PREVALENCE AND TITRE LEVELS OF ANTI- A AND ANTI- B ANTIBODIES AMONG BLOOD GROUP O DONORS AT KENYATTA NATIONAL HOSPITAL BLOOD TRANSFUSION UNIT, KENYA

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Prevalence and Titre Levels of Anti- A and Anti- B Antibodies among Blood Group O Donors at Kenyatta National Hospital Blood Transfusion Unit, Kenya

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2022

DECLARATION

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

Signature: ..... Date: .....

Hashim Musa Haji

This thesis has been submitted for examination with our approval as university supervisors

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DEDICATION

I dedicate this thesis to my family, and friends.

ACKNOWEDGEMENT

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LIST OF ABBREVIATIONS AND ACRONYMS

ABGAT	Anti-Blood Group Antibody Titre
AfSBT	The Africa Society of Blood Transfusion
Anti A	Antibodies against antigen A
Anti B	Antibodies against antigen B
CAT	Column Agglutination Test
EDTA	Ethylenediaminetetraacetic acid
ERC	Ethics Research Committee
HDN	Hemolytic Disease of New born
IgM/IgG	Immunoglobulin M/G
ISBT	The International Society of Blood Transfusion
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KNBTS	The Kenya National Blood Transfusion Services
KNH	Kenyatta National Hospital
MOH	Ministry of Health
RBCs	Red Blood Cells
SOPs	Standard Operating Procedures
SPRCA	Solid Phase Red Cell Adherence
STDs	sexually transmitted diseases
TTI:	Transfusion-transmitted infections
UoN	University of Nairobi

OPERATIONAL DEFINITIONS

ABO/Rhesus The different blood groups among the human population that are clinically important.

Antigen A protein that elicits antibodies production against it on stimulation of the immune system

Colloid/crystalloid A solution that is infused to support cardiac and restore normovolemic state in the human body.

Dysfunction Inability to perform function and activity of an organ optimally.

Immunohematology analyzer: A machine used to test antibodies and antigens in transfusion laboratories.

Invivo Within the circulatory of a living organism (human).

Organomegally Increased in size of an organ that usually happens when there is increased activity and function of an organ involved eg spleen.

Probiotics Bacteria and yeasts that are good for human health, especially the digestive system.

ABSTRACT

In many countries, blood group O is considered a universal donor; nevertheless, there have been reported cases of transfusion reactions due to transfusion of group O whole blood and its components in non O recipients. These adverse outcomes are attributed to high titres of naturally occurring immunoglobulin M (IgM) and immune immunoglobulin G (IgG) blood group antibodies in this donor blood. Antibody levels vary in diverse populations and are not known at the Kenyatta National Hospital (KNH) setting. This study aimed at determining the prevalence, and titre levels of IgM and IgG anti-A and anti-B antibodies amongst group O donors at Kenyatta National Hospital Blood Transfusion Unit. A cross-sectional study was adopted and carried out at KNH between February and April 2018. A sum of 233 group O donors took part in the study. A multi-stage convenience sampling method was used. A questionnaire was used to obtain demographics and specific known variables related to high antibody titre. Donor blood was collected and processed to obtain plasma which was afterwards titrated by the use of an immunohematology analyzer (Immucor Neo®) to detect IgM and IgG anti-A and anti-B antibody titres. Descriptive statistics was established on demographic variables. In assessing the relationship between high antibody titre and variables, Chi-square and Fisher exact tests were used. The blood donor population ages ranged from 18-58 years and the mean age was 29.3(SD ±8.7) years. The majority of them were male 183/233 (78.5%) with the most common age group was of 18-28 years consisting 122/233(52.4%). The prevalence of high titre among the group O donors was at76 % irrespective of antibody class (IgG and IgM) and specificity (anti-A and anti-B) using an antibody dilution cut off of ≥ 1:64. The prevalence of group O donors with titre ≥ 1:64 was 36% for IgM anti-A, 26.6% for IgM anti-B, 48.1% for IgG anti-A and32.2% for IgG anti-B. Age, gender, past transfusion history, and pregnancy were not related to high antibody titre. Nevertheless, and contrary to expectations, a higher antibody titre was detected among those who had not consumed yoghurt (P=0.03). This study sheds light on the significance of pre-transfusion ABO antibody titration at KNH. It is highly recommended that the use of group O donor blood be restricted to group O recipients. It should only be transfused to others groups in emergency times when a low antibody titre can be established.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Blood transfusion is a life-saving therapeutic intervention(Alan T. Tinmouth, 2008) administered when colloids and crystalloids fail to sustain cardiac and respiratory distress in acute anaemic patients in need of blood. Patients with very low haemoglobin may also be transfused depending on their clinical presentation. Blood components are also infused to restore coagulation factors such as platelets, albumin and many other constituents

The ABO and rhesus (Rh) are clinically the most significant blood types. Ensuring identical blood groups between donors and recipients are preferred for transfusion to recipients (Oyedeji, Adeyemo, Ogbenna, & Akanmu, 2015). However, this may not be attainable in emergency settings like in trauma and when there is a shortage of recipient blood type (Barty *et al*, 2017)

Globally, blood is a scarce resource and in sub-Sahara Africa(including Kenya), this is even a more rare commodity (Osaro & Adias, 2011). Group O is the most commonest blood group in Kenya of all the various blood groups forming almost 50% of the donor blood pool (Mwangi, 1999). Owing to its availability and it is given during medical emergencies when blood from other groups is lacking. The use of group O among non-blood group O is not uncommon in Kenya just like many other countries in the world (Zeller *et al*, 2017). ABO antibody titration test is not routinely performed in Kenya.

Blood group antibodies belong to two classes -natural IgM and immune IgG. Individuals naturally produce antibodies for which they don't possess their corresponding blood group antigens during infant life. These naturally occurring antibodies are in the form of IgM class. Carrying the pregnancy of a fetus that possesses a dissimilar blood group antigen from that of the mother or transfusion of blood that lacks a similar antigen to that of the recipient into the circulatory system of a recipient causes the production of immune IgG antibodies against that antigen.

This acquired antibody can cause hemolysis or delayed transfusion reaction in a recipient of a different blood group. IgG antibodies also can cross the placental barrier and result in Hemolytic Disease of the Newborn (HDN) (Bain, Bates & Laffan, 2017).

During the transfusion, the strength of reaction to these antibodies depends on the antibody titres, which are thought to be influenced by environmental factors, age, race, gender and geographical distribution of a population. According to the Africa Society of Blood Transfusion(AfSBT) standard guidelines, the cut-off for high titres has been defined as titres above1:64 (AfSBT,2014). Transfusion of group O blood with a high titre of anti-A and B antibodies have been associated with acute hemolysis and other unwanted transfusion-related reactions among non-O recipients. In Kenya, there is limited data available on the titres of naturally occurring and immune acquired ABO antibodies among donors of this blood group.

Consequently, the goal of this research was to establish the prevalence and levels of these antibodies among the blood group O donor population at KNH in order to inform policy on safe transfusion practices.

1.2 Problem statement

Globally, developed countries are moving towards newer, safer techniques and technologies of transfusion practices. However, Africa still lags in these efforts. The utilization of group O blood as a ‘universal donor’ is a widely accepted and practised phenomenon. This blood group has antibodies A and B. High titres of these antibodies in group O are associated with transfusion reaction when such blood is transfused to non-O blood group recipients.

The common pre-transfusion and compatibility test at KNH and nearly all the hospitals in Kenya after blood grouping is a major cross-match where patient serum or plasma are reacted with donor cells without prior ABO antibody titration of donor blood. This major cross-match technique does not detect at all these A and B antibodies in group O donor blood since, the donor cells are washed to remove plasma or serum. The goal of a major cross-match is to prevent haemolytic transfusion reaction due to ABO-incompatible red cells (Oberman, 1992). A minor

cross-match test which is sometimes done cannot quantify the strength or titre of these A and B antibodies.

At KNH during scarcity, blood group O is used in medical emergencies requiring transfusion when identical blood of the recipient is not available. This poses risks to non O recipients due to possible high titres of A and B antibodies in this blood. This may cause acute transfusion reaction and results in adverse clinical outcomes like anaemia, rejection of transplanted organs and various other undesired effects, and in the worst situation may even cause death (Berséus *et al*, 2013; Sadani *et al*, 2006). The risk is higher for patients with chronic diseases who frequently require blood transfusions such as those with haematological disorders, cancer, and those on kidney dialysis awaiting transplantation.

1.3 Justification for the study

Several studies have suggested that ABO antibody titres differ in diverse populations with regard to age, gender, race, diet, ethnic origin and environmental factors (Mavichak, Chiewsilp, Tubrod, & Ovataga, 2013). In Kenya, no studies have been conducted to assess the titres of naturally occurring and immune acquired antibodies among donors of this blood group and consequently, the levels of these antibodies are unknown, both in KNH and Kenya as a whole. Also, how these antibody titres relate with the above-stated factors are not known too.

ABO antibody titration of group O donor blood has been cited as an important consideration in ensuring improved safety in clinical transfusion (França *et al*, 2011; Sood, 2016), a procedure which is never performed in our setup.

