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A STUDY OF (a) THE PATTERNS OF UTILISATION OF BLOOD AND BLOOD PRODUCTS, AND (b) THE STATUS OF SUBGROUPS OF BLOOD GROUP A (A1 AND A2) AT THE KENYATTA NATIONAL HOSPITAL

BY

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A DISSERTATION SUBMITTED IN PART FULFILMENT FOR THE DEGREE OF MASTER OF MEDICINE (PATHOLOGY AND MICROBIOLOGY)

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DEDICATION

TO BOTH MY PARENTS, AND TO OCHIENG'

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LIST OF ABBREVIATIONS

- KNH** - **Kenyatta National Hospital**
- C/T Ratio** - **Crossmatch/Transfusion Ratio.**
- CPD-A** - **Citrate-Phosphate-Dextrose-Adenine.**
- AIDS** - **Acquired Immunodeficiency Syndrome.**
- MSBOS** - **Maximum Surgical Blood Order Schedule**
- T A H** - **Total Abdominal Hysterectomy.**
- rpm** - **Revolutions per minute.**

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SUMMARY

a) This study aimed at assessing the demand for and utilisation of blood by different wards at the Kenyatta National Hospital. Five hundred and five requests for blood received at the hospital's blood transfusion centre between July and November, 1990 were studied. The ratio of units of blood crossmatched for a patient to units transfused into the patient (crossmatch/transfusion ratio) was used to assess blood utilisation. For the paediatric requests, the amount of blood issued to a patient and the amount actually transfused into the patient was also considered.

Approximately thirty seven per cent (37.4%) of the requests were from the surgical wards; 34.9% were from the obstetric/gynaecologic wards and the rest (27.7%) were from general medical wards (adult and paediatric).

Blood was crossmatched for 51.3% of those requesting for it. The paediatric wards showed the lowest crossmatch/transfusion (C/T) ratio of 1.1/1 while the surgical and medical wards had C/T ratios of 2.0/1 and 1.5/1 respectively. In the paediatric wards, 61.7% of the patients were transfused with less than 500 mls of blood although each was

(x)

issued with a 500mls of blood pack.

The findings show that blood transfusion service is not adequate to meet the demands of the hospital. Surgical patients have the highest demand for blood. However, by comparing C/T ratios, the medical wards show better blood utilisation rates than the surgical wards. There are some surgical procedures which are associated with such low transfusion rates that pre-operative cross-matching for 2 units of blood may not be warranted. The 500ml blood packs issued to paediatric patients are often not fully utilised and any blood remaining in the pack is wasted.

b) Of the subgroups of blood group A, A_1 and A_2 are the most common. These subgroups are identified by mixing the cells with anti- A_1 serum (absorbed B) or anti- A_1 lectin obtained from the seeds of *dolichos biflorus*.

In this study, three hundred and thirty two blood group A and forty four hundred group AB (total three hundred and seventy six) samples received at the hospital's blood transfusion centre were subgrouped into A_1 and A_2 using commercial anti- A_1 prepared from the lectin *Dolichos biflorus*.

Subgroup A_1 was found to comprise 84.3% of group A and 79.5% of group AB samples. The ratios of A_1/A_2 in blood groups A and AB were found to be 5.4/1 and 3.9/1 respectively. The apparent difference in the ratio of A_1/A_2 in blood groups A and AB was not statistically significant. This result is in contrast to the findings of other investigators who have reported a higher proportion of non- A_1 antigens. It is recommended that a survey involving a larger population should be undertaken to verify these findings.

These 2 studies, though different, are both related to transfusion medicine. Because the study on blood utilisation consisted entirely of data collection and analysis, the second study was chosen to engage the author in a practical aspect of blood transfusion which is a requirement for this course.

INTRODUCTION AND BACKGROUND INFORMATION

. Blood is respected as essential for the maintenance of life. The earliest recorded administration of blood into the vein of a human was in (1642) (1). The year 1900 however, marked the beginning of the modern concept of blood transfusion when Karl Landsteiner published his classic studies on the three blood types in human beings (A,B.and O), to which his associates De Castello and Sturli added the fourth (AB) in 1901 (1,2,3,4).

This led to the recognition that serologic incompatibility between donor and recipient could account for a transfusion reaction and in 1908 Ottenberg performed the first pre- transfusion tests in vitro to determine compatibility(10. Later the discovery of the Rhesus factor by Landsteiner and Weiner in 1940 (1,2) and the advances in immunohaematology that followed, provided the immunologic understanding required for modern safe transfusion of blood. This would not have been possible without parallel advances in the techniques of drawing, storage and administration of blood. The use of sodium citrate as an anticoagulant was first described in 1914 by two independent authors- Hustin in Belgium and Agote in Buenos Aires(1).

In 1916 Rous and Turner showed that addition of glucose improved the preservation of citrated blood (1) and this made indirect transfusion of stored blood possible.

Loutit and Mollison in 1943 described an improved acid-citrate-dextrose anticoagulant preservation solution that made storage of whole blood possible for upto 21 days (1,2). However, it was only after the development of blood banks after world war II that the use of whole blood was practised on a wide scale(5).

The basic purpose of transfusing blood is to replace the loss of either whole blood or one or more of its components in a patient when that loss is likely to cause severe disturbance of one or more of the essential functions of the blood. Thanks to the progress that has been made in the technology of blood component preparation and storage, each unit of whole blood appropriately fractionated can now provide red blood cells, platelets, leucocytes, plasma, cryoprecipitate, factor VIII and IX concentrates, albumin and gamma globulin (6)

One study on the use of blood transfusion in different clinical conditions was conducted by Cooke in New South Wales (7). The use of blood was found to be as follows:-

Medical	- 33.2%
Obstetrical	12.0%
Traumatic	10.0%
Surgical	44.8%

This study showed that most of the blood was administered to surgical patients. It was found that the treatment of gastro-intestinal haemorrhage was the largest single indication for transfusion amounting to 9.3% of the blood used during the survey period. A large amount of blood (5.44%) was transfused to patients with leukemia and anaemia associated with other forms of malignant disease. This was an indication of the important part played, even then, by blood transfusion in the management of patients with neoplastic conditions.

