TITLE

DETERMINATION OF QUALITY PARAMETERS OF PLATELET CONCENTRATES AT KENYATTA NATIONAL HOSPITAL'S BLOOD TRANSFUSION UNIT

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF **MASTERS OF MEDICINE (HUMAN PATHOLOGY)** OF THE UNIVERSITY OF NAIROBI

SUBMITTED BY:

Dr. RICHARD N. NJOROGE, M.B.Ch.,B (U.o.N)

DEPARTMENT OF HUMAN PATHOLOGY
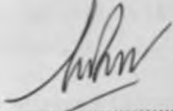
UNIVERSITY OF NAIROBI

Declaration

I declare that this is my original work and has not been presented for examination at any other institution.

signature 17.11. 2011

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Dr Richard N. Njoroge
Principle investigator
M.B.,Ch.B (UoN)
Resident. Department of Human Pathology
University of Nairobi

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Supervisors
This work has been submitted with our approval as university supervisors

Dr. RAJAB J
M.B.,ChB(UoN), MMed Path. (UoN) MPH (UoN)
Consultant Hematologist and Senior Lecturer
Thematic Unit of Haematology and Blood Transfusion
Department of Human Pathology, University of Nairobi

Signature................................Date... 18·11·11

Dr. GITHANGA J.
M.B.,ChB(UoN), MMed Path. (UoN)
Consultant Haematologist and Senior Lecturer
Thematic Unit of Haematology and Blood Transfusion
Department of Human Pathology
University of Nairobi

Signature.....................Date.... 18·11·11

Dr. MATURI P.
M.B.,ChB. MMed Path.(UoN)
Consultant Haematologist and Lecturer.
Thematic unit of Hematology and Blood Transfusion
Department of Human Pathology
University of Nairobi

Signature.............................Date......................

Contents

List of abbreviations.

AABB	American Association of Blood Banks
ABO	Blood group ABO system
BC	Buffy coat
BTU	Blood Transfusion Unit
BRM	Biological Response Modifiers
CCI	Calculated Corrected Increment
CFU	Colony Forming Units
CMV	Cytomegalovirus
EDTA	Ethylene diamine tetra acetic acid
FDA	Food and Drug Administration
FNHTR	Febrille-Non-Haemolytic-transfusion Reactions
KNH	Kenyatta National Hospital
MPV	Mean Platelet Volume
MPD	Mean Platelet Diameter
NBTS	National Blood Transfusion Service (Kenya)
PC	Platelet Concentrates
PRP	Platelet Rich Plasma
PSL	Platelet Storage Lesion
PLT	Platelets
PSL	Platelet Storage Lesion
RBC	Red blood cells
RhD	RhD antigen
rpm	revolutions per minute
SD	Standard Deviation
SDI	Standard deviation index
SOP	Standard operating procedures
TTI	Transfusion Transmitted Infections
WBC	White blood cells
WHO	World Health Organisation

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1.0 ABSTRACT

Background:

Platelet concentrates are increasingly being used as part of supportive therapy in the management of haematological malignancies.Platelet transfusion therapy is also indicated in thrombocytopenia due to various causes including all forms of bone marrow failure. Production of platelet concentrates is expensive and rigorous.They also deteroriate rapidly during storage and have a short shelf life.Therefore it is important to evaluate the quality of platelets concentrates transfused so as to prevent wastage.ensure efficacy and maximum benefit to the patient as well as to avoid exposing a patient to multiple transfusions.Efforts to formulate standards for platelet concentrates have resulted in minimum requirements being prescribed by American Association of Blood Banks (AABB).These have subsequently been adopted by Standards Committee of Kenya National Blood Transfusion Service (NBTS).These standards stipulate the methods and processes of platelet concentrate preparation, requirements for platelet counts,residual WBC content,volume and final pH.This study sought to find out if the platelet concentrates prepared Kenyatta National hospital's Blood Transfusion Unit (BTU) meet these standards.

Objectives: The objectives of this study were to assess the processes employed in preparation of the concentrates and to determine and describe several quality parameters of platelet concentrates prepared by the Kenyatta National Hospital (KNH) Blood Transfusion Unit, and to determine whether these parameters met the minimum standards set by standards committee of NBTS.

Methodology: This was a laboratory based cross-sectional study performed at KNH BTU between February and May 2010.The processes of platelet concentrate preparation (centrifugation, separation storage and agitation) were directly observed and scored on a Lickert Scale based on completion of the process. Platelet concentrate parameters (pH. volume and cell counts) were determined and recorded for comparison with NBTS standards. Data was analysed using means, median and standard deviations and presented in tables and figures. The student's t test was used to compare the degree of agreement of the characteristics of concentrates that met the minimum standards and those that did not meet the standards.

Results: A total of 78 platelet concentrates were analysed. Centrifugation of whole blood was performed according to specification for all (100% n=78) concentrates. Separation was achieved by 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. none fulfilled specification for residual WBC count,whereas 91% (71) and 95% (74) of the concentrates fulfilled the standards for volume and pH respectively. Only 6 (7.6%) of the concentrates did not have red cell contamination.

Conclusions and recommendations: The processes of separation,storage and agitation used in the preparation of platelets did not conform to the standards prescribed by NBTS.Only half of platelet concentrates prepared by the BTU fulfilled minimum specifications set by NBTS for platelet counts.None met criteria for residual WBC count.The concentrates fulfilled criteria for volume and pH. There is therefore need to strengthen the quality assurance programme for platelet concentrates at KNH BTU that should include process control. development and adherence to standard operating procedures,and internal and external quality control.In addition 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 .

2.0 Introduction

2.1 Background

Use of platelet concentrates continues to grow globally.This increase is partly due to increase in aggressive myelosuppressive therapy for haematological malignancies and solid tumours[1].Limited data is available on the clinical use of platelets in Kenya. Kenyatta National Hospital prepared and issued approximately 450 platelet concentrates in 2008. However the demand is likely to be much higher.

As for all other blood products, there are limitations, risks and controversies in the use of platelet concentrates. In general platelet transfusions are indicated for the prevention of and treatment of haemorrhage in patients with thrombocytopenia or platelet function defects. Although most platelets are infused due to thrombocytopenia arising from marrow suppression, occasionally platelet transfusion is indicated when thrombocytopenia is consequent to massive blood loss and transfusion, cardiopulmonary bypass, immune mediated, splenomegaly, or hereditary thrombocytopenia.

The cause of thrombocytopenia should be investigated and established before platelet transfusions are administered.Platelet transfusions are not always appropriate for treatment of thrombocytopenia, and in some instances are contraindicated,for example in thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome (HUS), and heparin induced thrombocytopenia (HIT).

