6}$/µl, $0.02 \pm 0.01 \times 10^{6}$/µl and $0.03 \pm 0.03 \times 10^{6}$/µl at day 1, day 3 and day 5 respectively, the mean of freshly prepared PC; WBC counts was $0.04 \pm 0.02 \times 10^{9}$/unit, $0.03 \pm 0.01 \times 10^{9}$/unit and $0.03 \pm 0.01 \times 10^{9}$/unit at day 1,3 and 5 respectively and overnight prepared PC; WBC counts was $0.06 \pm 0.06 \times 10^{9}$/unit, $0.04 \pm 0.03 \times 10^{9}$/unit and $0.04 \pm 0.03 \times 10^{9}$/unit at day 1, day 3 and day 5 respectively and the mean of the freshly prepared PC: pH level was 7.62±0.08, 7.59±0.13 and

7.48±0.20 at day 1,3 and 5 respectively and overnight PC; pH level was 7.48±0.07,7.37±0.13 and 7.27±0.28 at day 1, day 3 and day 5 respectively. PRP-derived PCs from overnight-held WB showed significantly different in vitro characteristics than freshly processed WB. However, determined by the one-sample t-test comparison with the quality requirement of PCs, the use of PCs from overnight-held WB was possible as the results of volume, platelet count, and pH were significantly higher compared to the quality requirement and specification (29).

Kasim *et al*. (2016) conducted a cross-sectional research on 46 platelet samples in Malaysia to assess the quality of PCs generated from overnight-held and immediately processed WB; 23 platelets were employed for each category, revealed that the means platelet count of freshly prepared was $7.1\pm18\text{x}10^{10}$/unit, $7.1\pm25\text{x}10^{10}$/unit and $7.1\pm34\text{x}10^{10}$/unit at day 1, 3 and 5 respectively and PC prepared overnight hold was $6.3\pm33\text{x}10^{10}$/unit, $6.6\pm25\text{x}10^{10}$/unit and $6.3\pm36\text{x}10^{10}$ /unit at day 1,3 and 5 respectively, the mean WBC counts of freshly prepared was $0.04\pm0.03\text{x}10^{9}$/unit, $0.03\pm0.03\text{x}10^{9}$/unit and $0.03\pm0.02\text{x}10^{9}$/unit at day 1, 3 and 5 respectively and overnight hold was $0.02\pm0.02\text{x}10^{9}$/unit, $0.03\pm0.02\text{x}10^{9}$/unit and $0.03\pm0.01\text{x}10^{9}$/unit at day 1,3and 5 respectively and the mean pH level of freshly prepared was 7.4±0.13, 7.4±0.21 and 7.3±0.21 at day 1, 3 and 5 respectively and overnight hold was 7.3±0.05, 7.5±0.18 and 7.4±0.15 at day 1,3 and 5 respectively. Their findings revealed that, with the exception of platelet count, all quality criteria met the acceptable requirement. They also conclude that a 24-hour delay in whole blood processing has no effect on some in vitro quality indices and function when compared to freshly manufactured PCs (35).

Siew *et al*. conducted a cross-sectional study on 30 platelet samples in Malaysia in 2019 to evaluate platelet quality using either a manual or automated procedure; 15 units of whole blood were mixed by an automated blood collection mixer and the other 15 units were mixed manually, showed that the mean platelet count of manual mixing was $5.4\text{x}10^{10}$/unit and $5.2\text{x}10^{10}$/unit at day 1 and 5 respectively and automated mixing was $5.7\text{x}10^{10}$/unit and $5.6\text{x}10^{10}$/unit at day 1 and day 5 respectively, the mean WBC counts of both manual and automated mixing was 0.03x109cell/unit at day 1 and day 5 respectively and the mean pH level were 7.50 and 7.44 for manual measured at day 1 and 5 respectively and 7.47 and 7.50 for automated measured at day 1 and 5 respectively. They found that the platelet count on day 1 was significantly larger than on day 5 ($p = 0.01$) for both mixing procedures, but there was no statistically significant difference in any of the PCs quality measures at either day 1 or day 5 of storage ($p > 0.05$). They discovered that both hand mixing and auto mating techniques produced equivalent PC quality (37).

An experimental study conducted in Iran by Ali in 2012 on 30 platelet samples to assessed quality of PC prepared by PRP and BC methods; the PRP method showed that the mean of volumes was 55.6±12.3 ml, the mean PC counts was 5.6 ± 2.1 x10^{10}/unit. The mean of platelet count done at day 0, 1, 3, and 5 were 5.8± 1.1 x10^{10}/unit, 5.7±2.1 x10^{10}/unit, 5.6±2.1 x10^{10}/unit and 5.4±3.4 x10^{10}/unit respectively, the mean of RBC contamination was 27 ± 2.1 × 10^{8}/unit, the mean WBC counts was 43 ± 0.48 ×10^{6}/unit and the mean pH level was 6.8 ± 0.1. They found that the mean platelet, RBC counts, and pH of BCs and PRP-PCs were comparable, with no statistically significant difference identified ($P > 0.05$). They also reported that the mean leukocyte counts BCs and PRP-PCs were comparable, with a statistically significant difference ($P < 0.05$) observed. They concluded that the Buffy coat product had more leucoreduction than the PRP PCs product (38).

Another cross-sectional study conducted in Iran in 2013 by Naghadeh HT *et al* on 20 platelet samples to assess platelet quality after 48 hours of agitation, then investigated quality control with and without continuous agitation for 6 hours, revealed that the swirling was seen in all PC, the mean and of PC volume was 56±8.5ml and the mean pH level was 7.22±0.16. They reported that the mean platelet counts and pH values of control and resting PC were not statistically different ($P >0.05$). They found that PC stored in permeable bags at 22-24°C for 42 hours and then rested for 6 hours had more stable pH and swirling than PC stored with continuous agitation during the whole 48-hour period. (39). Another experimental study conducted by Ali in Iran in 2015 among 25 platelet samples of PRP method PCs, 25 platelet samples for BCs and APCs preparation method; PRP method PCs, showed that the mean of PC volume was 50.6 ±15.3 ml, the mean platelet counts was 5.8±2.1x10^{10}/unit. The mean of platelet count done at day 0, 1, 3 and 5 were 5.7±1.9 x10^{10}/unit, 5.7±2.1 x10^{10}/unit, 5.9±1.8 x10^{10}/unit and 5.4±2.4 x10^{10}/unit respectively, the mean WBC counts was 41+0.48 × 10^{6}/unit and the mean pH level was 6.9 ±0.2. They showed that the mean platelet and WBC count were comparable, with a statistically significant difference ($P < 0.05$). Their tests revealed that all PCs product units met their stipulated quality control requirements of volume and cell count. Apheresis product showed higher leucoreduction than BCs and PCs products. There was no difference in mean pH between the three forms of platelet concentrate (40).

Raturi *et al*. conducted a retrospective analysis on 86 platelet samples in India in 2017 to analyze 1% monthly quality control of platelets manufactured using the PRP technique, showed that the swirling was seen in all PC, the mean volume of PC was 58.4±9.50ml, the mean platelet count was 5.70 ± 1.42 × 10^{10} per unit, the mean of WBC counts was 1.50±1.20x10^{7}/unit and the mean of pH level was

6.67±0.48. Although the quality of their platelet output was substandard by international standards, it met the requirements of their country (41).

An additional investigation on 119 samples of PRP-PC, BC-PC and APH-PC preparation methods; for PRP-PC on 36 platelets samples was performed in India in 2017 by Trivedi and colleagues to improve and increase platelet count, showed that the mean of PC volume was 73.04±4.35ml, the mean platelet count was $7.95 \pm 2.31 \times 10^{10}$/unit, the mean of WBC counts was $5.48 \pm 3.75 \times 10^{7}$/unit and the mean of pH level was 6.23±0.15. They found no statistically significant differences between the three PC types. The pH of all units was significantly higher than the recommended level. In terms of swirling, PC per unit, and pH, the PRP-PC and BC-PC units were comparable (22).

Biplabendu Talukdar *et al.* conducted a prospective study on 105 platelet samples in India in 2017 in order to evaluate platelet quality using different platelet production methods: 40 platelet samples obtained through the PRP method, showed that the mean volume of PC was 59.40±10.21ml, the mean platelet count was $2.1 \pm 0.9 \times 10^{10}$/unit, the mean of WBC counts was $1.7 \pm 1.0 \times 10^{7}$/unit and that the mean of pH level was 7. They found that the swirling, volume, platelet count per unit, WBC count, and pH of BC-PC and PRP-PC units were comparable. Volume variation was greater in BC-PC. Apheresis-PC units showed higher platelet count and swirling. They came to the conclusion that the ex-vivo quality of platelet concentrates produced by BC-PC, PRP-PC, and Apheresis-PC was acceptable (42).

An additional prospective study on 144 platelet samples was undertaken in India in 2017 by Gupta S, *et al.* to look at platelet quality gathered through three different platelet preparation methods; 48 platelets were produced by PRP method, showed that the mean volume was 58.45 ± 13.05 ml, the mean platelet count was $5.68 \pm 1.83\ 10^{10}$/unit, the mean of WBC counts was $7.6 \pm 4.87 \times 10^{7}$/unit and the mean of pH level was 6.9 ± 0.32. Their finding was that PRP-PC and BC-PC units for swirling were comparable. Apheresis-PC units showed less whirling than PRP-PC and BC-PC units. PRP-PC and BC-PC units had comparable mean volume and platelet count per unit; Apheresis-PC units had higher platelet counts than PRP-PC and BC-PC units. BC-PC units showed lower mean WBC contamination than PRP-PC units, while Apheresis-PC units had the lowest mean WBC contamination (28).

Thazha *et al*. did an ongoing study on 56 platelet samples in India in 2018 to examine platelet preparation, storage, and quality control, presented the observed swirling was found in all the assessed PC units, the PC units were an average volume of 62 ml, the average platelet count was

$4.8x10^{10}$/unit; 58% of the units had a platelet count greater than $4.5x10^{10}$/unit, 40% had a platelet count between 4.0 and $4.5x10^{10}$/unit, and 9% had a platelet count greater than $6.2x10^{10}$/unit, the average RBC contamination was 0.05 10^{12}/L, and the pH level of all units was greater than 6.2. They found that only a minority of patients with cancer required platelet transfusion; yet, platelets were more commonly transfused to cancer patients than to patients with other diseases (43).