Cooke noted that although obstetricians were among the first to use blood transfusion to the fullest extent, there had not been the same percentage increase in the amount of blood

used for obstetrical conditions as there had been in that used for medical and surgical conditions. Booth, Path and McGregor conducted a survey in Port Moresby, Papua New Guinea and compared the indications for blood transfusions with those found in Sydney by Cooke during the same period (1966-1967) (8). Their findings were as follows:-

Medical	28.2%
Surgical	28.9%
Traumatic	4.5%
Obstetrical	29.8%
Gynaecological	8.6%

In contrast to the Sydney findings, haematemesis required relatively small amounts of blood less than 2% of the overall compared to 9.3% in New South Wales. The largest single indication for transfusion was anaemia of pregnancy amounting to 12.6%. An earlier survey in this region (1963-64) had shown that anaemia of pregnancy accounted for 15.6% of the total blood used. The absolute fall in the blood used for these patients was considered to be a reflection of a greatly improved standard of antenatal care.

The proportion of blood used in traumatic cases was notably lower than in New South Wales and attributed to the lower incidence of serious road accidents in Port Moresby. 0.94% of the total blood was used for treatment of leukemia and anaemia of malignant neoplasm. This compares with 5.44% used for the same in New South Wales. More recent studies have indicated changes in utilisation of blood. There has been a notable increase in the proportion of blood administered to surgical patients. Friedman reported that during 1975-1977 at the University hospital, University of Michigan, Ann Arbor, Michigan, 66.4% of all units of blood were administered to surgery patients (9). Napier et al. in their study in South and Mid Wales, found that 40% of blood was used for surgical patients (10). There has been an associated decrease in the amount of blood used by obstetrical patients. Friedman reported 4.4% (9) and Napier et al. 10% (10) of blood was administered to obstetric and gynaecologic patients. There has also been an increase in the blood utilised by medical patients and Friedman et al. in 1974 found that 18.9% of all transfused units were given to patients with malignant neoplasm (II).

The advances in medicine have created a great increase in the demand for blood but the supply has not kept pace and hence there are always shortages. To combat these shortages initial research concentrated mainly on augmenting the supply, (by enhancing donor recruitment and using autologous blood) and on prolonging the shelf life of blood.

Storage of blood for upto 42 days is now possible with citrate-phosphate-dextrose adenine (CPD-A)(12). Liquid nitrogen frozen banks are also available for maximum storage (12). Increased awareness of diseases transmitted by blood transfusion, especially hepatitis and the acquired immunodeficiency syndrome (AIDS) has resulted in major changes in transfusion practices. Decreasing the demand for blood has become the desirable approach to reducing the stress on limited blood supplies. This can be achieved by:-

1. Reduction in outdateding of blood
2. Use of blood components
3. Elimination of unnecessary transfusions, and
4. Use of autologous blood(12)

Outdating of blood can be reduced by having an efficient inventory control, which includes implementing a first

in first out policy; multiple cross matching of short dated blood, and application of the type and screen procedure (12). The type and screen consists of ABO-Rhesus typing and screening for unexpected antibodies. It is advocated as a substitute for pre-operative cross-match of blood for those elective surgical procedures which rarely necessitate blood transfusion (10,13,14,15, 16,17). The use of blood components instead of whole blood is preferred for the reasons that more than one patient can benefit from one unit of blood and it avoids the risk of immunisation due to unwanted factors (12, 18). Apparent logical reasons for transfusions have often prevented a scientific analysis of the indications for transfusion. In 1964, Diethrich reported that 17% of units administered on the surgical service; 25% in the medical service and 38% on the obstetrical and gynaecologic service were without valid indication (19). Anaemia is considered by many to be an anomaly which should always be corrected and this has often led to inappropriate use of blood(20,21). Okafor et al. in a study on blood transfusion in obstetrics and gynaecology in Benin, Nigeria, found that 15.2% of patients were transfused for a chronic anaemia(20). Most of these were single

unit transfusions which they considered unnecessary.

It has then been emphasized that transfusion in chronic anaemia is not justified unless the patient is symptomatic (19,20,21). Single unit transfusions require special mention. The transfusion of less than 500ml of blood to an adult has been a subject of controversy for many years. Many authors have stated that most cases of single unit transfusions are not justifiable. Reece A.L. and Becket R.S. in 1962 found that 66% of single unit transfusions in a community hospital were regarded as questionable or unnecessary (22). Diethrich found that 70% single unit transfusions on the surgical service, 60% on the medical service and 66% on the Obstetric/Gynaecology service were not indicated (19). Condemnation of the use of single unit transfusions by authorities led to a decrease in the practice and Morton noted a decrease in the number of adult single transfusions from 36% in 1959-60 to 24% in 1967 (5). In addition, he found that the percentage of those transfusions judged unnecessary or questionable dropped from 72% to 59%. The American Medical Association

Committee on blood and blood banking authorities however stated that in indicated situations the transfusion of a single unit of blood was sound judgement and good medical practice.(22). The increasing demand for compatible blood has stimulated renewed interest in autologous transfusion. The description of autotransfusion dates back to 1818(23). When available autologous blood would be the safest that a person could receive. There is serologic compatibility and no risk of post transfusion infection (23,24). The disadvantage is that it presents logistic problems of collection and storage. Autologous blood is however indicated for patients with atypical antibodies to any cellular or plasma components which may cause clinical reactions including patients with multiple red cells alloantibodies. It is also indicated for patients of certain religious sects who do not accept homologous blood transfusion.

JUSTIFICATION OF STUDY

Blood is a resource which has always been in short supply..

Because of this many centres have formulated guidelines for blood transfusion practices aimed at maximising the usage of blood products.

This is a pilot study which is to act as an eye opener into the practice of blood transfusion in this hospital. Such knowledge would be first step in the process of setting up guidelines to suit our particular environment.

SPECIFIC OBJECTIVES

1. To determine the demand for blood and blood products by different wards at the Kenyatta National Hospital.
2. To assess the utilisation of blood and blood products by different wards at the hospital.

MATERIALS AND METHODS

The study was conducted in the Routine Laboratory of the Kenyatta National Hospital after due approval from the Hospital's Ethical and Research Committee. Kenyatta National Hospital is a teaching hospital affiliated with the College of Health Sciences of the University of Nairobi. It has approximately 2000 beds and functions as a general acute care and referral facility. The hospital runs a blood bank which processes and issues mainly whole blood. Most of this blood (71%) is supplied by the National Blood transfusion service. The rest comes from the hospital's Blood Donor Service (25).

