QUALITY DETERMINATION OF PLATELET CONCENTRATES PREPARED AT THE NAIROBI REGIONAL BLOOD TRANSFUSION CENTRE IN KENYA

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Quality Determination of Platelet Concentrates Prepared at the Nairobi Regional Blood Transfusion Centre in Kenya

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DECLARATION

This is my original work and has not been submitted for a degree in any other University.

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DEDICATION

This work is dedicated to my Parents Mr. Patroba Mayaka (The late), my beloved Mother Mrs. Miriam Mayaka for all the moral support which was accorded to me throughout the period of my studies

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LIST OF ABBREVIATIONS

AABB	American Association of Blood Bank
ABO	Blood group ABO system
BC	Buffy Coat
CFU	Colony forming units
CLSI	Clinical Laboratory Standard
CPDA	Citrate Phosphate Dextrose Adenine
EDTA	Ethylene diamine tetra acetic acid
FDA	Food and Drug Administration
HIT	Heparin induced thrombocytopenic
ITP	Immune thrombocytopenic
KNH	Kenyatta National Hospital
LF	Lactose Fermenters
MPV	Mean plate volume
NLF	Non Lactose Fermenters
NRBTC	Nairobi Regional Blood Transfusion Centre
PAS	Platelet Additive Solution
PC	Platelet Concentrate

PCI	Platelet count increament
PDW	Platelet distribution width
PLCR	Platelet large cell ration
PRCs	Packed Red Cells
PRP	Platelet Rich Plasma
PSL	Platelet storage lesion
QA	Quality Assurance
RDP	Random donor platelets
SAGM	Saline Adenine Glucose Mannitol Solution agar
SOP	Standard Operating Procedure
SSI	Surgical site infection
USA	United States of America
WB	Whole Blood
WBC	White Blood Cells

DEFINITION OF TERMINOLOGIES

Aseptic Technique: Refers to a procedure that is performed under sterile conditions

Blood component: is any therapeutic substance prepared from human blood. This includes whole Blood; blood components, and Plasma derivatives; whole blood is not commonly used in transfusion medicine.

Blood product: is any component of blood which is collected from a donor for use in blood Transfusion.

Staphylococcus: Is a genus of Gram-positive bacteria

Thrombocytopenia: It refers to low blood platelet count <150/µl

ABSTRACT

Blood component preparations are preferred to whole blood in transfused majority for instances. The overall benefits from any components is dependent on its quality. The platelet rich plasma-platelet concentrate (PRP-PC) are randomly prepared from platelet rich plasma which are derived from Whole Blood of donors. Platelets concentrates (PC) can be stored for 5 days at 22 ± 2°C with continuous agitation. The quality of these platelet concentrate preparations has not been evaluated at Nairobi Regional Blood Transfusion Centre (NRBTC). Therefore, the aim of this study was to determine the quality of Platelet Concentrate preparations at Nairobi Regional Blood Transfusion Centre, using various parameters which included: the platelet count, volume of the platelet concentrate, WBC count, contamination of Red Blood Cells and assessing bacterial contamination (on day 0, 1, day 3 and day 5) post preparation. The study was a cross-sectional study which started from January to April 2017 to assess various quality determinants of platelets prepared at the Nairobi Regional Blood Transfusion Centre. However a total of 91 platelet concentrates were prepared from random donors by platelet rich plasma method (PRP-PC). Only 87 (96%) met the requirement of the platelet count of >5.5 x 10^{10} the mean and SD for platelet count was 242.5±67.0 × 10^{10} with a median of 237× 10^{10}. The concentrates that met the specification of Residual White cell count of less than (<0.83) in the platelet concentrate were 76 (84%). The mean 0.51 ±0.49 and median 0.350 whereas 100% (91) of the platelet concentrates in this study fulfilled the standards for volume while 11 (12.0%) post platelet concentrates preparations had bacterial contamination respectively. All the platelet concentrates prepared at Nairobi Regional Blood Transfusion Centre were issued within three days of processing the blood bag and can store platelets up to 5 days. Only 86(95%) of the concentrates did not have red cell contamination, The mean and SD 0.99835±0.21003.The platelets concentrates preparation processes conforms to the standards set by Nairobi Regional Blood Transfusion Center which led to half of platelet concentrates to fulfil the minimum specifications set by Nairobi Regional Blood Transfusion Centre for platelet counts. However the criteria for volume was met. Hence there is need to strengthen the quality for the preparations of platelet concentrates at Nairobi Regional Blood Transfusion Centre. Therefore it is recommended that all platelet concentrates preparedd at Nairobi Regional Blood Transfusion Centre must be subjected to platelet count an culture before dispatch and only those concentrates that meet the criteria should be issued.

Keywords: platelets, quality, blood transfusion.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Platelets concentrates were first identified earlier in 1881, since then there has been understanding of their function and their clinical use internationally (Wallace et al, 1994, 1998). Platelet rich plasma- platelet concentrate, were prepared and there quality indicators were assessed at Nairobi Regional Blood Transfusion Centre, platelet concentrate are prepared from random whole blood donors and platelet concentrates are separated from platelet rich plasma derived from these donors. The Buffy coat poor-platelet concentrate method is rarely used due to the complexity of preparing them. The single donor platelets, apharesis-platelet concentrate collected from voluntary donors with help of an automated cell separator is not available. In Kenya, platelet concentrate rich plasma- platelet concentrate method is preferred while in in North America, the platelet rich plasma method is preferred and in Europe, the Buffy coat method is preferred (Richard et al, 20014). However, Maximum storage period before platelet concentrates preparations is within 8 hours at 20 to 24^0C, both methods are not significantly different in terms of yield (Perrota PL. et al, 2003).

The recommended shelf life of platelet concentrates is 5 days stored 22 ± 2°C with continuous agitation. It is known that platelets undergo various changes immediately after they are collected, during processing, storage and even when being transfused to patients. This may affect the effective dosage that a patient receives (Richard et al, 20014).

In the laboratory the platelet quality can be assessed by using several parameters (platelet count, volume, WBC count, RBC count, and bacterial contamination in platelets on day 0, day 1, day 3 and day 5) post preparation. (Holme S. et at, 1998)The American Association of Blood Banks 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. Also American Association of Blood Bank has prescribed minimum requirements for platelet concentrates processed by the PRP method (Gupta A, et al, 2010). In addition, AABB has restricted the basic minimum requirements to at least include platelet count, volume, red blood cell content, and residual white cell count in leuko-reduced products. These standards have since been adopted by the standards committee of National Blood Transfusion Service (Richard et al, 2014)

Platelet rich plasma, platelet concentrates are used mainly for transfusions to manage conditions such as thrombocytopenic bone marrow failure and malignancies, patients most commonly as therapy, there is increased consumption of platelet concentrates as in disseminated intravascular coagulopathy (DIC), and aplastic anemia . Other less frequent indications include thrombocytopenia due to qualitative defects example in quantitative defect there is decreased platelet count seen in such patients receiving chemotherapy (Murphy et al, 1996). Thrombocytopenia patients should be investigated and establish the cause before platelet concentrates transfusions are administered for they are not always appropriate for treatment of thrombocytopenia.

Other cases like immune thrombocytopenic purpura (ITP), thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome (HUS), and heparin induced thrombocytopenia (HIT) platelet transfusions ideal they are not recommended (Segnatchia et al 1997; Vassallo et al, 2006).

Table 1.1 shows the specifications for several parameters in Kenya, United States and Europe. In this study we have analyzed the quality of different platelet concentrates prepared at Nairobi Regional Blood Transfusion Centre as per the American Association of Blood Banks recommended quality standards which have been adopted by the NBTS (Van der Meer PF, et al, 2011).