Raveendran and his colleagues conducted a prospective study in India in 2019 on 64 platelet samples to study an impact of storage on platelets in PRP and PC, showed that the mean volume was 74 ml, the mean platelet count on days 0, 3, and 5 were 6.23×10^{13}/unit, 6.0×10^{13}/unit and $5.68x10^{13}$/unit respectively, the mean of WBC counts on days 0, 3 and 5 were $2.87x10^{8}$/unit,$3.53x10^{8}$/unit and $1.48x10^{8}$/unit respectively and the mean pH level was 7.1 and pH at days 0, 3 and 5 were 7.18±0.23, 7.10±0.26 and 7.00±0.30 respectively. They found that the majority of the prepared units were of the desired quality. All the parameters were assessed and the results obtained on both the units were well above the values of recommended norms. The quality of platelet concentrates were maintained well within the usual 5 days thus an extension of platelet storage time is recommended (23).

Latha along with colleagues carried out prospective research in India in 2019 on 48 platelet samples to assess the quality of PC produced by BC and PRP approach; the 24 platelet unit via the PRP method, revealed that the swirling was observed in all PC, the mean PC volume was 65.1±3.0ml, the mean platelet count was 5.5±0.2 x 10^{10}/unit, the mean of WBC counts was $14.7\pm12.6x10^{7}$/unit and the mean of pH level was 6.9 ± 0.1. They found that although both BC-PC and PRP-PC met the stated quality control standards, BC platelets exceeds PRP platelets in terms of higher platelet counts, larger PC volumes, and lower WBC counts. With BC-PC, production-related damage was reduced, and the quality of the storage platelets was enhanced. They conclude that it would be practicable to switch from PRP since the advantages—increased platelet output, stronger platelets, decreased bacterial contamination, and cost-effectiveness—outweigh the loss of packed RBCs (44).

A prospective comparative evaluation study conducted in India by Das S, Nikhil and Kalyani R in 2020 on 100 platelet samples to assess platelet quality prepared freshly and overnight storage;50 platelet unit was used for each category, showed that the mean platelet count prepared from Fresh Whole blood were 870.800 ± 78.892, 847.531 ± 79.652 and 814.653 ± 90.656 at day 1, 3 and 5 respectively and mean platelet count prepared from overnight stored at room temperature Whole blood were 664.314 ± 47.990, 642.140 ± 47.517 and 624.824 ± 59.759 on days 1, 3 and 5 respectively and the mean pH level prepared from fresh Whole blood were 7.230 ± 0.280, 7.185 ± 0.284 and 6.952 ± 0.904 on days 1, 3 and 5 respectively and mean of pH level prepared overnight

stored at room temperature Whole blood were 7.237 ± 0.248, 7.118 ± 0.296 and 6.972 ± 0.286 on days 1, 3 and 5 respectively. The researchers found that PRP-obtained PCs from overnight WB showed considerable changes in quality parameters compared to freshly processed WB. Their research shown that, when compared to the quality required for PCs to be transfused into patients with thrombocytopenia, the quality of PCs acquired from stored WB kept at RT and extracted by PRP was at the lowest level. They advise that while selecting whether to employ overnight WB at room temperature, the plasma quality and RBC must be taken into account (20).

Butale P *et al.* conducted prospective research in India in 2020 on 200 platelet samples aimed at examining the overall quality of PCs production through various methods.100 platelet samples produced with PRP, showed that the mean volume was 51.54±2.95ml.The percentage of passed volume was 77%, the mean of platelet count was 4.912 ± 1.36 $x10^{10}$ /unit, the mean RBC count was 0.056 ±0.02$x10^{9}$/unit, the mean of WBC counts was 0.404 ± 0.32$x10^{10}$/unit and the mean of pH level was 6.942 ±0.20. They found that Apheresis-PC units produced more platelets, improved other quality control parameters, and decreased the possibility of infection and alloimmunization in recipients. They advise that it be given priority among the other two techniques (45).

Sharma S *et al*. performed a review of data on the quality control of components of blood in India in 2022, showed that the mean volume platelet unit was 65.5 ml, the mean platelet count was 8.3±1.50$x10^{10}$/unit and the mean of RBC contamination was 0.077±0.09$x10^{12}$/l. They recommend that quality indicators be clearly established, often checked, and adequately recorded. Additionally, they found that quality control was a crucial tool for preventing the danger of transfusion-transmitted diseases and ensuring the patient receives the most benefit at the simplest price (46).

Bashir *et al.* (2014) fulfilled a comparative study on 42 samples in Pakistan to evaluate the variation in platelet storage with and without platelet additive solution; the quality of platelet storage without additive solution, showed that the swirling in PC was 100% and the mean platelet count of PC were 5.6 ±0.01$x10^{10}$/L,5.4±0.02$x10^{10}$/L and 5.0±0.03$x10^{10}$/L on days 0, 3 and 5 respectively. According to their findings, platelets with platelet additive solution in platelet concentrate storage bags have a longer shelf life and are more viable than platelets without platelet additive solution (47).

El-Danasoury *et al.* undertook a prospective, cross-sectional in Egypt in 2014 on 30 platelet samples focusing on the quality of platelets obtained from overnight stored blood at 20-24°c temperature and freshly prepared PC, showed that the mean of platelet count of overnight group was 5.73±0.49$x10^{10}$ and freshly prepared was 6.09 ±0.77$x10^{10}$ and the mean of pH level prepared after 8 hours were

7.34±0.05, 7.29±0.05 and 7.24±0.07 at day 1,3 and 5 respectively and prepared after overnight were 7.27±0.04, 7.20±0.05 and 7.14±0.06 at day 1,3 and 5 respectively. According to the platelet quality characteristics determined in their investigation, overnight storage of WB at room temperature before to manufacturing PCs by the PRP approach did not impact the quality of the PCs produced (15).

A cross-sectional study conducted in Egypt by Reham Abd Allah Selim *et al* in 2022 on 60 platelet samples to assess platelet quality at different day of storage; 45 of platelet taken prepared by PRP method, showed that the mean of platelet count was 742± $72x10^9$/L, $654±75x10^9$/L and $578±89x10^9$/L at days 1, 3 and 5 of storage respectively and the mean of pH level were 7.0 ± 0.2, 6.8±0.1 and 6.8±0.1 at days 1, 3 and 5 of storage respectively. They recommend that single donor platelets be used instead of random donor platelets for transfusion because they met the quality control criteria for platelet count, WBC count, pH, and swirling, whereas random donor platelets met the criteria for WBC count, pH, and swirling but not for platelet count (48).

Ahmed AS *et al.* conducted a comparative investigation in Egypt in 2010 on 20 platelet samples to analyze the quality of leucoreduced and non-leucoreduced platelets. For non-leucoreduced group; 10 platelet samples, showed that the mean of pH level were 7.02 ±0.04, 7.1±0.07 and 6.98±0.05 at day 1, day3 and day 5 respectively. According to their findings, leucoreduced PC varies in terms of several particular markers of platelet activation and immunological reactivity, and that pre-storage leucofiltration followed by storage in the currently utilized plastic bags is a safe approach for at least 5 days. However, current leucoreduction technologies are not sufficiently healthy to totally eliminate transfusion responses, and their capacity to achieve the goal of maximized yield and minimal transfusion reactions with platelet therapy still has to be improved (49). Another descriptive analysis study conducted in Kenya by Nancy Wanjiru Thuku *et al* in 2017 on 384 platelet samples to assess the platelet quality, showed that the mean of pH level was 7.3± 0.5. According to their findings, 65% of platelet concentrates satisfied platelet transfusion standards, whereas 35% did not. When platelet concentrates were prepared, there were higher numbers of white blood cells ($4.53.5x10^9$) than the $0.83x10^9$ suggested by Kenya National Blood Transfusion Services (50).

The majority of the literatures concern the quality of platelet concentrates prepared using several processes (PRP-PC, BC-PC, and Apheresis PC) and whole blood held for 8 hours to prepare platelet concentrates. The literature covers nearly all platelet concentrate quality criteria, and the evaluation days were days 1, 3, and 5. The limitations were that no literature was found that was similar to our study in Ethiopia, and no information about platelet quality at three distinct holding groups was found only within 8 hours and 24 hours, not 16 hours overall.

3. NULL HYPOTHESIS

The quality indicators in PCs of 8, 16, and 24-hour-held whole blood prepared by the EBTBS did not meet the standards set by the EBTBS.

4. OBJECTIVES

4. 1. General objective

To assess the standard platelet concentrates prepared from different time held whole blood at Ethiopian Blood and Tissue Bank Service; Addis Ababa, Ethiopia from August 1 to October 30, 2022.

4. 2. Specific objectives

- To determine the swirling, volume, platelet count, RBC count, WBC count and PH level of platelet concentrates prepared from 8-, 16- and 24-hours held whole blood.
- To compare platelet concentrate quality of prepared from 16- and 24- hours held whole blood with 8 hours held whole blood platelet concentrate.
- To compare platelet concentrate quality of prepared from 8-, 16- and 24- hours held whole blood with the set standard of EBTBS.

5. MATERIALS AND METHODS

5. 1. Study area

This research is being carried out at EBTBS, which is located in Addis Ababa, Ethiopia's capital city. Ethiopian Red Cross Society (ERCS) founded it in 1969. It was separated from ERCS in 2010 and was taken over by the Federal Ministry of Health (FMoH) (51). By proclamation authorized by the Council of Ministers in 2014, it became an autonomous organization and was designated National Blood Bank Service with legal entity proclamation No 330/2014. This agency was renamed 'Ethiopian Blood and Tissue Bank Service' in 2023 and is now governed by Regulation No. 528/2023.

EBTBS collect 350 and 450 ml blood unit from non-ruminated volunteer blood donors (NRVBDs) and from 450 ml unit prepared blood components (CRC, FFP and PC). Furthermore, the EBTBS procedure encompasses a thorough screening for Transfusion Transmissible Infections (TTIs), as well as blood group determination, and subsequently transfers these blood products to healthcare facilities free of charge. This initiative serves to supply blood and blood components to over one hundred healthcare establishments, both private and governmental.