Over a four month period between July and November, 1990 every 10th request for blood transfusion received at the Routine Laboratory was scrutinized. The 10th request was chosen with the aim of giving a sample size of approximately 400 requests. This sample size was deduced using the following mathematical formula:-

$$N = \frac{Z^2(p(I-P))}{C^2}$$

Where N= sample size

Z= Confidence unit factor

P= Estimated prevalence (0.48)

This was calculated from the previous year's records of blood grouped and number crossmatched for patients (25)

C= 1-0.95 (confidence level 95%)

The following data was collected from each request card: ward; indication for transfusion; Haemoglobin level; number of units requested component requested and the degree of urgency. Demographic data was also collected.

All the requests were followed up to find out the number of units that were crossmatched; how quickly the blood was available for issue to the ward; how soon the blood was collected by the ward personnel and how much of it was transfused into the patient. All this was recorded on a proforma sheet, (See appendix I).

RESULTS

505 blood request cards were scrutinized during the 4 month study period. 202 requests were from male patients and 303 from females giving a male to female ratio of 1:1.5. Their ages ranged from 1 day to 81 years with a mean of 30 years.

189 requests (37.4% of total) were from the surgical wards. This was followed by, in descending order, 176 requests (34.9%) from the obstetric/gynaecology unit, 78 (15.4%) from medical wards and 62 requests (12.3%) from the paediatric wards.

Blood was crossmatched for 50 (64.1%) and 39 (62.9%) of those requesting for it from the medical and paediatric wards respectively, compared with 111 (58.7%) and 59 (33.5%) from the surgical and obstetric/gynaecology wards respectively. In the obstetric unit, out of 81 requests, blood was crossmatched for only 13 (16%).

These findings are shown in Table I.

Table I: Number of blood requests and crossmatches in 505 patients at KNH

Ward	No. of requests	% of total requests	No. of crossmatches	% crossmatched of the requests from each ward
Paediatric	62	(12.3)	39	62.9
General Medicine	78	(15.4)	50	64.1
Surgery	189	(37.4)	111	58.7
Acute Gynaecology	50	(9.9)	17	34.0
Cold Gynaecology	45	(8.9)	29	64.4
Obstetrics	81	(16.0)	13	16.0
Overall Obstetrics and Gynaecology	176	(34.9)	59	33.5
Total	505	(100)	259	51.3

The gynaecology unit consists of two wards. One is an emergency ward (the acute gynaecology ward) and the other caters for cold gynaecology patients. There were 50 requests for blood from the acute gynaecology ward and 45 from the cold gynaecology ward. Blood was cross-matched for 17 (34.0%) and 29 (64.4%) of those requesting from the acute gynaecology and cold gynaecology wards respectively.

In response to these requests, 436 units of blood were crossmatched for 259 patients. However, only 245 units were ultimately transfused to 146 patients, meaning that not all the blood crossmatched was utilised.

The ratio of units of blood crossmatched to units transfused into patients, that is the crossmatch transfusion (C/T) ratio is an index that can be used to determine the efficiency of blood ordering. The overall C/T ratio for the hospital is 1.8. Those for the various wards range from 1.1 to 2.8. The high C/T ratios are from the surgically oriented wards with the obstetric ward having the highest ratio of 2.8.

In the gynaecology unit, the C/T ratio for the acute gynaecology ward is 1.6 while that for the cold gynaecology ward is 2.5.

These results are shown in Table 2.

Table 2: Analysis of crossmatches done, transfusions given and crossmatch: transfusion ratios.

Ward	No. of patients cross-matched	No. of patients transfused	No. of units crossmatched	No. of units transfused	Crossmatch: transfusion ratio
Paediatric	39	34	42	37	1.1
General Medicine	50	34	82	55	1.1
Surgery	111	54	197	101	2.0
Obstetrics	13	3	28	10	2.8
Acute Gynaecology	17	10	34	21	1.6
Cold Gynaecology	29	11	53	21	2.5
Overall Obstetrics and Gynaecology	59	24	115	52	2.2
Total	259	146	436	245	1.8

C/T ratios calculated for surgical procedures showed marked variation ranging from 1.5 for craniotomy to 17.0 for caesarian section. The higher the C/T ratio, the less the likelihood for blood transfusion during the particular surgical procedure. For plastic surgery and myomectomy, the blood issued was never utilised at all. In such cases the C/T ratio would be infinity. This is shown in Table 3.

Table 3: Number of patients crossmatched and the crossmatch/transfusion ratios for some surgical procedures

Procedure	No. of patients cross-matched	No. of units crossmatched	No. of units transfused	C/T Ratio
a) Surgery				
Exploratory laparotomy	10	18	9	2.0
Oesophagectomy	6	17	8	2.13
Craniotomy	8	24	16	1.5
Prostatectomy	6	13	4	3.25
Plastic Surgery	6	7	0	-
b) Obstetric & Gynaecologic Surgery				
Total abdominal hysterectomy	9	19	5	3.8
Myomectomy	4	9	0	-
Tuboplasty	6	10	3	3.3
Exploratory laparotomy	9	18	7	2.6
Caesarian Section	10	17	1	17

Blood transfusion requirements were correlated with diagnosis. 26 units of blood (10.6%) of the total were given to medical patients (both adult and paediatric) with anaemia whose aetiology had not been determined at the time of transfusion (i.e. unspecified anaemia). 14 of these units were administered to adults and 12 to paediatric patients. 20 units of blood which comprises 8.2% of all transfused units were transfused into patients with upper gastrointestinal haemorrhage all of whom were from the adult medical wards. 18 units of blood (7.3% of total) were administered to patients with anaemia associated with malignant neoplasia. Of these 18, 10 units were transfused into patients with carcinoma of the uterine cervix. Not included in this group are those patients with malignancies whose transfusions were related to surgical procedures. 10 units of blood (4.1% total) were transfused into patients with uraemia. 3 of these patients were being treated by dialysis. 6 units of blood were given to 3 patients with Aplastic anaemia. 2 of whom were adults and 1 was a paediatric patient. Patients with leukemia also received 6 units of blood. All of them (4 patients) had acute lymphoblastic leukemia. These results are given in Table 4.