Table 1.1: The quality of Platelet concentrate parameter specifications in different regions

Parameter	United States	KNBTS	Europe
Platelet count ($\times 10^{10}$)	>5.5	>5.5	>6.0
Volume	60ml	>40-70ml	>50
WBC	<0.83	<0.83	<0.2
RBC	<0.06	<0.06	<0.06

The data available on clinical use of platelet concentrates is limited in Kenya, Nairobi Regional Blood Transfusion Center (NRBTC) prepared and issued approximately 550 platelet concentrates in 2016, (Though Unpublished NRBTC records), however the demand is likely to be much higher. Determining platelet quality is important to confirm that the platelet concentrates (PCs) meet the requirements defined by national and international standards and to evaluate the effect of PCs transfused to the thrombocytopenic patient (Vassalo et al., 2006)

Platelet Concentrates there quality is affected by the preparation method of the PCs the storage conditions including duration of storage, the storage temperature, type of anticoagulant used, the concentration of PCs in the bag and the agitation (Rvera et al., 2005; Tynngard et al., 2009). The possibility of the PCs prepared from WB to be used for transfusion to thrombocytopenic patients can be detected by analyizing of several in vitro platelet quality parameters (Vassalo et al., 2006).This study focuses on quality assessment of PCs prepared from WB by determining the volume of platelet in a bag, platelet count, total red blood count (RBC), Residual white blood count (RWBC), and assess bacterial contamination on day 0, 1, 3 and day 5.

1.2 Statement of the Problem

The quality of these platelet concentrate preparations there quality was determined at Nairobi Regional Blood Transfusion Centre (NRBTC). The indicators in this study are, platelet count, red cell content, WBC count and volume of platelet concentrates were assessed at NRBTC. Platelet was considered quality if the platelet count was≥150×109 cells/l, RWBC ≤0.83×109 cells/l, RBC of ≤0.06×109 cells/l and volume ranged between 40-70ml respectively. In this case, the quality of platelet count was to check the quality in terms of platelet number, if not checked plasma might thought to be platelets, The quality of WBC count should be minimal (<0.83), the presence of WBC count above the stated number was known to cause of febrile transfusion reaction. RBC count should be minimal (<0.06), the presence of RBC count above the stated number is known to cause transfusion incompatibility if not cross matched. Platelets are stored at 20-24 ^{0}c which enhances bacterial growth, if contaminated platelet concentrates is transfused they are likely to cause septicemia. The quality of volume of platelet concentrate should range between 40-70ml to maintain the pH of 6.2. Currently, at NRBTC bank supply do not screen for bacterial contamination in the platelet concentrates which is risk for transmission to patients. However Bacterial Contamination of platelets is rare but may cause serious complication to patients after platelets transfusion. Bacterial contamination of platelet concentrates occurs because of the storage temperature for platelets (22± 2°c) and this may facilitate bacterial growth and the risk of transfusion associated sepsis is likely to go higher due to contamination. Thus due to the success of screening viral pathogens, it's a good approach of screening bacterial contamination being the most common infectious risk in transfusion and has become a matter of increasing concern and attention (Morel et al., 2003). However the proper laboratory techniques have been done to avoid platelet reactions before transfusion, still there are platelet transfusion reactions that have been reported and cause not known (Nor Raihan, 2014). In contrast, bacterial contamination has been shown to be the cause of transfusion reactions in some cases though the platelet was properly cross matched (Hillyer et al., 2003). In Tamale Teaching Hospital Ghana, the study findings shows that 17.5% grew isolates of various

bacteria from donated blood (Opoku-Okrah et al., 2009). If these is no intervention to this problem, then the risk of transfusing bacterial pathogens to the patients will still continue to be a problem to the society.

1.3 Justification

Platelet concentrates transfusion practice serves an important role in saving lives and improving the quality of life in a large range of clinical conditions (Silva et al., 2005). However, it is also a potential source of infection to the platelet recipient (Richard et al., 2014). Among the infections that can be transmitted through platelet concentrates transfusion, bacterial sepsis remain a major health-care concern being the most frequently reported cause of transfusion related to acute septic reactions (Walther et al., 2010).

Having limited studies in Kenya to check on the quality of the platelets prepared such as Volume, platelet levels residual WBC, RBC and bacterial contamination of the platelet concentrate on post preparations. The current study aims at assessing the quality of platelet concentrates at Nairobi Regional Blood Transfusion Centre to check on its quality before transfusion. For the platelets to give therapeutic effect (Thibault et al., 2006), the following were assessed. The quality of platelet count, red cell content, WBC count and volume of platelet concentrates are indicators that were assessed at NRBTC. RBC count should be (<0.06), the presence of RBC is known to cause transfusion reaction if platelet concentrates were not cross matched and counted as a contaminant Platelet concentrates transfusion serves an important role in thrombocytopenic patients to treat or prevent bleeding. For that reason, the findings were to help policy makers to formulate appropriate mechanisms of avoiding platelet contamination and improvement of its concentrate quality.

1.4 Null Hypothesis

Quality indicators (volume, platelet count, RBC count and residual WBC count) in platelet concentrates made by Nairobi regional blood transfusion Centre did not conform to the standards set by the Kenya National Blood Transfusion Service.

1.5 Broad Objective

To determine routine quality parameters of platelet concentrates prepared by Nairobi Regional Blood Transfusion Centre.

1.5.1 Specific Objectives

1. To determine the platelet count, red blood cell content, WBC count and volume of platelet concentrates prepared by Nairobi regional blood transfusion center
2. To determine the level of bacterial contamination in the platelet concentrates on day 0, 1, day 3 and day 5 post-preparation
3. To compare the parameters with the set standards by the American Association of blood banking (AABB).

CHAPTER TWO

LITERATURE REVIEW

2.1 Separated blood components for transfusion

Globally, approximately shows that 80 million units of blood are donated each year (WHO, 2002). Out of these, a total of 2 million units (2.5%) are donated in Sub-Saharan Africa, where the need for blood transfusion is greater because of many causes of anaemia including parasitic infectious diseases such as malaria (WHO, 2002). As such it is necessary that maximum utilization is made of the appropriate component instead of whole blood. However particular component needs to have acceptable level of quality.

2.2 Procedures for preparation and storage of platelet concentrates

With the exception of apheresis (a procedure in which blood is collected) 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 to either PRP or buffy coat platelets. Apheresis method is now preferred to both PRP and BC methods. However Unavailability of apheresis machine due to cost constraints means the former two manual methods are still in use. (Richard et al, 2014)

NRBTC 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 (Dijkstra et al., 2011).

2.3 Standards for Blood Banks and Transfusion Services

AABB recommends that each centrifuge should have functional calibration for both the light and heavy spins to yield the optimum number of platelets in a certain volume of PRP. 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 heavy spin) is recorded and included in the standard operating procedures (Apelseth et al., 2007).

AABB also recommends that separation should be performed using a plasma extractor immediately after the heavy 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 necessary to ensure viability. Cooled platelets become activated and aggregate and show reduced recovery because of rapid clearance post transfusion. These aggregates also result in low platelet count in automated cell counters. (Nor Raihan et al, 2014)

Storage at 4^0c for 24 hours results in irreversible loss of discoid shape and eliminates platelet capability after transfusion. Abnormal in vitro platelet function and significant decreases in viability occur concomitantly with losses in microtubule assembly after platelet cooling to 4^0. Therefore, platelets concentrates should be stored in a temperature regulated environment between 20-24^0C and in containers that allow full gaseous exchange. Continuous agitation is recommended to avoid spontaneous aggregation of platelets during storage. (Gullikson et al, 2012)

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 lactate dehydrogenase (LDH:3) 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, it is associated with more mechanical damage and loss of viability in platelets than horizontal agitation (Van der Meer et al., 2011; Walther et al., 2010).

2.3.1 Standards and Quality Control of Platelet Concentrates

Quality assurance programs have been developed to monitor the composition and viability of platelet products. Practical difficulties in quality checks, however mean that only a few platelet units can be sampled for these quality parameters. 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 (Tynngeard et al., 2009).