Different countries have adopted different methods of preparing platelet concentrates.In North America, the Platelet-Rich-Plasma (PRP) method is preferred while in Europe, the Buffy Coat (BC) Method is preferred.[2] Consequently, because of the inherent differences in these two methods, the quality standards required of the final product are different (table 1). A third method   is the apheresis platelet method which requires special equipment. At KNH BTU, the method in use is the Platelet Rich Plasma Method.Apheresis platelets have now replaced pooled platelets as first choice therapy where they are available.One unit of apheresis platelets, also called single donor platelets are equivalent in platelet count to 4-6 units of pooled PRP or BC platelets. Because apheresis platelets are obtained from one donor (unlike PRP and BC platelets which are obtained from pooled multiple donations), patients who require platelets can be transfused without exposing them to the risk of multiple donors. Apheresis platelets are not available in most resource constrained centres in Africa, including KNH. Hence, PRP and BC platelets continue to be used.

Platelets transfused immediately after preparation from fresh blood exhibit the same in vivo recovery and function as native platelets. Loss of function and recovery of stored platelets is therefore attributed to the Platelet Storage Lesion (PSL) which can be as high as 30% . PSL refers to   progressive decline in platelet viability due to   storage,which is only partly explained by platelet senescence and is reflected   by deleterious in-vitro changes in platelet morphology/activation state, metabolic capacity and physiologic responsiveness.[3]

Platelet Rich plasma PRP and buffy coat methods are not significantly different in platelet yield [4]. The main difference in these two methods is in regard to the Platelet Storage Lesion (PSL).Whereas the final quality parameters of the concentrates appear similar for both PRP and BC methods the initial quality parameters are fundamentally different. This has led to the conclusion that each method produces platelets with different initial characteristics. For example the Buffy Coat method produces platelets with significantly less in vitro activation and also have lesser residual white cell count.[5,6,7,8] PRP-PCs display higher in-vitro levels of markers of activation (surface CD62P and CD63 expression, glycoproteinIIb/IIIa activation,

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loss of membrane glycoprotein Ib, platelet factor 3 activity, b-thromboglobulin and Platelet Factor 4 release, etc) [4,5,10] These differences can affect in vivo function and recovery of transfused platelets. Stored Platelet concentrates therefore have numerous variables which affect the quality of final product and require stringent quality checks irrespective of method of preparation. [11,12]

Of the numerous molecules and parameters that indicate storage lesion and assess viability of platelet concentrates, only a few are used to monitor platelet quality in routine practice in clinical laboratories. The rest, such as membrane glycoproteins IIb/IIIb, Platelet Factor 3 activity, b-thrombomodulin[3], lactate and ATP are only employed settings.

The American Association of Blood Banks (AABB) is the body that 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[13]. In addition AABB has prescribed minimum requirements for platelet concentrates processed by the PRP method [11,12]. 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 leukoreduced products.The above minimum requirements by AABB have since been adopted by the standards committee of NBTS as the applicable standards in Kenya.

A proportion of platelet products should be sampled periodically and the results documented in a typical quality control programme.[14] There has, however been no consensus on the frequency of sampling or the number of units to be sampled in such a quality control program. It has been suggested that at least 1% of all products produced should be sampled during the period in question[14]. If the total number of concentrates produced is relatively small then the number of concentrates sampled for quality checks should be higher.

Most parameters assess in-vitro platelet functions. It is difficult to determine which *in vitro* platelet functions are essential for in-vivo hemostasis after transfusion.[3] Furthermore it has not been determined which of these in-vitro parameters are most critical in controlling or preventing bleeding after transfusion of stored platelets. It is therefore very important that the functional capacity of the platelet be preserved before transfusion.

Except for platelet count and pH, the utility of the other parameters in predicting in vivo platelet fuction and recovery is limited and are therefore only used to indicate the presence and severity of Platelet Storage Lesion (PSL) particularly in research settings. Due to these difficulties, platelet quality assurance programs are restricted to performing only the basic assessments

Table 1 shows the specifications for several parameters in the United States, Europe and Kenya.

Table 1: Platelet quality parameter specifications in different regions. [15,16]

Parameter	United States	KNBTS	Europe
Platelet count ($\times 10^{10}$)	>5.5	>5.5	>6.0
pH	> 6.0	>6.2	>6.2
WBC content ($\times 10^6$)	< 0.83	<0.83	<0.2
Volume (mls)	Tomaintain pH >6.2		>50

3.0 Literature review

Processes and procedures used in preparation and storage of concentrates

With the exception of apheresis platelets whose preparation is automated, both PRP and buffy coat methods involve manual methods that include whole blood collection,centrifugation, separation, and storage with agitation.Automation in the apheresis method ensures better process control which produces platelets concentrates whose quality is superior than either PRP or buffy coat platelets.Apheresis method is now preferred to both PRP and BC methods. Unavailability of apheresis due to cost constraints means the former two manual methods are still in widespread use.

KNH employs the PRP method. The percent platelet yield from whole blood depends on the speed and duration of centrifugation. Inadequate centrifugation contributes to lower platelet percentage yield and higher red cell contamination of the platelet concentrate because of inadequate pelleting[17]. Though various studies have suggested various speeds and centrifugal force for centrifugation[17]. AABB recommends that each centrifuge should have functional calibration for both the light and hard spins to yield the optimum number of platelets in a certain volume of PRP[18].

Functional calibration is usually performed by centrifuging a number of whole blood units at different speeds and durations and then calculating the percent yield in the final concentrate. The lowest speed that gives the highest yield and the least red cell content is considered the final calibration for the instrument. After calibration, the speed,duration and expected optimum platelet yield (usually >95% from the hard spin) should be recorded and included in the standard operating procedures[18].AABB recommends that separation should be performed using a plasma extractor immediately after the hard spin without any delay. Delay in separation allows the cellular components to mix, resulting in lower platelet counts and higher red cell residue.

Proper storage of platelets is crucial to ensure viability.Cooled platelets become activated and aggregate and show reduced recovery because of rapid clearance post transfusion.These aggregates may also result in low platelet count in automated cell counters. Storage at 4^0C for 24 hours results in irreversible loss of discoid shape and eliminates platelet capability for microtubule reassembly after transfusion. Abnormal in vitro platelet function and significant decreases in viability occur concomitantly with losses in microtubule assembly[19,20] after platelet cooling to 4 degrees centigrade. Therefore, platelets concentrates should be stored in a temperature regulated environment between 20-24 degrees centigrade[21,22] and in containers that allow full gaseous exchange.

Continuous agitation is recommended to avoid spontaneous aggregation of platelets during storage. The mode of agitation used should be vigorous to prevent aggregation but gentle enough to prevent mechanical damage and release of metabolic substances from platelets such as LDH[23,24], which indicate platelet fragmentation. Gentle horizontal platform agitation is recommended as the optimal method of agitation of stored platelets which avoids mechanical damage to platelets. Though circular agitation is more effective in preventing formation of aggregates [25] it is associated with more mechanical damage and loss of viability in platelets than horizontal agitation.