Table 4: The use of blood in different clinical conditions

Indication	No. of units Transfused	Percentage of Category	Overall Percentage
Medical:	106		43.3
Haematemesis	20	18.9	8.2
Anaemia of Malignant neoplasm	18	17.0	7.3
Aplastic anaemia	6	5.7	2.4
Uraemia	10	9.4	4.1
Leukemia	6	5.7	2.4
Hodgkin's lymphoma	1	0.9	0.4
Malaria	5	4.7	2.0
Haemolytic disease of newborn	2	1.9	0.8
Iron deficiency anaemia	1	0.9	0.4
Neonatal haemorrhage	3	2.8	1.2
Unspecified anaemia	26	24.5	10.6
Other medical conditions	8	7.5	3.3

Table 4 Cont'd

Indication	No. of Units Transfused	Percentage of Category	Overall Percentage
Surgical	101		41.2
Orthopaedic Surgery	7	6.9	2.9
Cardiothoracic Surgery	20	19.8	8.2
Neurosurgery	11	10.9	4.5
Urological surgery	11	10.9	4.5
Dental procedures	5	5.0	2.0
Injuries and burns	20	19.8	8.2
Other surgical procedures	27	26.7	11.0
Obstetric/ Gynaecology	38		15.5
Caesarian section	1	2.6	0.4
Postpartum haemorrhage	6	15.8	2.4
Incomplete abortion	12	31.6	4.9
Ectopic pregnancy	8	21.0	3.3
Anaemia in pregnancy	3	7.8	1.2
Gynaecological operations	8	21.0	3.3
Grand Total 245			100

In the surgical service, 20 units of blood were used in the management of injuries and burns. 11 units were administered to patients with burns and the rest were given to those with injuries. This group of patients utilised 19.8% of the blood transfused in the surgical service and this amounted to 8.2% of all the blood transfused in the study. 85% of the injuries were due to road traffic accidents and the affected patients had limb fractures. An equal amount of blood was transfused into patients undergoing cardiothoracic surgery. Most of these units (65%) were given to patients with carcinoma of the oesophagus. Equal amounts of blood (11 units each) were transfused into patients undergoing neuro- and urological operations. However, because the neurosurgical patients were fewer in number (4 compared to 7 urosurgical patients), the amount of blood transfused into each was on average more than that transfused into urosurgical patients. The patients in the Ear, Nose, and Throat (ENT), and eye wards had fairly low transfusions requirements. 4 units of blood were transfused into ENT patients, all of whom had Burkitts lymphoma.

In the eye ward, 2 units of blood were administered to two patients with retinoblastoma.

A large share of the blood used in the obstetric/gynaecology service (12 units) was given to patients with incomplete abortions. This amounted to 31.6% of the blood used in these wards and constituted 4.9% of all the units transfused in the study. On the other hand, caesarian section was associated with a low transfusion rate, blood used during this operation amounting to only 0.4% of all the units transfused in the study. Patients with ectopic pregnancy utilised 8 units (3.3% of the total units transfused) while 6 units of blood, constituting 2.4% of all transfused units were given to 2 patients with postpartum haemorrhage. One of these patients had a retained placenta and received 4 units of blood.

Patients undergoing gynaecological procedures used 8 units of blood which constituted 21% of the blood transfused in these wards. 5 of these units were administered to patients undergoing total abdominal hysterectomy and the rest were used during tuboplasty.

Table 5: Units of blood transfused into adult patients

Ward	No. of patients	Units of blood transfused				
		1	2	3	4	5
Medical	34	14	20			
Surgical	54	19	27	6	1	1
Obstetric/ Gynaecology	24	8	41		2	
Total	112	42	61	6	3	1

61 of the adult patients transfused (54.5%) received 2 units of blood while 42 (37.5%) received a single unit of blood. 14 (41.2%) of the transfusion in the medical wards were single unit transfusions. The respective percentages for the surgical and obstetric/gynaecology wards are 35.2 and 33.3.

4 patients received more than 3 units of blood. 2 of these were from the surgical wards and the other 2 from obstetric/gynaecological wards. Of the surgical patients I received 5 units of blood while undergoing craniotomy for removal of a brain tumour while the other one was transfused with 4 units during oesophagectomy for carcinoma of the oesophagus. Of the obstetric/gynaecology patients, both received 4 units of blood each. One patient had post partum hæmorrhage due to a retained placenta and the other had septic abortion with disseminated intravascular coagulation.

Table 6: Amount of blood transfused into paediatric patients

Amount of blood (mls)	No. of patients	%
0-250	15	(44.1)
300-450	6	(17.6)
500	9	(26.5)
> 500	4	(11.8)
Total	34	(100)

21 patients in the paediatric ward (61.7%) were transfused with less than 500 mls of blood, 15 of them(44.1%) receiving less than 250mls.

DISCUSSION

One of the methods of determining the efficiency of blood ordering is to determine the ratio of units of blood crossmatched to units transfused i.e. the crossmatch/transfusion (C/T) ratio (26). For a hospital providing a full range of clinical services, the C/T ratio should be about 2.5/1 (26). In this study the overall C/T ratio was 1.8/1. In 1976, Rouault and Gruenhagen found the C/T ratio for the University of Southern California Medical Centre to be 4/1 (26). Napier et al. in a study involving 17 hospitals in Wales, found C/T ratios ranging from 1.3 to 4.2 (10). They suggested that the hospitals having low C/T ratios may be crossmatching blood for a smaller proportion of patients more readily. Both these factors may have contributed to the results found in this study. Most of the blood transfused was administered to patients in the surgical wards (41.3%) with those from Obstetrics/Gynaecology taking 21.2%. These relate well with results from other studies. In 1967 Cooke found that 42.5% of units in New South Wales were administered to surgical patients and 18.3% to obstetric/gynaecological patients (7).

Napier et al. reported similar findings in 1985 (10).

Friedman in his study found that 66% and 4.4% of all units were transfused into surgical and obstetric/gynaecologic patients respectively(9). Many of the units crossmatched are however not transfused into patients as is indicated by the high C/T ratios especially in the obstetric and cold gynaecology wards (2.8 and 2.5 respectively).

The usual preparation for many surgical procedures calls for typing and crossmatching for 2 units of blood. Some of these procedures however, are associated with low transfusion rates and hence the blood may not be utilised. This leads to a situation where excess blood is crossmatched, a situation that is undesirable because it increases outdating of blood and increases laboratory costs (costs of reagents). Furthermore, the blood is unavailable for patients with legitimate requirements and its availability during the operation may serve as a stimulus for unnecessary transfusion (9,15, 17). In order to prevent this practice, new systems for ordering blood have been developed.