Statistical process control in the production of blood components including platelets has been introduced. In one such study, (Gullikson et al, 2012) it was found that manual methods of platelet production are more difficult to control than automated methods, but the better results can be achieved by using experienced personnel if manual methods are used. Available (Richard et al, 2014) recommendations however shows that quality control programs are more likely to be successful in a national or regional transfusion service than in a hospital based service.

2.3.2 Platelet count and platelet indices in concentrates

According to National Blood Transfusion Service/American Association of Blood Banking recommendations, the platelet count in a unit should be at least $5.5\times I0^{10}$ cells/ ml at the end of the maximal storage period or at issue 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. (Nor Raihan et al 2014)

Doses of platelets, which are determined by platelet count in individual units, have not been standardized. However, a single dose of platelets contains 5.5×10^{10} 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. It is therefore critical that platelet counts and indices be determined in quality control programs since dosage is influenced by platelet count per concentrate. (Gullikson et al, 2012)

Other specifications of platelet components indices 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 (Thibault et al., 2006).

Fresh platelets are discoid in morphology. They slowly convert to spherical shape upon storage which is reflected in a higher MPV. 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) (Thibault et al., 2006).

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. Currently platelet counts and indices are routinely determined by automated cell count employing various principles. Cell counts are usually performed in EDTA which disperses reversible aggregates that occur in citrate after centrifugation (Walther et al., 2010).

2.3.3 Red blood cell content in prepared platelet concentrate

Even in the most refined platelet concentrate are found at least 1.5 mls of contaminating red cells which can immunize an RhD-negative recipient. 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. (Dijkstra-Tiekstra et al, 2011)

Previously, the practice 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. (Richard et al, 2014)

2.3.4 Platelet concentrate volume

Sufficient plasma volume in a platelet unit is required to maintain platelet concentrate, insufficient plasma volume results in lower pH at the end of allowable storage period. The typical final volume is 40-70ml per concentrate but concentrates may be divided into smaller volumes for use in neonates. (Gupta et al, 2011)

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. (Richard et al, 2014) A balance must therefore be struck between sufficient volumes 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.

2.3.5 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. These differ between countries like 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 (Richard et al, 2014).

A drawback is that leuco reduction is still an unaffordable technology in many developing countries including Kenya. Leuco reduction adds to the total unit cost of each product and introduces logistical challenges. Therefore non-leuco-reduced platelet concentrates continue to be transfused in these resource poor settings. (Gupta et al., 2011).

2.4 Bacterial Contamination of platelet concentrates

2.4.1 Blood transfusion and bacterial contamination in Africa

In African countries example, Tanzania blood transfusion services is high because of endemicity of infections causing anemia, malnutrition, surgical and obstetrical emergencies due to blood loss (Emmanuel et al, 2010). There was a high number of voluntary donors from 20% to 80% in 2006 to 2009 respectively which increase number of blood collection from 52,000 units in 2006 to 114,000 units in 2008 (WHO, 2002).

In Africa, there are infectious diseases found to be associated with transfusion of blood and blood components. It was found out that in Cameroon significant Salmonella antibody there titers were more than 10% of apparently donated blood (BiplabenduTalukdar et al, 2017). Although in Nigeria, a study showed that out 200 samples, 106 (53%) were found to be Widal-positive , S. typhi (D) was the commonest bacteria which shown high titers in reaction (48.6%) in donated blood (Tedd et al., 2010).

In East Africa, bacterial contamination of pediatric patients, whole blood transfusions in Kenyan and in Dar es salam, Tanzania hospital showed that 44 bacterial contaminants were isolated from 38 blood packs-an overall contamination frequency of 8.8% (95% confidence interval, 6.1%-11.4%). During platelet culture the bacteria isolated were Gram-negative which accounted to Sixty four percent. Many of these isolates are mostly are found in the environment and the most likely source of contamination was considered to be the hospital blood bank (Tedd et al., 2010). Bacterial contamination of platelets are significant but not recognized hazard of platelet transfusion for children in sub-Saharan Africa (Hassall et al., 1993).

The study conducted in Kenya on quality assurance program of platelet concentrates showed only 51% of platelet concentrates had platelet count of $>5.5\times10^{10}$ against NBTS requirement of >95%. The wide variation in the platelet count was ranging from 0.89-21.50×10^{10} which showed a wide variation in the counts. Taking into account that 30% of the platelets failed to recover on account of platelet storage lesion (Washitani et al., 1988; Gullikso et al., 2012) this suggests that half of the platelets unit transfusions may not have the expected therapeutic effect on the patients. The mean and the standard deviation for platelet count was $6.63\pm4.73\times10^{10}$ was well above the minimum threshold. The SD showed a significant number of units that have extremely low platelet count, becauseonly 51% meet the criteria for minimum platelet count was recommended that all platelet concentrate prepared at Kenyatta National Hospital be subjected to platelet count before issue so that those concentrates that meet the quality standards to be issued to patients for transfusion (Richard et al., 2014).

2.4.2 Blood transfusion and bacterial contamination in settings outside Africa

Each year, approximately 13,898,000 units of prepared red blood cells or whole blood are transfused in the United States alone. This means one unit of the component is being transfused every 2-3 seconds. Despite this large number, sepsis associated with the transfusion of bacterially contaminated red blood cells components is generally regarded as a very rare event (Biplabendu Talukdar et al 2017). From 1976 through September

1998, 26 fatalities thought to be secondary to contaminated whole blood or red cells were reported to the U.S Food and Drug Administration (FDA) (Jafari et al., 2002), approximately one unit of red blood cell-related death per year has been reported.

The study done and reported by reported to the FDA showed that majority of death were caused by *Yersinia enterocolitica*. Another study done in New Zealand reported also the incidence of *Y. enterocolitica* contamination with the rate of 1 in 65,000 and the fatality rate of 1 in 104,000 were from red blood cell units transfused (Sen et al., 2000).

Recent passive reporting studies of bacterially contaminated red blood cells from the United States, France, and the United Kingdom that caused signs of infections show a relative small number of Yersinia cases (Hoppe et at., 1992). For the reported deaths, one was due to a coagulase-negative Staphylococcus (Emmanuel et al., 2010).

Transfusion products, example platelet concentrates, bacterial contamination is a long standing problem which has been partially controlled with modern phlebotomy practices, refrigeration of red blood cells, freezing of plasma, and improved materials for transfusion product for collection and storage. In transfusion set up, it has been an alarming that bacterial contamination of platelet concentrates has been acknowledged as the most frequent infectious risk of transfusion approximately 1 of 2,000–3,000 whole-blood derived from random donor platelets, (hereafter RDP), and apheresis-derived, single donor platelets (Walther et al., 2010).

In Canada, the estimated risk of contamination of blood products with bacterial agents is 1 in 5,000 for platelets and 1 in 30,000 for red blood cells (Kleinman, 2006). It has been proposed that the higher incidence of bacterial transmission is due to the difference in the storage temperatures; also the duration of storage, has a direct correlation with the bacterial contamination (Yomtovian et al., 1993).

In USA bacterial contamination is the second common cause of death from transfusion with mortality rates for platelet-related sepsis ranging from 1:20,000 to 1:85,000 donor

exposures (Ness et al., 2001). Estimates of severe morbidity and mortality range from 100 to 150 were transfused to individuals each year (Biplabendu Talukdar et al 2017). From 1976 to September 1998, 51 fatalities thought to be secondary to contaminated platelets were reported to the U.S. FDA (Jafari et al., 2002), the gram-negative organisms accounted for the majority of deaths (59.7%). In other similar cases from United Kingdom, the United States, France showed that gram-positive organisms were implicated in 41 (71%) of 58 of cases but gram-negative organisms (mostly members of the Enterobacteriaceae) account for the majority (82%) of 11) of the fatalities (Biplabendu Talukdar et al 2017).