5. 2. Study design and period

A cross-sectional study was undertaken at the Ethiopian Blood and Tissue Bank Service in Addis Ababa, Ethiopia, from August 1 to October 30, 2022.

5. 3. Population

5. 3. 1. Sample source

Blood units were obtained from NRVBDs at mobile and static clinics during the research period.

5. 3. 2. Study samples

A total of 450 ml of blood units which fulfilled the EBTBS standard with weight of 721 to 821 gm are transported to processing site within acceptable temperature during the research period.

5. 4. Inclusion and exclusion criteria

5. 4. 1. Inclusion criteria

Blood units collected with a volume of 450 ml ±45ml from donors aged between 18-65 years, having greater or equal to 50 kg weight and a hemoglobin grater or equal to 12.5 gm/dl were involved in the this research.

5. 4. 2. Exclusion criteria

Blood units with less than 450ml ±45ml and PCs containing TTIs were not included.

5. 5. Study variables

5. 5. 1. Dependent variable

- Platelet concentrate's quality indicators

5.5.2. Independent variables

- Whole blood holding time
- Age of platelet concentrate (Day 1, day 3 and day 5)

5. 6. Sample size calculation and sampling method

5. 6. 1. Sample size calculation

According to EBTBS, standards quality control should be done for 1% of each blood components monthly including platelet concentrates. As a baseline last year (July 2021 to June 2022) at EBTBS 49,852 units of PCs were prepared. Through this year the PC preparation would have increased by 10% then total platelet concentrate would be 54,837 units. Therefore, sample size calculation would be based on the PC amount estimated monthly as follows.

- Monthly platelet concentrate $= \frac{\text{Total platelet concentrate prepared in year}}{12}$

$$= \frac{54837 \text{ units}}{12}$$

$$= 4569.75$$

- 1% of monthly platelet concentrate = 4569.75x0.01

  = 45.6975 approximately= 46
- 10% of 46 units were added to get total sample size monthly
- Sample size = 46 +10% of 46

  = 46+4.6

  = 51
- Therefore, sample size of monthly quality control of platelet concentrate will be 51 units and for three month in our study period will be 153 units. That is 51 units for each of 8, 16 and 24-hours held whole blood was used.

- Where: 12 is month a year

  Total platelet concentrate prepared is 54837 units

  1% is quality control done for prepared blood components

  10% was Contingency

5. 6 .2. Sampling method

A convenient method of sampling was implemented.

5. 7. Measurement and data collection

5. 7. 1. Data collection procedure

Whole blood was obtained from healthy donors following brief medical history and clinical assessment in line to EBTBS standards. A triple blood bag system (MITRA, INDIA) was used to collect WB. A total of 450 ± 45 ml of blood was collected into a primary bag, which contained 63 ml of a citrate phosphate dextrose adenine-1 anticoagulant preservative. The collection process would not exceed 12 min to avoid the possibility of platelet activation. Blood was gently agitated during collection to ensure adequate mixing with the anticoagulant-preservative mixture in the bag.

A total of 153 units of WB were collected; 51 units were kept at room temperature and prepared PC within 8-hours (current standard), 51 units were kept at room temperature and prepared PC within 16-hours (new tested standard one), and 51 units were kept at room temperature and prepared PC within 24-hours (new tested standard two). The controlled atmosphere and an air-conditioned room were used, with the temperature maintained between 20 and 24°C.

After storage, WB bags were processed into packed RBC, FFP, and PCs using a room temperature adjusted centrifuge (ROTO SILENTA 630 RS) according to the PRP technique.

The time and speed to prepare the blood components were set. Before centrifugation, the WB bags were turned upside down multiple times to mix their contents. PRP-PC was made utilizing a two-centrifugation method: a soft spin followed by a hard spin. PRP was isolated from WB in the soft spin by light spin centrifugation of WB units at 2789 RPM for 3 minutes, with centrifuge brakes switched off and the slow stop option with a slowing curve of 4. Using a manual extractor, PRP was swiftly transferred into the empty PC bag. The separation was not delayed to prevent cells from additional sedimenting. Platelets were next concentrated from PRP in the hard spin by heavy spin centrifugation at 3561 RPM for 10 minutes, and platelet-poor plasma was expressed into the FFP bag, with a limit that 50-70 ml remained in the platelet bag. PC bags were left undisturbed with the label side down for 1 hour before being gently agitated manually and stored on a platelet agitator with constant horizontal agitation. All centrifugation and storage procedures were completed at temperatures ranging from 20 to 24°c.

The prepared platelet concentrate was visually examined for morphological characteristics, screened for the absence of TTIs, and weighed to determine the volume before being stored at 20-24^{0}C for five days in a platelet agitator and incubator (Helmer, India) using a horizontal agitation rate of 70±2 per minute. At day 1, day 3, and day 5, 5ml of platelet concentrate was taken out in an aseptic manner after proper mixing for laboratory testing.

5.7. 2. Laboratory analysis

The laboratory analysis was performed by trained laboratory technologist who managed the sample collection, handling, transport of sample, component production, visual inspection of platelet concentrate, PH measurement and operating the test on ABX Micros 60s hematology analyzer. These were also supervised by the principal investigator.

Principles: The ABX Micros 60s principle of automated cell counting and sizing can be used in whole blood analysis. Each of the cells suspended in a conductive liquid (Diluent) functions as an insulator. As every single cell flows through the aperture, it for a time increases the resistance of the electrical path between two submerged electrodes on either side of the aperture. This results in a measurable electrical pulse. While the number of pulses indicates particle count, the amplitude of an electrical pulse is proportional to cell volume. These pulses are transmitted to the signal conditioner for analog to digital conversion. Pulse counts and digitized pulse measurements are submitted to the system manager for processing by the algorithms that generate the reported parameter values, flags, and histograms. The lysing items disintegrate the RBC cell membrane and let out the hemoglobin contained inside the cell. The hemoglobin released by the lysing reagents bonds with the potassium cyanide from the lysing reagents to produce a chromogenous cyamogenous molecule. This compound is then measured using a spectrophotometer at 550nm through the optical portions of the WBC/HGH chamber.

The concentrated platelet unit was inspected visually to check for the presence of excessive platelet aggregation and contamination. Platelet aggregates over excess were removed. Pink or red pigmentation on the units were an indication of red cell contamination, and such units were to be given only once the cross-matching tests finished. Observe the swirling appearance of platelet by carefully pressing the bag against a good light source. The presence or absence of swirling was reported.

Volume of PC unit estimated by weighing the bag containing PC using a digital scale with the method described below (17).
Volume = PC weighs minus unfilled bag weights divided by 1.03
Where 1.03 represents a specific gravity of PC produced via the PRP approach.

A three-part ABX micro 60s hematology analyzer blood cells counting was utilized to calculate the unit's total platelet count as stated bellow (17).

The total amount of platelets per unit was determined by multiplying the platelet count per micro litter of PC by 10^3 times the volume of the PC unit.

The WBC and RBC counts of PCs were determined by examining the number of pulses created over their lower limit using a three-part ABX micro 60s hematology analyser blood cells counting equipment.

A pH meter (Pen type pH meter, P301) was used to measure the pH level of the PC unit. The conductor's pH scale was put into the sampled PCs volume, and the solution was agitated. Before determining the pH level of the PC, the pH reading must be kept constant.

5.7.3. Quality control

To ensure all procedures were done according to standard, which could affect the quality of platelet concentrates, the preparation room temperature was controlled. Specifications were fulfilled for blood component preparation machines, equipment, and materials. A trained laboratory technologist on SOP performed the component preparation, visual check of platelet concentrates, hematological test, and pH test. Validation of processes, procedures, equipment, and materials was done. Regular maintenance schedules for equipment management and calibration are also performed. Documentation of all processes and procedures was done for traceability, and aseptic techniques were used to prevent bacterial contamination through any procedure.

5. 8. Data collection quality

5.8.1. Pre-analytical

The quality of the gathered data was ensured by properly filling out the standardized data collecting form. The obtained data was reviewed for completeness and supervised by the lead investigator during the whole data collection and entry process.

5. 8. 2. Analytical

Data quality was maintained by collecting and processing data in accordance with the type of analysis applied.

5. 8. 3. Post analytical

The principal investigator double-checked the results of the data analysis to ensure that the correct data was analyzed.

5. 9. Data analysis and interpretation

Pre-prepared data sheets were used to enter data. SPSS version 26 Statistical packages were used to analyze the data. Data were presented as mean, standard deviation, median, interquartile range, frequency, and percentages. The Mann-Whitney U test, the Wilcoxon Signed-Rank test, and

Friedman's test were employed for pairwise group comparisons, within group pairwise comparisons, and triple measure within group median comparisons, respectively. The significance level was set at P value less than 0.05. Bonferroni type adjustment for p value was done in pairwise comparison within groups. PCs were divided into two categories: those that satisfied the specified standard for each quality indicator and those that were insufficient to meet the required minimum requirement for each of the variables.

5. 10. Operational definition

Whole blood (WB): is a blood collected from non-ruminated blood donor which contains about 450 ml blood units.

Group 1: PC prepared within 8 hours of WB collection.

Group 2: PC prepared between 9 and 16 hours following WB collection.

Group 3: PC prepared between 17 and 24 hours following WB collection.

Platelet concentrate's quality indicators: include platelet concentrates of swirling, volume, platelet count, RBC count, WBC count and pH level.

Swirling status: The existence or absence of a swirl or a cloud in the platelet bag to verify sufficient and quality of platelets available for transfusion as viewed by light on days 1, 3, and 5

5. 11. Ethical consideration

The investigation was carried out after an ethical letter was acquired from the Department of Medical Laboratory Sciences, College of Health Sciences, and Addis Ababa University's Departmental Research and Ethics Review Committee (DRERC). EBTBS received a formal letter requesting authorization to perform the study. After approval was obtained from the EBTBS, data was collected. The study was conducted with at most confidentiality, utilizing non-identifying codes and prohibiting access to unauthorized individual.

