THE UNIVERSITY OF ZAMBIA
SCHOOL OF MEDICINE
DEPARTMENT OF ANAESTHESIA AND CRITICAL CARE

THE VIABILITY OF WHOLE BLOOD AND PACKED CELLS AT THE TIME OF TRANSFUSION AT THE UNIVERSITY TEACHING HOSPITAL IN LUSAKA ZAMBIA
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A dissertation submitted in partial fulfilment of the requirement for the award of the degree of Masters of Medicine in Anaesthesia and Critical Care.
DECLARATION

I declare that this dissertation herein presented for the Degree of Master of Medicine in Anaesthesia and Critical Care is the work of my own and has not been previously submitted either wholly or in part for any other Degree at this or any other University nor is it being currently submitted for any other Degree.

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Date : .................................

Examiner 03 : .................................
Signature : .................................
Date : .................................
DEDICATION

To my beloved wife and children who have always been understanding and encouraged me to do more. To my mum and dad who have always believed in me and made sacrifices to make me the man I am today.
ACKNOWLEDGEMENTS

I would like to thank my supervisors Dr. Joseph Mulenga and Dr. Dylan Bould for their invaluable guidance. I wish to thank my Head of Department of Anaesthesia and Critical Care, Dr. Feruza Ismailova for allowing me some time to work on my thesis. I wish to sincerely thank Mr. Patrick Kaonga for his help with data analysis and for his training me to be able to analyze and interpret data. I also wish to thank Mr. Joseph Ngulube for his professional invaluable help in handling the samples and ensuring no time lag for quality results. I would also like to thank Dr. Kowa for his invaluable help with interpretation of the positive blood cultures. I thank the nurses from the various wards who notified me whenever there was a blood transfusion about to happen. Last but not the least I would love to thank you the physicians and surgeons for their corporation in managing patients with positive blood culture results.
**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>PH</td>
<td>Preponderance of Hydrogen Ions</td>
</tr>
<tr>
<td>TA</td>
<td>Transfusion-Associated Dyspnoea</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
</tr>
<tr>
<td>UTH</td>
<td>University Teaching Hospital</td>
</tr>
<tr>
<td>2, 3 DPG</td>
<td>2, 3 Diphosphoglycerate</td>
</tr>
<tr>
<td>K+</td>
<td>Potassium</td>
</tr>
<tr>
<td>RBCs</td>
<td>Red Blood Cells</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>FFP</td>
<td>Fresh Frozen Plasma</td>
</tr>
<tr>
<td>TTI</td>
<td>Transfusion Transmitted Infection</td>
</tr>
<tr>
<td>QC</td>
<td>Quality Control</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
</tr>
<tr>
<td>ZNBTS</td>
<td>Zambia National Blood Transfusion Service</td>
</tr>
<tr>
<td>UNZA</td>
<td>University Of Zambia</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>ATCC</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td>S. Aureus</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>E. coli</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>H. influenza</td>
<td>Haemophilus influenza</td>
</tr>
<tr>
<td>LF</td>
<td>Lactose fermenting</td>
</tr>
<tr>
<td>LIS</td>
<td>Laboratory information system</td>
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**ABSTRACT**

**Introduction.** In low- and middle-income countries (LMICs) the maintenance of standards relating to the transport and storage of blood may be more difficult to achieve. Bacteria contamination and hyperkalemia represent two significant risks. The objectives of this study were to assess the viability of transfused blood defined by negative blood culture and potassium concentration of less than 42mmol/l.

**Methods.** Samples from 84 units of whole blood and packed cells were collected aseptically and analyzed to determine the presence of bacteria by culturing and the level of potassium. Method of storage/transport once the units left the blood bank (cool box or room temperature) was also recorded.

**Results.** 8 samples (10.5%) showed a positive culture and the organisms isolated included Pseudomonas fluorescens, Corynabacterium, Acinetobacter baumannii and Staphylococcus capitis. Only 12.5% of the culture positive units were stored in cooler boxes compared to 35.5% of the culture negative units. The mean potassium content was 12.25mmol/l (±7.4SD). None of these were outside the expected range for stored blood. However, blood stored at room temperature was found to have a higher potassium concentration than blood stored in a cool box. The median time between blood leaving the blood bank to the time the transfusion was actually commenced was 6(IQR±6) hours and a range of 1-14hours.

**Conclusions.** Under the prevailing circumstances at the University Teaching Hospital, bacterial contamination remains a significant risk in recipients of whole blood and packed cells. The current practice, therefore, needs improvement. This study recommends continued medical education in the transport and storage of blood and blood products.
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<td>5</td>
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1.0 INTRODUCTION

Since the 17th century, transfusion medicine has drastically evolved and blood transfusion has become a lifesaving intervention. An estimated 80 million units of blood are donated annually worldwide. Of these, only 38% come from the “Low- and Middle-Income Countries.” The shortfall has a particular impact on women with complications of pregnancy, trauma victims and children with severe life-threatening anaemia. A single unit of whole blood costs about US $40 to produce. Hence, stringent measures have to be instituted in the storage and transport of this precious scarce resource to ensure its effective use and cost effectiveness.

The recognized key hazards of transfusion include viral and bacterial infections, immunomodulation (accumulation of non-specific soluble immune mediators in stored blood), specific hazards (transfusion-associated circulatory overload and transfusion-related acute lung injury), immediate haemolytic transfusion reaction, delayed haemolytic transfusion reaction, acute non-haemolytic transfusion reactions (febrile or allergic, reactions with febrile and allergic features and hypotensive reactions), transfusion-associated dyspnoea (TAD), post-transfusion purpura, transfusion-associated graft vs. host disease and transfusion-related iron overload. This has led to strides being made regarding haemovigilance to reduce and prevent transfusion related complications. Transfusion of infected blood contributes to an ever-widening pool of infection in the general population with far-reaching consequences for society as a whole. Hazards of transfusion increase with increasing dose and age of whole blood and blood products.