2.4.3 Sources of bacterial contamination that compromises the quality of platelet concentrates

Staphylococcus epidermidis and *Bacillus aureas* organisms are mostly recovered from donated blood (implicated in bacterial contamination of platelets). These contamination occurs during phlebotomy, due to incomplete disinfection and/ or skin core removal. These normally organisms do not grow at 1 to 6^0c but can survive well and multiply readily at the platelet storage temperature of 20 to 24^0c (Emmanuel et al., 2010; Tedd et al., 2010).

In the case of gram negative bacterial contamination, asymptomatic donors with transient bacteremia are presumed to be cases of contamination eg *Y. enterocolitica* contamination of red blood cells, is implicated donors typically are found to have elevated titers to Y. enterocolitica, implying recent infection (Emmanuel et al, 2010).

Common skin microbial infections include *Staphylococcus aureas, Staphylococcus epidermidis, Micrococcus* species (a common blood contamination). The organisms *Bacillus* species and Gram negative organisms such as *Yersinia enterococolitica, Escherichia coli, Campylobacter jejuni, Enterobacter* species, and *Salmonella* species are some of the bacterial isolates reported from donated blood. The most common

infective agents to be transmitted through platelet concentrates transfusion are, bacteria (Emmanuel et al, 2010).

2.4.4 Reduction of post transfusion bacteria sepsis

Platelet concentrate bacterial growth inhibition indicates any chemical of natural origin (from any type of cell) which has the effect to kill or inhibit the growth of other types cells. Donor screening on bacterial contamination refers to screening and selecting blood donors using the policies, standards, SOPs and septic agents to minimize risk of bacterial contamination, further the questionnaire may include epidermiological screening including risk for contracting and transmitting bacterial infection. Quality to be maintained, skin preparation on disinfection to reduce the skin bacterial load is mandatory. This refers to standard practice ensuring preoperative skin disinfection which helps to prevent the incidence of surgical site infection (SSI). (Tedd et al., 2010)

2.4.5 Importance of Screening Platelet Concentrates for Bacterial Pathogens

The incorporation of manual screening for bacterial culture of platelet concentrate in addition to use of aseptic techniques during blood collection is the approach with the most potential to increase the efficiency and effectiveness of safe use of platelet concentrates by increasing the quality of the component is required to provide platelets which are safe (Tedd et al., 2010). For that reason, it provides platelet concentrates which are safe and for transfusion into patients who require the platelet concentrate as one of the component, (Biplabendu Talukdar et al., 2017).

However, platelet concentrates for transfusion can be a potential source of infection by a variety of transmissible agents due to human error which occurs during the processing in the laboratory, collection, and transportation of the platelets for administration of concentrates to the patient, which leads to contamination of platelet concentrates with infectious agents (Hillyer et al., 2003).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

This study was conducted at Nairobi Regional Blood Transfusion Centre (NRBTC) located at the National Public Health Laboratories-KNH grounds in Kenya.

3.2 Study Population

The study population was 91 platelet concentrates (units) from male and female who donated blood at Nairobi Regional Blood Transfusion Centre.

3.3 Study design

This was a cross-sectional study at Nairobi Regional Blood Transfusion Centre.

3.4 Sample size determination

NBTS recommends that 95% of all units shall meet quality standards. Therefore sample size calculation was based on the estimated proportion of concentrates not meeting the quality standards (5%) using Fisher's for finite population;

$$n \geq \frac{N Z^2{}_{\alpha/2} P(1-P)}{d^2(N-1) + Z^2{}_{\alpha/2} P(1-P)}$$

Where:

n= minimum sample size 91 required

N=Total estimated population of concentrates in the cold room (N=91)

$Z_{\alpha/2}$= Critical value for standard normal distribution at α-level of significance (α=0.05, $Z_{\alpha/2}$=1.96)

P=Estimated proportion of poor quality platelet concentrates (P= 0.05

d=Margin of error (d=0.05)

The minimum sample size required is; n=91 units

3.5 Inclusion criteria

All platelet concentrates prepared in the period January 2017 and April 2017 ready for issue were included in the study.

3.5.1 Exclusion criteria

Platelet concentrates that had transfusion transmissible infections in the period of January 2017 to April 2017 were excluded in the study.

3.6 Platelet concentrate specimen collection

Platelet Concentrate preparations is derived from Platelet-rich plasma (PRP) (Richard et al, 2014). Briefl**y,** the unique laboratory identification number was used to select 91 of platelet bags from the agitator containing a maximum of 290 platelet bags. Platelet concentrates, each concentrates was assigned identification number for the purposes of the study. The date and time of the platelet concentrates preparations were recorded. The platelet concentrates were then weighed and there volume was determined. The pilot tube was sealed then cut with sterile scissors and 1ml of the unit drawn into two EDTA 75x 15 mm tubes. The tube was then resealed using the tube sealer. The collected samples were taken to Kenyatta National Hospital laboratory for platelet count and National public health laboratory, Nairobi for microbiological investigation.

The platelet bag was sterilized from outside with methylated spirit then was brought at the bench which was sterilized with 10% Sodium hypochlorite, commercially prepared solution. The pilot tubes were folded into four parts the quarter closest to the bag was chosen as a site for puncture. Electrical tube sealer and sterilized needle were used to draw the platelet sample, and they were also sterilized before and after drawing the concentrates for culture. Both broth (Bact/ALERT SN–Aerobic commercially from the manufacturer) and 40 ml bottles already sterilized at 121^{o}C for 15 minutes were supplied by (Biomerieux inc. Durham, NC 27704). (Biplabendu Talukdar et al 2017)

The total volume of 10mls of platelet was drawn and delivered into the bottle of 40mls capacity. Then the cap of the bottle was sterilized with methylated spirit and was packed into a carrier. The bottles were incubated at 37^{0}C for 24 hours.

3.7 Platelet counts

Platelet count, residual WBC count and contaminating RBC count were determined using the Coulter* AcT™5Diff Haematology Analyzer (Beckton-Dickinser) located at the laboratory of the Department of Haematology KNH.

The following formula was used to obtain total cell count for WBC, RBC and Platelet for each concentrate. Cell count/ unit= (Sample cell count/1× Volume (ml) ÷1000

3.8 Platelet concentrates volume formula

The volume of platelet concentrate preparations was determined using the following method:

Volume= weight of concentrate (a)-weight of empty bag (s) ÷1.03

Where 1.03 = specific gravity of plasma

3.9 Testing of platelet concentrates for bacterial contamination:

Culture was performed on Day 0, day 1, 3 and day 5 post preparation of the PCs. Bacterial growth examination was performed by using automated blood culture system (Bact/ALERT SN –Aerobic). The plastic flip-top from the culture bottle was removed and disinfected with an alcohol pad. 10 ml of PCs was obtained by using syringe with aseptic technique and it was transferred into the culture bottle. The bottles were incubated at 37^0C.

The bottle was loaded into the automated blood culture system by scanning the bottle barcode label. The cultured bottle had to be incubated at 37ºC in the automated blood culture system for seven days. If there was positive culture, light was flashing from the automated blood culture system. The cultured units were examined by the principal investigator and two supervisors. Microbiological features of bacterial contamination were described and displayed using photomicrographs as prescribed by (Emmanuel et al., 2010).

The cultured blood bottles of platelet concentrates were observed daily for bacterial growth. After incubation a loopful of each broth suspension was then sub cultured onto MacConkey agar plates every day in three consecutive days. The MacConkey agar plates of platelet concentrates were incubated aerobically at 37ºC for (18-24 hours). After overnight incubation the plates were inspected for any bacteria growth.

3.10 Identification of bacteria

The bacterial growths in the platelet concentrate if any were identified by their colonial morphology characteristics, gram stain reactions, and biochemical test and sugar test (lactose and non-lactose fermentation). (Emmanuel et al, 2010)

3.10.1 Colonial Morphology

On a sheep blood agar the *Staphylococcus* species which appeared as white, non-pigmented colonies without hemolysis after incubation measuring 2.5-6 mm.