5. 12. Dissemination of the result

The findings would be presented and submitted to Addis Ababa University's Department of Medical Laboratory Sciences, College of Health Science. The findings would also be presented at Addis Ababa University's annual research conference and submitted for publication in a peer-reviewed journal.

6. RESULTS

6.1. Quality parameters of stored platelet concentrate

This study was conducted on a total of 153 PC units taken from 25 female and 128 male NRVBDs whose ages ranged from 18 to 61 years, which were divided into three groups based on their storage at 8, 16, and 24 hours storage at room temperature. Each group included 51 PC units. Each group was tested for six quality parameters. Accordingly, swirling was visually observed in all units tested. Other quality parameters: volume, platelet count, RBC count, WBC counts and pH level of PCs were analyzed and the results are shown in Table 1. The means of volume were within the ranges of 50-70ml for all three groups. The means of platelet count of 8, 16 and 24hrs held prepared PCs were above $\geq 5.5x10^{10}$/unit at day 1, 3 and 5. The means of WBC count was within an acceptable range $<0.2x10^{9}$ at all group in all point of test. The means of pH level were in acceptable range 6.4-7.4 at day 1, 3 and 5. Thus, these four quality parameters evaluated across all three groups met the standards established by EBTBS in terms of mean values. However, the RBC count was only met in group 1 on days 1, and in group 2 at day 1.

The mean volume, platelet count, and WBC count fell from group 1 to group 2 and then again from group 2 to group 3. RBC count and pH level, on the other hand, increased from group 1 to groups 2 and 3.The mean platelet count fell from day 1 to day 3 and again from day 3 to day 5 (figure 1). The mean RBC count increased from day 1 to day 3 and again from day 3 to day 5 (figure 2). The mean WBC count fell from day 1 to days 3 and 5. The mean pH level increased from day 1 to days 3 and 5.

Table 1. Distribution of laboratory findings of PCs quality indicators of group 1, group 2 and group 3 at Ethiopian Blood and Tissue Bank Service, Addis Ababa, Ethiopia, from August 1 to October 30, 2022.

Parameters (Standards)	Sampling days	*Group	N	Mean± SD	95% CI Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Volume (50-70ml)	1	1	51	67.7±2.15	67.1	68.3	61.7	71.8
		2	51	67.0±2.19	66.4	67.7	61.5	70.9
		3	51	66.4±2.15	65.8	67.0	60.3	70.9
Platelet count /unit of 10^{10} ($\geq 5.5 \times 10^{10}$)**	1	1	51	6.77±2.13	6.17	7.37	3.82	16.4
		2	51	7.61±3.12	6.73	8.49	7.26	20.5
		3	51	7.17±2.28	6.53	7.81	2.70	13.0
	3	1	51	6.62±2.13	6.02	7.22	3.81	16.8
		2	51	7.45±3.14	6.57	8.33	6.83	20.4
		3	51	7.18±2.28	6.54	7.83	2.30	13.0
	5	1	51	6.27±1.83	5.76	6.79	3.53	13.6
		2	51	7.24±3.20	6.34	8.14	6.71	21.0
		3	51	6.95±2.33	6.30	7.61	2.50	13.0
RBC count/unit of 10^9 ($\leq 1.0 \times 10^9$)	1	1	51	1.0±0.54	0.872	1.17	0.00	2.05
		2	51	1.0±0.54	0.872	1.17	0.00	2.05
		3	51	1.1±0.95	0.876	1.41	0.00	6.00
	3	1	51	1.2±0.65	1.00	1.37	0.616	3.69
		2	51	1.2±0.65	1.00	1.37	0.616	3.69
		3	51	1.4±0.14	1.1	1.65	0.635	5.84
	5	1	51	1.5±0.94	1.28	1.81	0.616	6.76
		2	51	1.5±0.94	1.28	1.81	0.616	6.76
		3	51	1.9±1.2	1.57	2.22	0.647	5.84
WBC count /unit of 10^9 ($<0.2 \times 10^9$)	1	1	51	0.016±0.006	0.014	0.017	0.006	0.034
		2	51	0.016±0.006	0.014	0.017	0.006	0.034
		3	51	0.014±0.006	0.012	0.015	0.006	0.030
	3	1	51	0.013±0.005	0.012	0.015	0.006	0.031
		2	51	0.013±0.005	0.012	0.015	0.006	0.031
		3	51	0.014±0.005	0.013	0.015	0.006	0.024
	5	1	51	0.015±0.006	0.013	0.016	0.006	0.037
		2	51	0.015±0.006	0.013	0.016	0.006	0.037
		3	51	0.014±0.004	0.013	0.015	0.006	0.026
PH level (6.4-7.4)	1	1	51	7.11±0.19	7.06	7.17	6.60	7.40
		2	51	7.11±0.19	7.06	7.17	6.60	7.40
		3	51	7.11±0.13	7.07	7.15	6.90	7.50
	3	1	51	7.14±0.17	7.09	7.19	6.70	7.50
		2	51	7.14±0.17	7.09	7.19	6.70	7.50
		3	51	7.18±0.13	7.14	7.22	6.90	7.70
	5	1	51	7.12±0.17	7.08	7.17	6.60	7.40
		2	51	7.12±0.17	7.08	7.17	6.60	7.40
		3	51	7.17±0.12	7.14	7.21	6.90	7.40

* Group 1 for PCs prepared within 8 hr., Group 2 for PCs prepared within 16 hr., and Group 3 for PCs prepared within 24 hr.

** platelet count is calculated using volume of platelet concentrate before sample taken for test

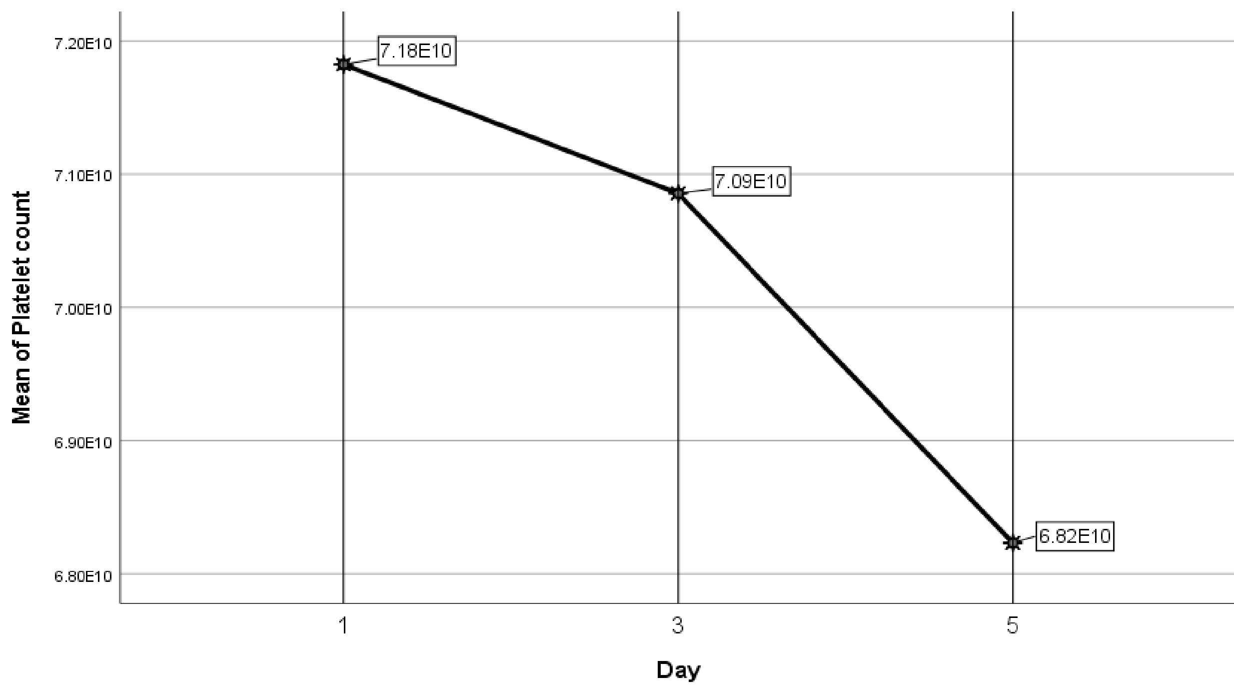

Figure 1: Mean plots of platelet count at day 1, 3 and 5 of three groups at Ethiopian Blood and Tissue Bank Service, Addis Ababa, Ethiopia, from August 1 to October 30, 2022.

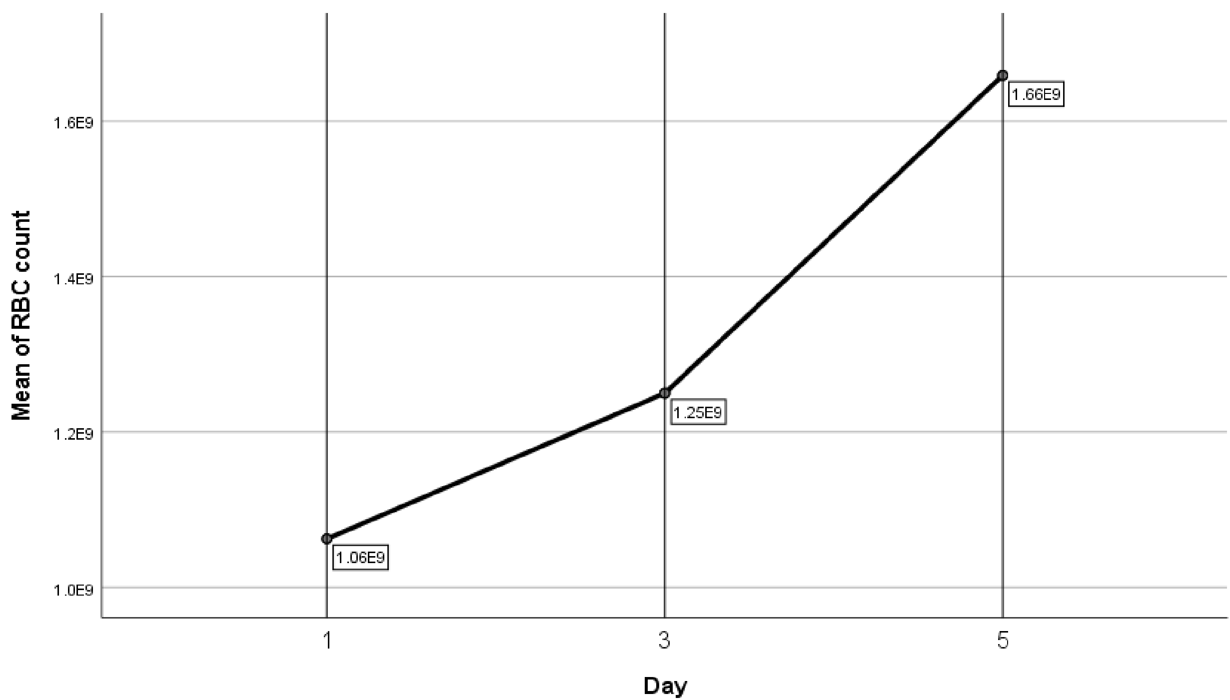

Figure 2: Mean plots of RBC count at day 1, 3 and 5 of three groups at Ethiopian Blood and Tissue Bank Service, Addis Ababa, Ethiopia, from August 1 to October 30, 2022.