A five year evaluation of transfusion outcome in a resource-limited setting of Cameroon revealed that blood transfusion is still unsafe in many resource limited communities of developing countries. Of the 40,134 donations, only 80% of the donated blood was considered safe for distribution with 20% of donated blood being rejected for positive HIV or Hepatitis B antigen results. More than 50% of transfusions within the hospital were associated with an unfavourable outcome the most common being febrile reactions (40.1%) followed by urticaria (19.4%). Acute intravascular hemolysis, circulatory overload and deaths occurred in 0.01%, 0.04% and 0.14% of cases respectively.

Storage of whole blood, packed cells and other blood products at room temperature for longer hours prior to transfusion favours the growth of bacteria and contributes to the unfavourable outcomes of blood transfusion. It has become evident that stored red blood cells/whole blood undergo time-dependent
metabolic, biochemical and molecular changes. These “storage lesions” may be responsible for the many adverse effects of red blood cell/whole blood transfusion. Also clinically, the age of red blood cells/whole blood has been associated with multiple organ failure, post-operative pneumonia and wound infection. This also results in prolonged hospital stay and hence increased cost of patient management. The constellation of changes to the red blood cells that occur during storage, including metabolic, biochemical and molecular changes, which eventually culminate in irreversible damage and ultimately limit the storage period, have come to be widely referred to as the “storage lesion”.

To remain viable, whole blood and/or packed cells should retain the same properties as they have during their normal circulation in the body. To help with this, the blood storage conditions/environment should closely approximate those of the natural habitat.

2.0 LITERATURE REVIEW

Preservation and long term storage of whole blood and packed red blood cells (RBCs) is cardinal to ensure the ready availability of safe blood supply for transfusion medicine. Blood collection and storage systems that have been licensed by the Food and Drug Administration (FDA), allow packed cells to be stored up to 42 days, while the median duration of storage of transfused red cell units in the United States is 15 days.

The physiological parameters of blood such as extracellular sodium (Na+), potassium (K+), chloride (Cl-), pH, 2,3-DPG and ATP undergo changes during storage of blood. According to a study by Ulgen and Sedzi in 2006, blood bank storage resulted in a rise in K+ and a fall in the Na+, Cl-, pH, 2,3-DPG and ATP on days 10 and 21 of storage in the blood bank. These values vary the longer the blood is stored and especially when the recommended storage and transport conditions are not adhered to. With the increasing storage times the active uptake of potassium into red cells decreases; therefore, a unit of red cells stored for 4 – 5 weeks may contain 5 – 30 mmol/L of extracellular potassium. The administration of such a unit may cause cardiac arrhythmias in susceptible patients such as neonates and those with renal failure or major tissue injury.

According to the findings of a study by Murphy et al, patients who receive transfusion have a dose related-increased risk of death, organ dysfunction, infection, length of critical care, and hospital stay. According to a further retrospective study done in 2008 by Koch and colleagues in which 2872 cardiac surgical
patients who received red cells that were less than 14 days old were compared with 3130 cardiac surgical patients who received red cells that were greater than 14 days old, it was found that in addition to the dose-related effects, transfusion of older blood significantly increased the risk of postoperative complications and a reduction in short and long term survival. The pathophysiology of these findings may be related to structural and functional/metabolic changes in red cells during storage. The metabolic changes such as deprivation of 2, 3 diphosphoglycerate (2, 3 DPG), acidosis and hyperkalaemia are likely to occur in recipients of massive transfusion.

**Hyperkaleemia**

Sodium and Potassium disorders are on the list of common metabolic electrolyte derangements seen by anaesthetists. If untreated these quickly become life threatening. Potassium is the most abundant cation in the intracellular fluid, with a concentration around 150mmol/l. Only 1% of the total body extracellular potassium is found in the plasma and the concentrations are kept between 3.5 and 4.5 mmol/L. Hyperkalaemia is a plasma concentration of greater than 5.5mmol/L. This may be caused by either an overall increase in the total body potassium or an acute shift of potassium from the intracellular to the extracellular compartment. This can be caused by acidosis (in which H+ is taken into the cell in exchange for K+), insulin deficiency, digitalis toxicity, beta blockers, exercise, suxamethonium administration, excessive potassium input (as is the case in massive blood transfusion and transfusion of old blood) and impaired renal excretion. Increases of potassium above 6.0mmol/L can prove fatal as can result in life-threatening arrhythmias, paraesthesia, weakness, paralysis (including respiratory muscle paralysis), decreased renal production of ammonia, an increased renal retention of H+ and a subsequent metabolic acidosis, natriuresis and increased levels of aldosterone and insulin. These signs and symptoms of hyperkalaemia may be difficult to recognise on a general ward at the University Teaching Hospital due to the non-availability of frequent monitoring and monitoring equipment and thus difficult to recognise hyperkalaemia that may result from transfusion of old blood as a contributory effect or causative factor in the morbidity and mortality of post transfusion patients. The increased amount of potassium in transfused red cell units most results from the hemolysis of the red cells. Causes of hemolysis related to the manufacturing and subsequent handling processes include: temperature extremes, excessive centrifugation, tubing stripping, tubing heat sealers, bacterial contamination, mishandling and use of incompatible solutions. A published case of cardiac arrest during blood transfusion reported a potassium concentration of 120 mmol/L in the blood unit tested after the arrest. According to two recent studies,
even under proper storage conditions, the potassium in whole blood stored in CPDA-1 will increase from 5.1 mmol/L on day 0 to 78.5 mmol/L on day 35 while that of red blood cells (RBCs) stored in Citrate-phosphate-dextrose SAGM will increase from 2.1 mmol/L on day 0 to 45.3 ± 3.7 mmol/L on day 42. A study in Ghana by Clement et al., analysed the effects of blood storage on the concentrations of potassium and sodium in stored blood. Similar to the work done by Wallas (1979, 2010), their study revealed a steep rise in the plasma potassium concentrations. The study saw a potassium rise from 3.31 mmol/l on day 0 to a potassium level of 14.98 mmol/l on day 20. According to a study by Bailey and Bove, plasma levels of potassium may increase by 0.5-1.0 mmol/l per day of refrigeration. During blood storage under the recommended settings, the increase in the plasma potassium is attributed to the leakage of potassium from the cells into the surrounding plasma along a concentration gradient. This is due to the failure of the sodium-potassium ATPase pump. It can therefore, be deduced from these studies that a potassium level of 42 mmol/L in a unit of blood is allowable/expected by day 42 of storage. However, it remains the discretion of the managing doctors whether to transfuse such a unit having in mind the recipient’s age and condition. Because of this, some Hospital have policies that do not transfuse blood that is older than 7 days to infants.