The International Society of Blood Transfusion (ISBT) and the Africa Society of Blood Transfusion (AfSBT) requires that health institutions assess the titres of anti-A, anti B and anti-AB antibodies of blood group O donors to enhance transfusion safety to patients and is also a critical step in attaining accreditation to these bodies.

1.4 Research questions

1. What is the prevalence of IgM anti-A and anti-B antibodies titres among blood group O donors at KNH?
2. What is the prevalence of IgG anti-A and anti-B antibodies titres amongst blood group O donors at KNH?
3. What is the relationship between high antibody titre and associated factors such as age, gender, history of transfusion, pregnancy and probiotics use (yoghurt)

1.5 Objectives

1.5.1 General objectives

To assess the prevalence, titre levels and association of factors related to a high titre of anti- A and anti-B antibodies amongst donors of blood group O at KNH.

1.5.2 Specific objectives

1. . To establish the prevalence of IgM anti-A and anti B antibodies titre amongst donors of blood group O at KNH.
2. . To find out the prevalence of IgG anti-A and anti B antibodies titre amongst donors of blood group O at KNH
3. To interrogate the relationship between high antibody titre and associated factors such as age, gender, history of transfusion, past pregnancy and probiotic use (yoghurt).

CHAPTER TWO

LITERATURE REVIEW

2.1 History and milestones of blood transfusion

Even though venesection was a procedure conducted during the time of Hippocrates in 400 BC through the 19th century to treat ailments in Europe. Nonetheless, clinical transfusion only came into practice in the early 19th century because the basics of physiology of blood was a knowledge that was lacking then (Giangrande, 2000). The sequence and chronology of understanding the human circulatory system and anatomy took a considerable amount of time in the ancient past.

An Arabian physician, Ibn Al Nafis in 1242(1210-1288) emerged as the first person to correctly describe the cardiovascular circulatory system (West, 2008). Years later Spanish and Italian physicians, Michael Servetus (1511-1553) and Realdo Colombo (1516-1559) proved his assertion (Severinghaus, 2016). Thereafter the concept of transfusion started to come into the minds of ancient doctors. In 1615, A chemist Andreas Libavius postulated on how an old, sick, emaciated man can be rejuvenated by a way of transferring blood from a young healthy gentleman by opening up and attaching a silver tubing to the artery of a young man and doing the same on an old man- This formed the basis of the first description of transfusion. (Chandler *et al*,2012).

Practical transfusion experimentation started with using animal models like dogs to gauge the effectiveness in saving a life by Richard lower in 1666. Personalities like William Harvey, Christopher Wren and Robert Boyle attempted to transfuse dogs in the sixteen century which eventually led to the success achieved by James Bundell in the early 18th century in transfusing dogs (Learoyd, 2012).

Transfusion between animals and humans was attempted to gauge its effectiveness in life-saving efforts. For instance, lower “transfused blood from a lamb into the bloodstream of a clergyman named Arthur Coga. However, the practice was subsequently abandoned for hundreds of years” due to safety concerns (Fastag *et al*, 2013).

Richard Lower also demonstrated the physiological role of the lung in oxygenating the blood within the circulatory system. In 1667, Jean-Denis tried to transfuse blood from animals to humans and finally, transfusion between humans was first successfully attempted by James Blundell in 1818. Blundell, an English obstetrician transfused a woman experiencing postpartum haemorrhage and showed significant success (Dzik, 2018).

In early 1900, Karl Landsteiner indicated that adding serum from given people would cause clumping of the red cells of others and recognized it as an occurrence that had an immunological basis. He originally recognized only three blood groups terming them A, B and C. Group C serum subjects clumped the cells of groups A and B (Giangrande, 2000). This led to the finding of the existence of ABO and rhesus blood groups. Karl also briefly described the role of blood group antibodies in transfusion medicine (Heier, 2014; Branch, 2015).

In 1915, Richard Lewinsohn developed a preservative (0.2% sodium citrate) that would keep blood in its fluid state. Later preservatives such as citrate-phosphate-dextrose and citrate-phosphate-dextrose with adenine that kept blood viable and for long period at cold temperatures were discovered.

In 1945, Coombs, Mourant, and Race developed antihuman globulin, a reagent that can be used to identify the presence of IgG antibodies in the blood. This is a technique that is now been used in the diagnosis of autoimmune haemolytic anaemia, haemolytic disease of the new born and in the investigation of transfusion reaction (Hillyer, 2013).

With industrialization and the revolution of the world, techniques and technologies that would produce blood components and fractionated products were invented. In the modern-day, transfusion is slowly moving from manual to automation from the donation process through testing(Townsend and Li, 2019; Bajpai, Kaur and Gupta, 2012).

The earliest form of blood donor services in the world was started in the United Kingdom (UK) by Percy Oliver in the year 1921. He established the so-called British Red Cross Blood Transfusion Service- An organisation that recruited and kept a list

of donors whose blood was grouped and screened for transfusion transmissible infections, particularly syphilis. These donors were called on short notice to give their blood to save other citizens' lives. They were either telephoned or summoned by the police. In 1948 a National Blood Transfusion Service was set up in the UK (Giangrande, 2000).

The first record of transfusion in Africa was reported in the 1920s (Schneider, 2013). Mostly it was a hospital-based service with no centrally organized system. The different colonial governments had different ways of organising transfusion services within the countries they ruled. By the Second World War, few African countries had some level of centralised transfusion services.

In the 1930s, transfusion in Kenya was organized only during surgical circumstances in hospital setups with slightly more structured blood transfusion services emerging in the 1950s as the demand for blood increased.

With the emergence of HIV/AIDS, the cost of transfusion service increased and safety concerns regarding blood became apparent in Kenya. The need for centrally organised transfusion services became more pronounced during the time of the Nairobi terrorist bomb blast in 1997 (Oduor, 2009). A centralized national blood transfusion service was thereafter started in 2000 with the formation and legislation of the Kenya National Blood Transfusion Services (KNBTS). The mandate of KNBTS was "to recruit Donors, collect, process, screen and distribute blood and blood products to all transfusing hospitals in Kenya" (KNBTS 2014). It has currently six regional centres and eleven satellite centres.

2.2 Challenges of blood donation and impact on transfusion services

Globally, blood and blood products are a limited resource. According to the World Health Organization report of 2016, 112.5 million pints of blood were collected globally in 2013 and of this, 5.6 million was from Africa which is way below the recommended donation of 1% of each country's population (World Health Organization, 2016).

For instance in Nigeria against a demand of one and half million pints of blood required to meet the country's blood needs annually, only half a million pints are collected from donors which mostly consists of family replacement and commercially remunerated donors (Aneke & Okocha, 2017).

In Kenya the year 2018, approximately 164,275 pints were donated which is equivalent to 0.37% of her population (KNBTS, 2019). Kenya's population in the year 2019 was 47,564,296 (KNBS, 2019). Some of the peculiar challenges facing blood donor programs in Sub Sahara Africa, including Kenya, are poor infrastructure, over-reliance on international donor funds, a limited financial resource to carry out donor campaigns, a shrinking pool of voluntary non remunerated donors, inadequately trained personnel and changing dynamics of transfusion transmissible infections (TTIs). As a result of these factors and as the demand for transfusion of blood increases, the supply drastically continues to decrease (Osaro & Adias, 2011).

This blood shortage has resulted in delays which consequently resulted in high mortality among patients requiring transfusion in Kenya (Thomas *et al*, 2017). Surprisingly, even with the scarcity of this precious life-saving product, inappropriate blood use has been common (Lackritz *et al*, 1993).

Most of the voluntary donors in Kenya are school and college-going students-Over dependence on students has resulted in perennial shortages especially when learning centres are closed for holidays (Uyoga & Maitland, 2019).

The challenge posed by transfusion transmissible infections continues to be a major public health threat worldwide impacting blood donation and supply with the growing trend of emerging and evolving pathogens which require continuous redefining of laboratory testing standards and techniques (Dean *et al*, 2018). TTIs prevalence among different countries vary globally (Song *et al*, 2014; Flichman *et al*, 2014; Schmunis, 1998). In Africa, some countries such as Benin and Nigeria have been reported to have transfusion-transmitted malaria of over 30% (Tagny, Owusu-Ofori, Mbanya, & Deneys, 2010). TTIs screened in Kenya include HIV, Hepatitis B,C and Syphilis. In Kenya, the prevalence of TTIs ranging from 9.4 -to 14.1% have

been described in Kisumu, Siaya and Nakuru regional transfusion centres (Onyango *et al*, 2018; Bartonjo, Oundo and Ng'ang'a, 2019).

2.3 Group O blood and risk of its utilization

Similar to many other countries,(Oluwadare and Shonekan, 2008; Dass *et al*, 2001), In Kenya, group O is the most commonly available blood type (Mwangi, 1999). The use of group O blood and its components in non-group O patients, is linked with acute hemolytic transfusion reactions and other unfavorable transfusion-related outcomes (Berséus *et al*, 2013).

Blood components derived from group O such as platelet concentrate containing high ABO antibody titre have also been implicated in transfusion reactions when used in non O recipients (Cassandra D. Josephson, Castillejo, Grima, & Hillyer, 2010).

In emergency and resource-limited settings, the utilization of uncrossing matched group O whole blood with low ABO antibody titre is used among ABO incompatible patients since such a setting can be chaotic and delays caused by conducting compatibility testing of the patient can result in fatal outcomes for patient (Strandenes *et al*, 2014). This helps overcome the challenge of whole blood specific group and is useful in cases where the blood type of a patient is not known.