6.2. Compliance with the standard of PCs quality indicators

Table 2 displays the results of our samples. The volume calculated prior to sampling was satisfactory for each of the three groups. The swirling and WBC counts were acceptable for all three groups on the first, third, and fifth days of assessment. Swirling could be found in all samples at each point in time. On day 5, only one unit in Group 1 failed to meet to the WBC count. The platelet counts fulfilled the indicated quality requirement in all three groups, except as they were only slightly below the standard in group 2 at day 5. The RBC count of PCs, on the other hand, was below the standard in all three groups, as it was only acceptable on day 1 of both groups 1 and 3. The pH levels values for all groups remained accepted on all days.

Table 2. Compliance with the EBTBS's standards for PCs quality indicators for three groups at day 1, 3 and 5 at Ethiopian Blood and Tissue Bank Service, Addis Ababa, Ethiopia, from August 1 to October 30, 2022.

Parameters	Required Standards Meet	Sampling days	Group 1 N (51)		Group 2 N (51)		Group 3 N (51)	
			Meet standards N (%)	Did not meet standards N (%)	Meet standards N (%)	Did not meet standards N (%)	Meet standards N (%)	Did not meet standards N (%)
Swirling	≥75%	1	51(100)	0	51(100)	0	51(100)	0
		3	51(100)	0	51(100)	0	51(100)	0
		5	51(100)	0	51(100)	0	51(100)	0
Volume	≥75%	1	44(86)	7(14)	48(94)	3(6)	49(96)	2(4)
PC/unit	≥75%	1	40(78.43)	11(21.57)	42(82.35)	9(17.65)	40(78.43)	11(21.57)
		3	40(78.43)	11(21.57)	39(76.5)	12(23.5)	40(78.43)	11(21.57)
		5	39(76.5)	12(23.5)	38(74.51)	13(25.49)	39(76.5)	12(23.5)
RBC/unit	≥75%	1	43(84.31)	8(15.69)	30(58.82)	21(41.18)	43(84.31)	8(15.69)
		3	36(70.59)	15(29.41)	25(49.02)	26(50.98)	23(45.09)	28(54.9)
		5	17(33.33)	34(66.67)	12(23.53)	39(76.47)	11(21.57)	40(78.43)
WBC/unit	≥75%	1	51(100)	0	51(100)	0	51(100)	0
		3	51(100)	0	51(100)	0	51(100)	0
		5	50(98.04)	1(1.96)	51(100)	0	51(100)	0
pH level	≥75%	1	50(98.04)	1(1.96)	51(100)	0	50(98.04)	1(1.96)
		3	49(96.08)	2(3.92)	50(98.04)	1(1.96)	51(100)	0
		5	48(94.12)	3(5.88)	51(100)	0	51(100)	0

6.3 Comparison of median values of PCs quality indicators within groups

The frequency distributions for the platelet count, RBC count, WBC count, and pH readings were not considered normally distributed; consequently, the non-parametric statistical tests for repeated measure (Friedman's test) and for two related samples (Wilcoxon Signed-Ranks test) were used for assessing the medians in groups for every day of sampling.

All three groups had significantly different median values for platelet count, RBC count, and WBC count ($p<0.05$). Group 3 had a significant difference in median pH levels ($p<0.05$). There was no significant variation in the median pH level between groups 1 and 2 ($p>0.05$) (Table 3).

Table 3. Comparison of triple measure median values of PCs quality indicators within groups at Ethiopian Blood and Tissue Bank Service, Addis Ababa, Ethiopia, from August 1 to October 30, 2022.

Parameters	Sampling day	Group 1[a] N (51)			Group 2[a] N (51)			Group 3[a] N (51)		
		X^2	Df	P-value	X^2	Df	p-value	X^2	Df	P- value
Platelet count	1	38.926	2	<0.001	48.039	2	<0.001	24.128	2	<0.001
	3									
	5									
RBC count	1	38.994	2	<0.001	39.500	2	<0.001	24.365	2	<0.001
	3									
	5									
WBC count	1	6.025	2	0.049	14.519	2	0.001	8.220	2	0.016
	3									
	5									
pH level	1	0.889	2	0.641	1.556	2	0.459	7.581	2	0.023
	3									
	5									

a. Friedman test

Significant variation in platelet count median values were seen in all three groups between Day 1 with 3, Day 1 comparable 5, and Day 3 against 5, with one exception of Group 3 between Day 1 with 3 ($p>0.025$). There were notable variations in RBC count medians between Day 1 vs. 3, Day 1 in comparison 5, and Day 3 vs. 5, with the exception in Group 2 for Day 1 vs. 3 ($p>0.025$). The results showed no significant change in median WBC count values between Day 1 vs. 3, Day 1 vs. 5, and Day 3 vs. 5 in group 3 ($p>0.025$), however there was a significant difference between Day 1 vs. 3 in group 1 as well as between Day 1 vs. 3 and Day 1 vs. 5 in group 2 ($p<0.025$). Significant variations in pH median values were observed in group 3 ($p<0.025$) between Day 1 vs. 3 and Day 1 vs. 5 (Table 4).

Table 4. Paired Comparison of median values of PCs quality indicators within groups at Ethiopian Blood and Tissue Bank Service, Addis Ababa, Ethiopia, from August 1 to October 30, 2022.

Parameters	Sampling day	Group 1[a] N (51)			Group 2[a] N (51)			Group 3[a] N (51)		
		Median difference	Z – value	P-value	Median difference	Z- value	P-value	Median difference	Z - value	P-value
Platelet count	1 vs. 3	0.1	-2.962[b]	0.003	0	-3.824[b]	< 0.001	-0.1	-0.070[b]	0.944
	1 vs. 5	0.4	-5.062[b]	< 0.001	0.1	-5.165[b]	< 0.001	0	-3.862[b]	< 0.001
	3 vs. 5	0.3	-4.793[b]	< 0.001	0.1	-4.426[b]	< 0.001	0.1	-4.996[b]	< 0.001
RBC count	1 vs. 3	0	-2.757[c]	0.006	-6.1	-1.851[c]	0.064	-0.3	-3.055[c]	0.002
	1 vs. 5	-6.2	-5.540[c]	< 0.001	-7.1	-4.704[c]	< 0.001	-0.4	-5.779[c]	< 0.001
	3 vs. 5	-6.2	-4.626[c]	< 0.001	-0.1	-4.153[c]	< 0.001	-0.1	-4.251[c]	< 0.001
WBC count	1 vs. 3	0	-2.377[b]	0.017	0.1	-3.216[b]	0.001	-0.3	-0.801[c]	0.423
	1 vs. 5	0	-.360[b]	0.719	0	-1.147[b]	0.251	-0.3	-1.176[c]	0.239
	3 vs. 5	0	-1.704[c]	0.088	-0.1	-2.670[c]	0.008	0	-0.075[b]	0.940
pH level	1 vs. 3	0	-1.126[b]	0.260	0.1	-1.233[c]	0.218	-0.1	-3.018[c]	0.003
	1 vs. 5	0	-1.017[c]	0.309	0	-0.363[c]	0.717	-0.1	-2.862[c]	0.004
	3 vs. 5	0	0.000[d]	0.021	-0.1	-0.879[b]	0.380	0	0.265[b]	0.807

a. Wilcoxon signed ranks test b. Based on positive ranks c. Based on negative ranks d. The sum of negative ranks equals the sum of positive ranks

6.4. Comparison of median values of PCs quality indicators between groups

The frequency distributions for the volume, platelet count, RBC count, WBC count, and pH readings were not considered normally distributed; thus, the non-parametric statistical analysis for two independent samples (Mann-Whitney U test) was applied for evaluating the medians between the groups to each day of sampling.

The medians for volume, Platelet count, RBC count, WBC count, and pH level variables did not vary significantly different between groups 1 and 2, group 1 and 3, and group 2 and 3 at all dates (p>0.05), with the exception of volume between groups 1 and 3 at day 1, WBC count among a group 1 and 3 at day 1, and group 2 and 3 at day 1 (p<0.05). (Table 5)

Table 5. Paired comparison of median values of PCs quality indicators between group 1 and 2, group 1 and 3, and group 2 and 3 with respective days at Ethiopian Blood and Tissue Bank Service, Addis Ababa, Ethiopia, from August 1 to October 30, 2022.