3.1 Standards and Quality Control of Platelet Concentrates

Quality assurance programs have been developed to monitor the composition and viability of platelet products.[11,13] Practical difficulties in quality checks, however mean that only a few platelet units can be sampled for these quality parameters. The quality of the rest, therefore, has to be inferred from physical inspection,especially for swirling phenomenon and hue of the unit,which can be subjective.[26] These quality checks are performed either at the end of storage time or at issue. Since the products obtained by the various methods of platelet concentrate preparation are similar,these controls can be applied to any method.[27]

Statistical process control in the production of blood components including platelets has been introduced.[28] In one such study, it was found that manual methods of platelet production are more difficult to control than automated methods, but that better results can be achieved by using experienced personel if manual methods are used.Available recommendations however recognise that quality control programs are more likely to be successful in a national or regional transfusion service than in a hospital based service.[28]

3.2 Platelet count and platelet indices in concentrates

According to NBTS/AABB recommendations, the platelet count in a unit should be at least 5.5×10^{10} at the end of the maximal storage period or at issue (table 1). The platelet count in a unit influences the corrected count increment (CCI) with higher CCI achieved from higher counts, provided patient's clinical factors remain constant.

Doses of platelets, which are determined by platelet count in individual units, have not been standardised. However in general, a single dose of platelets contains 3×10^{11} platelets, corresponding to a single unit of apheresis donor platelets or 5 to 6 units of random donor whole blood platelet concentrates. Because of this lack of dosage standardization the doses are often determined based on factors unrelated to efficacy, such as cost and availability.[29] It is therefore critical that platelet counts and indices be determined in quality control programmes since dosage is influenced by platelet count per concentrate.

Other platelet indices of interest include mean platelet volume (MPV), platelet distribution width (PDW) and platelet large cell ratio (PLCR). These have great utility in assessing platelet storage lesion (PSL) and have been used as markers for the quality control of PCs, as these reflect storage-induced shape changes in platelets. In particular the indices PDW and MPV, correlate with pH [30] and are therefore of great potential as markers of platelet quality. In general MPV rises with a fall in pH as the platelets assume a spherical shape.

Fresh platelets are discoid in morphology. They slowly convert to spherical shape upon storage which is reflected in a higher MPV. This results in loss of swirling hence loss of viability. Different types of collection and processing and shortcomings in operational performance, can introduce heterogeneity in platelets quality, as indicated by changes in the platelet cellular indices MPV and PDW. In the presence of small aggregates there is a right shift in MPV (high mean MPV). Larger aggregates may appear as pseudo-erythrocytes or pseudo-leucocytes in the RBC and WBC histograms. Addition of EDTA during sampling disperses the aggregates and this is reflected in a fall in MPV. Therefore MPV is only useful as a marker of platelet storage lesion if the post-storage MPV is compared to pre-storage MPV in quality control programmes.[31,32] Currently platelet counts and indices are routinely determined by automated cell counters employing various principles. Cell counts are usually performed in EDTA which disperses reversible aggregates that occur in citrate after centrifugation.[31,32]

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pH is the simplest parameter indicator of the platelet storage lesion and probably the most important quality parameter that gives an indication of viability and potential recovery of platelet concentrates at the end of the storage period. pH has been identified as the parameter having the highest correlation with recovery and survival platelets.[33] In absence of oxygen stored platelets revert to glycolytic metabolism with increased generation of lactic acid and consequent fall in pH within 3 days of preparation.[34,35]

Platelet viability is markedly affected by pH.The final pH of platelet concentrate and hence in-vivo recovery and survival will depend on the type of storage container, storage conditions[36] and the volume of residual plasma. Therefore platelet storage bags must allow for free gaseous exchange. Platelets must be stored in sufficient plasma, whose bicarbonate content acts as a buffer, to maintain pH at greater than 6.2. Depletion of bicarbonate by high lactic acid level, typically at 20-25 mmol/l, lowers pH and results in loss of platelet viability.

Several methods of determining pH are in use, and most employ pH meter. The methods recommended by AABB use a pH meter. However use of blood gas analyzer is equally reproducible. The major limitation in these methods is that they require obtaining the sample from the bag, which may result in contamination.

A novel method that measures pH products without sampling has been developed. This is a new pH detector system that measures pH in platelet concentrates (PCs) via a special port containing optical sensing technology. The pH can be determined in a sterile way at any moment without sampling of the PC. This method, which requires a special container showed good correlation of pH between this method and blood gas analyzer method.[17]

Platelet pH, when assessed with plasma glucose levels was found to have a lower sensitivity in detecting bacterial contamination compared with cultures, Gram stain (10^5-10^6 CFU/mL), fluorescence microscopy with acridine orange stain (10^4-10^5 CFU/mL), chemiluminescence's detection of ribosomal RNA (10^3-10^4 CFU/mL), and automated bacterial culture (1 CFU/sample volume.[18] In one study, concentrates were found to have normal pH despite contamination with some bacterial species such as *E. cloacae.*[38] In that study, pH was determined by dipstick method.

In another study, the use of commercially available multiple-reagent urine dipsticks was evaluated in assessing bacterial contamination of platelets. It was found that urine dipsticks for pH and glucose can be used to rapidly and inexpensively detect bacterial contamination in platelet products, which potentially will reduce morbidity and mortality at minimal cost.[39]

3.4 Red cell content

Even in the most refined platelet concentrate will be found at least 1.5mls of contaminating red cells which can immunize an RhD-negative recipient[40].FDA guidelines require that if a unit of apheresis platelets contains more than 2mls of red cells, then a sample of donor red cells should be collected for compatibility testing before transfusion of the unit.

Previously, the practise in many transfusion centres was to issue non group matched platelets. Recognition of the risk of alloimmunization by residual contaminating red cells in a platelet concentrate led to AABB to recommend that each centre formulates a standard policy to be followed if the need arises to transfuse ABO incompatible platelet concentrates.

High cell counts including erythrocytes accelerate the platelet storage lesion due to increased metabolism. Beutler and Kuhl, and Taylor et al have found that low erythrocyte and leucocyte count appear to have no effect on the glucose consumption, lactate production or fall in pH.

[41] Therefore low cell count ensures that the viability of the platelets during *in vitro* storage is not compromised.[41,42]

3.5 Volume

Sufficient plasma volume in a platelet unit is required to maintain platelet concentrate pH.[43]Insufficient plasma volume results in lower pH at the end of allowable storage period. The typical final volume is 40-60ml per concentrate[44] but concentrates may be divided into smaller volumes for use in neonates.[45]

Although sufficient volume is required to buffer pH, large volumes of group O plasma in the platelet product may cause significant haemolysis of recipient red cells particularly where the donor has high titre Anti-A and anti-B isoagglutinins. All published reports of haemolysis following platelet transfusion have documented that the donor platelets were invariably group O [46] resulting in minor-incompatibility-associated haemolytic transfusion reaction.
A balance must therefore be struck between sufficient volume to buffer pH, and lesser volumes to minimize adverse effects. Reduction in volume is therefore meant to minimize amount of alloantibody transfused to the recipient and is important because determination of anti-A and anti-B titres is not performed in routine transfusion practice, even where group matching is performed.