3.10.2 Gram staining of bacterial colonies

Gram staining was done using a sterile wire loop where a colony of the positive culture growth from the platelet concentrates was picked and emulsified on the sterile saline on a clean glass slide to form a thin film which was heat fixed before Gram staining was performed; The film was placed on a staining rack over a sink and was covered with 0.5% gentian violet for 1 minute, then it was washed with a thin stream of clean water to remove the excess stain. Then the film was covered with Lugol's iodine and left for 1 minute, the smear was washed in a thin stream of clean water and also decolorized with 50% acetone alcohol solution slowly, one drop at a time till no more blue colour comes out of the smear, the stain was counterstained with dilute Neutral red for 30 seconds. Then washed in a thin stream of clean water to remove excess stain and was air dried. A drop of immersion oil was added on the smear, and was placed on the microscope stage. This was examined under oil immersion objective (100 X magnifications) to examine the presence of Gram positive or Gram negative bacteria. These microscopically appeared as gram-positive cocci 0.5-1.5mm in diameter occurring singly, in pairs or short chains, and as irregular grape-like clusters indicating the presence of Staphylococcus species. This procedure was as according to (Emmanuel et al., 2010).

3.10.3 Biochemical testing of *Staphylococcus* species

Bacteria colonies of Staphylococcus were identified by using Catalase, oxidase, Coagulase and Novobiocin tests.

310.4 Catalase Test

Catalase Test was used to identify these species; by using sterile glass rod a good growth of the test organism was picked from the media and immersed in the test tube containing 2-3mls of hydrogen peroxide solution. Immediate bubbling was observed which indicated the presence Staphylococcus species because they do produce the enzyme

catalase which breaks down the hydrogen peroxide into water and oxygen. This distinguishes them from Streptococci by (Tedd, C, et al, 2010)

3.10.5 Coagulase test

Coagulase test: A colony of growth was picked from the media and mixed with plasma after 2-3 mins coagulation was observed. Coagulase test was used to differentiate Staphylococcus aureus which produces the enzyme coagulase, from Staphylococcus epidermidis and Staphylococcus saprophyticus which do not produce coagulase. (Biplabendu Talukdar et al 2017)

3.10.6 Novobiocin Test

Novobiocin Test by using Kirby-Bauer (Biplabendu Talukdar et al, 2017) test technique to inoculate each Mueller-Hinton plate with one of the three Staphylococcus species; A diluted stock culture was prepared by transferring 0.25 ml of stock culture into a labeled, sterile tube containing 5 ml mixed well by finger vortexing, a sterile swab was dipped into the diluted culture, the excess inoculum was removed by pressing the saturated swab against the inner surface of the tube, the swab was used to streak the surface of the plate in a horizontal direction, then the plate was turned at 90° and the surface was streaked in the other direction.

Then when the surface of the plate appears dry, a Novobiocin disc was placed at the centre of the Mueller-Hinton plate. And the plates were incubated for 48 hours. After incubation, the zone size was measured. Any measurement equal to or less than 16 mm and 17mm or larger indicated resistance and susceptibility respectively. Therefore, Novobiocin test was used to identify Staphylococcus epidermidis from Staphylococcus saprophyticus. Therefore the test identified Staphylococcus epidermidis.

3.11 Data management and analysis

Data was entered in pre-prepared data sheets. These were presented in figures and tables representing the means, medians, standard deviation, and percentages. STATA version 13 Statistical package was used analyze the samples. After the analysis platelet concentrates were categorized 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.

3.12 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.

3.12.1 Pre-analytical process Specimen collection

The samples were obtained directly from the bag through the tubing after thorough mixing at the time of issue. EDTA vacutainers were used for samples taken for cell counts. A unique number was used to label each tube immediately after sampling. The samples for cell counts were held for at least one hour at room temperature to allow EDTA to disperse any reversible aggregates. Samples for culture were cultured within one hour of collection. 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

3.12.2 Analytical process

The control results for cell counts for the day were examined and deemed satisfactory before each run was made. Whereby the internal control and self-calibration results for the blood haematological analyzer were examined and recorded before analysis. The quality of the platelet concentrate was maintained and the correct sample number was

keyed into the haematological analyzer just before analysis. The sample runs were then carried out only when the control results were acceptable. One sample for cell counts from each batch was analyzed by a different instrument Cell Dyn-3000 located at the Department of Hematology and the results of inter-laboratory quality control for cell counts compared using coefficient of variation.

3.13 Ethical Review

Ethical review and approval was obtained from the KNH/UoN ERCP672/09/2016. Sample privacy and confidentiality was strictly observed that all procedures was performed in a standard manner, and maximize benefit the donors. The recommendation was sent back to NRBTC to guides them so that they make decision.

CHAPTER FOUR

RESULTS

4.1 Introduction

Ninety-one (N=91) prepared platelet concentrates were examined for quality. The quality indicators in this study included platelet count, platelet concentrate volume, residual white blood cell count, red blood cell count, and level of bacterial contamination post preparation.

Table 4.1: Platelet concentrate quality assessment using five quality indicators

	Platelet parameters				**Bacterial Contamination**		
	PLT 10*9/I	V ML	RWBC (10^{10}/L)	RBC (10^{9}/L)	*S* Aureus	*S* Epidermids	*S* Soprophyticus
MEAN	242.4945	62.96703	0.509868	0.199835	0.0549451	0.043956044	0.021978022
Stad Dev	67.00354	5.309217	0.487404	0.21003	0.2291354	0.20613307	0.147423952
Range	399	25	3.168	0.964	1	1	1
Max	451	70	3.19	0.964	1	1	1
Min	52	45	0.022	0	0	0	0
Range	399	25	3.168	0.964	1	1	1
Poor quality (n)	**4**	**0**	**15**	**5**			
Poor quality (%)	**4%**	**0%**	**16%**	**5%**			
Good quality (n)	**87**	**91**	**76**	**86**			
Good quality (%)	**96%**	**100%**	**84%**	**95%**			
TOTAL	**91**	**91**	**91**	**91**			

4.2 Platelet concentrate count

Platelet concentrates were considered to have met quality standards if the platelet count was $\geq 150x10^9$ cells/l, only of platelet concentrates 87(96%) were of good quality (Gullikson et al., 2012). Not all the 91 platelet concentrates met had the recommended platelet count (Richard et al., 2014) (Table 4.1). The mean platelet count was 242.4945 while the range was 399 (Appendix V)

4.3 Platelet concentrate volume

Platelet concentrate volume was considered to have met good quality standards if the platelet ranged between 40-70ml 91(100%) (Gupta et al., 2011) All the 91 platelet concentrates met the recommended platelet volume (Richard et al., 2014) (Table 4.1). The mean platelet count was 62.96703 and SD was 5.309217 while the range was –25 (Appendix V)

4.4 Residual white cell count

White blood cell count was considered to have met the recommended standards if the white cell count was $\leq 0.83x10^9$ cells/l (Richard et al., 2014). Out of 91 units, 76(84%) had the recommended good quality of white blood cell count (Gullikson et al., 2012) (Table 4.1). The mean RWBC was 0.509868, SD was 0.487404 and range was 3.168 (Appendix V)

4.5 Red blood cell content

Red blood cell content was considered to fall within the recommended range if the count was $\leq 0.06x10^9$ cells/l (Dijkstra et al., 2011). Out of the 91 concentrates, 86(95%) had the required RBC count (Richard et al., 2014, Gullikson et al 2012) (Table 4.1). The mean RBC count was 0.199835 and SD was 0.21003 while the range was 0.964 (Appendix V)

4.6 Bacterial contamination in the platelet concentrates on post preparation.

The 91 prepared platelet concentrates were assessed for bacterial contaminations on day 0, 1 and 3, Out of 91 platelet concentrates 80(88%) samples were negative for bacterial culture test (Tedd et al., 2010). At day 5, 11/91 (12.1%) tested positive for bacteria culture (Table 4.1). The mean for bacterial contamination in platelet concentrates S Aureus was 0.0549451, S Epidermidis was 0.043956044 and S Saprophyticus 0.021978022 (Emmanuel et al., 2012).