Parameters	Sampling day	*Group 1 vs. Group 2			*Group 1 vs. Group 3			*Group 2 vs. Group 3		
		Median difference	Z-value	P-value	Median difference	Z-value	P-value	Median difference	Z-value	P-value
Volume	1	0.48	-1.480	0.139	1.26	-2.882	0.004	0.78	-1.630	0.103
Platelet count	1	-0.5	-1.663	0.096	-1	-1.285	0.199	-0.4	-0.459	0.647
	3	-0.6	-1.529	0.126	-1.1	-1.857	0.063	-0.5	-0.104	0.917
	5	-0.8	-1.747	0.081	-1.3	-1.811	0.070	-0.5	-0.003	0.997
RBC count	1	-0.1	0.000	1.000	-3.2	-1.309	0.190	-3.1	-1.309	0.190
	3	-0.62	0.000	1.000	-0.62	-0.244	0.807	0	-0.244	0.807
	5	-0.1	0.000	1.000	-0.1	-1.014	0.311	0	-1.014	0.311
WBC count	1	-0.4	0.000	1.000	0	-2.450	0.014	0.4	-2.450	0.014
	3	-0.3	0.000	1.000	-0.3	-0.472	0.637	0	-0.472	0.637
	5	-0.4	0.000	1.000	-0.3	-0.867	0.386	0.1	-0.867	0.386
pH level	1	0.1	0.000	1.000	0.2	-0.905	0.365	0.1	-0.905	0.365
	3	0.2	0.000	1.000	0.1	-1.117	0.264	-0.1	-1.117	0.264
	5	0.1	0.000	1.000	0.1	-1.290	0.197	0	-1.290	0.197
*Mann-Whitney U test between groups										

7. DISCUSSION

Testing all blood products for quality characteristics is a crucial tool for ensuring that the blood products generated meet the standard requirements. EBTBS has developed a standard guideline of testing quality criteria as a method of controlling problems that may impact blood product quality and ensuring safe blood and blood product transfusion. As a result, quality assured blood products would be manufactured on a constant basis to meet demand. In this study, there was also adherence to the EBTBS standards requirements, i.e., >75% of units tested must fall within the value stated for each parameter. To meet the EBTBS's quality standards, a minimum of 75% of units assessed must meet the requirements.

The objective of this cross-sectional study was to assess platelet concentrates qualities; such as swirling, volume, platelet count, RBC count, WBC count and pH level prepared after WB storage 8, 16 and 24 hours by PRP method. The use of extended-storage WB (greater than 8 hours) for component preparation provides various operational benefits to blood bank activities. Component production, including PC preparation, can be facilitated logistically through overnight-held WB, resulting in cost reductions. Furthermore, regardless of the distance between the collection site and the processing center, PCs could be prepared from every unit of WB, and PCs could be processed in a single shift, reducing operating expenses. As a result, the number of WB shipments from collecting sites may be greatly decreased, and the workload in the laboratory can be distributed more equally throughout time because component preparation will only be required during working hours.

In the present study, the presence of swirling was seen in all groups of PCs prepared by PRP method. Several similar studies done by Trivedi., *et al* (44), Latha *et al* (42), and Naghadeh HT *et al* (39) in India, Coêlho., *et al* in Amazon, Brazil (4), Ali., in Iran (40) and Raturi *et al* in India (41) reported a 100% presence of swirling just like our study. In general, evaluation of swirling is carried out by visual inspection and is an excellent procedure for quality control of PCs. Swirling can be visually inspected to determine platelet shape; swirling suggests discoid morphology, while its absence suggests spherical morphology. However, in a study done by Bashir, et al (47), they identified a decrease in swirling in PCs. They also reported that the percentage of PCs with positive swirling results dropped after 5 days of storage which is considered to be due to storage lesions occurring during preservation.

The present research additionally showed that the mean volume was between 50 and 70ml. Several more research conducted in Brazil (4), in Malaysia (29, 35), in Iran (38, 40), in India (20, 23) and in Egypt (15, 48) back with our findings. Platelets prepared from WB are often preserved in donor plasma, which acts as a buffering agent. PCs are typically suspended in 50 to 70ml plasma for metabolism, platelet clumping prevention, and pH maintenance (41). However, our result was lower than study conducted in India (22-23). This might be because of the technique used to separate and weight by a standardized electrical balance.

According to our research, all groups evaluated on days 1, 3, and 5 had acceptable mean for volume, platelet count, and WBC count. However, in groups 1, 2, and 3 the mean platelet concentrate count fell during day one until day five (figure 1). This result lines up to those studies carried out at the Amazon blood bank in Brazil (4), two experimental studies carried out in Iran (38,40), a prospective comparative evaluation study carried out in India (20,23), a prospective, controlled, and a cross-sectional analysis done in Malaysia (29,35) and a cross-sectional one performed in Egypt(15,48). But our finding on platelet count mean was higher than the study conducted in India (42-43, 45). This could be due to donor variables, phlebotomy procedure, time period of PC separation, transportation, and storage conditions.

Furthermore, in the present study mean of pH level of group one showed slightly greater than groups two and three and it was in range of 6.4-7.4. In our study the overall pH level were in acceptable range which is similar to the studies conducted in India (20, 22-23, 28, 42, 45), Malaysia (29), Egypt (15, 48, 49) and Kenya (50) and lower than the study conducted in Brazil (4) and Malaysia (37). This might be because we used high-quality blood and anticoagulant preservative solution, performed the whole blood collection in an aseptic manner, and finished it in 12 minutes. We also stored the platelet concentrates in a controlled incubator that was constantly agitated, which might have an impact on the pH level.

Swirling, volume, WBC count and pH level all met the EBTBS requirement in the current study, which required that at least 75% of the units examined adhere to the standards. 100% of the Swirling, volume, WBC count and pH level were met in groups 1, 2 and 3. Platelet count was met the EBTBS requirement in our finding, that required at least 75% of assessed unit obey to the standards except in group 2 slightly below the standard at day 5. 100% of the platelet count was met in groups 1, 2 and 3 except in group 2 at day 5. This result is consistent with those studies carried out in the Malaysian National Blood Bank Centre in Kuala Lumpur, Malaysia (29), Six different studies carried out in India (20, 22-23,41,44-45), two studies done in Egypt (15,49) and better to studies performed in

Malaysia (35) and two research in India (28,43). This could be due to donor variability, procedure and personnel expertise, blood collection and component preparation material utilized, and PC storage conditions.

In this study the RBC count was not fulfilled the EBTBS standard. However, 100% of the RBC count was met in groups 1 and 3 at day 1 only. Therefore, RBC count in group 2 fell short of the minimum criterion (75%), achieving 58.82%, 49.02%, and 23.53% on days 1, 3, and 5. RBCs in PCs in our study are the results of a small amount of RBCs flowing through the PCs bag during the first separation process after the first spin (soft spin). Due to of the improper separating of PRP from the red cells resulting from soft spin, RBCs may settle at the bottom with platelets in hard spin. Our finding on RBC count is lower than those study carried out in Malaysia (29), in Iran (38) and three studies in India (43, 45-46). This could be as a result of RBCs passing through the initial separation process between red blood cells and platelet-rich plasma, improper PRP separation from red blood cells due to soft spin, RBCs settling at the bottom with platelets in hard spin, or platelets counting as RBC due to platelet cell size.

To figure out the date on which changes become major for platelet count, RBC count, WBC count and pH level, we assessed outcomes for successive dates in each group. For group 1 and group 2 platelet counts, the difference was significant between day 1 and day 3, between day 1 and day 5, and between day 3 and day 5, but the values of group 3 were significantly increased when comparing between day 1 to day 5 and day 3 to day 5. For group 1 and group 3 RBC counts, the difference was significant between day 1 and day 3, between day 1 and day 5, and between day 3 and day 5, but the values of group 2 were significantly increased when comparing day 1 to day 5 and day 3 with day 5. For group 2 WBC counts, the difference was significant between day 1 and day 3 and day 3 and day 5, whereas group 1 values were significantly higher only when comparing day 1 and day 3 and the remainder of group 3 values were not significantly different when comparing day 1 and 3, day 1 and day 5, and day 3 and day 5. The difference in pH level values for group 3 was significantly higher between day 1 and day 3 and between day 1 and day 5, however the difference in pH level values for group 1 and group 2 when comparing day 1 and day 3, day 1 and day 5, and day 3 and day 5 were not significant. A similar study done by Das S, Nikhil, and Kalyani R (20) revealed that there was significant difference in platelet count overnight versus freshly prepared platelet concentrate assessed at days 1, 3 and 5. Furthermore, they also found out that pH level showed an insignificant difference between fresh and overnight.

In the present study, three groups were included and compared each other for the value of quality parameters showed significant difference in volume between groups 1 and 3, WBC count between groups 1 and 3, and group 2 and group 3 on day 1. There were no significant variations in platelet count, RBC count, or pH level between groups 1 and 2, groups 1 and 3, and groups 2 and 3 on day 1, day 3, and day 5. In general, extending the PC preparation period to 24 hours had no effect on group difference in volume, platelet count, RBC count, WBC count, or pH level. Although a significant difference in WBC counts throughout groups, the results were within the allowable range needed by EBTBS. Our results were consistent with those of Shabani M and his colleagues (29) and Kasim *et al* (38).

A number of conditions influence the quality of PCs such as donor variables, technique used and personal skills. Blood collection, processing, and storage conditions are significantly influence quality of PCs. Recent studies have shown that although PC preparation has increased from eight hours to twenty-four hours by the PRP method, there is no quality difference in platelet production (15). Our study also showed that the PC prepared after more than eight hours of hold WB did not show any difference in the quality with respect to swirling, volume, platelet count per unit, RBC count, pH level and had least WBC contamination as compared to PC prepared within 8 hours. This is consistent with the findings of research conducted in Malaysia in 2014 and 2016 (29, 35), in India (20) and in Egypt (15).

8. STRENGTH AND LIMITATIONS OF THE STUDY

8. 1. Strength of the study

The study's strength is that it was conducted over a three-month period, with samples collected from both a static and mobile blood collection team, which may compensate for variations in PC quality. The study covered quality assessment parameters and enough samples for each PC preparation hours. Most of the available studies in literature had used PC quality parameters like swirling, volume, platelet count, RBC count, WBC count and pH level testing for platelet quality. This was one of the most studies that tested the platelet quality using swirling, volume, platelet count, RBC count, WBC count, and pH level.

To the best of our knowledge, this is the first study of its kind in Ethiopia, and it could serve as a starting point for future research and policymakers.

8. 2. Limitation of the study

Even though, the PC is prepared under a closed system, the sterility test was not included which could have identified bacterial contamination throughout storage in PCs. Furthermore, P-selectin molecule (CD62P) test was not included which could have indicated the viability of platelet cell in the blood unit better than swirling and pH level.