Boral, Rouault and Gruenhagen are among those who advocated for the type and screen approach (14,15,26,27) while Friedman (9) put forward a maximum surgical Blood Order schedule (MSBOS) which is a list of the commonly performed surgical procedures with the maximum number of units of blood which will be crossmatched for each. The primary goal of these systems is to make pre-operative blood orders coincide more closely with the number of units of blood which would actually be transfused into patients during or immediately after the surgical procedures. In this study, the C/T ratios for total abdominal hysterectomy (TAH) and caesarian section were 3.8/1 and 17/1 respectively. Napier et al, found C/T ratios of 4.8/1 and 9.6/1 for TAH and caesarian section respectively (10). These are some of the procedures for which the type and screen is advocated (9,10,14,15,16,20,26). However, in order to convince surgeons to change their pre-surgical blood orders from a 'type and crossmatch' to a 'type and hold', it is necessary to guarantee blood availability within a short time should blood be unexpectedly required.

In the cardiothoracic unit, the C/T ratio was 1.5/1.

This is slightly lower than what has been found by other investigators. Boyd et al. in 1979 found a ratio of 2.0/1 at the Upstate Medical Centre, New York (14)., while Boral et al. at the USAF Medical Centre, Texas, reported a ratio of 2.5/1 for cardiopulmonary patients(16). The variation in these ratios may be a reflection of differences in surgical expertise as well as differences in the procedures undertaken in these centres. 65% of the blood used in the cardiothoracic unit was administered to patients undergoing oesophagectomy for carcinoma of the oesophagus. These patients received an average of 3 units of blood each. In their study, Boyd et al. reported similar results (14). Napier et al. in their maximum surgical blood order tariff recommended 4 units of blood to be crossmatched for patients undergoing oesophagogastrectomy(10). Those patients who underwent craniotomy also received an average of 3 units of blood each. The recommended number of units to be crossmatched for this procedure is 2 units(26). The demand for blood by the cardiothoracic and neurological units is expected to rise because with increasing number of specialist surgeons, more patients are undergoing these delicate operations.

12.2% of all the blood was used in the management of obstetrical patients. Cooke in his study in 1967 reported similar findings (7). In Port Moresby, Booth et al. found 30% of the total was used for obstetrical indications (8), and they noted a fall in the use of blood for management of anaemia in pregnancy from 15.6% in 1963-1964 to 12.6% in 1966-1967(8). They attributed this fall to improved standards of antenatal care. Many of the obstetric patients here have been attending antenatal clinic in this hospital which may explain the low transfusion rate in these patients. Only one patient had anaemia in pregnancy and she received 3 units of blood which amounted to 1.2% of all blood transfused in the study. Compared to the other surgically oriented wards, the acute gynaecology ward had a low C/T ratio (1.6/1) which approximates that found in the medical wards (1.5/1). This may be due to the fact that it is an emergency ward that deals mainly with cases of incomplete abortions and ectopic pregnancies. Incomplete abortions are said to constitute upto 60% all emergency gynaecology admission at the KNH (28). Blood transfused into these patients comprised of 31.6% of all the

blood transfused in the obstetric/gynaecology unit. This constituted 4.9% of all the blood transfused in the study, a figure that correlates with the findings of Booth et al. who reported 3.3% of all units were transfused into patients with abortion (8). 4-5 cases of ectopic pregnancy are operated at the KNH every week (29). This condition is said to be the most common of all acute gynaecologic conditions requiring immediate abdominal surgery. 21% of the blood transfused in the obstetric/gynaecologic unit was administered to patients with ectopic pregnancy. This is much higher than 4.6% found by Cooke in New South Wales (7) although his study was done more than 20 years ago, and the incidence of ectopic pregnancy may have changed since then.

Medical patients are not included in maximum blood order schedules since a high percentage of blood cross-matched for them is ultimately transfused. In this study, 74% of the units crossmatched for medical patients were transfused. Friedman et al. found the proportion to be 46% in their study (30). This however, does not necessarily imply good transfusion practice. In a prospective study of blood transfusion in a large general hospital, Diethrich concluded that 25% of units administered to medical patients, 18% of units administered to surgical patients and 39% of units administered to obstetric/gynaecologic patients were without valid indication (19). The necessity for transfusing patients in the medical wards is encountered in two major clinical settings-acute blood loss and chronic anaemia. 31% of the units crossmatched in the adult medical wards were for patients with acute upper gastrointestinal haemorrhage. This was also the largest single indication for transfusion accounting for 8.2% of all the blood transfused in the study. This compares well with Cooke's study where 9.3% were administered to patients with upper gastrointestinal haemorrhage (7) and contrasts with Booth et al who found percentages of 2.3 and 1 during the periods 1963-1964 and

and 1966-1967 respectively (8). This emphasizes the differences in clinical indications for blood transfusions in different regions. Blood transfusion plays an important part in the management of patients with neoplastic conditions. It may be used during operations for resection of tumours or may be transfused to those with anaemia due to malignant tumours. In this study, 7.3% of all units were administered to patients with anaemia of malignant neoplasia. Most of these patients had cancer of the uterine cervix. This is the commonest female genital malignancy in Kenya (31). The management includes radiotherapy and blood transfusion is important as a supportive measure. In their study, Friedman et al. found 18.9% of all blood was given to patients with malignant neoplasm (14). Their percentage however included those patients transfused during surgical resection of the tumour. Blood transfusion is also an important supportive measure for patients with end-stage renal disease receiving dialysis treatment. Here 4.1% of all units were given to patients with uraemia, some of whom were on dialysis. Cooke found 3.3% of all blood was transfused into patients with nephritic anaemia (7).

Although it has been said that anaemia in the tropics is frequently attributable to multiple causes and thus many patients transfused for anaemia cannot be accurately classified(8), the importance of searching for the cause of anaemia cannot be over emphasized and it is only then that appropriate therapy can be instituted thus reducing the need for blood transfusion. A high proportion of blood (10.6% of the total) was transfused into patients with anaemia whose aetiology had not been determined at the time of transfusion (i.e. unspecified anaemia). One of the criteria Graham-Stewart used while considering whether transfusions were clinically justified was that where anaemia was not due to acute blood loss, it should be treated by transfusion only if it will not respond to drugs (32). Some of these transfusions may therefore not be justifiable. Cooke in 1967 and Booth et al. found that 1.6% and 5.8% respectively were given to such patients (7,8). There is a significant proportion of adult patients - 41% from medical, 35% from the surgical and 33.3% from the obstetric and gynaecology wards who received only one unit of blood. Early reviews of single unit transfusions revealed that a high proportion of them were unnecessary. In his study, Diethrich found that 70% of the single units transfused on the surgical service, 60% on the medical service, and 66% on the obstetric/gynaecology service were without positive indication (19). Other authors support the practice stating that it more often reflects appropriate use of blood than misuse (9,33). When reviewing transfusion practices, more emphasis should be placed on whether or not blood

transfusion was warranted at all. Here it is likely that the transfusion of one unit was appropriate since for all those who received one unit of blood, the clinicians had requested for 2, but were issued with only one unit of blood.