Some laboratories have developed a policy of using ABO–incompatible platelet concentrates; however most have not included a method to minimize transfusion of anti A or anti B. Such methods should include performing isoagglutinnin titres for group O products[46]. If compatible platelets are unavailable, plasma may be removed by centrifugation [44] and replaced by saline or albumin, especially in cases where a donor antibody is directed against an antigen present in the recipient. Alternatively the platelets may be suspended in group AB-plasma, which lacks anti-AB isoagglutinnins.This also prevents IgA present in donor plasma from precipitating anaphylactic reactions in the IgA deficient recipient.

Deplasmatisation of platelets is achieved by centrifugation and subsequent removal of plasma, without washing. The final volume of the concentrate depends on the patient needs as prescribed by the physician. Platelet loss during volume reduction by centrifugation depends on the speed and duration of centrifugation, and ranges from 5-55%. AABB technical committee recommends recentrifugation at 2000 rpm for 10 min with only 5-20% platelet loss after 1-hour rest period. However even at higher centrifuge speeds, in vivo platelet survival is normal for the residual platelets. When platelets are spun for longer times at lower speeds followed by a shorter rest period, platelet loss is less than 15%.[45]

3.6   Residual WBC content and leukocyte reduced platelets.

There are specific standards that have been adopted to define the levels of residual leukocytes in leukoreduced and non-leukoreduced platelet units.These have also been adopted by AABB.[15,16]These differ between countries,with Europe having more stringent standards because they employ the buffy coat and apheresis methods.

Many febrile reactions, which occur in 4-30% of platelet transfusions, are as a result of biologically active products contained in the PCs or produced by the recipient. These biologic response modifiers BRMs are now known to be mainly cytokines, and are mainly elaborated by contaminating WBCs and from platelets themselves. Other BRMs include complement fragments, interleukins, chemokines, arachidonic acid metabolites, kininogens and histamines.[47,48]

Leukoreduction decreases the incidence and severity of FNHTR by minimizing the production of cytokines by residual WBCs. For example levels of IL-8 in PCs prepared by PRP method and leukoreduced immediately after preparation are much lower than in non-leukoreduced products.[48] Levels of anaphylatoxins such as c3a and c5a and other biologic response modifiers are also reduced after leukoreduction. Leukoreduction also reduces the rate of alloimmunization among hemato-oncology patients, which is crucial in candidates for allogeneic bone marrow transplantation.

The incidence of FNHTR is estimated at 0.5-1.4 % of non-leukoreduced red cell transfusions and accounts for 43-75 % of all transfusion reactions.[49] Prestorage leukoreduction is now recommended because bedside leukoreduction has no effect on incidence of FNHTR. Leukoreduction filters are polyester fibre matrix filters. Specific filters have been developed to leukoreduce platelets without affecting the platelet count.

One study has found the prevalence of anti-CMV antibodies among donors at NBTS in Nairobi to be 97% and 3.5% for IgG and IgM respectively[50]. This prevalence of anti CMV antibodies means that availability of CMV negative blood is constrained. Therefore leukoreduction is necessary to reduce the risk of transmission and reactivation of CMV infection, and post transfusion immunosuppression associated with transfusion of CMV infected blood to vulnerable patients such as neonates, allogeneic transplant recipients and the immunosuppressed.

A drawback is that leukoreduction is still an unaffordable technology in many developing countries including Kenya. Leukoreduction adds to the total unit cost of each product and introduces logistical challenges. Therefore non-leukoreduced platelet concentrates continue to be transfused in these resource poor settings.

4.0 Rationale

Use of specific blood components for the treatment of specific disorders is increasingly being promoted. In this regard the need for platelet transfusion has increased at the Kenyatta National Hospital because of improved patient care and use of intensive therapeutic regimens in oncology and haematological practice. Therefore there is a need to assess the quality of the platelet concentrates prepared at KNH with a view to ascertain the quality of the concentrates available for patient use.

Production of platelet concentrates is a costly and rigorous process from the point of collection, separation, storage and administration, and even in the selection of eligible recipients, which means high standards of quality are required to ensure efficacy and safety. This can be achieved by determination of simple platelet parameters such as platelet counts, pH, volume, red cell count and WBC count in the concentrates, which is an effective way of assessing quality of concentrates before issue.

5.0 Hypothesis
Quality parameters (pH,volume,platelet count and residual WBC count) in platelet concentrates made by KNH conform to the standards set by the Standards Committee of NBTS.

6.0 Objectives
To determine whether some routine quality parameters of platelet concentrates prepared by KNH blood transfusion unit meet the minimum standards set by Standards Committee of NBTS .

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Specific objectives

1. To evaluate the processes and practices used in the preparation of platelet products at KNH.

2. To determine the platelet count, pH, red cell content, WBC content and volumes of platelet concentrates prepared by KNH blood transfusion unit.

3. To compare these parameters with the standards set by AABB as adopted by NBTS

4. To make recommendations for enhancing quality assurance for platelet concentrates at KNH based on the findings of this study.

7.0 Methodology

Study time frame
Data was collected and analysed between January 2010 to May 2010

7.1 Study design

This was a laboratory-based cross-sectional study.

7.2 Study area
This study was conducted at the Blood Transfusion Unit (BTU) of Kenyatta National Hospital.

7.3 Sample size
NBTS recommends that 95% of all units shall meet quality standards. A sample size based on the assumption that 5% of the units do not meet the set standards was selected.
Since there has been no similar study in Kenya it was assumed that 95% of the samples meet the NBTS standards.

$$ n = (Z_\alpha)^2 P(1-P) \Big/ D^2 $$

Where:
n is the total sample size,
P is the assumed prevalence of the Samples meeting NBTS Quality Recommendations (0.95),
z_α value is the desired significance criterion (95% = 1.96),
D is the study precision set at 5% (0.05)

Substituting the above into the equation .
n = 72.
In the study period, 78 platelet concentrates were sampled.

7.4 Sampling process and inclusion criteria

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

7.5 Data collection on the process of producing platelet products

The processes of spinning, separation, agitation and storage of components was 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.
These data sheets and the scoring system are shown in appendix 2.

7.6 Collection of specimen

The unique laboratory identification number and ABO/RhD group were recorded for each concentrate. Each concentrate was then assigned another identification number for the purposes of the study. The date and time of concentrate preparation were recorded. The concentrates were then weighed and 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 the bag sealer and released for patient use. The samples were transported to the laboratory for analysis.

7.7 Laboratory methods:
Cell counts

Platelet count, residual WBC count and contaminating RBC count were determined by Coulter® AcT™5Diff Haematology Analyzer (Beckton–Dickinser) located at the laboratory of the Department of Paediatrics. University of Nairobi (appendix 4). The following formula was used to obtain the total cell count for WBC, RBC and Platelets for each concentrate.

$$\text{Cell count/unit} = \frac{\text{sample cell count/l} \cdot \text{volume (mls)}}{1000}$$

Determination of pH

pH was determined by an automated Bayer BG analyzer Rapid*lab* 348 model located at intensive care unit. KNH. This instrument determines pH based on respective electrodes (Ready Sensor ™ for pH reference electrode and reference cassette. appendix 5)

Determination of volume

Volume was determined using the following method:

$$\text{Volume} = \frac{\text{weight of concentrate (g)-weight of empty bag (g)}}{1.06}$$

Where 1.06 = specific gravity of plasma.