9. CONCLUSION AND RECOMMENDATION

9. 1. Conclusion

The quality characteristics of the groups 2 and 3 were equivalent to those of the 8-hour-held PC samples in group 1. Except for RBC count, all parameters met the EBTBS's standard criterion. In general, this study demonstrates that a delay in WB processing of up to 16 and 24 hours has no significant effect on quality measures when compared to PCs prepared from WB after stored for 8 hours.

9. 2. Recommendation

Even though, this study has limitations on sterility and P-selectin molecule (CD62P) test, it can be used as a reference for further study on PC quality. However, due to a lack of data and some restrictions throughout the study period, it is premature to accept the findings that PCs prepared from 16 and 24 hours held WB which has same quality with PCs prepared from 8 hours held WB. As a result, additional research may be required to either back up or modify the current study's conclusions.

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11. ANNEXES

Annex I. Blood donor questionnaire

IMPORTANT: DO NOT DONATE BLOOD IF YOU MAY HAVE BEEN EXPOSED TO HIV/AIDS, HEPATITIS OR SYPHILIS.

PLEASE FILL THIS FORM PROPERLY.

SECTION 1. Donor identification Information

Donor Code ________________________

Sex ……… Date of Birth (DD/MM/YY)……………. Age ………

Marital Status: Single ☐ Married ☐ Others ☐……………………………………………

………

SECTION 2. HEALTH QUESTIONNAIRE

All donors must complete this section. Your answers will be treated confidentially. Please read all questions carefully and answer honestly. Please TICK '✓' your answers.

S.N	Questions	Yes	No	Staff
1	Are you feeling well today?			
2	Have you had something to eat or drink in the last 4 hours?			
3	Have you ever donated blood?			
	3.1 If yes, When?			
4	Have you ever been deferred as a blood donor?			
	4.1 If Yes ,reason for deferral			
5	Are you involved in any of the following activities			
	5.1Within the next 12 hours, Train or Heavy truck driving, heavy machine operating?			
	5.2For the next 72 hours, flying an airplane?			
	5.3 Are you an Athelet? if so are you participating in a regular training or athletic programme?			
6	In the past 5 days: have you taken Aspirin?			
7	In the past 2 weeks: have you taken Antibiotics?			
8	In the past 28 days: have you been vaccinated?			
9	In the past 4 weeks: Have you had Typhoid fever?			
10	In the past 6 weeks: Have you had Typhus?			
11	In the past 6 months: have you had Malaria?			
12	Have you ever had:			

	12.1 Heart, circulatory problems, bleeding disorder or polycythemia			
	12.2 kidney, Lung, High blood pressure ,Diabetes, Epilepsy			
	12.3 have you had yellow jaundice, liver disease, or hepatitis B/C?			
	12.4Have you had sexually transmitted disease?(Syphilis, gonorrhea, and others)			
13	In the past one year			
	13.1Recieved blood or blood products, tattoo, Ear or skin piercing, acupunctured, and accidental needle injury/blood splash?			
	13.2Have you had unsafe sexual intercourse?			
	13.3Have you or your sexual partner ever used recreational, street drugs by nose, mouse or injection needle?			
	13.4 have you ever had sexual contact with anyone who has HIV/AIDS or Hepatitis B/C in his/her blood?			
	13.5 Have you had major surgical procedure or, are you scheduled to have surgery?			
14	Do you consider your blood safe to be transfused to a patient?			
15	Do you consent for post blood donation counseling service rendered by the institution?			
16	FOR WOMEN ONLY			
	16.1 Are you pregnant? Have you started antenatal care?			
	16.2 In the past 6 months have you had miscarriage or abortion?			
	16.3 Are you breastfeeding?			

Section 3: Declaration

1. I have read and understood the pamphlet “basic brochure on blood donation”.

2. To the best of my Knowledge all the information supplied is the truth

3. I understand that if I have not answered these questions truthfully this could endanger for patient.

4. I consent to my blood being tested to syphilis, hepatitis B, hepatitis C and HIV.

5. I accept that donation data may be used on occasion for scientific research the objective of which is to improve the safety of the blood supplied to patients and the donors’ health and wellbeing.

6. I confirm that I am 18 years of age or older.

7. I understand that all the information on this form will be kept confidentially.

Donor’s Pack Number ……………………………… (Filled by Blood Bank Staff)
Donor’s Signature…………………… Date …………………
Remarks: ……………………………………………………………………………
Screener Staff ……………………… Signature ……………………………… Date ……………….
FOR OFFICE USE ONLY
Counseled by……………………………………Signature ………………………
Donor reaction: YES………………..NO……………

Annex II. በደም ለጋሾች የሚሞላ መጠይቅ (Amharic version)

በዛሬው ዕለት ደም ለመለገስ ስለመጡ እናመሰግናለን!

ልብ ይበሉ፡-

እንደ ኤች አይ ቪ/ኤድስ፣ቂጥኝ እና የጉበት ቫይረስ ዓይነት በደም ሊተላለፉ ለሚችሉ በሽታ አምጪ ተሐዋስያን ተጋልጫለሁ ካሉ እባክዎ ደም አይለግሱ፡፡

ለእርስዎም ሆነ ለደም ተቀባዩ እጅግ በጣም ጠቃሚነት ስለላው እባክዎ መረጃዎቹን በአግባቡ ይሙሉ፡፡

ክፍል1 የደም ለጋሹ/ሿ መለያ፡-

የደም ለጋሹ/ሿ መለያ ቁጥር________________

ጾታ………...የልደት ቀን (ቀን/ወር/ዓ.ም) ……………….ዕድሜ……………ሥራ፡………………

የጋብቻ ሁኔታ ፡- ☐ ያገባ ☐ ያላገባ ☐ ሌላ

ክፍል 2: የጤና ምርመራ መጠይቅ

ቀጥሎ ያሉትን ጥያቄዎች ሁሉም ደም ለጋሾች ሊሞሏቸው የሚገቡ ሲሆን የመረጃዎቹ ምስጢራዊነት የተጠበቀ ነው፡፡እባክዎትን ጥያቄዎቹን በጥንቃቄ ያንብቡና በታመኝነት ይመልሱ፡፡ እባክዎትን መልሱን የ " ✓" ምልክት በማድረግ ያመልክቱ፡፡

ተ.ቁ	መጠይቅ	አዎ	አይደለም	ባለሙያ
1	በዛሬው ዕለት ጥሩ የጤንነት ስሜት ይሰማዎታልን?			
2	ባለፉት 4 ሰዓታት ውስጥ የሚበላ ወይም የሚጠጣ ነገር ወስደዋል?			
3	ደም ለግሰዉ ያዉቃሉ?			
	3.1 አዎ ከሆነ መልስዎ መቼ?			
4	ደም ለመለገስመጥ ተዉ ሳይለግሱ ተመልሰው ያውቃሉ?			
	4.1 አዎ ከሆነ መልስዎ፤ ምክንያቱ ምን ነበር?			
5	ከዚህ ቀጥሎ በተዘረዘሩት የስራ መስኮች ይሰማራሉ?			
	5.1.በሚቀጥሉት 12 ሰዓት ውስጥ በከባድ መኪና ሾፌርነት ወይም የኤሌክትሪክ ሃይል በሚጠቀሙ ማሽኖች ይሠራሉ?			
	5.2. በሚቀጥሉት 72 ሰዓት ውስጥ አውሮፕላን ያበራሉ?			
	5.3. አትሌት ነዎት፤ከሆኑስ ተከታታይ ልምምድ ያደርጋሉ?			
6	ባለፈዉ 5 ቀናት ዉስጥ አስፕሪን ወሰደዋል?			

7	ባለፈው 2 ሳምንታት ውስጥ እንደ አንቲባዩቲክስ አይነት መድሐኒት ወስደዋል?			
8	ባለፈው 28 ቀናት ውስጥ ክትባት ተከትበው ያውቃሉ?			
9	ባለፈው 4 ሳምንታት ታይፎይድ (Typhoid fever) ታመዉ ነበር?			
10	ባለፈው 6 ሳምንታት ውስጥ የተስቦ በሽታ (typhus) ታመዉ ነበር?			
11	ባለፈው 6 ወር የወባ በሽታ ይዞዎት ያዉቃል?			
12	በሚከተሉት በሽታ ታመው ያውቃሉ?			
	12.1 የልብ፤የደም ዝዉዉር ወይም የደም መርጋት ችግር የቀይ ደም ሴል መብዛት/polycythemia/			
	12.2. የኩላሊት፤የሳንባ፤የደም ግፊት፤የስኳር፤የሚጥል በሽታ			
	12.3. የጉበት በሽታ ታመው ያውቃሉን (አይኖት ቢጫ ሆኖ ወይንም የጉበት በሽታ እንዳለብህ/ሽ ተነግሩህ/ሽ ያቃል?)			
	12.4 በግብረ ስጋ ግንኑነት የሚተላለፉ በሽታ ይዞዎት ያዉቃል? (ቂጥኝ፤ጨብጥናሌሎችም)			
13	ባለፈዉ 1 አመት ዉስጥ			
	13.1 ደምና የደም ተዋፅኦ መዉሰድ፤ንቅሳት መነቀስ፤ጆሮ መበሳት ወይም ቆዳ መበሳት፤የደረቅ መርፌ ህክምና ማድረግ፡ በስራ ቦታ የድንገተኛ መርፌ መወጋትእና የደም መረጨት አጋጥመወታል?			
	13.2. ከትዳር ጓደኛዎ ወይም ፍቅረኛዎ ውጪጥንቃቁ በጎደለዉ ወይም ተጋለጭነት ባለዉ መልኩ የግብረ ስጋ ግንኙነት ፈጽመዋል?			
	13.3.እርሰዎ ወይም የትዳር ጓደኛዎ ወይም ፍቅረኛዎ አደንዛዥ እጽ ወይምመድሃኒት ይጠቀማሉ?			
	13.4. ኤች አይ ቪ ቫይረስ በደሙ ካለዉ ሰዉ እና የጉበት በሽታ ካለው ሰው ጋር የግብረ ስጋ ግንኙነት ፈጽመዋል? (Discordant)			
	13.5 ከባድ የቀዶ ጥገና አድርገዋል፤ ወይም ቀጠሮ አለዎት?			
14	ደሞዎ ለሌላ ሰው ቢሰጥ ጤናማ ነው ብለው ያስባሉ?			
15	የደምዎን ጤናማነት በተመለከተ ለማወቅና የምክር አገልገሎት ለመቀበል ይፈልጋሉ?			
16	ለሴቶች ብቻ፡-			
	16.1ነፍሰጡር ነዎት? የእርግዝና ክትትል ሕክምና እየተደረገልዎት			

	ነው?			
	16.2 ባለፈው 6 ወራት የፅንስ ማቋረጥ ወይም ማስወረድ ገጥሞዎታል?			
	16.3 ጡት በማጥባት ላይ ነዎት?			