**Bacterial contamination**

Bacterial contamination represents the greatest risk for the recipient receiving transfusion. Gram–positive bacteria usually found on the skin, constitute the most common bacterial contaminants of blood products. Contamination occurs when the bacteria on the skin is passed into the collected blood through the collection needle. Gram-negative bacteria cause a spectrum of infections including pneumonia, bloodstream infections, wound or surgical site infections, and meningitis. Escherichia coli (E. coli) and other similar bacteria, may contaminate the donation when blood is collected from donors who have bacterial infection without symptoms.

Storing and transporting whole blood and packed cells between +2 and +6 degrees Celsius keeps the growth of any bacterial contamination in the unit of blood to a minimum. If blood is stored above +6 degrees Celsius, bacteria that may have inadvertently entered the unit during collection may grow to such an extent that transfusion of the contaminated blood could be fatal.

In a case of septic transfusion reaction reported to the American Red Cross, the donor of the implicated donation was healthy and asymptomatic on the day of donation, but reported a remote history of bacterial...
endocarditis. In this case, the reaction was attributed to coagulase-negative Staphylococcus contamination. According to the findings of the British committee for standards in haematology guidelines, the risk with whole blood and red cell concentrates was found to be 1:500000. This risk is multiplied if whole blood and packed cells are stored/transported in sub-optimal conditions. As the on-set of sepsis due to transfusion of culture positive blood is not immediate and may happen during the postoperative period in surgical patients, the symptoms may be attributed to surgery or anaesthesia, thereby masking the real cause of morbidity and mortality.

A five year evaluation of transfusion outcome in a resource-limited setting of Cameroon revealed that blood transfusion is still unsafe in many resource limited communities of developing countries. Of the 40,134 donations, only 80% of the donated blood was considered safe for distribution with 20% of donated blood being rejected for positive HIV or Hepatitis B antigen results. More than 50% of transfusions within the hospital were associated with an unfavourable outcome the most common being febrile reactions (40.1%) followed by urticaria (19.4%). Acute intravascular hemolysis, circulatory overload and deaths occurred in 0.01%, 0.04% and 0.14% of cases respectively.

According to a study done by AP Gibb et al in 1995, Yersinia enterocolitica and Pseudomonas fluorescens remain the most common bacterial contaminants. These two organisms share the ability to proliferate at 4°C. Storage of whole blood, packed cells and other blood products at room temperature for longer hours prior to transfusion will therefore favour the growth of bacteria and contributes to the unfavourable outcomes of blood transfusion.

While transmission of bacteria during blood component transfusion is rare, transfusion transmitted infections can be severe and life threatening with a mortality rate of about 20-30%. This results in an estimated 100-150 every year deaths among recipients of blood components in the United States. Transmission of bacteria with blood component transfusion does not necessarily guarantee sepsis in the recipient. Whether one gets sepsis from blood component transfusion is dependent on a number of factors such as the volume of the blood component transfused, bacterial concentration in blood component, immune and general condition of the recipient, extent of surgery, type of invasive diagnostic procedure, intensity of recipient monitoring and whether the recipient is already on antibiotics. Sources of bacterial infection can either be endogenous or exogenous. Blood donor bacteremia is an example of
an endogenous source of blood sample/unit contamination. The presence of bacteria on the donor’s skin during venipuncture at the time of collection is another cause of bacterial contamination of blood and blood components. The risk of transmitting an infection via who blood and packed cell transfusion to a large extent depends on the conditions and the time a component has been stored before it is transfused into a patient.

3.0 STATEMENT OF THE PROBLEM

The World Health Organization (WHO) has given guidelines on storage and transport of blood and blood products to ensure transfusion of viable, safe blood and blood products. These guidelines are to be observed at all the three interfaces of blood collection, blood screening and testing and the clinical interface. These guidelines are well adhered to at the two initial points of collection and testing. The clinical interface, however, faces challenges in adhering to these stipulated guidelines especially in resource limited settings such as University Teaching Hospital (UTH) in Zambia. The discrepancy between the demand and supply of blood has fostered the development of certain practices that may hamper blood transfusion safety and/or viability. The non-availability of proper blood carriage equipment such as coolers, the non-availability of blood storage fridges in the theatres and wards and the high temperatures experienced in some wards and theatres impact negatively on the viability of blood and blood products to be transfused. The often prolonged time interval between the time the blood leaves the blood bank to the time the blood is actually transfused or returned to the blood bank, if not used, and the possibility of recirculation of returned unused blood also compounds this challenge. Studies have shown that the longer whole blood stays out of the fridge at the advised temperatures, the more dangerous it becomes and the less viable it may be. Yet little is known at Zambia’s University Teaching Hospital regarding the viability of whole blood and packed cells at the time of transfusion under the prevailing circumstances.

3.1 STUDY JUSTIFICATION

The safety of blood transfusion is still a daunting challenge in many resource limited communities of developing countries. The results of this study will help reinforce the current existing practice (low resource setting) or foster a change in the current practice. If the research shows that the current practice is dangerous, then this is the evidence needed to implement
change for patient safety. However, if the research shows that the blood is still viable at later time intervals, then this finding could prove crucial for the transfusion practices in low resource settings. Depending on the results, the study will also improve communication between the blood bank and the clinical setting regarding the prompt supply of safe blood products in recommended storage setting. The overall result may be improved patient safety and reduced hospital stay.

3.2 RESEARCH QUESTION

Is cross matched whole blood and packed cells viable at the time of transfusion at the University Teaching Hospital (UTH) in Lusaka, Zambia?

3.3 HYPOTHESIS

All cross matched whole blood and packed cells are viable at the time of transfusion at the University Teaching Hospital in Lusaka, Zambia.