2.4 Antibody A, B titre and blood transfusion reaction.

Antibodies A and B are naturally produced by humans during the first few months of existence. Immune anti-A and anti B are developed following insult of the immune system by a foreign and lacking blood group antigen into the circulatory system of an individual.

The natural antibodies, after production, rise in titre with the increase in age till teenage and thereafter are thought to decline in old age though data on this are not consistent (Saphire, Rudolph, Hackleman, & Stone, 1993).

Transfusion reactions mostly occur because of transfusion of non-compatible blood between a recipient and a donor and are due to an immunologic reaction due to corresponding antigen and antibody interaction (Davenport, 2016).

The reactions can either be acute (occurring almost immediately up to 24 hours) or delayed taking place after forty-eight hours (Frazier, Higgins, Bugajski, Jones, & Brown, 2017). Acute is often fatal and life-threatening while delayed produce symptoms that may be detrimental to health if not diagnosed and treated in good time.

An individual with blood group O has no antigens on their red cells but have got antibodies A, B and AB in their serum. High titres of these antibodies are associated with hemolytic transfusion reactions when the blood of such a donor is transfused into a recipient who has the corresponding blood group antigen e.g. when group O blood is transfused to non O recipient (Berséus *et al*, 2013; Barjas-Castro *et al*, 2003). The extent of these transfusion reactions is often dependent on the titres of these antibodies.

2.5 Factors associated with high antibody titre

Some studies have shown that the titres of these antibodies vary with age and gender. (Mavichak *et al*, 2013) has shown that high antibody titre, to some extent, was associated more with female donors than male ones and that young age was also attributed to high ABO titre.

The influence of environmental factors and lifestyle have also been suggested as many people turn to take diets that contain probiotics which are known to stimulate high production of these antibodies (Daniel-Johnson *et al*, 2009).

Differences in ethnic origin and temporal factor are believed to play a big role. For instance in Japan, a decreasing trend in ABO antibody titre were reported over fifteen years (Mazda, Yabe, NaThalang, Thammavong, & Tadokoro, 2007)

2.6 Prevalence of ABO antibodies

Studies in different populations across the world have revealed varying levels of ABO antibodies titres. In Nigeria, several studies have reported a prevalence of hemolysin ranging from 20-80% (Oyedeji *et al*, 2015). One study at the Lagos university teaching Hospital analysed 350 samples from group O donors and found a prevalence of hemolysin of 30.3% while those with a titre of 1:8 were 18.6%

(Oyedeji *et al*, 2015). Another study carried out in North Eastern Nigeria whose aim "was to determine the prevalence and haemolytic significance of alpha- and beta-haemolysins in" their "voluntary group O donor population" found a high overall prevalence of hemolysin at 55.4% though the lytic antibodies with titres of 1:16 and 1:32 were very low (Kagu *et al*, 2011).

In Thailand, a study involving 100 group O donors found that the "overall titer ≤ 64 for anti-A was 84% while overall titer ≤ 64 for anti-B titer was 73%." (Mavichak *et al*, 2013). A previous study reported a prevalence of 69% of hemolysin and a high titre of IgM and IgG antibodies among Thai donors (Khampanon *et al*, 2012)

A Brazilian study in 2011 showed a "prevalence for the titers were: anti-A,B < 128 = 86.9% and > 128 = 2.16%; Anti-A > 128 = 9.29% and anti-B > 128 = 4.81%." (França *et al*, 2011). A more recent publication using a cut off ≥128 for IgM and ≥256 for IgG antibody titre reported a prevalence of 30.5% among donors in Belo Horizonte, Brazil (Godin *et al*, 2016)

In Zimbabwe, hemolysin prevalence of 18.6% was demonstrated and this "study has shown that high titres of IgG anti-A and anti-B of up to 64 and greater were present in more than 60% of strongly haemolytic sera" (Adewuyi *et al*, 1994) among their donors.

2.7 Recommendation for donor blood titration

Some studies have recommended limiting the use of group O to O recipient only (Oyedeji *et al*, 2015),while some allow in situations where there are emergencies (Victorine *et al*, 2016; Berséus *et al*, 2013).Others advocate for the use of low titre blood for transfusion in such instances (Strandenes *et al*, 2014).

Even with the advancement in kidney transplantation in current times and in order to overcome the challenge of ABO-incompatible immunological barriers. achieving baseline low anti-blood group antibody titre (ABGAT) among recipients, following the post-transplant patient and ensuring that ABGAT is maintained at a low level is necessary for achieving good clinical outcomes (Shah *et al*, 2017; Tobian, Shirey and King, 2011)

International and regional transfusion society standards recommend antibody titration of group O donor blood to enhance transfusion safety. It is also a requirement for accreditation to these bodies(AfSBT-AABB team of documentation, 2014). Therefore, a baseline understanding of titre levels within our donor population is of great importance.

2.8 Methods of titration

Two methods are generally available, manual titration and automation, using one of the three principles namely, tube agglutination, column gel agglutination test (CAT), and Solid Phase Red Cell Adherence Technique (SPRCA).

The tube agglutination method is the gold standard. A comparison between automation in the case of the Immucor neo machine and tube method has shown excellent concordance rate in matters of transfusion serology (Joo *et al*, 2011).

CAT and SPRCA have been easily adapted into automated systems (Ching, 2012). Automation in immunohematology has improved the quality of results and reliability by removing inter-operator variability (Bajpai *et al*, 2012). Results from the fully-automated machine Immucor Gamma GalileoNeo is comparable to the standard tube method (Joo *et al*,2011; Bruce *et al*, 2013).

2.9 Blood group antigens

Antigens are structurally made up of either protein or sugar molecules. It is this blood group antigen that determines a person’s blood type. Genes within the red cells dictate what type of protein is to be manufactured and it is these proteins that play a critical role in maintaining red cells' integrity, cell adherence and agglutination. 600 red cell antigens have been identified so far (Schenkel-Brunner, 1995).

Although there are about 33 blood groups system that has been classified (Mitra, Mishra, Girija, 2014).Of these systems, ABO and Rh are mainly important in clinical transfusion. The ABO genes are inherited from parents according to Mendelian law and are located on chromosome 9. The A and B antigens are dominant while O is recessive and only expressed in the absence of the other genes. The appearance of the A and B genes depends on another gene referred to as the H gene, which is also

inherited. Therefore, human blood groups are determined by the type of antigen present on the surface of their red cells. For instance, a blood group A individual has A antigen on their RBCs surface, group B individual has B antigen, while group O don't possess any A or B antigen.

2.10 Blood group antibodies

ABO blood group antibodies are developed naturally. Immune blood group antibodies may be acquired through transfusion of mismatched blood, immunization or pregnancy of a fetus with a different blood group from that of the mother.

2.10.1. Naturally occurring blood group antibodies (IgM)

Human beings naturally produce antibodies that are against the blood group antigen for which they do not possess it early in life. For example, group A persons develop anti-B, group B individuals develop anti-A, group O form both anti-A and anti B antibodies, while group AB develops no antibodies in their plasma or serum (Mitra, *et al*, 2014).

Neonates do not have detectable ABO antibodies of their own except that of maternal origin (Daniels, 2007). Naturally occurring antibodies begin to form in their bodies at around 3-6 months without any specific antigenic stimulus. These antibodies belong to the immunoglobulin M (IgM) type and interact best at room temperature. They are often referred to as complete antibodies because they easily agglutinate RBCs with the corresponding antigen in saline environment (Mehta, 2009).

These antibodies rise in titre to peak levels by the age of 5-10 years (Geoff Daniels, 2007) and then slowly decrease with progressing age.