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

Functions of circulatory transfused platelets depend upon ex-vivo storage lesion and also in the status of the in- vivo environment of transfused individuals Platelet concentrate (Richard et al., 2014). These platelet concentrates are transfused within 24 to 48 hours of donation, and they have high recovery, with good survival and preserved function. This concurs with other studies done in US (Nor Raihan et al., 2014).

The majority of the platelet concentrates prepared were issued on day 1. According to records at the NRBTC, most of the platelet concentrates were issued within 24 hours of processing, however the bag used allowed storage for up to 5 days. Several studies shows that platelet concentrates transfused within 24-48 hours have little platelet storage lesson compared to stored platelets (Tedd et al., 2010). At NRBTC, blood components including platelet concentrates are processed and issued on demand.

Only 87(96%) out of the 91 platelet concentrates prepared, showed platelet count of $>5.5\times10^{10}$ against NRBTC requirement of the set standards. In this study, the mean and standard deviation for platelet count was 242.4945×10^{10} which was the threshold. The SD was 67.00354 showed a number of platelet units with normal platelet count of 96% that met the criteria for the minimum platelet count. This can be due to standardization in preparation procedures, especially in centrifugation and separation. A total of 4(4%) of the platelet concentrates were poor quality that returned platelet count of less than $<5.5\times10^{10}$ per bag (Richard et al., 2014). There parameters were compared with those returned higher counts.

The mean volume of platelet concentrates in this study was 62.96703 and SD of 5.309217 mls, a total of 91(100%) of the concentrates were between 40-70mls, therefore meeting the NRBTC standards set. In another study by (Fasola et al., 2002) the mean

volume of the platelet concentrates was considerably low (18.52ml) whereas Singh (Singh et al., 2003) reported a mean of 62.30±22.68ml. Other similar studies have shown that as little as 35-40mls of plasma can maintain adequate buffer in platelet concentrates, (Adam et al., 1987).

AABB has not specified the minimum of platelet concentrates volume, simply stating that the final volume of platelet concentrate to be adequate to buffer the pH to >6.2, however NRBTC has set the minimum platelet volume of 40 mls. Higher volumes platelet concentrates may be necessary in this setting to maintain the adequate buffer since neither leuco-reduction nor testing for pH was not performed on platelet concentrates in this study. These results was exposing the recipients to high volumes of plasma instead of platelet, and may lead to minor incompatibility reactions if platelets are not group matched, anaphylaxis will occur due to IgA, or other allergic reactions due to plasma proteins (Fung et al., 2007). More studies are required to establish the minimum volume required to maintain platelet viability where leuco reduction is not performed.

There was a difference in the RWBC and RBC counts between these platelet concentrates (Richard et al., 2014). In a similar study at tertiary Hospital in Nigeria where platelet concentrates were prepared by PRP method, (Fasola et al., 2002) it was found that only 35% of concentrates met the minimum 5.5×10^{10} platelet count threshold. The mean ±SD for that study was 4.17±3.95 as wide range as in this study. Sing RP (Singh et al., 2003) reported a mean value of 7.6±2.97 which is well above the 5.5×10^{10} threshold in another study in which he assessed PRP, BC and apharesis platelets.

These studies are demonstrating such wide variations of the means reported for the same method in various regions suggesting the variability in platelet concentrate production method between regions. Low residual WBC counts the in platelet concentrate preparations are desirable in order to reduce the side effects of the residual leucocytes like as febrile reactions and to reduce the platelet storage lesson which can be enhanced by high leukocyte count (Seghatchian et al., 1991). In this study the concentrates had

RWBC 15(23.1%) contaminant however RBC contaminant was high 86(95%). and 5(5%) of RBC did meet the criteria of the standards set by (AABB 2003), this level of RBC contamination were considerably higher than the results reported from similar studies (Richard et al., 2014).

(Sing et al., 2003) reported mean RWBC count of 40.5±4, 8×106 for PRP method which is far much higher than these levels established in this study (0.51±0.49×10.6) even though no leuco-reduction performed in this study. These study for RWBC count, the platelet counts suggests marked in there counts due to variability in process which is higher than those reported by (Fasiola et al., 2002) found that 30% of platelet concentrates in his study met the criteria with no any red blood cell contamination.

Currently NRBTC does have the guidelines for residual red cell contamination. It shows that as low as 0.5 mls of red cells in a platelet unit are sufficient to cause RHD and ABO alloimmunisation (Menitove et al., 2002) especially if continue issuing platelets that are not cross matched has been adopted. AABB has recommended that each transfusion facility to formulate there policy which should be followed if they have to transfuse ABO group incompatible of platelet concentrates. NRBTC has adopted the policy of issuing only ABO compatible platelet concentrates (Dijkstra et al., 2011).

However, there are instances where by ABO group incompatible platelet concentrates may need to be used in case of emergency settings or when there are no compatible platelets available. In such cases, this high level of red blood cell contamination of platelet concentrates may be a cause for concern for further adverse reaction other than those rare effects which would arise from transfusion group matched red blood cells (Richard et al., 2014).

As illustrated in this study, the general effect of the red blood cell counts, residual WBC count, platelet count, volume and bacterial contamination on day 0, day 1, 3, and day 5 post platelet preparations expected of very high platelet count in a single unit of the platelet concentrate, they appear to accelerate the storage lesson, especially if other

parameters such as adequate RWBC, presence of bacterial contamination, and RBC count are not fulfilled (Richard et al., 2014).

In this study, bacterial contamination was assessed on day 0, day 1, 3 and day 5 post platelet preparation respectively. The aim of this study was to determine the quality platelet concentrates prepared in NRBTC, we noted that there were low bacterial contamination in this center, which was about 11(12.0%) and most of them were nonpathogenic bacteria**.** This finding was considered low compared to other studies like in Ghana but other researchers reported levels as high as 17.5% (Opoku et al., 2009; Mac Donald et al., 1998; Morel et al., 2003) which is higher than the values obtained in this study.

This study findings showed the organisms of Staphylococcus epidermidis, Staphylococcus aureas, and Staphylococcus saprophyticus were isolated which is similar to the study conducted by other researchers which categorized the platelet units into group 1 to VIII totaling to 214 units in group VI, only three (1.4%) had bacterial contamination, with two occurrences of coagulase-negative Staphylococcus species plus which were an unclassified gram-negative rods and one instance of coagulase-negative Staphylococcus species (Emmanuel et al, 2010; Biplabendu Talukdar et al., 2017).

In total 523 of blood units were cultured and out of (309 from Group V and 214 Group VI), 2.9% (assuming 12 units contaminated in Group V) and evidence showed the presence of bacterial contamination. A total of 43 units in Group VII, only two (4.7%) showed evidence of bacterial contamination, where one unit grew-variable rods, and the other grew an Enterococcus species. Out of 54 units Group VIII none was positive for bacterial growth or signs of contamination. 97 of blood units were cultured from 13,641 units produced, 2(2.1%) showed evidence of contamination. (Tedd et al., 2010; Biplabendu Talukdar et al., 2017).

Although the mean prevalence of bacterial contamination in whole blood derived, RDP platelets was 33.9 per 100,000 units and for apheresis-derived, platelet units is 51.0 per 100,000 units. For RBCs was 2.6 per 100,000 units (Tedd et al., 2010; Emmanuel et al., 2010).

The NRBTC itself does not have data on blood bank bacteraemia, platelet transfusion sepsis or mortality records for comparison, as reported by others studies (Biplabendu Talukdar et al., 2017) because it has been perceived that transfusion related sepsis or mortality are rare phenomena. Probably low quantity of inoculum of bacteria occurs in the platelet concentrates only results in transient bacteraemia on transfusion (Emmanuel et al., 2010, Biplabendu Talukdar et al., 2017) or because of antibiotic coverage (Emmanuel et al., 2010) which masks the symptoms. Proper blood donor skin disinfection has long been recognized as the only way to reduce blood contamination (Tedd et al, 2010).