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7.8 Data management and analysis

Data was entered in pre-prepared data sheets. Variables collected included Lickert scores on centrifugation, separation, storage and agitation, proportions of blood groups ABO and RhD, duration of storage in hours, volume of each concentrate, pH, red cell counts, platelet counts, and white cell count in each concentrate. These were presented in figures and tables representing the means, medians, standard deviation, and percentages. SPSS version 11.5 statistical package was used for data analysis. After the analysis, Platelet concentrates were categorised into two: one category which met the specific standard for each parameter and another category for those that did not meet the minimum requirement for each parameter. A student's t-test was used to compare the means of the various parameters between the two categories with a p-value of <0.05 being considered significant.

8.0   Quality assurance

Quality assurance measures were instituted to ensure that the results obtained were accurate. These included pre-analytical, analytical and post-analytical quality control measures as outlined below.

8.1 Pre-analytical process
Specimen collection

- The samples were obtained directly from the bag through the tubing after thorough mixing at the time of issue.
- E.D.T.A vacutainers were used for samples taken for cell counts.
- A unique number was used to label each tube immeadiately after sampling.
- The samples for cell counts were held for at least one hour at room temperature to allow E.D.TA to disperse any reversible aggregates.
- Samples for pH were analysed within one hour of collection.

8.2 Specimen transport and storage

- Samples were held standing in a rack after collection
- In the laboratory samples for cell counts were placed in a mixing rotator rack for 30 minutes before analysis

8.3 Analytical process

The control results for cell counts for the day were examined and deemed satisfactory before each run was made.
- The internal control and self calibration results for the blood gas analyser were examined and recorded before analysis.
- The correct sample number was keyed into the instrument just before analysis.
- Sample runs were then carried out only when the control results were acceptable.
- One sample for cell counts from each batch was analysed by a different instrument, Cell Dyn-3000 located at the Deparment of Hematology and Blood Transfusion and the results of inter-laboratory quality control for cell counts compared using coefficient of variation.

9.0 Ethical Concerns

This study was approved by the Ethics Committee of Kenyatta National Hospital.

The major ethical considerations included the following:

- Ensuring that no contamination occured during sampling as outlined in the methodology.

- Ensuring that there was no delay in releasing the concentrates after sampling.

- Any platelet concentrate that showed obvious signs of contamination or clumping was sampled but was brought to the attention of the technologist in charge for appropriate action.

10.0 Results

The sample size was achieved in a period of four months, and all the samples were processed and analysed in accordance with the methodology outlined in this study.

10.1 Blood groups

A total of 78 concentrates were sampled. 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 in the platelet concentrates.

Blood Group	Frequency	Proportion (%)
A+	18	23.1
B-	5	6.4
O+	54	69.2
O-	1	1.3
Total	78	100

10.2 Age of platelets concentrates

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.

Table 3
Duration of storage of platelets (days)

Age of platelets	Number	Proportion (%)
Day 1	61	78
Day 2	8	10
Day 3	9	12
Total	78	100%

10.3 Process Assessment

Centrifugation for all 78 concentrates was peformed 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 one 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 4. Lickert scores for the processes of platelet concentrate preparation.

Processes	Activity	Score	Number of concentrates	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 extactor)	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 extractor 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	

Key :The details of the Lickert scores are recorded in appendix 1

10.4   Results of cell counts, volume and pH

Fourty (51%) of concentrates recorded a platelet count of $>5.5 \times 10.^{10}$

The mean and SD for platelet count was $6.63\pm4.73\times 10^{10}$ with a median of 5.58×10^{10} and a range of $0.89-21.50 \times10^{10}$. The Mean+SD, median and range of residual WBC count are 545 ± 429, 4.40 and $0.08-18.9$ (10^6) respectively.

The results for RBC count, pH and Volume are shown in Table 5.

Table 5: Cell counts, Volume and pH of platelets concentrates

Parameter ($n=78$)	Mean	Median	Range	NBTS standard	% Meeting criteria
Platelet Count (10^{10})	6.63 ± 4.73	5.58	0.89-21.50	5.5	51.3
WBC Count (10^6)	545 ± 429	440	8.0 -1890	<0. 83	0
RBC Count (10^9)	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	6.000 — 7.804	>6.2	95

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10.5 Characteristics of the Platelet Concentrates that did not meet the criteria for minimum platelet count.

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

The WBC and RBC count were significantly lower in this group compared to the group that met the criteria (p-value- <0.001). The pH in this group was significantly higher (P-value <0.001) (Table 4).

Table 6: Comparison of the concentrates with platelet count $<5.5\times10^{10}$ and $>5.5\times10^{10}$ 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	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

10.6 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 $24.3 \pm 20.3 \times 10^3$ which was significantly higher than the in the other group(p-value <0.001).

The mean pH was 6.4 ± 0.4 which was significantly lower than the rest of the concentrates. There was no significant difference in platelet counts and residual WBC counts in these concentrates (Table 5).

Table 7: Comparison of parameters of platelet concentrates with volume <60 and >60mls

	Volume	WBC Count (10^6)	RBC Count (10^9)	pH	PLT Count (10^{10})
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Category 1	Volume > 60mls (71)	530 ± 430	6.0 ± 5.7	7.2 ± 0.4	6.0 ± 4.1
Category 2	Volume < 60mls (7)	770 ± 440	24.3 ± 20.3	6.4 ± 0.4	13.8 ± 6.0
	p-value	0.1637	<0.0001	<0.0001	0.5579

10.7 Characteristics of the concentrates that did not meet criteria for PH

A total of four concentrates (5%) recorded pH below 6.2.

The mean volume for these concentrates was 58.7 ± 11.8, which is significantly lower than in the concentrates of comparable category (p-value 0.0019). In these concentrates the mean platelet count and red cell count were significantly higher at $15.33 \pm 5.3 \times (10^{10})$ and $34.15 \pm 20.0 \times (10^9)$ (p - value <0.001).