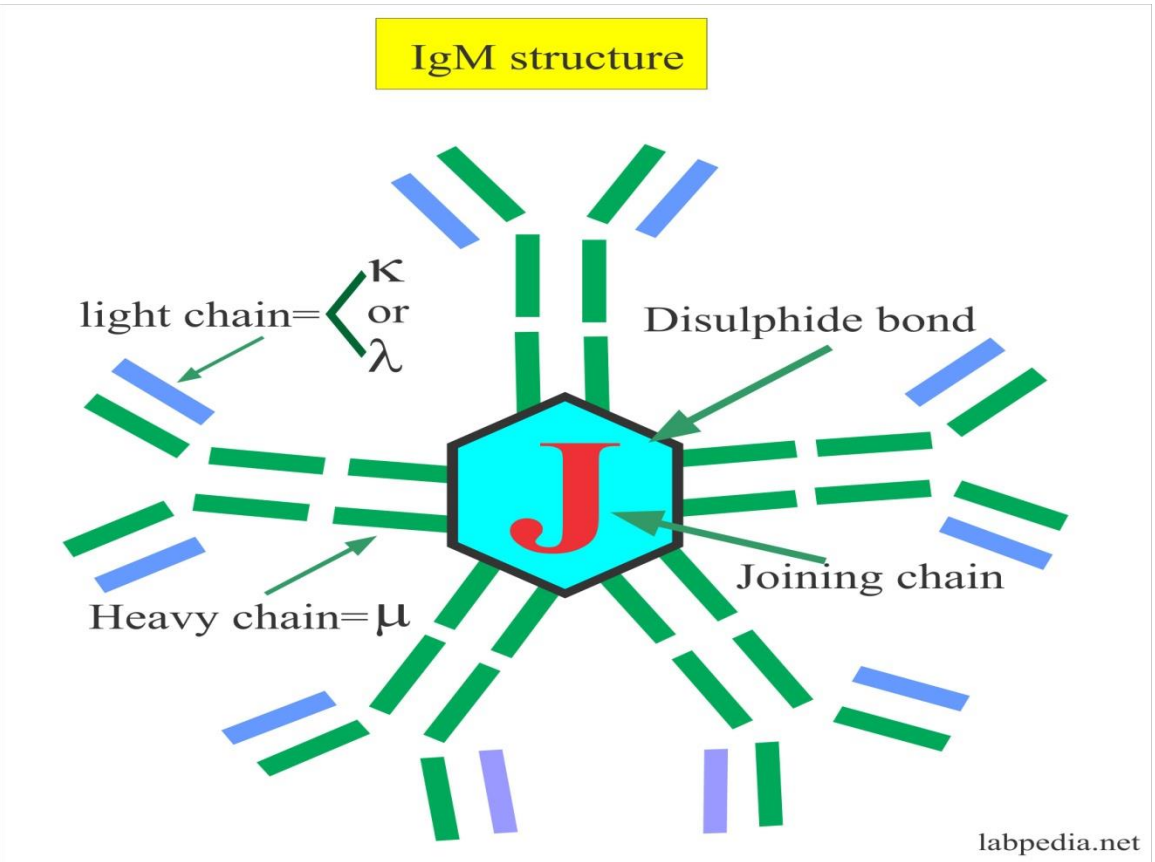

Figure2.1: Structure of an IgM molecule

Structure of IgM molecule showing the the J chain, light and heavy chains. Adapted from labpedia.net

2.10.2 Immune blood group antibodies

These types of antibodies are formed after immunization as a consequence of the introduction of a lacking antigen during transfusion or pregnancy of an ABO blood group incompatible fetus (Sood Ramnik, 2010). These are IgG antibodies, which react optimally at 37^0C and can cross the placental barrier and cause HDN (Dean L., 2005). The features that may suggest HDN include increased unconjugated bilirubin in the fatal blood; raised count of juvenile red blood cells referred to as reticulocytes and nucleated RBCs (Hoffbrand, Catovsky, & Tuddenham, 2007). The laboratory findings will show morphological changes in the RBCs and the Direct Antiglobulin test will be positive.

IgG antibodies do not cause agglutination in the saline environment but are known to cause sensitization of RBCs in vivo. The coated RBCs are then cleared by the reticulo endothelial system resulting in extra vascular hemolysis (Harewood & Samip, 2017)

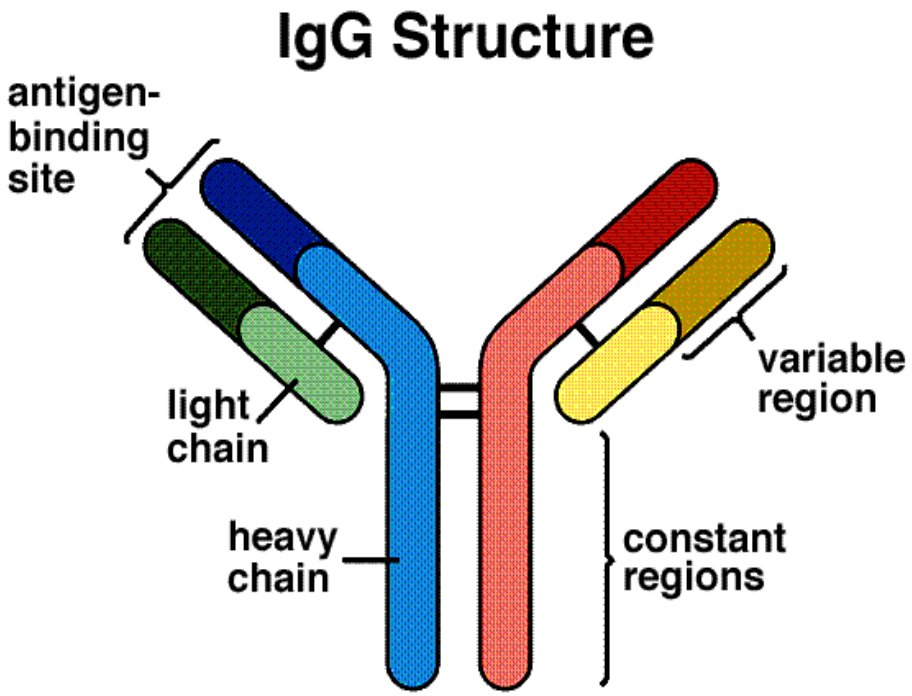

Figure 2.2: Structure of an IgG molecule

Structure shows the antigen binding sites, constant and variable region of the IgG antibody

Source: microbenotes.com/immunoglobulin-g-igg-structure-

2.10.3 Detection of IgM and IgG antibodies

IgM antibodies are detected by direct agglutination whereby 3-5% of specific red cells suspension is added to serum or plasma whereas IgG antibodies are tested indirectly using comb's cells and antihuman globulin reagent (Hoffbrand *et al*, 2007)

To achieve end titre determination, plasma is serially diluted in saline before the addition of red cell suspension and the presence or absence of agglutinating

antibodies can be detected visually, spectrophotometrically or using the camera as in Immucor Neo.

The method of testing for agglutination can either be manual or automated using tube, gel or micro titre plates.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

A laboratory-based cross-sectional study was adopted.

3.2 Setting and site description

This study was done at Kenyatta National Hospital transfusion department situated in the upper hill area of Nairobi, Kenya. KNH was selected since it is the largest Kenyan referral hospital and the department receives a considerable number of blood donors with diverse socio-cultural background.

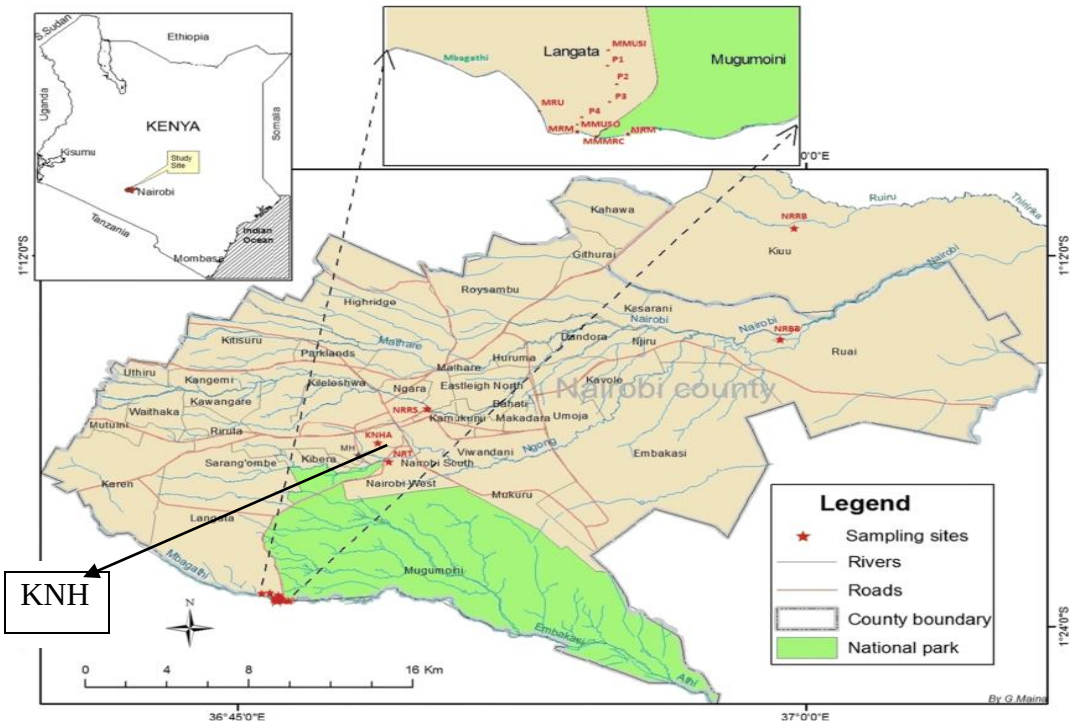

Figure 3.1: Map of Nairobi showing the study location

Source : Ngigi, A. N., Magu, M. M., & Muendo, B. M. (2020). Occurrence of antibiotics residues in hospital wastewater, wastewater treatment plant, and in surface water in Nairobi County, Kenya. *Environmental monitoring and assessment*, *192*(1), 1-16.