The cultures performed on the platelet concentrates was considered with an aim of studying two aspects of blood components contamination: the presence of transient of bacteraemia in blood donors is due to collection of contaminated blood with normal flora on the skin with the needle prick in the first milliliter of collected blood. In this case, the study findings showed the contamination of 11(12.0%) and the only positive samples were contaminated with common skin contaminant known as normal (Staphylococcus epidermidis, Staphylococcus aureus, and Staphylococcus saprophyticcus).

Culture method were performed using Bact/ALERT and aerobic bottles was not able to prevent transfusion of the contaminated platelet which were identified in this study. In other studies have shown screening methods proposed to reduce the risk of sepsis due to contaminated platelets which lacks sensitivity or specificity (Biplabendu Talukdar et al., 2017) (Tedd et al., 2010) or too expensive. Therefore, it has been suggested that with regulatory agencies, pathogen inactivation systems are suitable for cellular components and they should be a more effective approach to reduce the risk of transfusion which is

associated with sepsis that have approach based upon the screening tests which is currently available (Blajchman et al., 2005). In this study the isolates obtained in this were skin associated organisms and they are considered as contaminants that are either related to procedure used during donor bleeding, or taking platelet sample for culture (Tedd et al, 2010).

5.2 Conclusion

In conclusion, the quality of platelet count, volume of platelet concentrates, RWBC count, red cell content, concentrates were assessed at NRBTC, the study findings demonstrated the need to adhere to SOPs in the blood donor blood collection and preparations of platelet concentrates**.** This study has also demonstrates that bacteraemia prepared platelet concentrate for platelet count can form an integral part of a rapid, simple and practical method for validation of collection and improve its quality. Currently at NRBTC bank supply do not assess the level of bacterial contamination in the platelet concentrates risk for transmission to patients. Implementation of quality using the SOPs will help in continuous quality checks as well as entrench a standardization and harmonization program on platelet quality monitoring. The study findings in this study were compared with the standards set by American Association of blood banking (AABB) though not all the platelet concentrates met the criteria.

5.3 Recommendation

This study recommends the quality of platelet count, volume of platelet concentrates, RWBC count, red cell content, have not been assessed at NRBTC hence, there is need to improve on the existing SOPs at the blood storage centers. RBC count should be (<0.06), the presence of RBC is known to cause transfusion incompatibility if platelet concentrates is not cross matched and counted as a contaminant. Also the study findings shows need to improve on the prevention control measures for blood collection and storage procedure to adhere on their SOPs in order to improve safety precautions, protocols/ quality assurance especially during blood collection and any other procedures

involving during blood collection to reduce the risk of contaminating blood units with bacteria. Recommendations advocate enhanced testing or pathogen reduction inactivation strategies (or both) further improve platelet safety.

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APPENDICES

Appendix I: Data Collection Sheet

Date of Sampling Date of processing Data of processing

Sample number KNBTS no

Platelet Count WBC

PDW

MPV

PH ABO/D

Volume

Cell count/unit sample cell count/1<volume (mls)

1000

Comments

Signed: **Signed:**

Technologist **investigator**

DateDate

Appendices II: Procedure on sample collection

1. All whole blood per unit requiring separation for platelets was processed for RBC, platelet count and volume of the unit was performed before spinning by the principal Investigator.
2. Platelet concentrate was analyzed for:

- Volume
- Sorting using Transmissible transmitted infections (TTIs) work sheet separate infected samples.
- Analyzing
- Labelling- quality controlled by the principal investigator

3. All the platelet concentrate bags was clumped prior to identification and separation of study samples.
4. For study specific platelet concentrates:
5. All platelet concentrates was arranged/ accorded study numbers as they arrived for processing.
6. At least 4 samples was picked at every 2^{nd} sample from a consecutive batch.
7. One tube of 2 ml EDTA vacutainer and blood agar culture bottle 10ml was labelled for each platelet concentrate bags picked.
8. Each platelet concentrate bag was appropriately mixed and a 2 ml sample aliquot was labelled into each vacutainer using a 5cc and 10cc syringe and needle 23 gauge.
9. Each study unit bag was sealed appropriately for further management
10. Each vial was tested / processed for

- Cultures using macConkey Agar and blood agar bottles were used
- Platelet count using haematological analyzer
- WBC count using Haematological analyzer

11. The culture plates was incubated at $35^0c \pm 2^0c$

12. The plates was read every 12 hours for 48 hour
13. The plates was read every 12 hours for 48 hours
14. On the 5^{th} day culture plates was incubated at $35^0c \pm 2^0c$
15. The plates was read analyzed on the 5^{th} day
16. The remaining samples was discarded.

Appendices III: Cell Counts

Platelet count, residual WBC count and Red cells were determined by Coulter Hematology Analyzer (Beckton–Dickinser). The following formula was used to obtain the total cell count for WBC and Platelets for each concentrate

$$\text{Cell count/unit} = (\text{sample cell count}/1 \times \text{Volume (ml)}) \div 1000$$

Volume

Volume was determined using the following formula:

$$\text{Volume} = (\text{weight of concentrate} - [\text{weight of empty bag(g)}]) \div 1.03$$

(*Where 1.03=specific gravity of plasma*)

Appendices IV: Culture Media

MacConkey Agar Description

1. MacConkeys agar showed both lactose and non-lactose fermenting colonies. Lactose fermenting colonies were pink whereas non- lactose fermenting ones were colorless.

Preparation of MacConkey Agar

1. Suspend the measured amount of powder in 1L purified water and mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121^0c for 15 minutes.

Expected Colony characteristics in MacConkey Agar

Lactose- fermenting organisms grows as pink to brick red colonies with or without a zone of precipitation bile.

Non-lactose fermenting organisms grow as colorless or clear colonies.

Procedure for Gram stain.

A drop of normal saline was placed on a clean glass slide. Using sterile wire loop (by flaming) a colony of the culture was emulsified on the sterile saline to form a thin film and this was heat fixed and Gram staining was performed; The film was placed on a staining rack over a sink and it was covered with 0.5% gentian violate for 1 minute, it was washed with a thin stream of clean water to remove the excess stain. Then the film was covered with lugols iodine and left for 1 minutes, the smear was washed in a thin stream of clean water and also decolorized with 50% acetone alcohol solution slowly, one drop at a time and stop as soon as no more blue color comes out of the smear, then it Was counterstained with dilute carbol fuchsin for 30 seconds. The smear was washed in a thin stream of clean water to remove excess stain and it was air dried on a draining

rack. A drop of immersion oil was added on the smear, and was examined with power (100 X magnifications) for the presence of Gram positive or Gram-negative.

Novabiocin disc. A diluted stock culture was prepared by transferring 0.25 ml of stock culture into a labeled, sterile tube containing 5 ml. mixed well by finger vortexing, a sterile swab was dipped into the diluted culture, the excess inoculum was removed by pressing the saturated swab against the inner surface of the tube, the swab was used to streak the surface of the plate in a horizontal direction, then the plate was turned at 90° and the surface was streaked in the other direction. Then when the surface of the plate appears dry, a Novobiocin disc was placed at the center of the Mueller-Hinton plate. And the plates was incubated for 48 hours at 37^0c. After incubation, the zone size was measured. Any measurement equal to or less than 16 mm indicated resistance to Novobiocin. A zone 17 mm or larger indicated susceptibility to Novobiocin.