Table 8. Comparison of parameters of platelet concentrates that had pH < 6.2 and those >6.2

|	pH (>6.2)	Duration of storage (Days)	WBC Count (10^6)	RBC Count (10^9)	PLT Count (1010)	Volume (ml)
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Category1	pH=> 6.2(n=74)	2.1 ± 0.4	527 ± 420	5.92 ± 5.3	6.16 ± 4.2	75.5 ± 10.1
Category2	pH< 6.2(n=4)	2.5 ± 0.6	891 ± 520	34.15 ± 20.0	15.33 ± 5.3	58.7 ± 11.8
	p-value	0.06	0.09	<0.0001	0.0001	0.0019

10.8 Figures showing the relationship between various parameters

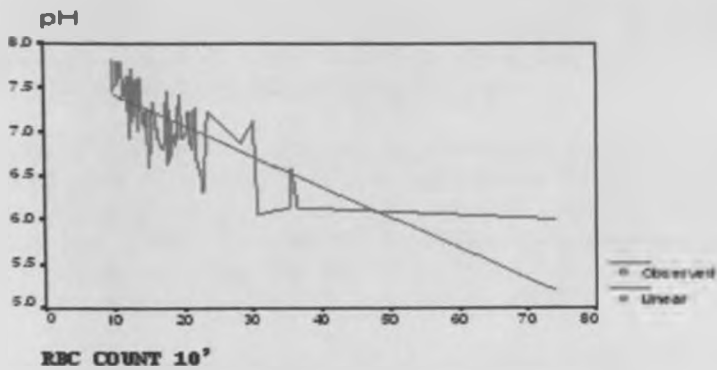**Figure 1:** Figure showing relationship between platelet count and pH.
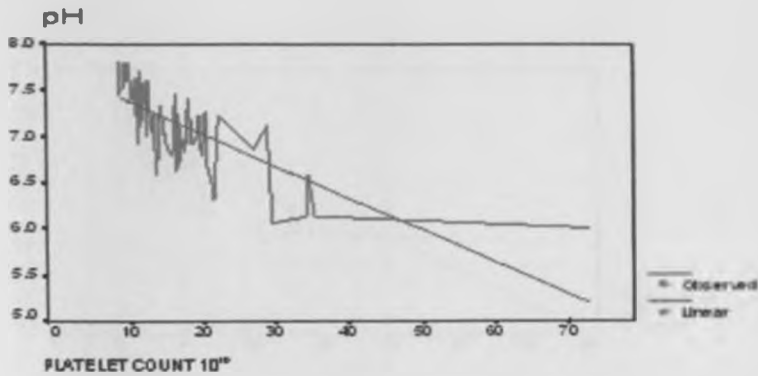
The total platelet count in the 78 concentrates was plotted against corresponding pH as shown in the figure above High platelet count in a platelet concentrate leads to a gradual fall in pH.

**Figure 2:** Figure showing relationship between residual WBC count and pH.
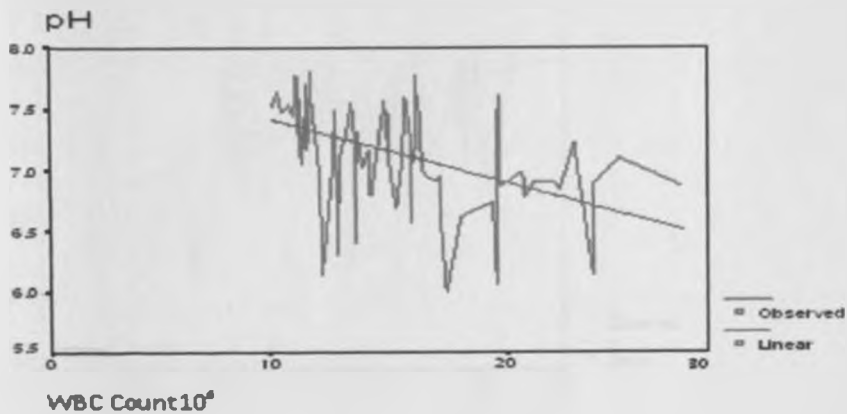
The total residual WBC count in the 78 concentrates was plotted against corresponding pH as shown in the in the above figure. High numbers of residual WBC in platelet concentrates lead to a fall in pH.

Figure 3: Figure showing relationship between contaminating red cell count and pH.

The total red cell count in each of the 78 concentrates was plotted against pH in the above figure. High numbers of residual RBC in platelet concentrates lead to a gradual fall in pH.

Figure 4 Figure showing relationship between volume and pH.
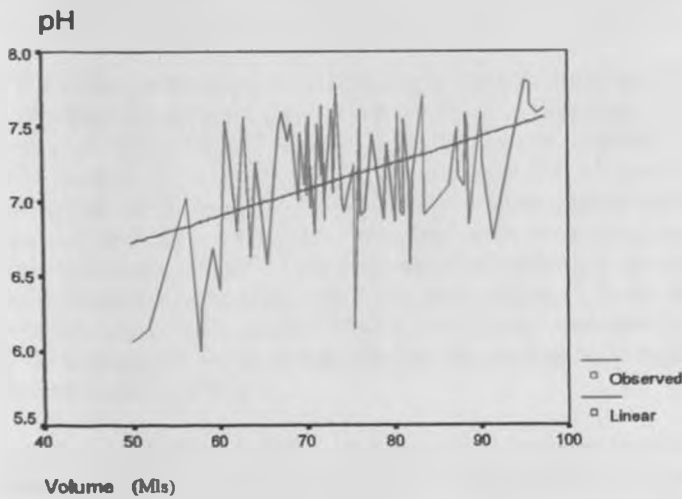
The volume of each the 78 concentrates was plotted against pH The figure shows that lower volumes result in lower pH confirming the lower buffering capacity of small volumes in platelet concentrates.

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11.0 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 transfusion unit. KNH manages various haematological conditions in which platelet transfusion is indicated such as thrombocytopenia, bone marrow failure and hematological and solid malignancies in adult and paediatric patients.

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 compared 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 KNBTS as adopted from AABB.

Of the 78 concentrates sampled, 69.2 % were group O-RhD+ve, 23.1% were A-RhD+ve, 6.4% were B-RhD+ve and only one unit (1.3%) was O-RhD-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[?]

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 upto 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 were consistent for all concentrates, 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 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.[17 18] Lack of functional calibration of the centrifuge for speed and duration of centrifugation may affect the final products[11]. 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 cellular and plasma compartments and allow the cells to mix. This may result in higher red cell contamination in the final platelet concentrate as was seen 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 requirement for storage at 20-24 degrees centigrade. Lower temperatures cause platelet activation, aggregation and subsequent reduced recovery after transfusion. In addition lower temperatures result in difficulties in resuspension of platelet pellet and may result in lower platelet count[25]. Since no markers of platelet activation were perfomed in this study, its not possible to ascertain what deleterious effect, if any, this storage had on these concentrates.

Only 51% of all concentrates showed platelet count of $> 5.5 \times 10^{10}$ against NBTS requirement of >95%. The range of platelet counts was $0.89\text{-}21.50 \times 10^{10}$, showing a wide variation in the counts. Taking into account that 30% of the platelets fail to recover on account of platelet storage lesion this suggests that approximately half of the platelets unit transfusions did not have the expected therapeutic effect on the recipients[21,22,51,52]. The mean and standard deviation for platelet count was $6.63 \pm 4.73 \times 10^{10}$ which was well above the minimum threshold. The large SD shows that 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.

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 platelet counts. In the concentrates with lower platelet count, the WBC and RBC counts were lower while pH was higher. The converse was true in those concentrates with platelet count above 5.5×10^{10}. 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.

In a similar study at a tertiary hospital in Nigeria where platelet concentrates were prepared by PRP method, Fasola[53] 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 showing a range as wide as in this study. Singh RP [6], in a study in India, 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 also demonstrates that there is a wide variation in the techniques used for the same method of platelet concentrate preparation in various countries.