3.4 OBJECTIVES

3.4.1 General objectives

To assess the viability of whole blood at the time of transfusion at the University Teaching Hospital (UTH) in Lusaka, Zambia

3.4.2 Specific objectives

1. To determine bacterial contamination (blood culture and gram stain)
2. To determine the common bacterial contaminants
3. To determine the levels of extracellular potassium

4.0 METHODOLOGY

4.1 Study design

This is an observational study, a service evaluation of how transfusion medicine is conducted in this setting.
4.2 Study site

This hospital-based study will be conducted in theatres (emergency and elective theatres), blood bank complexes, medical and surgical wards, with the exclusion of the paediatrics wards, at the University Teaching Hospital (UTH) in Lusaka. The University Teaching Hospital of Lusaka serves as a general hospital for the population living in and around Lusaka as well as the national referral and teaching hospital of Zambia.

4.3 Target population

All cross matched blood and packed cells to be transfused

4.4 Eligibility criteria

4.4.1 Inclusion criteria

All cross matched transfused whole blood and packed cells

4.4.2 Exclusion criteria

Non-viable cross matched whole blood and packed cells by visual inspection (presence of clots and agglutinins in cross matched blood) and non-transfused cross matched whole blood and packed cells.

4.5 Sampling method

Convenient sampling method was used.

4.5.1 Sample size

The prevalence formula was used to calculate the sample size.

\[ N = \frac{Z^2 \times P \times (1-P)}{E^2} \]

Where:

N = sample required

Z = Z statistic = 1.96 (95% CI), P = expected prevalence 0.1

E = confidence interval 0.05

At 80% power

N = 136
Primary outcome was blood viability which mandated that all viability tests are met successfully namely: negative gram staining and blood culture results and potassium of less than 42 mmol/L. Table 1 illustrates this composite outcome.

<table>
<thead>
<tr>
<th>Item</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Potassium</strong> (extracellular)</td>
<td>&lt;42mmol/l  &lt;42mmol/l  &lt;42mmol/l  &lt;42mmol/l  &gt;42mmol/l</td>
</tr>
<tr>
<td><strong>B/Culture</strong></td>
<td>Negative  Negative  Positive  Negative  Negative</td>
</tr>
<tr>
<td><strong>G/Staining</strong></td>
<td>Negative  Positive  Negative  Positive  Negative</td>
</tr>
<tr>
<td><strong>Viability</strong></td>
<td>Yes  No  No  No  No</td>
</tr>
</tbody>
</table>

4.5.2 Sample collection

The sample was collected just before the blood transfusion was commenced. Sterility was maintained throughout the sampling procedure. This was achieved by using sterile gloves, sterile Y-blood giving sets and sterile needles and syringes. The blood sample was collected from the main blood bag using the one sterile part of the Y-blood giving set that ensured the collection of the sample with no contamination. The samples were then immediately put in the appropriate sample collection bottles (blood culture and EDTA bottles) and taken to the lab immediately. Once the samples reached the laboratory, they were immediately attended to (see appendix for details). All samples were collected by the principal investigator himself.

4.5.3 Data collection tool

The data collection tool (find attached) was administered by the principal investigator. The principal investigator was called by the ward managers once there was a blood transfusion taking place. The data collection tool was then administered to the managing doctor and in cases were
the doctor was unavailable, the patient file was reviewed for the notes of the instructions for the transfusion and other details were gotten from the nurses, if necessary.

4.5.4 Data analysis

Data analysis was done using the statistical software graph pad prism version 7.01 by Graph pad and the tests of significance used included man Whitney test for non-parametric data to calculate the p-value when comparing two groups (blood stored in cooler boxes vs. that stored at room temperature. A p-value ≤0.05 was taken as significant. The spearman correlation coefficients, r, was also calculated.

Key: BT-Blood transfusion
5.0 ETHICAL CONSIDERATIONS

Approval was sought from University of Zambia Biomedical Research Committee (UNZA-BREC). A standardised questionnaire was used to recruit Participants. Considering that the study was observational there were no anticipated adverse events to the participants.

Informed consent was obtained from all participants and they were informed that they could withdraw from the study at any time. The consent was explained in the language the patient was familiar with. All patients were treated with respect. The data findings were kept confidential for all patients and no name was used in the data set only numbers which each patient was assigned.

No sample in any form was withdrawn or taken from the patients receiving the transfusion. The principal investigator was not involved in selecting or determining which of the patients was to receive blood transfusion. The decision to transfuse was made by the managing physicians. However, feedback regarding abnormal test results was communicated to the attending medical team. To avoid contamination of blood to be transfused, y-shaped giving sets were used to take the sample. Sterility was maintained throughout the sampling procedure.
6.0 RESULTS

A total of 84 blood units were sampled. Of the patients to be transfused, 44 were females and 40 were males. The youngest recipient of the transfusion was 8 years and the oldest was 84 years old with an average of 46 years.

Transportation and storage of transfused blood

28 of the transfused blood units were transported and stored in cooler boxes, while 56 were transported and stored at room temperature immediately before commencement of transfusion. A significant number of the blood units were stored under room temperature (p=0.0053).

Vein to vein interval

The mean number of days between donations to transfusion (vein to vein) was 7 (±3SD) days. The shortest vein to vein period was 1 day while the longest was 24 days with possibility of recirculation. Re-circulation in this case means that some of the blood units may have been issued out of the blood bank to the clinical interface (ward or operating theatre) then returned because it was not used. This blood would then be re-issued to a different recipient. All the while, the unit was subjected to the poor transport and storage conditions at the clinical interface. The average time taken to commence transfusion once the blood left the blood bank under the above described conditions was 6 hours while the shortest was 1 hour and 14 hours for the longest.

Case profile for transfused patients

Among the patients transfused in this study, 50% were obstetric surgical cases, 30% were general surgical cases while 20% were medical cases. 5% of the cases were being attended to on the intensive care unit.

Potassium levels

The levels of extracellular potassium increased with increasing days of storage. The measured potassium levels were a minimum of 4.1mmol/l, a mean of 12.25mmol/l (±7.4SD) and a maximum of 36.01mmol/l in a unit of whole blood that was stored for 24 days. There was a positive correlation between the levels of extracellular potassium and the length of storage of whole blood and packed cells (r=0.89, p=0.0001). Figure 1 (a) below illustrates this finding.
There was also a significant rise in the levels of extracellular potassium depending on the number of hours the blood stayed out of the blood bank before the transfusion was commenced (p=0.005, r=0.37 as illustrated in figure 1 (b) above.