3.3 Study population

Between February and April 2018, every blood donor presenting to the KNH Transfusion department for donation was approached for recruitment into the study. All donors 18 years and above, who consented to participate in the study and met the Kenya National Blood Transfusion Services (KNBTS) inclusion criteria for blood donation were recruited. A total of 461 consented of which 233 were group O donors

3.3.1 Inclusion criteria

To be included as a study participant an individual had to meet all of the following criteria:

- Fit to donate blood according to KNBTS standard guideline for donation
- Aged 18 years and above (both male and female)
- Able to give consent

3.3.2 Exclusion criteria

Individuals who had any of the following characteristics were excluded and not enrolled in the study:

- Individuals below 18 years
- Those declining to give consent
- And those who were not fit to donate blood according to the inclusion criteria of KNBTS

3.3.3 Criteria in KNBTS guideline for donation

KNBTS questionnaire (appendix xi**)** was utilized to determine suitability as a blood donor. Some of the exclusion or inclusions according to KNBTS guidelines included:

That a donor must have been of good general health on the day of donation, must have eaten a meal within six hours before donating blood and not have been ill, been treated, vaccinated or on medication in the past six months before donating.

Female participants who were breastfeeding, pregnant or on menses were not allowed to donate blood. Donors who have received transfusion in the past twelve

months and with a history of fainting were not allowed too. Similarly, those who had conditions such heart ailment, lung diseases, cancer together with those with any chronic diseases such as diabetes, hypertension, tuberculosis, bleeding disorders etc were barred from donating

Donors were requested to undertake self-risk assessments for Sexually Transmitted Diseases (STDs) through the questionnaire and at the end, they were free to exclude themselves if they feel so. Some of the questions on the self-assessment included risky sexual history, exposure contaminated piercing objects and lifestyle habits such use of the drug of abuse eg bhang, cocaine. Finally, donors were asked if they felt that their blood was safe for transfusion based on their assessments. Those whose responses were 'no' were rejected.

Thereafter a basic physical examination was undertaken including observation of their eyes for jaundice and their skin for signs of excessive scars which may raise suspicion for drug abuse. If they passed this basic examination, the donors were then weighed, blood pressure was taken and haemoglobin levels measured. Acceptable ranges for these measurements were: 45kg to 100kg for weight, 100-140 mmHg (systolic) and 60-90 mmHg (diastolic) for normal blood pressure and haemoglobin of between12.5g/L and 18g/L. If all the parameters were within the acceptable ranges, consent was taken and necessary steps for phlebotomy was carried out.

3.4 Sample size determination

Using a confidence level of 95%, prevalence of high titre at 18.6% (Oyedeji *et al*, 2015), and precision of ± 5%, the sample size was estimated at 233. Fisher et al formula was used to calculate the size of the sample as indicated below.

$$n = \frac{Z^2 P(1-P)}{\delta^2}$$

Where;

n = is the required sample size

P = estimated occurrence of high titre of anti-A and anti B was on based expected prevalence of 18.6%

δ = Degree of precision. The study used a 0.05 margin of error

Z = Standard error for confidence interval at 95% = (1.96)

Using this formula;

$$n = \frac{1.96^2 \times 0.186(1-0.186)}{0.05^2} = 233$$

3.5 Sampling Method

Consenting individuals were consecutively recruited in a multistage convenient sampling method till the desired sample size was reached. In the first stage, all donors presenting were screened and later, in a second stage, group O blood was selected after testing for the blood group was conducted. A total of 461 donors were screened before the sample size was achieved.

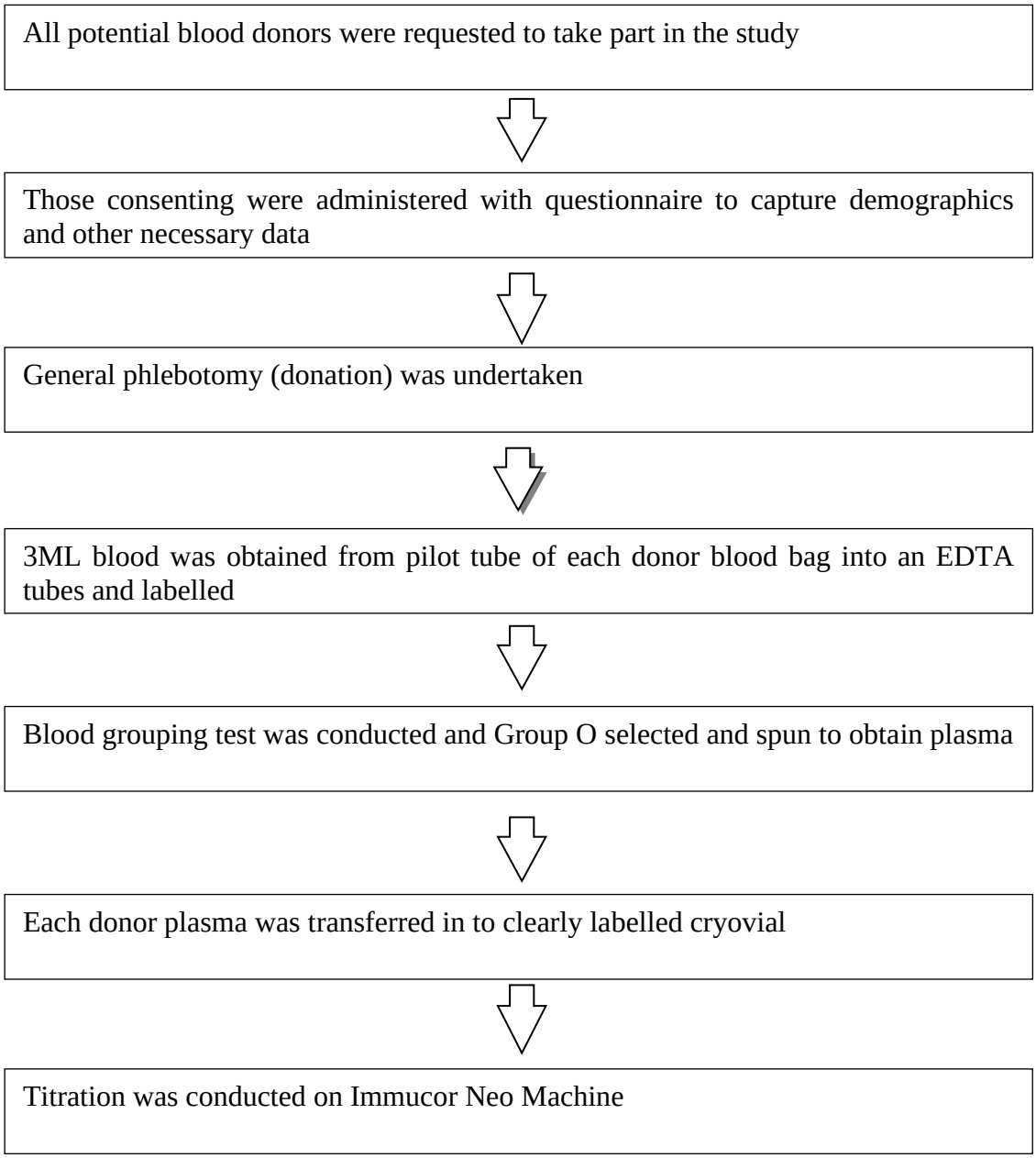

Figure 3.2: Flow chart of study processes

3.6 Data tools

Data such as age, gender, history of pregnancy for the female participant, history of transfusion and use of yoghurt was collected using a questionnaire. The questions were made available in English and Kiswahili. The tool was pretested and the necessary adjustment in cooperated. The questionnaire is found in appendix VI and VII.

3.7 Study Equipment and Other Consumables

Some of the equipment and consumables used in the study include EDTA tubes for collecting blood from donors. Cryovials tube for preserving plasma once separated from the whole blood, Disposable plastic pipette, micro centrifuge, and standard bench top centrifuge for separating plasma from the whole blood, minus 70-degree freezer, Neo Immucor machine and reagents.

3.8 Laboratory procedure

A blood sample of 3ML was removed from the blood bag pilot tube of the donor into an EDTA tube at the end of the donation process when other samples for routine tests are similarly taken. Upon grouping, group O samples were selected, centrifuged to get haemoglobin-free plasma and preserved at minus 70°C for analysis a labelled cryovials. The methodology of testing for both anti-A and anti B titre was performed using an automated technology assay adapted from the ImmucorGamma Galileo Neo user manual. A titre of 1:64 and above was considered a high titre. A detailed methodology of testing is found in appendix X.

3.9 Procedure for IgM anti-A and anti B titre testing

The principle of testing was direct agglutination using the camera. All required reagents were loaded into the machine including; Microtitre plates, Diluent, A and B cells. The plate was then identified through Barcode reading. The machine checks whether the wells of the plates are clean. Thereafter 50ul of Phosphate buffered saline is added to all the wells selected for testing the samples. This was followed by the addition of 100ul of plasma to the first well of each sample and then the machine serially double dilutes or titrates this plasma. Reagent cells (A&B) were stirred for 30 seconds to prepare 3-5% cells suspension and then 15ul of the respective cells (A&B) were added to selected wells depending on the set assay

The barcode of the plate was read and at room temperature, the plate is then incubated for 10minutes. The plates were initially centrifuged for 60 seconds at 300 RPM and for a second time for 30 seconds at 300 RPM. It was shaken for 60 seconds

and finally centrifuged again for 20 seconds at 135 RPM. At the end of this stage plate results for agglutination were read by the camera, graded and titre reported.

3.10 Procedure for IgG testing for anti-A and anti B titre

The principle of testing for IgG was indirect agglutination and end titres were determined through red cells adherence. The microtitre plates for this procedure were called a select plate and they had red cell binding agent coated on their internal surface.