Low residual WBC counts are desired to minimize side effects of residual leucocytes and to minimize the platelet storage lesion which can be enhanced by high leukocyte count.[54] All the concentrates in this study had high WBC contamination (mean WBC count of $545 \pm 429 \times 10^{6}$), 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 [6] reported mean WBC count of $40.5 \pm 4.8 \times 10^{6}$ for the PRP method which is far lower than the levels established in this study 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 is due to variability in process.

Only 6 (7.6%) of the concentrates did not have detectable red cell contamination. Fasola[53] in Nigeria reported that 30% of concentrates in his study did not have any red cell contamination. The proportion of concentrates with red cell contamination in this study is much higher (92%). Currently NBTS does not have guidelines for residual red cell contamination even though 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 alloimmunization [11] especially if platelets are issued without group matching.

AABB has recommended that each transfusion centre 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 and RhD negative products to RhD negative females. Therefore this high level of red cell contamination of platelet concentrates may not cause further adverse effects other than those rare effects which would arise from transfusing group 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 cell-plasma interface during separation.

The results of this study confirm that lower volumes result in undesirable lower pH than higher final volumes. In the study by Fasola [53] the mean volume was considerably low (18.52mls) whereas Singh [n] reported a mean of 62.30±22.68 ml. NBTS has set a minimum volume of 60mls whereas AABB has not specified the minimum volume,simply stating that the final volume should be adequate to buffer the pH to >6.2. This requirement for higher volume by NBTS may be due to lack of leukoreduction. There has been no consensus as to the final volume. 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. Therefore its apparent that the 60 mls threshold set by NBTS is considerably higher than the volume required to buffer the pH.[55] This higher volume may be necessary in this setting so as to maintain an adequate buffer since neither leukoreduction nor testing for pH is performed on platelet concentrates.

High volumes of platelet concentrates, 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.[46] Further studies are required to establish the minimum volume required to maintain platelet viability and pH where leukoreduction is not perfomed. Alternatively a shift to additive storage solutions as substitutes for plasma may be advised.

A total of 74 (95%) of the 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 0.7 [n] .These results are similar to other reported values. Fasola 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, (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 emphasises the need for stringent controls to ensure the high platelet count is not achieved at the expense of platelet viability.

There was no significant difference in duration of storage (p-value 0.06), between those concentrates with pH below 6.2 and above 6.2, possibly because all concentrates were issued within 72 hours.These results are similar to reported values. For example, Fasola in Nigeria reported that 100% of concentrates recorded pH 7.25 or higher[53] whereas Singh reported pH values of 6.7±0.26 (mean±SD) with a range of 6.5-7.0.There was a strong correlation between pH and volume (p-value <0.001,) and as expected a strong negative correlation between pH and cell counts (p-value <0.001). It is expected that higher volumes buffer the pH whereas high cell counts result in lower pH. Therefore these results confirm high cell counts increase the platelet storage lesion as indicated by the fall in pH.

The negative effects of platelet storage on platelet viability in concentrates prepared by PRP method without additive solutions during collection is as expected (see figure 2-5). This in effect illustrates the various interventions that may be used to prevent the deleterious effect of the platelet storage lesion. These include use of additive solutions,leukoreduction and strict maintenance of the volume within the recommended limits.

In summary, 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 standard operating procedures (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 pre-release testing of platelet concentrates. Furthermore in transfusion units where few platelet concentrates are processed and issued, it is possible to sample all units and subject them to pre-release quality checks. Implementation of these measures will help in continuous quality improvement as well as entrench a standardization and harmonization program for platelet quality monitoring.

13.0   Conclusions

1. The processes of centrifugation, separation, storage and agitation were not performed according to recommended standard operating procedures.
2. Only 51% of concentrates fulfilled the minimum requirement for platelet count .
3. The recommended criteria for residual WBC count were not met in any of the platelet concentrates.
4. All the concentrates met the criteria for volume and pH.
5. Red cell contamination of the platelet concentrates was very high in this study.
6. Red cells,contaminating WBC and volume had an effect on the on the platelet storage lesion as indicated by pH that is consistent with expected results.

14.0   Recommendations

1. There is need to strengthen the quality assurance programme for platelet concentrates at KNH, in particular the in-process control with stringent adherence to standard operating procedures, and compliance with recommended processing methods.
2. All platelet concentrates processed at KNH BTU should be subjected to platelet count before issue.
3. A quality assurance program should include post transfusion platelet counts check to establish the proportion of platelet transfusions which achieve desired platelet count increment.
4. Studies are required to determine the minimum red cell content acceptable for platelets concentrates.
5. Further studies should be performed at KNH to evaluate the in-vivo platelet recovery and function after transfusion in view of the inadequacies revealed in this study.
6. Further studies are required to characterize clinical effects, including adverse events, if any, following platelet transfusions at KNH, since this study has revealed some deficiencies in the quality of platelet concentrates.

14.0   Study limitations

In this study cell counts were performed in EDTA tubes after transfer from citrate. EDTA facilitates dispersal of the reversible aggregates which may form in citrate.No cell counts were performed in citrate.Therefore it was not possible to compare the differences in cell counts in citrate from those in EDTA.This study therefore assumed that EDTA dispersed all the reversible aggregates, if any, present in the original citrated sample.

15.0   **References**

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Appendix 1
DATA COLLECTION SHEET PARAMETERS

Date of Sampling ☐   Date of Processing ☐

Sample Number ☐   BTU Number ☐

Platelet Count ☐   WBC ☐

PDW ☐   RBC ☐

MPV ☐

PH ☐   ABO/D

☐

Volume ☐

Cell count/unit= <u>sample cell count/l × volume (mls)</u>
                           1000

<u>Comments</u>
☐
<u>Signed</u>:   <u>Signed</u>:

Technologist ☐   Investigator ☐

Date ☐   Date ☐

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Appendix 2

Data Recording Sheet for Processes
Sample Number .... Date... ....

1.Centrifugation.

First Centrifugation at 1800 rpm in a temperature regulated calibrated centrifuge, for a minimum of 6min, and second centrifugation at 3800 for 8min

Lickert scores for centrifugation

centrifugation	Lickert score Score	Number of concentrates (n)	comment
Completed	2		
Incomplete	1		
Not done	0		

Key:
       Score 2 - Completed - centrifugation performed according to recommendation in a calibrated centrifuge

       Score 1 - Incomplete- centrifugation not performed as stipulated: either centrifuge not calibrated or recommended speed and duration not used.