There was a significant difference (p=0.02) in the levels of potassium between the blood units that were transported and stored at room temperature to those that were stored in the cooler boxes prior to commencement of transfusion. The median was 13.04 (IQR±18) for the samples stored at room temperature while the median for those in cooler boxes was 8.3 (IQR ±5) (see figure 2). However, there was no significant difference (p=0.9) in the levels of potassium between packed cells (median 9.8 (IQR ±5)) and whole blood (median 9.1(IQR ± 8)). This was because the units were subjected to similar transport and storage conditions. (See fig. 3). The Mann Whitney was used to calculate the p value in the above cases.
**Blood culture**

This study revealed the presence of four different organisms which included Pseudomonas fluorescens, staphylococcus capitis, Acinetobacter and Corynabacterium. These were mixed growths. The most common bacterial contaminant in this case was staphylococcus capitis followed by Pseudomonas fluorescens. Of the 84 blood units sampled, 10.5% (8) had positive blood cultures. The sensitivities of these bacteria are attached in appendix VI.

**Table 2.** Isolated bacteria and sensitivities

<table>
<thead>
<tr>
<th>Antibiotic susceptibility</th>
<th>Pseudomonas fluorescens</th>
<th>Acinetobacter baumannii</th>
<th>Staphylococcus capitis</th>
<th>Corynabacterium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tazobactam</td>
<td>S</td>
<td>S</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>R</td>
<td>S</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>R</td>
<td>R</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>-</td>
<td>R</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>-</td>
<td>-</td>
<td>R</td>
<td>-</td>
</tr>
<tr>
<td>Penicillin</td>
<td>-</td>
<td>-</td>
<td>R</td>
<td>-</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>-</td>
<td>-</td>
<td>R</td>
<td>-</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>-</td>
<td>-</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>-</td>
<td>-</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>-</td>
<td>-</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>-</td>
<td>-</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>Dalfopristin</td>
<td>-</td>
<td>-</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>Linezolid</td>
<td>-</td>
<td>-</td>
<td>S</td>
<td>-</td>
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<td>Vancomycin</td>
<td>-</td>
<td>-</td>
<td>S</td>
<td>-</td>
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<tr>
<td>Tetracycline</td>
<td>-</td>
<td>-</td>
<td>R</td>
<td>-</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>-</td>
<td>-</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>-</td>
<td>-</td>
<td>I</td>
<td>-</td>
</tr>
</tbody>
</table>
An attempt was made to follow up those that received blood units with positive cultures. One patient died on the second day after his operation. His death could not be attributed to sepsis due to the contaminated unit due to the short period between the transfusion and his death. Also this was an 88 year old ASA 3-4 patient with many co-morbidities. The unit he received had positive culture for Pseudomonas fluorescens and staphylococcus capitis. The other successful follow up was a split skin graft patient who received a unit with positive culture for Acineobacter and Corynabacterium. This one was discharged with no signs of sepsis on day 5. The rest of the patients were lost to follow up. This was mostly because by the time the culture results were coming out the patients could not be located to which ward they had been taken and others where probably discharged. Even if the majority did not get sepsis from being transfused with contaminated blood, these cases are simply near misses and therefore, occurrence of these near missed should be reduced they can turn fatal.

Table 3. Illustrates this.

<table>
<thead>
<tr>
<th>Unit no.</th>
<th>Organism Isolated</th>
<th>Result of follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Staph. Capitis, Pseudomonas fluorescens</td>
<td>Died day 3 from co-morbidities</td>
</tr>
<tr>
<td>2</td>
<td>Acinetobacter, Corynabacterium</td>
<td>Discharged day 5 with no sepsis</td>
</tr>
<tr>
<td>3</td>
<td>Staph. Capitis, Pseudomonas fluorescens</td>
<td>Lost to follow up</td>
</tr>
<tr>
<td>4</td>
<td>Staph. Capitis, Acinetobacter</td>
<td>Lost to follow up</td>
</tr>
<tr>
<td>5</td>
<td>Staph. Capitis, Acinetobacter</td>
<td>Lost to follow up</td>
</tr>
<tr>
<td>6</td>
<td>Corynabacterium, Pseudomonas fluorescens</td>
<td>Lost to follow up</td>
</tr>
<tr>
<td>7</td>
<td>Staph. Capitis, Corynabacterium</td>
<td>Loss to follow up</td>
</tr>
<tr>
<td>8</td>
<td>Pseudomonas fluorescens</td>
<td>Loss to follow up</td>
</tr>
</tbody>
</table>

There was a significant difference (p=0.03) in the number of days of storage between the culture positive and culture negative samples. The blood culture negative units were stored for lesser number of days (median 2.5(IQR±2)) than the culture positive units (median 4.5(IQR±8)). The two results reveal that the transport and storage conditions immediately before whole blood and packed cells are transfused are just as key to the safety of blood component as are the long term storage conditions.
7.0 DISCUSSION

The objective of this study was to assess the viability of whole blood and packed cells at the time of transfusion at the University Teaching Hospital under the current practice. To be considered viable, the electrical and physiological properties of stored blood should be maintained as in vivo. This means that physiological parameters such as extracellular sodium (Na+), potassium (K+), chloride (Cl-), pH, 2,3-DPG and ATP are to be within or near normal values. Also there should be no bacterial contamination. In this study, viability was assessed by measuring the potassium levels and bacterial contaminants. Viable blood was defined as one with a negative blood culture with extracellular potassium of less than 42mmol/l.

This was deduced from the studies done by (i) Clement et al (ii) Klein et al (iii) Sedzi et al and (iv) Bailey and Bove that revealed an allowable/expected level of potassium on day 45 of storage of whole blood and/or packed cells to be 42mmol/L. 