The paediatric wards had the lowest C/T ratio (1.1/1) which compared well with the results of Boyd et al. (1.2/1)(14). The units administered in these wards were however not fully utilised. About 60% of the paediatric patients were transfused with less than 500mls of blood. Whenever a unit of blood is opened to give a small part to a youngster, the rest of the unit cannot be used for human transfusion and is therefore wasted. The finding by Morton that 10% of children transfused received blood from more than one unit and many of those using one unit received only a portion of it is pertinent to this problem(5). Based on these findings, the Red Cross, which was the supplier of blood then, decided that the additional expense involved in collecting a certain number of smaller units was justified by the potential saving of this valuable commodity in short supply (5). Due to inavailability of these paediatric packets, small units of blood are not collected here. Anaemia due to malaria infection was the largest single indication for blood transfusion in the paediatric wards and 2.0% of all units transfused in the study were administered to such patients. This reflects the morbidity caused to children by this parasite. 1.2% of all units and 0.8% were administered to neonates for neonatal anaemia and for exchange transfusion respectively. As more babies of low gestational age continue to survive thanks to the specialist care available at the new

born unit, it is expected that the demand for blood transfusion as supportive treatment, will also increase.

Most blood transfusion centres keep blood in the assigned(crossmatched) state for a maximum of 48 hours (10,13,14,15,16). In this study we found that blood remained in the assigned state for upto 12 days (mean of 2.8 days). The risk of wastage due to outdateding of blood is increased when there is a delay in re-cross-matching of blood which is not transfused.

For the duration of the study, there was no reported surgical procedure where autologous blood was used. Autologous blood pre-operative deposit is a practice which has received much support from among others, the American Medical Association (34). Apart from being a safe practice, it represents a source of blood for both the national and hospital blood inventory (34,35,36). Upto 4 units of blood can be safely drawn within 10 days before the operation with con-

current iron supplementation by either the oral or intravenous route (34,36,37). Most programmes require that haemoglobin level be above 11g/dl or haematocrit above 34% before each phlebotomy (34,36). There are reports of successful autologous donations by young children and elderly patients (34). Other diseases that usually preclude blood donation do not exclude autologous blood donation for example cardiovascular disease (36). Pregnant women may also safely donate during the second trimester (37). Intra-operative autotransfusion is also effective for many trauma and surgical patients. This practice is however associated with adverse effects if not well utilised and the filtering devices required may be expensive (23,34).

It has been recommended that a blood transfusion service should be organized so that it is one step ahead in the development of hospital services (38). We

however noted that the only blood product that was supplied by the hospital transfusion service during period was whole blood.

It was also noted that there were no requests for blood products from the clinicians looking after the patients. This may however be stemming from the knowledge that such products are not available. This falls short of the 1984 recommendations of the international society of blood transfusion which stated that for developing countries, at the state hospital level, it should be possible to provide whole blood, red cell concentrates, platelets, cryoprecipitate as well as plasma (39). This finding therefore shows that not only is the transfusion service lagging behind in development but it is also contributing to inefficient use of its scarce resources.

CONSTRAINTS:

The objectives of the study were to determine the demand for and assess the utilisation of blood and blood products. However, due to the inability of the hospital's blood transfusion service to provide any blood products, the study was limited to the utilisation of whole blood.

CONCLUSIONS

1. This study shows that the blood supplied by the hospital's transfusions service is not adequate to meet the demands of the hospital. Only 51% of all patients who requested had blood cross-matched for them.
2. The surgical wards make the highest demand on the hospital's transfusion service. There are however some surgical procedures which are associated with such low transfusion rates that pre-operative cross-matching of 2 units of blood may not be warranted.
3. By comparison of C/T ratios, the medical wards (both adult and paediatric) show better blood utilisation rates than the surgical wards.
4. Lack of paediatric blood packs is contributing to blood wastage since a 500ml blood pack is often not fully utilised by paediatric patients.
5. Crossmatched blood remains in the assigned state for unduly long period (average 2.8 days). This may contribute to wastage of blood due to increased outdating.
6. Blood products such as packed red cells and platelet concentrates are not routinely provided by the hospital's blood transfusion service.

RECOMMENDATIONS

1. A larger study should be conducted to establish blood requirements for the common surgical procedures undertaken at the hospital with the aim of formulating maximum surgical blood order schedules. This might reduce the number of unnecessary cross-matches done and will form the basis for future evaluation of transfusion practices.
2. The hospital bank should expand its services to include regular provision of blood components especially packed red cells and plasma. This would make more patients benefit from one unit of blood.
3. A certain number of smaller units of blood should be prepared with the paediatric transfusion in mind. This would save on this very valuable commodity.
4. Transfusion of blood into a non bleeding patient before the cause of anaemia is known should be discouraged since some of these patients may be adequately managed by drug therapy.

5. A program for autologous blood transfusion should be considered. Not only would such a program reduce the demand for blood, it would also increase the homologous blood inventory.

6. Most centres keep blood in the assigned state for a maximum of 48 hours. The hospital blood transfusion centre should adopt this policy. Releasing the blood to the general pool would make it available for some other needy patient and would reduce the outdating of blood.

STATUS OF SUBGROUPS OF BLOOD GROUP A(A₁ AND A₂)

11 years after Karl Landsteiner's discovery of the ABO blood groups, Von Dingern and Hursfeld noted that when blood group O serum, which has anti A antibodies, is treated with certain blood group A cells, the absorbed serum would still cause agglutination of other group A and AB blood (4,40,41).

These findings, which suggested subdivision of A and AB were confirmed by among others, Coca and Klein in 1923 and the subgroups were known as A₁, A₂, A₁b and A₂B (4,40,42). Since then other subgroups and variants of the A antigen have been found which include A₃, A₄, A_X, A_M, A_Z and A_O (4,40, 42, 43). Most of these variants are extremely rare and give very weak reactions with standard anti-A sera. The basis for the subdivision of A erythrocytes into A₁ and A₂ subgroups has been a subject of uncertainty and controversy ever since its discovery. Most investigators agree that there is a quantitative difference between the two subgroups, A₁ cells having been found to have two thirds to three fourths more A antigen sites than A₂ cells (2,4,40,42). Some investigators also suggested that A₁ red cells differed from A₂ cells in a qualitative manner (2,4,40, 42,43). The A antigen is said to have 2 components, A and A₁.