       Score 0 – Centrifugation not performed

2.Separation

Separation with plasma extractor within 60 minutes of completion of centrifugation

Lickert scores for separation:

Separation	Lickert Score	Number of concentrates separated	Percentage of units separated	comment
Within 60 min, use of plasma extractor	2			
Delayed(60min) + use of extractor	1			
Plasma extractor not used.	0			

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3.Storage & Agitation:

Continuous agitation and temperature regulation at 22-24^{0}C

Lickert scores for storage and agitation:

Activity	Score	Number	pecentage	comment
Complete	2			
Incomplete	1			
Not done	0			

Key:Score 2-complete- units stored under temperature regulation and continuous agitation

Score 1- incomplete: Either agitation or temperature regulation was not performed

Score 0- not done:Neither continuous agitation nor temperature regulation was performed

Procedure for preparation of platelet concentrates at KNH

1. The PRP method is used. The shelf life of platelets at KNH is 24 hours.
   These are prepared at the blood transfusion unit .

2. Blood is collected in a JMS 450 ml bag system containing 63mls of CPDA-1, with a 300ml capacity transfer bag (JMS Singapore PTE Ltd). This is collected over 5-8min to prevent platelet clumping. A bag agitator is not used.
   A sample for screening for TTIs is also obtained at that sitting.

3. A sample for ABO/RhD grouping is then obtained from the tubing after separation from the donor. The tubing is then cut and the unit sealed.

4. Immediately or as soon as possible, the units are balanced on a weighing machine and then centrifuged at 1800rpm for 15 min at 22^0c in a 6 pax capacity temperature regulated *himac CR 7B3* centrifuge (Hitachi Corporation).

5. The units are removed from the centrifuge and platelet rich plasma pressed manually into the second pilot bag by *Teruflex ACS 201* plasma separator (Terumo).

6. They are then subjected to a second centrifugation at 3800 rpm. Platelet poor plasma is then expressed into the 3rd bag to obtain the platelet concentrate.

7. The amount of plasma left in the bag is not measured and depends on the subjective judgment of the technologist.

8. The platelets are then placed on the agitator and are recorded in the register awaiting issue.

Procedure for Automated cell count and indices- Coulter[a] A[c]T[TM]5diff Haematology Analyzer

Procedure:

1. Check the waste container to determine if it needs to be emptied
2. Verify that the printer is connected and ready
3. Press start button and wait for the instrument to perform start up routine which include a rinse cycle and a background count on a reagent without any blood specimen.
4. When the start up results are checked and passed, run the controls
5. If the background counts are not within the acceptable limits after the first start-up cycle, the instrument will automatically perform start-up up to 2 more times. If the third attempt fails, the message STARTUP FAILED is displayed.
6. The background count limits are
   WBC= $0.3 \times 10^3/\mu l^3$
   RBC=$0.03 \times 10^6/\mu l^3$
   HB=0.3g/dl
   Platelet=$7.0 \times 10^7/\mu l^3$
7. The green LED will illuminate and analyzer is ready to analyze samples.
8. Enter the sample number (sample ID). Mix the sample well and open the vaccutainer
9. Put the probe in the tube and Press the suck button.
10. The machine will suck and when the LED illuminates, the tube is removed.
11. The machine automatically analyzes the sample and displays the results on the LCD and prints the results.
12. The probe returns to its position for the next sample.

Running controls for Coulter[*] A[c]T[TM]5diff Haematology Analyzer

This procedure was performed daily. The control reagent used was Coulter[*] A[c]T[TM]5diff Control Plus supplied by the manufacturer. Three levels (normal, low and high) quality control reagents were supplied and were run concurrently with the samples daily. The QC results must be within the QC range for each analyte.

1. Remove the control reagent from the refrigerator and warm it at ambient temperature for 15 min.
2. Mix the tube by rolling between the palms and inverting 8 times each. Inspect the tube to ensure adequate mixing.
3. Run the controls in order of the samples from low normal and lastly high. Check that the control readings are within the accepted range as provided by the manufacturer.
4. Enter the QC results in the Levey-Jennings charts in the computer. If within the range begin the sample analysis. If not repeat the control, and if still out of range investigate source of error(s).
5. The end of day shut down procedure is performed at the end of analysis.

Reproducibility check

This was to ensure consistent measurements.
One fresh normal properly mixed whole blood was analyzed at least 6 times and the results printed and tabulated. These are the parameter reproducibility specifications:

Parameter	Coefficient of variation (CV%)
WBC	<2.0%
RBC	<2.0%
HCT	<2.0%
PLT	<5.0%

If the CV% was greater than those listed for any parameter then there was an instrument problem. No instrument problem was noted.

Appendix 5
Procedure for pH analysis
instrument Bayer Rapid*lab* 348 BG analyzer

Principle:
The analytic principle for pH in this instrument is use of potentiometric silver/silver chloride electrode with glass membrane.

The analytic principle for pCO2 in this instrument is potentiometric, based on the electrode described by Severinghaus and Bradley.

The analytic principle for pO2 is amperometric, based upon the electrode described by Clark.

The instrument generates the following measured parameters:

pH, PCO_2, PO_2, Na⁻, Ca⁻⁻ and Cl-

The instrument generates the following calculated parameters:

HCO3, BE, s(O2) {% oxygen saturation}, Hct (hematocrit) and Hb (haemoglobin)

Calibration for Rapid*lab* 348 BG analyzer

This was performed according to manufacturer's instructions.
For this analysis, the instrument was calibrated using **Cal-Pak for Ciba-Corning 348 calibrating solution** supplied by Mission Diagnostic Reagents for Bayer instruments. The instrument was set to automatic calibration. There are two levels of calibration. One point calibration calibrates for pH at 7.382. Na+ at 14.0 mmol and K+ at 4.0mmol and was performed every 30minutes.
Two point calibration calibrates for pH at 6.8. Na+ at 11.0mmol and K+ at 8.0mmol.

Quality control for Rapid*lab* 348 BG analyzer
This was performed daily and results plotted on a Levey-Jennings chart.
The reagents were supplied by the instrument manufacturer and the batches were either Level 3(high). level 2(normal) or level 1(low).
These control reagents have a respective assigned mean and range.
The standard deviation for these control reagents was calculated as follows:
Since the acceptable Range is 4×SD, then:

SD= Assigned Range:
           4

The daily control runs were made in the morning. The results for pH. PCo2, and PO2 were compared with the assigned mean and SDI (standard deviation index) calculated using the above derived SD. The SDI for each parameter was then plotted on a Levey–Jennings chart.
For the control result to be acceptable. they must have been within 2SD of the assigned mean.

Manufacturer's Procedure for Rapid*lab* 348 BG analyzer

1. The sample is mixed within the syringe by rotation. ensuring there are no gas bubbles.
2. The sample hub on the instrument is opened.
3. The sample is introduced ensuring the probe reaches into the sample to avoid bubbles.
4. The instrument sucks the sample automatically.
5. The instrument will beeps when the sampling is complete.
6. The syringe is removed from the sample hub and the probe is closed.
7. Sample analysis continue automatically and the results printed

Ref: KNH/UON-ERC

RESEARCH PROPOSAL - DETERMINATION OF QUALITY PARAMETERS OF PLATELET PRODUCTS AT KENYATTA NATIONAL HOSPITAL'S BLOOD TRANSFUSION UNIT

Yours sincerely

DR. L. MUCHIRI
AG. SECRETARY, KNH/UON-ERC