The results of this study indicate that 10.5% of the sampled blood units were not viable as they had positive blood cultures. *Pseudomonas fluorescens* was among the most common bacterial contaminants in this study after *Staphylococcus Capitis*. This is similar to the findings of a study done by AP Gibb et al in 1995, which found *Yersinia enterocolitica* and *Pseudomonas fluorescens* as the most common bacterial contaminants. Under the prevailing transport and storage conditions at the clinical interface of The University Teaching Hospital, it is no surprise that *Pseudomonas fluorescens* was among the most common bacterial contaminants as it is able to proliferate at temperatures above 4°C. As indicated by this study, storage and transport of whole blood and packed cells once they leave the blood bank can hamper the safety of blood and blood products if the recommended transport and storage conditions are not closely adhered to. This study revealed that 56% units were transported and stored at room temperature. These transport and storage conditions encourage the growth and multiplication of bacteria that may have inadvertently entered a unit at the time of donation. Transporting and storing whole blood and packed cells at between +2 and +6 degrees Celsius keeps the growth of any bacterial contamination in the unit of blood to a minimum. The conditions to which whole blood and packed cells are subjected to at the University Teaching Hospital once they leave the blood bank hampers the safety of these products and can be a source of infections in the recipients of non-viable whole blood and packed cells.
**Staphylococcus Capitis**

This is a gram positive cocci found on human skin and mucosa. Recent reports indicate emergence of this bacterial species as significant pathogen causing nosocomial infections, meningitis, prosthetic valve endocarditis and late onset sepsis which is now resistant to some important antibiotics such as linezolid\(^2^8\). This rarely cause sepsis in recipients because they do not proliferate in whole blood and packed cells when stored \(+2 - +6^\circ\text{C}\).\(^2^7\) Under the current prevailing conditions at the clinical interface, this is a potential cause of sepsis in the recipients.

**Pseudomonas fluorescens**

This is a gram-negative bacteria commonly found in water and soil. It is able proliferate in temperature at and above \(4^\circ\text{C}\). It is one of the two most important causes of sepsis due to transfusion of contaminated blood with a propensity to cause an endotoxic shock. Similar to the results of a study done by A Gibb et al, the findings of this study shows that storage of whole blood and packed cells at room temperature even for six hours promotes the growth of bacteria that may have inadvertently entered the unit at the time of collection. This confirm that, the transport and storage conditions to which whole blood and packed cells are subjected to once they leave the blood bank, are very important. The results of this study also confirm this fact as the immediate transport and storage conditions, and not the long term storage of blood under the ideal conditions in the blood bank, seemed to have play a major role as to whether a unit had a negative or positive blood culture result as shown in figure 1 (a) and (b). Contamination of whole blood and packed cells by this species is likely due to an exogenous source, most likely the donor’s skin. Therefore, stringent aseptic techniques especially as regards skin cleaning is are key to avoiding contamination by this organism.\(^2^6\)

**Acinetobacter baumannii**

This is a Gram negative bacillus and is aerobic in nature. It has a high affinity for aquatic environments but is also known to colonize the integumentary and respiratory systems. It has recently been recognized as an emerging opportunistic bacterial pathogen closely associated with nosocomial infections. A rise in the incidence of multi drug resistant strains have been reported. Its incidence is high among immunocompromised subjects, especially in those with a hospital stay of 90 days or more. This pathogen, together with Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa and Enterobacter spp., have become the most serious causes of concern to the World Health
Organization among the multi drug resistant pathogens. Therefore, the isolation of this pathogen among the units sampled in this study raises serious concerns, especially if the blood units were to be transfused in immunocompromised individuals.29

**Corynabacterium**

This organism exists as part of normal skin flora. Until recently, Corynabacterium was considered simple skin contaminant with no potential for pathogenicity. According to a case report by Weiss et al, this organism has been isolated in infectious processes in the respiratory and urinary systems. A multi resistant susceptibility profile has characterised these organisms. Weiss et al reports a case of meningitis caused by Corynabacterium in a 23 year old subject. The multi resistant susceptibility of this organism was confirmed by this study (see the appendix VI). This organism flourishes at temperatures of 4°C and above.30 Thus, the current transfusion practice at the clinical interface at the university teaching hospital predisposes recipients to possibility of transfusion transmitted infection by this virulent organism.

The fact that there was no direct case of sepsis resulting from transfusion of infected blood units does not in any way mean that presence of bacteria in transfused blood is acceptable. These cases basically represent the near misses and the presence of bacteria in transfused blood is dangerous and can have serious implications on the recipients. These can also be cases of delayed onset sepsis.27

**Potassium levels**

Potassium is the major intracellular cation while sodium is the major extracellular cation. Various homeostatic mechanisms are involved in ensuring that this balance is maintained. While the potassium on day zero was unknown, our study revealed a maximum level of extracellular potassium of 36.01 mmol/l and an average of 12.25 mmol/l. Being the major intracellular cation, the acceptable level of extracellular potassium ranges from 3.5-5.5 mmol/l. The apparent accelerated increase in the extracellular potassium levels in our study confirms that the current transfusion practice at the clinical interface may encourage haemolysis which could explain the high levels of potassium observed in this research. Some of the sampled units of whole blood and packed cells may have been recirculated and hence exposed to the unfavourable transport and storage conditions more than once. Efforts to trace sampled blood units that were suspected to have been recirculated was difficult as some of the returned units may have been re-issued without being entered in the blood bank database as returned. Hyperkalaemia is one of the recognised complications of massive blood transfusion. Although the results of this study indicate that the
levels of potassium encountered where within the expected levels for the length of storage of less than 45 days, Hyperkalemia remains a significant risk of massive transfusion.\textsuperscript{33, 34} The current lack of intensive patient monitoring on the wards makes the discovery of this complication difficult. In addition to the already established sodium-potassium ATPase failure in stored blood, this research suggest that poor transport and storage conditions, such as temperatures above 4°C, once whole blood and packed cells leave the blood bank further cause a disruption in the integrity of the red cell membrane and may result in haemolysis and thus cause a further rise in the levels of extracellular potassium in whole blood and packed cells prior to transfusion.

**Transportation and storage of transfused blood**

The ZNBTS has gone out of its way to assist improve the transport and storage conditions at the clinical interface by procuring a few cooler boxes for the transport and storage of blood before being transfused once it leaves the blood bank. However, the clinical interface still faces a challenge in adhering to the stipulated use and maintenance of a cold chain using these cooler boxes. Sometimes the cooler boxes are warmer than room temperature because the ice packs are not replaced on time. Currently, there are workshops and simulations going on to train nurses and physicians on the proper use of these cooler boxes. This, therefore, needs to be continued and supported.