The process began by loading all required reagents into the machine including; Microtitre select plates, LISS, Diluent, indicator cells, A and B cells. Identification of the plate was done through bar code reading. Thereafter the machine checks for the existence of the selected strips and verifies whether the wells of the plate were clean. This is done by a camera.

The procedure started with the addition of 50ul of Phosphate buffered saline to all the selected strips for testing the samples. Reagent cells (A&B) were stirred for 30 seconds to prepare 3-5% then 20ul of the respective cells (A&B) were added to selected strips depending on the set assay

Plates were moved to centrifuge. The plate is centrifuged 60 seconds at 135 RPM, shaken for 20 seconds, centrifuged again for 60 seconds at 150 RPM and in the end shaken for 30 seconds. This allowed for the RBCs to adhere to the binding agent on the bottom of the wells and form a monolayer

The plate was moved to the washer where it was washed twice to remove any unbound red cells. This was followed by the plate being moved to the reader where monolayer formation is verified by the camera reader. Any contamination and improper washing is detected at this level

Thereafter 50ul of Phosphate buffered saline was added to all the selected strips for testing the samples followed by the addition of 100ul of plasma to the first well of every strip for a specified sample and the machine serially titrated the plasma.

To enhance the reaction machines added 100ul of LISS to all the selected wells/strips and then the machine conducted well-filled verification to check the correct volume of LISS has been added.

The plate was then incubated for 30 minutes at a warm temperature (39^0). This allowed the antibodies in the plasma and red cell antigen to bind. At end of the incubation period, the plate was moved to the washer where the plate was washed two times to remove unbound antibodies, LISS and plasma. Thereafter the plate was moved to the reader where the machine checked for second monolayer formation. Any contamination and improper washing were detected at this level.

Indicator cells were stirred for 30 seconds to prepare cell suspension and 55ul of the indicator cells were added to selected strips/wells. The barcode of the plate was read and moved to a centrifuge where the plate was centrifuged for 90 seconds at 225RPM. Finally, at the end of this procedure, the plate results for red cells adherence were read, graded and titre reported

3.11 Quality assurance

All reagents were preserved and stored following the instructions of the manufacturer. There was also strict adherence to the Standard Operating Procedures (SOPs) during all the procedures. There was physical checking of all reagents for signs of deterioration and expiry.

Known positive controls and negative controls were included in every batch of the test. Machine plate results were visually examined to ensure the validity of the results.

3.12 Ethical considerations

Consent was sought to conduct the study from the Kenyatta National Hospital/The University of Nairobi Ethics and Research Committee (KNH-ERC/A/51). Serial numbers were used instead of donor names during laboratory analysis to conceal donors' identities. The data generated was keyed into a password-protected Microsoft excel database only accessible to the investigator. The study was of no

cost to the donor, and the donors did not suffer any pain since samples were obtained from the pilot tube of the donors' blood bag and not directly from participants.

3.12.1 Potential Benefits of the study

There were no direct benefits for donors but data generated from the study will help in contributing to policy formulation and raising the awareness of the risk associated with high ABO antibody titre among group O donor blood and contributes to transfusion safety.

3.12.2 Dissemination of information

The study finding was disseminated through scientific publication in the Journal of Medical Science and Clinical Research AND presented at Kenyatta National Hospital journal club forum on 26 Aug 2018.

3.13 Data management and analysis

Data was entered into Microsoft Excel, cleaned and verified. SPSS version 21 was used to do data analysis. Access was restricted only to authorized persons.

Descriptive analysis of the blood donors was conducted by calculating mean, SD, and frequency distributions. The categorical variables namely donor's gender, history of transfusion, pregnancy and yoghurt use were analyzed by calculating percentages in each category and these was presented percentages with the corresponding frequencies using frequency tables. In the next stage, the overall prevalence of high titre levels defined as titres greater 1:64 was calculated. Prevalence of high titre for both IgM, IgG anti-A and B antibodies was done independently. To determine whether associations exist between high titre levels, gender and age categories, a Chi-square was performed. In trying to establish the relationship between high titre, history of transfusion, pregnancy and use of yoghurt fisher, the exact test was used.

3.14: Study limitation

Results obtained from this study may not reflect a true frequency of ABO antibody titre amongst donors of group O in the whole country since the study was limited to Kenyatta National Hospital within Nairobi County.

CHAPTER FOUR

RESULTS

4.1 Descriptive characteristics of blood donors at KNH

4.1.1 Donor demographic characteristics

A total of 461 donors consented to participate in the study, out of which 233 (51%) satisfied the inclusion criteria of being blood group O. The age range of donor population was from 18-58 years and a mean age of 29.3 (SD ±8.7) years. Age was stratified into groups and frequencies were determined. Almost half of the donors of group O who took part in the study were youthful of 18-28 years of age. The donor age above 48 years had the least number of participants (3.4%). Male participants were the majority comprising 79 % while females made up not more than a quarter 21 %. The male: female ratio was 3.7:1 as shown in Table 4.1.

Table 4.1: Donor Distribution by age and gender

Variable	Frequency (%)
Mean age in years (SD)	29.3 (8.7)
Min-Max	18-58
Categories, n (%)	
18-28	122 (52.4)
29-38	77 (33)
39-48	26 (11.2)
49-58	8 (3.4)
Gender	
Male	183 (79)
Female	50 (21)

4.2 Prevalence of high Anti A, Anti B antibody titre at KNH

Based on a cut off titre of 1:64 the overall frequency of high antibody titre was estimated at 76 % irrespective of antibody specificity and class (Table 4.2). The proportion with minimum titre ≥ 1:64 for IgM anti-A was 36%, IgM anti-B 26.6%, IgG anti-A 48.1% and IgG anti-B 32.2% as shown in tables 4.3 and 4.4 respectively.

Table 4.2: Overall Prevalence of High Antibody Titre

Variable	Frequency (%)
Titre	
≥64	176 (76)
<64	57 (24)

Table 4.3: Prevalence of IgM anti A and B antibodies

Variable	Frequency (%)
IgM anti A	
≥64	84 (36.1)
<64	149 (63.9)
IgM anti B	
≥64	62 (26.6)
<64	171 (73.4)

Table 4.4: Prevalence of IgG anti A and B Antibodies

Variable	Frequency (%)
IgG anti A	
≥64	112 (48.1)
<64	121 (51.9)
IgG anti B	
≥64	75 (32.2)
<64	158 (67.8)

4.3 Association between the high titre and associated factors

Donor age was stratified into groups. Chi-square and Fisher exact tests were used to measure variables associated with high antibody titres such as age, diet, gender, history of pregnancy and transfusion. High titre was not related to any age group, gender, history of transfusion or pregnancy. Some association was detected between antibody titre and yoghurt consumption as shown in Table 4.5

Table 4.5: Relationship between Age, Gender, History of Pregnancy, Transfusion Yoghurt Intake and antibody titre.

Variable	Titre ≥64	<64	OR (95% CI)	Chi-square statistics	P-value
Age group					
18-28	92 (75.4)	30 (24.6)	1.0	X^2=1.992,	**0.574**
29-38	57 (74.0)	20 (26.0)	0.9 (0.5-1.8)	3df	
39-48	22 (84.6)	4 (15.4)	1.8 (0.6-5.6)		
49-58	5 (62.5)	3 (37.5)	0.5 (0.1-2.4)		
Gender					
Male	134 (73.2)	49 (26.8)	0.5 (0.2-1.2)	X^2=2.468,	**0.116**
Female	42 (84.0)	8 (16.0)	1.0	1df	
History of pregnancy					
Yes	21 (87.5)	3 (12.5)	0.6 (0.1-2.8)	X^2=0.421,	**0.704[a]**
No	21 (80.8)	5 (19.2)	1.0	1df	
Yoghurt intake					
Yes	147 (73.1)	54 (26.9)	0.3(0.1-0.96)	X^2=4.570,	**0.033**
No	29 (90.6)	3 (9.4)	1.0	1df	
History of blood transfusion					
Yes	2 (40.0)	3 (60.0)	0.2 (0.0-1.3)	X^2=3.492,	**0.096[a]**
No	174 (76.3)	54 (23.7)	1.0	1df	

[a] fisher's exact test p-value

4.4 Association between titre and Yoghurt intake among Gender

To find out where the significance was, the data was further stratified by gender and analysed.

Nevertheless, and contrary to expectation, the high antibody titre was detected amongst males who had not consumed yoghurt (p-value 0.029) as shown below in Table 4.6

Table 4.6: Association between titre and Yoghurt intake among Gender

Variable	Titre ≥64	<64	OR (95% CI)	Chi-square statistics	P-value
Male					
Yoghurt intake					
Yes	108 (70.1)	46 (29.8)	0.3 (0.1-0.9)	X^2=4.745,	**0.029**
No	26 (89.7)	3 (10.3)	1.0	1df	
Female					
Yoghurt intake			-		
Yes	39 (92.9)	8 (100.0)		X^2=0.608,	**1.000**
No	3 (7.1)	0		1df	

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

In Kenya, blood group O is the most common blood type of the diverse blood collected constituting almost 50% of all types of blood (Mwangi, 1999). This was also true in this study, where from a total of 461 participants, 233(51%) were from blood group O.