ክፍል3፡ የስምምነት መግለጫ

1. መጠይቆችን እና 'ደሙን የሚለግሱት ለትክክለኛ ምክንያት ነው'? የሚለውን ማብራሪያ አንብቤ ተረድቻለሁ፡፡
2. እስከ ማውቀው ድረስ የሰጠሁት መረጃ ትክክል ነው፡፡
3. መጠይቁን በትክክለኛዉና በታማኝነት ባልሞላ ደም ለሚጠቀሙ ታካሚዎች ጉዳት እንዳለዉ ተገንዝቤአለሁ፡፡
4. በለገስኩት ደም የቂጥኝ፣ የጉበት በሽታ ቢ(B) ፡ የጉበት በሽታ ሲ(C) እና የኤች አይ ቪኤድስ አምጭ ተሐዋስያን ምርመራ መደረጉ አሰፈላጊነቱን ተረድቻለሁ፡፡
5. የደም ልገሳውን ተግባር ጤናማ ለማድረግ አስከታሰበ ድረስ የደም ናሙና ውጤት ለሳይንሳዊ ምርምር ተግባር እንዲውል ፈቅጃለሁ፡፡
6. እድሜዬ ከ18-65 አመት መሆኑን አረጋግጣለሁ፡፡
7. በዚህ ቃለ መጠይቅ ላይ የሰጠሁት መረጃ በሚመለከተው አካል ምስጢራዊነቱ በተጠበቀ መልኩ እንደሚቀመጥ ተገንዝቤአለሁ፡፡

   ፊርማ ....

   አስተያየት፡...

(በደምባንክ ባለሙያ የሚሞላ)

የምርመራ አገልግሎት የሠጠው ባለሙያ ስም.........................ፊርማ------------------ቀን-----------

የደም ለጋሹ/ሿ ልገሳ መለያ ቁጥር

ለቢሮ አገልግሎት ብቻ

የምክር አገልግሎት የሠጠው ባለሙያ ስም.......................ፊርማ...............በደም ለጋሹ/ሿ ላይ የታየ ችግር አለ...የለም..................

Annex III, Standard operating procedures (SOPs) for specimen collection, handling and transport

Procedure

1. Documentation of Sample Movement

   1.1. It is important to track the movement of platelet concentrate samples from the point of collection from the donor, during processing, storage, distribution and/or use, and disposal.

   1.2. The time and date of collection should be recorded on the report form; the time the samples are received in the laboratory and the time of testing should be recorded by the laboratory.

   1.3. Platelet concentrate samples that are to be stored prior to shipment to an off-site laboratory or another site must be stored under appropriate conditions (at 20-24°c) with a continuous agitation.

2. Collection and handling of samples must be handled in accordance with the requirements specified in the study protocol

   2.1. Select the appropriate container to collect and store the sample

   2.2. Accurately label the sample

   2.3. Ensure that the immediate sample handling (methods and time requirements) complies with the study protocol

3. Storage of samples:

   3.1. The platelet concentrates sample store at room temperature with agitation for a maximum of 5 days.

4. Transport of samples

   4.1. Platelet concentrate are transport to test laboratory with room temperature environment and the principal investigator must:

   4.1.1. Ensure that ethical approval and participant consent is in place prior to transporting to a third party.

   4.1.2. Ensure specimens are handled, packed and shipped in accordance with the protocol need for the test not affected.

   4.1.3. Ensure that documentation (e.g., Receipts, shipping records, order forms, etc.) related to handling and shipment specimens is maintained and filed in the respective Site Investigator File.

5. Sample transport by personnel who should trained and qualified.

6. Disposal of samples

   6.1. Waste must be disposed of according to the Laboratory Waste Disposal system.

Annex IV. SOP for preparation of CRC, PC & FFP

Procedural steps

1. Receive collected 450ml whole blood less than 6 hours/24 hours
   1.1. Check for any abnormality (clotted, hemolyzed, leakage and etc) and proper labeling
2. Prepare platelet rich plasma (PRP)
   2.1. Put each blood units in cups
   2.2. Balance the cups using pieces of dry rubber
   2.3. Keep equally balanced cups of blood opposite to each other in Centrifuge and allow settling for one hour
   2.4. Centrifuge the blood at 2789 RPM /RCF 2496 g for 3 minutes on Roto silenta 630 RS centrifuge at 22^0c
   2.5. Take out the cups with the blood carefully & place on the table
   2.6. Put the blood on plasma extractor without disturbing
   2.7. Break the seal valve tubing connecting to the satellite bag
   2.8. Express 225ml-250ml PRP and clip the bag
   2.9. Weigh the PRP and CRC bags
   2.10. Transfer SAGM solution into CRC bag
   2.11. Seal the tube between the primary bag and satellite bag in three places
   2.12. Detach between the two seals and separates the packed red cells from PRP
   2.13. Arrange CRC units in their pack number sequence
   2.14. Record the weight of packed red cells
   2.15. Store the CRC at 2-6^0c within 30 minutes
3. Preparing platelet concentrate
   3.1. Place the platelet rich plasma in cups
   3.2. Weigh & balance using pieces of dry rubber
   3.3 Place balanced cups opposite to each other in centrifuge
   3.4. Centrifuge the PRP blood at 3561 RPM/4069g RCF for 10 minutes at 22 ^{0}C on Roto Silenta 630 Rs centrifuge.
   3.5. Gently removes the bags from the cups and hang on the hooks
   3.6. Transfer platelet poor plasma into satellite bag by leave approximately 100ml Platelet concentrates
   3.7. Clip the segment

3.8. Place the platelet concentrate bag gently on weighing balance to adjust plasma volume by transferring excess plasma into the plasma bag and leave 50– 70 ml plasma in the platelet concentrate bag

3.9. Seal the tube between the platelet bag & plasma bag in three places

3.10. Detach the two components apart

3.11. Weigh and record the Platelet concentrates and FFP

3.12. Discard the component outside the standard weight

3.13. Label the platelet concentrate and FFP with expiry date

3.14. Leave the platelet concentrates at room temperature on the table for 1 hour keeping the Label side down.

3.15. Place the platelet concentrates on agitator / platelet incubator

3 .16. Store the Fresh Frozen Plasma below -18^0 c in deep freezer

Annex V. Procedure for ABX MICRO 60S hematology analyser

Procedure:

1. Check operation of the machine, ensuring it is clean and that all required supplies are present in sufficient quantities.
2. Switch the instrument on by pressing the ON/OFF switch, located on the back of the instrument.
3. The instrument performs an initialization phase for the internal electronics. Please wait.
4. Once the initialization phase is complete, the ABX Micros 60s will automatically run a startup cycle.
5. If the ABX does not automatically run a startup cycle after the initialization phase is completed, press "Startup" button in the "Status" area to initiate a startup cycle.
6. Then, the instrument will perform a blank cycle for a reference blank count (an analysis cycle based on reagents without any blood sample).
7. Check and verify that the reference blank counts do not exceed the following parameter limits: WBC < 0.3, RBC < 0.02, HGB < 0.3, PLT < 10 then: Press "OK" button to validate blank results.
8. Perform quality control analysis on 3 levels of control blood material (low, normal and high) to verify that the instrument is performing within the specified ranges of the quality control material.
   - 8.1. Entering sample ID
   - 8.2. Mix the sample gently and thoroughly.
   - 8.3. Remove the cap from the sample tube.
   - 8.4. Place the sample beneath the sampling needle.
   - 8.5. Raise up the tube so that the sampling needle lowers into the blood and press the manual sample bar.
   - 8.5.1 The analysis cycle will begin.
   - 8.6. When the analysis is completed, the "Sample analysis" dialog box is closed and results are displayed in the "Result display" menu for print out.
   - 8.7. Dilute the sample if White blood cell counts ≥100,000 /mm3 and platelet counts ≥1,000,000 /mm3 are outside the linearity specifications of the instrument
9. Quality control procedures:
   - 9.1. At the beginning of each work shift all parameters are tested with blood control.
   - 9.2. The 3 levels include: Abnormal Low, Normal and Abnormal High

9.3. Controls are stored at 2-8ºC and brought to room temperature on a roller mixer before use.

9.4. Controls are gently inverted many times according to the manufacturer's instruction before use.

9. 5. From the RUN screen, press (SPECIMEN TYPE).

9.6. Use the arrow key on the keyboard to move the cursor to the appropriate QC file (i.e., low, normal or high) and press the (QC SPECIMEN) key.

9.7. Control values must be within three standard deviation, otherwise the measurement has to be repeated, if the control still out of range:

9.7.1. Check operation of the machine, ensuring it is clean and that all required supplies are present in sufficient quantities.

9.7.2. Check reagents for expiration dates and lot numbers.

Annex VI. Data collection format

Blood unit code	Blood collection date	Weight	Platelet preparation date	Platelet Weight	Platelet expiry date	Remark

Annex VII. Dummy table for laboratory result reporting form

Date	Sample ID	Result														Remark
		Volume	PC count			RBC contamination			WBC contamination			PH Value				
			Day 1	Day 3	Day 5	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5	

Annex VIII. Visual inspection (Swirling) of platelet concentrate results

ID	Day 1		Day 3		Day 5		Remark
	Swirling		Swirling		Swirling		
	Present	Absent	Present	Absent	Present	Absent	

Annex IX. Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in in this or any other university and that all sources of materials used for thesis have been properly acknowledged.

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This thesis has been submitted with our approval as advisors.

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Signiture: ______________________

Date: ______________________

Place: Addis Ababa, Ethiopia

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