**8.0 STUDY LIMITATIONS AND STRENGTHS**

**8.1 Limitations**

1. Lack of funding. This research was solely funded by the principal investigator with no support from any institution. Therefore, other parameters that could be used to assess viability such as the pH, ATP and 2, 3 DPG, chloride and sodium were not analysed.

2. Sensitivity of study implied principal investigator collects all samples to ensure sterility and avoid contamination of samples. In an institution where service provision supersedes academic activities, this meant that the principal investigator could not follow up all notifications by ward managers where transfusion were being carried out.

3. The erratic availability of electrolyte reagents in the laboratory meant that samples could only be collected after confirming the availability of the reagents and hence causing a further reduction in the sample size.
4. The initial aimed sample size could not be attained due to the above mentioned reasons. A bigger sample size is required to ascertain the most common bacterial contaminants in the Zambian donor population.

8.2 Strengths
Data collection was very systematic and the principal investigator collected all samples. The principal investigator ensured that sterility was maintained at all points of sample collection. Once collected, the principal investigator took the samples to the laboratory for analysis immediately. The same laboratory personnel was used to ensure uniformity and accurate results of culture and potassium. The arrangement with the laboratory personnel ensured that the research samples were attended to once they reached the laboratory. See appendix for further clarification on the steps taken as regards laboratory sample analysis.

9.0 CONCLUSION
The evidence revealed by this study indicates that the current transfusion practice at the clinical interface needs to be improved. Evidence presented here confirms that storing whole blood and packed cells at room temperature encourages the growth of bacteria that may have inadvertently entered a blood unit to proliferate and thus a potential cause of sepsis and mortality of the recipients. This has been shown by the 10.5\% non-viable blood units that had positive cultures. The maintenance of a sterile conditions during blood collection from donors should also be closely adhered to. Though the levels of potassium encountered in this study are within the expected, the apparent accelerated rise in the level of extracellular potassium under the current prevailing transport and storage conditions at the clinical interface raises concern regarding the safety of the whole blood and packed cells that are to be transfused. While transfusing hyperkalaemic whole blood or packed cells could be a transient factor given the recipients renal function and sodium-potassium ATPase pump are normal, transfusion of such blood components is of serious concern in patients with renal failure or those undergoing dialysis.
10.0 RECOMMENDATIONS

1. UTH management, as well as other ZNBTS beneficiaries to ensure close adherence to set standards of transfusion practice as regards transport and storage of blood and blood products.

2. UTH management to consider acquiring equipment for proper storage of blood in theatres and wards.

3. Continued medical education on the use of blood and blood products as regards what blood products should be ordered and how much should be ordered. Especially that the ZNBTS has the capability and capacity to produce various blood components. (on-going)

4. A further funded study should be done to include patient follow up and look at the various other parameters that can be used to confirm viability of blood and blood products. Also this study should have a larger sample size to help in identifying the most common bacterial contaminants of blood and blood products among the Zambian donor population.

5. Temperature monitors in blood cooler boxes to ensure maintenance of the cold chain.
11.0 REFERENCES

3. Continuing Education in Anaesthesia, Critical Care & Pain | Volume 14 Number 3 2014
5. O Wenker, Hazards of blood transfusion, the internet journal of anesthesiology. 2008 volume 1 number 3.

16. CDC report, March 2013- Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Healthcare Quality Promotion (DHQP)


18. Eder AF, Mindy Goldman, How do I investigate septic transfusion reactions and blood donors with culture-positive platelet donations? TRANSFUSION 2011; 51:1662-1668


Appendix I

Participant Information Sheet

Viability of whole blood and packed cells at the time of transfusion study

Principal investigator: Dr. Abel Mwale

Sponsor: Self

Introduction

I, Dr. Abel Mwale from the department of anaesthesia in the School of Medicine at the University of Zambia, will be carrying out a study on the assessment of the viability of whole blood and packed cells at the time of transfusion study at the University Teaching Hospital in Lusaka, Zambia. This is to be carried out in the theatres (both elective and emergency theaters) as well as adult medical and surgical wards at University Teaching Hospital.

Purpose and method of the study

The purpose of the study is to obtain information that will be used to ascertain the safety of blood and blood products. This information will aid in ensuring that safe blood and blood products are administered to patients. This will also help in ensuring that once the blood or blood products leave the blood bank they are stored under optimal conditions that ensure maintenance of the viability. Eventually this should lead to improved care of patients needing blood transfusion.

Procedures

The study will involve measuring the amount of sodium, potassium, preponderance of hydrogen ions (pH), and gram staining and blood cultures in blood that is to be transfused. You will be required to answer basic demographic questions such as your sex and age. Other information will be; the reason for transfusion and the type of operation or illness and any history of blood transfusion. No sample of any form will be collected from you as a participant. The samples will only be collected from the blood to be transfused just before the transfusion is commenced.
Potential risks

There are no foreseeable risks attributable to participating in this study.

Potential benefits

There are no direct benefits to you for participating in this study but information obtained from you and the results of the tests done on the transfused blood will inform the pre-operative storage and safety of transfused blood. This should ensure transfusion of safe blood products. The benefits of this study to the community is the transfusion of safe blood products and reduction in the morbidity that comes with transfusion of unsafe blood products such as reduced hospital stay and improved quality of life.

Rights as a research participant

Your participation in this study is entirely voluntary. You may decide to withdraw from the study at any time. Such a decision will not affect the medical or surgical care given to you.

Confidentiality

A unique identifier only known to the study personnel will be used instead of your name. Personal information about you will not be released to anyone and will not be used in any publications from this study.

Remuneration

There will be no payment for your participation in this study.

Further Information

If you have any questions or concerns regarding ethical issues in the conducting of this study, you may contact:

The Principal Investigator Dr. Abel Mwale Department of Anaesthesia University of Zambia School of Medicine

Lusaka, Zambia

Tel: +260977 748829
Appendix II

Informed Consent Form

Consent to participate in the ‘blood viability study’.