Subgroup A_I has both components while subgroup A_2

is devoid of the A_I component (2,4,42).

The finding of anti A_I antibodies in the sera of some A_2 and A_2^B subjects provided evidence in support of a qualitative difference between A_I and A_2 (2,4,40,42,43, 44,45). These antibodies have been found in 1-8% of group A_2 subjects and 25-30% of group A_2^B subjects (2,4,42,44,46).

A_I and A_2 subgroups can be identified by mixing the cells with anti A_I serum absorbed from group B serum, or with anti- A_I lectin obtained from the seeds of *Dolichos biflorus*. A_I and A_I^B cells will react with these reagents and clump whereas A_2 and A_2^B cells will not.

The subdivision into A_I and A_2 has been of great forensic importance, especially in resolving cases of disputed parentage. As far as transfusions are concerned, this subdivision has usually been ignored.

This is because anti A_I normally acts as a cold agglutinin and rarely reacts with the appropriate red cell

antigens at temperatures above 30°C. There are however reported cases of haemolytic transfusion reactions caused by anti A_I. These are usually delayed transfusion reactions and are more likely to occur following multiple transfusions(2). Young in 1944 reported 2 cases where group A₂ patients developed anti A_I antibodies after receiving 21 units of blood group A. and 10 units of blood group O(41). The antibodies were active against A_I cells at room temperature and occasionally at 37°C (41). Boorman et al. in 1946 (47) and Lundberg and McGeiniss (48) also reported such cases and showed that it was possible to obtain by immunisation, either by transfusion or by pregnancy, agglutinins specific and active at 37°C for A_I blood in recipients of subgroups A₂ and A₂B. The significance of subgroups A_I and A₂ is not limited to incompatibility between them. Serious reactions may occur due to failure to recognise subgroup A₂. This

failure is usually caused by using typing sera with low titres of iso-agglutinins leading to misgrouping of A_2 as blood group O and A_2B as group B. There has been a report of a severe reaction after administration to a patient of blood group B with blood supposedly of the same group (49). A subsequent check showed the mistake in blood grouping and that the donor's blood was actually of group AB.

It has therefore been recommended that A_2B and A_1B red cells should be used to assess anti-A and anti-B $\frac{1}{2}$ respectively before these reagents are introduced into routine work because the weak A of A_2B and the weaker B of A_1B represent the weakest commonly encountered examples of these antigens in routine grouping (50). The World Health Organization (WHO) further recommended that in serum grouping, group A_1 red cells should be used as standard red cells, the reason being that since group A_2 red cells are only weakly agglutinated by the anti A

antibodies of group B and O subjects, there would be a risk of doubtful agglutination should A_2 red cells be used and the results would be difficult to interpret (51).

There are considerable ethnic differences in the incidence of the A subgroups. In caucasians, 75-80% of all group A individuals belong to subgroup A_1 . In a study of North London blood donors, subgroup A_2 was found to comprise 25% of all group A, while 19.5% of all group AB was found to be A_2B (4,42). The A_2 subgroup has been found to be absent in the Chinese, Vietnamese and Australian Aborigenes while a high proportion of it (34%) has been noted in the Lapps (2). In Africa, the earliest published work on blood group distribution is said to be that by Ronald Elsdon-Dew in 1936(52,53). Results of studies conducted later on the ABO blood groups indicated that Africans had a higher incidence of the non A_1 antigen (54). Kiango and Luwa in Dar-es-Salaam,

found that 36.8% of all A samples were non A_1 subgroup (54). A study of Rhodesian tribal groups in 1969 showed that the proportion of A_2 varied among different tribes, ranging from 14% to 30% of all group A, while A_2B ranged from 44%-67% of all group AB samples (55).

In Kenya, information on the subgroups of A is scanty, the 1951 study by Allison et al. is said to have included the subgroups A_1 and A_2 but this was limited to only three Kenyan tribes (53). Beecher's report on the ABO blood group distribution in 6 Kenyan tribes namely Kikuyu, Wakamba, Gusii, Abaluhya, Luo and Nandi did not include the A subgroups and neither did Amolo and Chhanyara when they studied the ABO blood group distribution in Western Kenya in 1981 (53,56). This lack of published information on the A subgroups in Kenya and knowing their importance in the quality assurance in blood grouping kindled the interest in this study. The subgroups A_1 and A_2 were specifically chosen because they are the most common and most important of the subdivisions.

Specific Objectives

1. To collect group A and AB blood samples and subgroup them into A_1 , A_2 and A_1B , A_2B respectively.
2. To calculate the proportions of subgroups A_1 to A_2 and A_1B to A_2B in the samples tested.
3. To compare the proportions of subgroup A_1 to A_2 and A_1B to A_2B with the findings of other investigators in Africa.

MATERIALS AND METHODS

Between November, 1990 and January, 1991, all samples of blood received in the Routine Laboratory and grouped as A and AB were subclassified into A₁, A₂, A₁B and A₂B.

Criteria for exclusion

1. All blood samples from infants were excluded from the study. This is because the A antigen is not fully developed till about one year of age (2,44,46).
2. All blood samples which showed significant red cell haemolysis. Such samples can give a false negative reaction.

LABORATORY METHODS

The blood samples were allowed to clot at room temperature and the serum was then separated. The cells were washed thrice in physiological saline and a 3-5% red cell suspension in physiological saline was prepared.

The following procedures were done:-

- a) Repeat ABO grouping using commercial anti A and anti B sera.

b) Identification of A_1 and A_2 using commercial anti A_1 prepared from the lectin *Dolichos biflorus*. This was done by the blood grouping method described by Dacie and Lewis (46) (see appendix II)

Both these procedures were done by the author herself. For quality assurance, known A_1 and A_2 red cells were tested along with each batch of blood samples being tested.

Statistical analysis was done using chi-squared tables.

RESULTS

Over the study period, a total of 376 group A and Ab blood samples were subgrouped into A₁, A₂, A₁B and A₂B. 332 samples (88.3%) were of blood group A while 44 (11.7%) were group AB.