Appendices V: Raw Data

		Platelet parameters				Bacterial Contamination			
S NO	DONOR NO	PLT 10*9/l	V mL	RWBC (10^{10}/L)	RBC (10^{9}/L)	*S Aureus*	*S Epidermids*	*S Saprophyticus*	
1	105412	198	58	**0.339**	0.222	0	0	0	**1**
2	105414	**201**	56	**0.346**	0.29	0	0	0	**1**
3	105415	**228**	62	0.872	0.294	1	0	0	0
4	106576	**158**	56	**0.396**	0.44	0	0	0	
5	105404	**174**	45	**0.326**	0.231	0	0	0	
6	105402	**230**	64	**0.659**	0.323	0	0	0	
7	105406	**226**	64	**0.342**	0.964	0	0	0	
8	105400	**217**	64	**0.52**	0.409	0	1	0	
9	105392	**327**	63	**0.587**	0.694	0	0	0	
10	105388	**350**	56	**0.312**	0.343	0	0	0	
11	105311	**157**	55	**0.228**	0.786	0	0	0	
12	105395	**284**	65	**0.123**	0.122	0	0	0	
13	105396	**203**	56	**0.224**	0.483	0	0	0	
14	105409	**237**	67	**0.369**	0.295	0	0	0	
15	105304	**320**	66	**0.571**	0.824	0	0	0	
16	105405	**192**	55	**0.123**	0.34	1	0	0	
17	105408	**285**	64	**0.312**	0.19	0	0	0	
18	105420	**207**	56	**0.242**	0.362	0	0	1	
19	105419	**220**	62	**0.372**	0.079	0	0	0	
20	105499	**340**	66	**0.231**	0.223	0	0	0	
21	105406	**237**	65	**0.081**	0.48	0	0	0	
22	105387	125	61	**0.112**	0.571	0	0	0	
23	105418	**232**	62	**0.52**	0.424	0	0	0	
24	105414	**168**	64	1.35	0.257	0	0	0	
25	105417	**158**	68	**0.184**	0.201	0	0	0	
26	105615	**331**	60	**0.037**	0.542	0	0	0	
27	105596	**422**	65	**0.381**	0.181	0	0	0	
28	105603	**251**	61	**0.088**	0.528	0	0	0	
29	115593	**295**	69	**0.453**	0.272	1	0	0	
30	105585	**196**	69	**0.613**	0.391	0	0	0	
31	105581	**218**	65	1.036	0.23	0	0	0	
32	105586	**330**	70	**0.528**	0.172	0	0	0	
33	105595	**304**	67	**0.242**	0.206	0	0	0	
34	105594	**191**	49	1.03	0.022	0	0	0	
35	105589	**357**	62	1.06	0.221	0	0	0	

36	105604	**259**	45	0.903	0.279	0	0	0
37	105602	**279**	64	1.41	0.429	0	0	0
38	105590	**233**	66	**0.365**	0.228	0	0	0
39	105588	**222**	63	**0.456**	0.121	0	0	0
40	105592	**192**	50	1.49	0.04	0	0	1
41	105597	**272**	70	**0.791**	0.355	0	0	0
42	105613	141	64	**0.444**	0.331	0	0	0
43	105587	**189**	69	1.37	0.06	0	1	0
44	105367	**270**	70	**0.258**	0.8	0	0	0
45	105368	**190**	64	**0.237**	0.05	0	0	0
46	105360	**220**	64	**0.374**	0.04	0	0	0
47	105355	**170**	70	**0.773**	0.06	0	0	0
48	105359	**302**	67	**0.395**	0.01	0	0	0
49	105356	**202**	70	**0.342**	0.08	0	0	0
50	105365	**189**	62	**0.083**	0	0	0	0
51	105372	**230**	70	**0.216**	0.06	1	0	0
52	105370	**245**	66	**0.119**	0.12	0	0	0
53	105369	**300**	67	**0.288**	0.05	0	0	0
54	105366	**197**	61	**0.224**	0.18	0	0	0
55	105361	**200**	69	**0.165**	0.04	0	0	0
56	105371	**310**	70	**0.442**	0	0	0	0
57	105362	**198**	67	**0.304**	0.08	0	0	0
58	105358	**280**	68	**0.447**	0.04	0	0	0
59	105373	**250**	51	**0.529**	0.04	0	0	0
60	105737	**276**	64	**0.32**	0.11	0	0	0
61	105735	**165**	60	**0.18**	0.07	0	0	0
62	105738	**263**	62	**0.22**	0.2	0	0	0
63	105740	**249**	65	1.18	0.06	0	0	0
64	105745	52	61	3.19	0.03	0	0	0
65	105736	**238**	62	0.88	0.1	0	0	0
66	105741	**451**	66	**0.74**	0.07	0	0	0
67	105743	**178**	62	**0.4**	0.11	0	0	0
68	105742	**250**	62	1.71	0.03	0	0	0
69	105750	**201**	67	**0.17**	0.03	0	0	0
70	105751	119	68	**0.38**	0.05	0	0	0
71	105749	**285**	60	**0.52**	0.02	0	1	0
72	105748	**374**	63	**0.17**	0.02	0	0	0
73	105752	**275**	66	**0.28**	0.01	0	0	0
74	105753	**252**	66	1.81	0.08	0	0	0
75	105744	**179**	61	**0.34**	0.12	0	0	0
76	105754	**239**	64	**0.34**	0.05	0	0	0

77	105739	**291**	66	**0.41**	0.18	0	0	0	
78	105755	**242**	61	**0.29**	0.04	0	0	0	
79	105746	**385**	64	**0.21**	0	0	0	0	
80	105747	**250**	56	**0.26**	0.04	1	0	0	
81	105494	**245**	63	**0.35**	0.08	0	0	0	
82	105764	**306**	66	**0.022**	0.04	0	0	0	
83	105763	**170**	67	**0.231**	0.04	0	0	0	
84	105761	**214**	55	1.81	0.07	0	0	0	
85	105762	**307**	64	**0.43**	0.02	0	0	0	
86	105760	**238**	63	**0.23**	0.17	0	0	0	
87	105759	**196**	64	**0.53**	0.01	0	0	0	
88	105758	**260**	66	**0.231**	0	0	0	0	
89	105767	**279**	62	**0.23**	0.02	0	0	0	
90	105765	**191**	64	**0.32**	0.02	0	1	0	
91	105766	**338**	66	**0.38**	0.01	0	0	0	
Poor quality (n)		4							
Poor quality (%)		**4.4**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Good quality (n)		87							
Good quality (%)		**95.6**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

MEAN	242.4945	62.96703	0.509868	0.199835	0.0549451	0.043956044	0.021978022
Stad Dev	67.00354	5.309217	0.487404	0.21003	0.2291354	0.20613307	0.147423952
Range	399	25	3.168	0.964	1	1	1
max	451	70	3.19	0.964	1	1	1
min	52	45	0.022	0	0	0	0
Range	399	25	3.168	0.964	1	1	1

Poor quality (n)	4	0	15	5
Poor quality (%)	4%	0%	16%	5%
Good quality (n)	87	91	76	86
Good quality (%)	96%	100%	84%	95%
TOTAL	91	91	91	91

	Platelet parameters	Bacterial Contamination

	PLT 10*9/l	V mL	RWBC (10^{10}/L)	RBC (10^9/L)	*S Aureus*	*S Epidermids*	*S Saprophyticus*
MEAN	242.4945	62.96703	0.509868	0.199835	0.0549451	0.043956044	0.021978022
Stad Dev	67.00354	5.309217	0.487404	0.21003	0.2291354	0.206133307	0.147423952
Range	399	25	3.168	0.964	1	1	1
max	451	70	3.19	0.964	1	1	1
min	52	45	0.022	0	0	0	0
Range	399	25	3.168	0.964	1	1	1

Poor quality (n)	**4**	**0**	**15**	**5**
Poor quality (%)	**4%**	**0%**	**16%**	**5%**
Good quality (n)	**87**	**91**	**76**	**86**
Good quality (%)	**96%**	**100%**	**84%**	**95%**
TOTAL	**91**	**91**	**91**	**91**