Of the 233 group O donors, only 50(21%) were female. This is similar to other studies where very low female donations were usually reported elsewhere (Arslan, 2007). This finding may be explained by the many factors that contribute to female donor deferrals due to their unique natural circumstance such as menses, lactation and pregnancy at the time of presenting for donation. Moreover, low haemoglobin among the female gender tops the list of deferral (Agnihotri, 2010).

The overall prevalence of anti-A and anti B antibody titre in this study was 76% irrespective of antibody specificity (anti-A, anti-B) and class (IgM, IgB). Khampanon *et al.*, (2012) reported a high occurrence of anti-A IgM antibody titre of 75.7% and 80.0% due to anti-B IgM antibody in Thailand, while the occurrences as a result of anti-A IgG and anti-B IgG antibody titre were 93.0% and 95.3%, respectively. The study suggested that environmental factors, enteric bacteria, intestinal parasites and mosquitoes bites may have contributed to this high prevalence of antibody titre (Khampanon *et al*, 2012). Relatively, all these factors are present in Kenya. Contrary, Oyedeji *et al*, in Nigeria, did a study in 2015 and reported a lower occurrence of haemolysin of 18.6% while in 2001, Olawumi *et al.*, (2001) found a prevalence of 2.0 for anti-A and 2.8 for anti B with titres of 1:8 and above (Olawumi and Olatunji, 2001;Oyedeji *et al*, 2015). The disparity between their results and those in this study can be clearly explained by the method used in testing. They evaluated haemolysin using visual and spectrophotometric end point determination of titre, whilst the testing in this study used highly sensitive and fully automated technology looking at both IgM and IgG antibody titre.

The prevalence of IgM anti-A and anti B antibodies titre in this study was 36% and 26.6% respectively. This is similar to a study by (Kannan *et al*, 2020) that reported a frequency of IgM anti-A of 36 % and IgM anti B of 32% in southern India. This study used a critical cut off titre of 1:64 and saline tube method of testing. A prevalence of lower titre were reported in other studies. (França *et al*, 2011) demonstrated a frequency of 9.29% for anti-A and 4.81% for anti B antibodies. The cut-off dilution used by Franca was greater than 1:128 for prevalence determination.

The difference in the wider range of prevalence is attributed due to the different methodology of testing and the cut off for titres. Our study used a cut off dilution of 1:64 while the other study used 1:128. More so our study employed fully automated technology while Franca study testing was manual tube method

Very few studies have looked at the prevalence of IgG blood group antibodies and measures of association. Our study showed a prevalence of 48.1% for anti-A and 32.2% for anti-B. (C. D. Josephson, Mullis, Van Demark, & Hillyer, 2004) demonstrated a general prevalence of 28% at 1:256. High prevalence of IgG anti-A and B of 93%and 95% respectively were reported by (Khampanon *et al*, 2012) using a cut off ≥1:64. The difference in methodology of testing may also explain the finding. There is no standard agreed-upon method of testing and as such establishing one would greatly reduce inter-laboratory variance(Lee *et al*, 2011)

Factors such as age, gender, probiotic use, incompatible transfusion and incompatible pregnancy have been associated with antibody titre. This study did not demonstrate any significant relationship between high titre and gender. Similarly, a study that was done by Khampanon *et al.*, (2012) in the Thailand National, Blood Centre found no relationship between IgM, IgG titre ≥64 and gender (P >0.05) (Khampanon *et al*, 2012). A related study by Mavichak done in the same setting reported some trend of higher anti-A and anti-B titres in females than males though it was not statistically significant (Mavichak *et al*, 2013). Contrarily noteworthy relationships of high titre amongst gender have been reported in other studies (Godin *et al*, 2016: França *et al*, 2011). They associated high antibody titre with the female gender.

Similar to some studies, this study did not establish any significant relationship between high titre and age. Nevertheless, there are literatures that have described association between titre and age (Godin *et al*, 2016) mainly pointing out higher frequency amongst the young age group.

Although the past incompatible transfusion history and pregnancy have been related to high antibody titre (Khampanon *et al*, 2012), this study attempted to find out whether generally history of past transfusion and pregnancy influenced antibody titre. However, no significance was associated to these factors. This can be explained possibly by the small number of individuals who were transfused with only 5 participants (2%) reporting ever transfused. It's also worth noting to know that the study did not establish the nature of ABO pregnancy and the type of blood amongst those transfused.

The use of probiotics for long have been related to stimulating production of high antibody titre (Daniel-Johnson *et al*, 2009), this study also evaluated the role of this dietary item(yoghurt) in relation to high antibody titre and contrary to expectations, high antibody titre was detected among donors who had not consumed yoghurt (P= 0.03).

The male participants who had not taken yoghurt reported high antibody titre when compared with their female counterparts. Lifestyle difference among gender may explain these results.

5.2 Conclusion

The study made the following conclusions.

1. The Overall prevalence of IgM, IgG anti-A and anti B Antibodies titre amongst group O donors at KNH was high at 76%, therefore this study emphasizes the significance of pre-transfusion ABO antibody titration.

2. High titre was not associated with any age group, gender, past transfusion, and pregnancy.

3. Male gender who did not use yoghurt were associated with high antibody titre.

5.3 Recommendation

The study recommended the following: -

1. That the use of group O donor blood and components should be restricted to group O recipients. Its uses should be limited to emergencies for other groups and only when low antibody titre can be established to minimize the risk of a reaction or sensitization among non-group O recipients. ABO antibody titration should be undertaken for group O blood and blood pints segregated and labelled high and low antibody titre.

2. Further multi-site study is recommended to establish a national prevalence and regional transfusion centre with safe antibody titre levels.

3. There is a need to have a policy and awareness on the risks of this high ABO antibody titre

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APPENDICES

Appendix I: Research Publication

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Journal Of Medical Science And Clinical Research

Prevalence of High Titre Anti-A and Anti-B Antibodies among Blood Group O Donors at Kenyatta National Hospital Blood Transfusion Unit, Kenya

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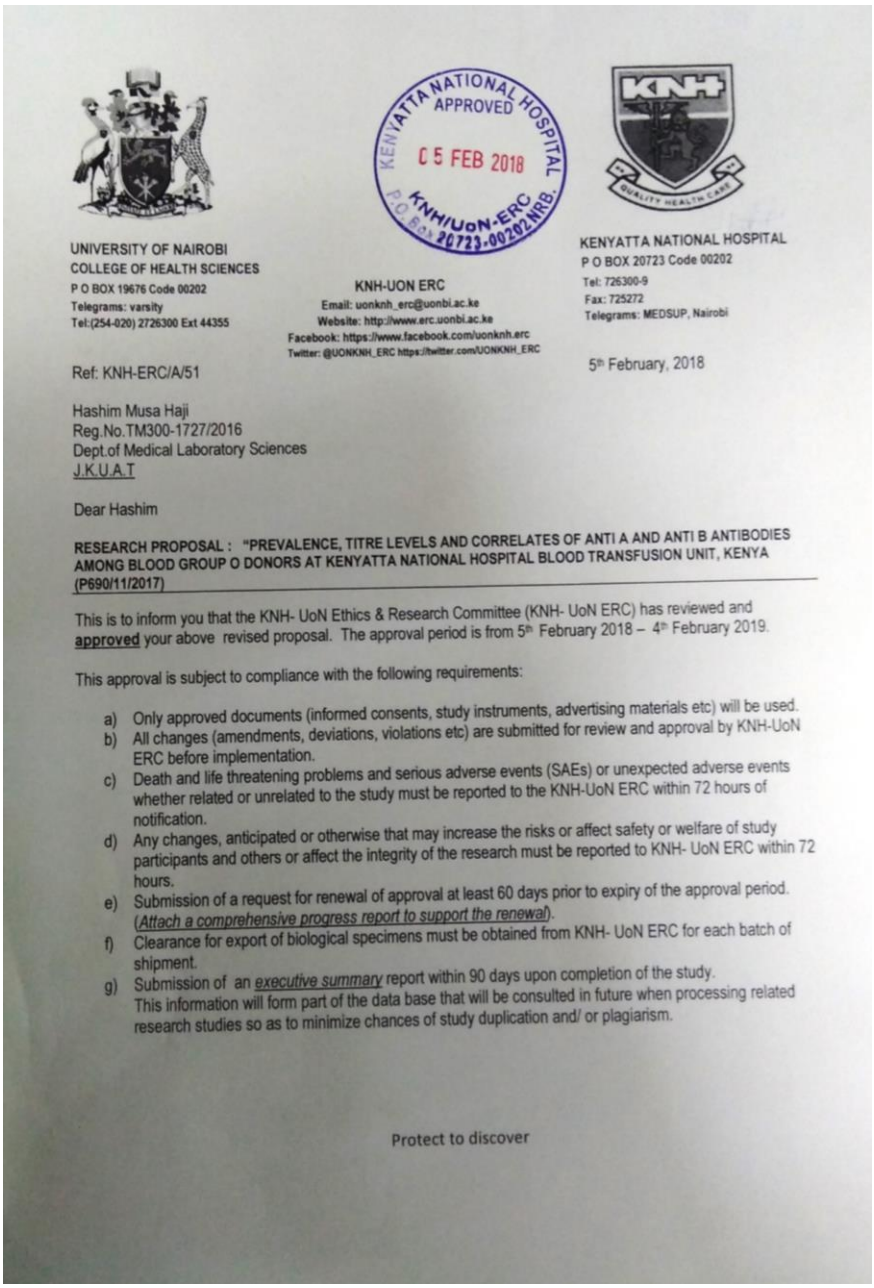
Abstract
Introduction: *High titres of naturally occurring (IgM) and immune (IgG) blood group antibodies in group O blood are attributed to acute transfusion reactions when this blood is transfused to non O recipients. The objective of this study was to determine the prevalence, and titre levels of IgM and IgG anti-A& B antibodies among group O donors at Kenyatta National Hospital (KNH) Blood Transfusion Unit.*
Method: *It was a cross-sectional study involving 233 group O donors. A questionnaire was administered to capture demographics and other variables that are associated with high antibody titre. A 3ml donor blood was collected from each donor and processed to obtain plasma which was titrated using an immunohematology analyzer (Immucor Neo®).Descriptive statistics was performed on demographics, Chi-square and fisher exact were used to assess association between high titre and these variables.*
Results: *The age of donor population ranged from 18-58 years with a mean of 29.3(SD ±8.7) years. Majority were male 183 (78.5%). The most common age group was 18-28 years constituting 122(52.4%)). Using a cut of ≥ 1:64, the prevalence of high titre among the group O donors was 75.5% irrespective of antibody class (IgG and IgM) and specificity (anti-A and anti-B). The percentage with titre ≥ 1:64 for IgM anti-A was 36%, IgM anti-B 26.6%, IgG anti-A 48.1% and IgG anti-B 32.2%. Age, sex, previous history of transfusion, and pregnancy were not associated with high antibody titre. However higher antibody titre was detected among those who did not consume yoghurt (P=0.03).*
Conclusion: *The study sheds light on the importance of pre-transfusion ABO antibody titration at KNH.*
Keywords: *High titre, anti A, anti B antibodies, group O donors, blood transfusion.*