I, _________________________________________________ hereby confirm that the nature of this clinical study has been explained to me. I am aware that my personal details will be kept confidential and I understand that I may, voluntarily, at any point withdraw my participation from the study without suffering any consequence. I have been given sufficient time to ask questions and seek clarifications, and I have agreed to participate in this research.

I have received a signed copy of this agreement

___________________________________________________   ___________________
Signature and name of participant                                                       Date

________________________________________________    ______________
Witness (Name and Signature)                                                            Date
## Appendix III: QUESTIONNAIRE

<table>
<thead>
<tr>
<th>STUDY NO.</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>TIME BLOOD LEFT BLOOD BANK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NUMBER OF UNITS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UNIT NUMBERS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOW WAS THIS BLOOD TRANSPORTED?</td>
<td>ROOM TEMPERATURE</td>
<td>COOLER</td>
</tr>
<tr>
<td>WHERE WAS THIS BLOOD STORED/IN THEATRE/WARD BEFORE TRANSFUSION?</td>
<td>ROOM TEMPERATURE</td>
<td>COOLER/FRIDGE</td>
</tr>
<tr>
<td>HOW MANY UNITS DO YOU PLAN TO TRANSFUSE AND OVER WHAT PERIOD?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO YOU THINK THIS BLOOD IS VIABLE?</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>AGE AND SEX OF PATIENT BEING TRANSFUSED</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYPE OF OPERATION/PROCEDURE BEING DONE ON PATIENT BEING TRANSFUSED:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HISTORY OF TRANSFUSION</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>IF YES, WHEN WAS THE LAST TIME YOU RECEIVED BLOOD TRANSFUSION?</td>
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Appendix IV

Quality control for blood culture

Instrument/consumables/data tools

1. Bactec FX 200 – For incubation of Blood culture bottles
2. VITEK 2 Compact – For identification of bacteria/fungi & antimicrobial susceptibility testing
3. Blood agar base (for blood agar & Chocolate agar)
4. Sheep Blood (for blood agar & Chocolate agar)
5. MacConkey agar
6. DisaLab (lab information system)
7. Mueller Hinton agar

Table 4: Quality control for manual antimicrobial susceptibility testing

<table>
<thead>
<tr>
<th>QC organism</th>
<th>Antibiotic</th>
<th>Zone diameter (mm)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa ATTC</td>
<td>Ciprofloxacin</td>
<td>26</td>
<td>Pass</td>
</tr>
<tr>
<td></td>
<td>Gentamicin</td>
<td>19</td>
<td>Pass</td>
</tr>
<tr>
<td></td>
<td>Ticarcillin</td>
<td>25</td>
<td>Pass</td>
</tr>
<tr>
<td></td>
<td>Piperacillin/Tazobactum</td>
<td>20</td>
<td>Pass</td>
</tr>
<tr>
<td>Staphylococcus aureus ATCC 25923</td>
<td>Cotrimoxazole</td>
<td>30</td>
<td>Pass</td>
</tr>
</tbody>
</table>

Table 5: Quality control for media

(a) Sterility:

<table>
<thead>
<tr>
<th>Medium type</th>
<th>Incubation time</th>
<th>Observation</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood agar</td>
<td>24 hours</td>
<td>No growth</td>
<td>Pass</td>
</tr>
<tr>
<td>Chocolate agar</td>
<td>24 hours</td>
<td>No growth</td>
<td>Pass</td>
</tr>
<tr>
<td>Medium type</td>
<td>Qc organism</td>
<td>Incubation time</td>
<td>Expected result</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------------------------</td>
<td>-----------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>Blood agar</td>
<td>S. aureus ATCC 25923</td>
<td>24 hours</td>
<td>Growth – Golden yellow colonies</td>
</tr>
<tr>
<td>Chocolate agar</td>
<td>H. influenza ATCC 10211</td>
<td>24 hours</td>
<td>Growth – Watery colonies</td>
</tr>
<tr>
<td>MacConkey agar</td>
<td>E.coli ATCC 25922</td>
<td>24 hours</td>
<td>Growth – Pink (LF) colonies</td>
</tr>
</tbody>
</table>

Key:
1. ATCC – American Type Culture Collection
2. S. aureus – Staphylococcus aureus
3. E. coli – Escherichia coli
4. H. influenza – Haemophilus influenza
5. LF – Lactose fermenting
Appendix V

Standard operating procedure (SOP) for blood culture processing

Day one:

1. Sample received in the lab.
2. Sample registered in the LIS (DisaLab). NB. Generate a bar code and stick it onto the specimen
3. Sample received in work area.
4. Sample loaded into the Bactec FX 200 by way of scanning the bar code of the bottle and the bar code of the specimen.

Day two:

1. Check in the Bactec machine for a “FLAG”. NB. Red for POSITIVE, green for NEGATIVE.
2. If POSITIVE, remove the specimen from the machine and subculture on Blood, Chocolate, and MacConkey agar. Incubate the Blood and Chocolate agar in the CO2 incubator, the MacConkey agar in the normal incubator.
3. Perform gram stain reaction on the specimen.
4. If NEGATIVE, reincubate and check each day, until day five, for a NEGATIVE flag.
   a. On day five, report finding as NEGATIVE flag and Culture: No growth.
   b. Enter data in the LIS
   c. Authorize the entered data and report to the clinician.

Day three:

1. Read the agar plates from the POSITIVE culture.
2. Set up purity plate(s), regardless of the number of colony type.

Day four:

1. Set up identification tests side by side with antimicrobial susceptibility testing, based on the gram stain reaction from the purity plate(s).

Day five:

✓ Read the ID and AST result.
✓ Report findings.
✓ Enter the data in LIS.
✓ Authorize the entered data and send the report to the clinician.
Convenient sampling here means that the blood units were sampled depending on availability of the blood units to be transfused and the availability of the PI. The principal investigator was the sole data collector for this project so he could only follow up blood units depending on his availability (in an institution where service provision precedes academics). The ward managers, the nurses, would alert the PI of a possible transfusion and the PI would follow up on the call if able to. Only blood that was actually being transfused was sampled.