104 samples were from male patients and 272 were from females. This gave a male female ratio of 1/2.6. Blood samples received in the laboratory for blood grouping from antenatal clinic mothers were also included in the study. This is the cause of large proportion of samples from females. Most samples were found to be of the A₁ subgroup 84.3% and 79.5% in groups A and AB respectively.

Table 7 summarises the distribution of these subgroups.

Table 7: Proportion of subgroups A1 and A2 in blood groups A and AB

Subgroup	Number	%
A1	280	(74.5)
A2	52	(13.8)
A1B	35	(9.3)
A2B	9	(2.4)
Total	376	(100)

In males, 92 samples were of blood group A and 13 of group AB giving the total of 104 samples. Only about 9% of blood group A samples from males were of the A_2 subtype, the rest being subtype A_1 . Of the group AB samples from males, 76% were of subtype A_1B and 24% of A_2B .

In the females, A_1 comprised about 82% of group A samples while the proportion of A_1B was about 81% in group AB.

The proportion of subgroups A_1 , A_1B and A_2B are almost equal in males and females. There is however a notable difference in the proportion of subtype A_2 in males and females (7.7% and 16.2% of all samples tested respectively). This low proportion of subgroup A_2 in males has led to a low male to female ratio of subgroup A_2 (0.5/1) and also a high ratio of A_1/A_2 in the males (10/1).

TABLE 8: Proportion of subgroups A_1 and A_2 in males and females

Subgroup	Males		Females		Male/Female RATIO
	Number	(%)	Number	(%)	
AI	83	(79.8)	197	(72.4)	1.1/I
A2	8	(7.7)	44	(16.2)	0.5/I
AIB	10	(9.6)	25	(9.2)	1/I
A2B	3	(2.9)	6	(2.2)	1.3
Total	104	(100)	272	100	0.4/I

TABLE 9: Ratio of A_1 to A_2 in males and females of blood groups A and AB.

Subgroup	Males	Females	Total
AI	83	197	280
A2	8	44	52
Ratio AI/A2	10/I	4.5/I	5.4/I
AIB	10	25	35
A2B	3	6	9
Ratio AIB/A2B	3.3/I	4.2/I	3.9/I

DISCUSSION

Out of 376 blood samples, 332 (88.3%) were of blood A and 44 (11.7%) were of group AB. This gives the ratio A:AB of 7.5:1. which correlates well with the distribution of the ABO blood groups reported by other investigators in Kenya and other African populations. Beecher in 1964 (53) reported the ratio of A:AB in some Kenyan tribes as ranging from 6:1 to 10:1 while Nhonoli and Kiangu found it to be 7.6:1 among Tanzanians (52).

The results of Ezielo in Zambia and Osamo et al, in Nigeria showed a similar trend (57,58). In the United Kingdom, the ratio of A:AB was found to be 13.7/1. This value is high compared to the African figures and reflects the racial differences in the ABO blood groups. Infact blood group variation was one of the characteristics that was used in the division of man into racial groups (58).

African have been said to have a higher incidence of

non A_1 antigen (37). In caucasians, A_1 has been found to comprise 75-80% of all blood group A (2,42,44,46). Kiango and Luwa reported the incidence of non A_1 in Tanzania as 36.6%(54). In this study, the A_1 subgroup comprised 84.3% of all blood group A samples. This result, conforms to what has been found that the proportion of A_1 among some Rhodesian tribes ranged from 70% to 86% (55). The disparity in the results of this study and that of Kiango and Luwa may in part be due to ethnic variation in the presence of subgroup A_2 . As we mentioned earlier A_2 is absent in some populations (2).

It is well known that the presence of the B gene weakens the A gene (55). In Africans, the B antigen is said to be much stronger than in caucasians thus increasing this suppressor effect (2,55). Some of the weaker A genes therefore, when combined with B, are weakened to a degree where they fail to

produce a reaction with anti A_1 . This, in Africans leads to a low ratio of $A_1B:A_2B$ as compared to the ratio $A_1:A_2$. Weiner reported that the ratios of $A_1:A_2$ and $A_1B:A_2B$ in the negroid population were 3.5:1 and 1.5:1 respectively (2). The results of this study did not conform to this. Here, 79.5% of blood group AB samples were of the A_1 subgroup giving the ratio $A_1B:A_2B$ of 3.8/1. The ratio $A_1:A_2$ was found to be 5.4/1. Statistical analysis done using chi-squared tables showed that the difference observed in the ratios of $A_1:A_2$ and $A_1B:A_2B$ in this study was not statistically significant ($\chi^2 = 0.55$). In his Rhodesian study, Lowe found that the ratio of $A_1B:A_2B$ ranged from 0.5/1 while that of $A_1:A_2$ ranged from 2.5/1 to 6/1 (55) there is a notable difference in the proportions of A_1 in males and females in this study. The ratios of $A_1:A_2$ and $A_1B:A_2B$ in males were 10:1 and 3.3:1 respectively while in females the ratios were

4.5:1 and 4.2:1 for groups A and AB respectively.

The disparity in the results of the male sub-population may perhaps be attributed to the small sample size since there were fewer blood samples tested from males (104 compared to 272 samples from females).

CONCLUSIONS

1. In the studied population, A_1 is the predominant subgroup, comprising 84.3% of group A and 79.5% of group AB samples.
2. The ratio of A_1/A_2 is 5.4/1, in contrast to findings of other investigators, there is no difference in the ratio of A_1/A_2 in blood groups A and AB.

RECOMMENDATIONS

An expanded study should be undertaken to verify these results, especially the finding that there is no difference in the ratio A_1/A_2 in blood groups A and AB which is not keeping with what has been reported by other investigators in Africa. This study should also include finding the incidence of anti- A_1 antibodies in blood group A₁ and A₂B subjects.

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Appendix II

Identification of A₁ and A₂

Using a commercial reagent dropper or a pasteur pipette, add 1 drop of commercial anti-A₁ into tubes followed by 1 drop of the 3-5% suspension of the red cells in saline.

Mix the suspensions by tapping the tubes.

Incubate at room temperature for 1-1½ hours.

Examine the tubes for macroscopic or microscopic agglutination.

For microscopic reading, hold the tube vertically, introduce a pasteur pipette, with its tip cut at 90°. Carefully draw up a column of supernatant about 1cm in length and then, without introducing an air bubble, draw up a 1-2mm column of red cells by placing the tip of the pipette, in the button of red cells. Gently expel the supernatant and cells onto a slide over an area of about 2 x 1 cm. Examine under a light microscope at x40 magnification.