Appendix II: Approval Letter by Ethics and Research Committee



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Ref: KNH-ERC/A/51

5th February, 2018

Hashim Musa Haji
Reg.No.TM300-1727/2016
Dept.of Medical Laboratory Sciences
J.K.U.A.T

Dear Hashim

RESEARCH PROPOSAL : "PREVALENCE, TITRE LEVELS AND CORRELATES OF ANTI A AND ANTI B ANTIBODIES AMONG BLOOD GROUP O DONORS AT KENYATTA NATIONAL HOSPITAL BLOOD TRANSFUSION UNIT, KENYA (P690/11/2017)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above revised proposal. The approval period is from 5th February 2018 – 4th February 2019.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

Protect to discover

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Yours sincerely,

PROF. M. L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director, CS, KNH
The Chairperson, KNH-UON ERC
The Assistant Director, Health Information, KNH
Supervisors: Dr.Fatma Abdalla, Thematic unit of Haematology and Blood Transfusion, UoN
Dr.Amos Mbugua, Dept.of Medical Laboratory, JKUAT

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Appendix III: Summarized Data

Serial no	Age in years	Gender	History of blood transfusion	History of pregnancy	Yoghurt intake	Antibody titres			
						IgM anti A	IgM anti B	IgG anti A	IgG anti B
1	23	Male	No		Yes	128	128	128	64
2	19	Male	No		Yes	16	8	64	32
3	21	Male	No		Yes	16	32	64	128
4	19	Female	No	No	Yes	8	32	64	128
5	22	Male	No		Yes	16	32	64	32
6	20	Male	No		Yes	1	8	0	0
7	46	Male	No		Yes	8	4	64	32
8	36	Female	No	Yes	Yes	16	32	64	32
9	36	Female	No	Yes	Yes	8	8	128	128
10	32	Male	No		No	64	16	64	64
11	31	Male	Yes		Yes	4	4	32	32
12	21	Female	No	No	Yes	64	128	128	32
13	48	Male	No		No	16	16	128	128
14	36	Male	No		No	4	4	128	64
15	37	Male	No		Yes	32	16	64	16
16	37	Male	No		Yes	16	4	64	64
17	18	Female	No	No	Yes	32	8	8	8
18	20	Male	No		Yes	64	32	64	128
19	26	Male	No		Yes	64	8	128	8
20	53	Male	No		Yes	16	4	64	32
21	44	Male	No		Yes	16	8	32	128
22	30	Female	No	Yes	Yes	16	16	64	32
23	42	Female	No	Yes	Yes	8	8	128	16
24	19	Male	No		Yes	8	32	32	32
25	22	Female	No	No	Yes	32	64	4	8
26	35	Male	No		Yes	64	64	128	128
27	32	Male	No		Yes	32	128	32	32
28	37	Male	No		Yes	32	16	64	128
29	25	Male	No		Yes	32	16	128	64
30	31	Male	No		Yes	64	16	128	128
31	21	Female	No	No	Yes	32	128	32	8
32	23	Male	No		No	16	8	128	128
33	26	Male	No		No	128	64	64	32
34	32	Male	No		Yes	8	8	32	16
35	29	Male	No		Yes	16	8	32	16

36	21	Male	No		Yes	64	128	4	16
37	36	Male	No		Yes	16	8	32	32
38	48	Male	No		Yes	128	64	128	128
39	23	Male	No		Yes	64	16	128	128
40	35	Female	No	Yes	Yes	32	32	32	32
41	31	Male	No		Yes	32	64	16	128
42	29	Male	No		Yes	16	16	64	64
43	34	Female	No	Yes	Yes	64	128	32	128
44	25	Male	No		Yes	32	64	8	32
45	18	Male	No		Yes	64	32	32	64
46	28	Male	No		Yes	128	128	32	64
47	18	Male	No		No	64	64	64	16
48	30	Female	No	Yes	Yes	16	16	64	64
49	19	Male	No		Yes	16	64	64	128
50	29	Male	No		No	64	16	8	16
51	30	Male	No		No	32	16	64	64
52	19	Female	No	No	Yes	64	16	64	32
53	24	Female	No	No	Yes	64	64	32	128
54	19	Female	No	No	Yes	64	64	2	64
55	18	Female	No	No	Yes	16	32	16	16
56	26	Male	No		Yes	32	16	8	16
57	29	Female	No	Yes	Yes	8	64	8	16
58	25	Female	No	Yes	Yes	64	64	4	8
59	33	Male	No		Yes	16	8	16	4
60	40	Male	No		Yes	64	64	2	16
61	31	Male	No		Yes	16	64	128	8
62	31	Male	No		No	16	16	128	64
63	20	Male	No		No	64	32	64	64
64	29	Male	No		Yes	8	16	8	4
65	25	Female	No	No	Yes	32	64	64	32
66	24	Female	No	No	Yes	32	128	64	128
67	49	Male	No		Yes	8	8	8	64
68	22	Male	No		Yes	32	16	16	32
69	30	Male	No		Yes	32	32	8	16
70	31	Female	No	Yes	No	128	64	4	8
71	30	Male	No		Yes	64	16	128	64
72	22	Female	No	No	Yes	4	32	8	16
73	20	Male	No		Yes	32	64	8	32
74	23	Male	No		Yes	16	16	64	8
75	20	Male	No		No	128	16	128	16
76	38	Male	No		Yes	16	16	32	128
77	22	Male	No		Yes	64	64	16	16
78	20	Female	No	No	Yes	32	64	32	16
79	21	Male	No		No	4	16	8	8

80	50	Male	No		Yes	32	32	32	64
81	54	Male	No		Yes	32	4	32	2
82	21	Female	No	No	Yes	16	16	8	32
83	39	Male	No		Yes	32	8	64	8
84	21	Male	No		Yes	8	8	8	8
85	26	Male	No		No	32	16	64	128
86	29	Male	No		Yes	64	16	64	64
87	31	Male	No		Yes	32	16	64	128
88	28	Male	No		Yes	16	8	32	32
89	42	Male	No		No	64	8	32	16
90	35	Male	No		Yes	32	8	4	8
91	19	Male	No		Yes	32	8	32	128
92	40	Female	No	Yes	No	8	32	32	128
93	37	Male	No		Yes	32	32	32	64
94	43	Male	No		Yes	32	32	32	8
95	28	Male	No		Yes	16	8	32	32
96	27	Male	No		Yes	64	8	8	16
97	21	Male	No		Yes	64	64	16	8
98	18	Male	No		Yes	8	16	8	0
99	43	Male	No		No	16	4	32	16
100	24	Male	No		Yes	32	16	8	4
101	24	Female	No	No	Yes	8	32	8	16
102	23	Male	No		Yes	64	32	8	32
103	31	Male	No		Yes	32	8	4	16
104	26	Male	No		Yes	32	16	0	4
105	23	Male	No		Yes	8	4	0	2
106	35	Female	No	Yes	Yes	1	8	0	4
107	29	Male	No		Yes	32	32	64	32
108	54	Male	No		No	16	4	32	1
109	35	Male	No		Yes	16	8	16	4
110	24	Male	No		Yes	32	8	8	16
111	27	Male	No		Yes	64	4	16	4
112	25	Male	Yes		Yes	64	32	32	32
113	26	Male	No		Yes	8	4	4	2
114	21	Male	No		Yes	8	16	64	64
115	30	Male	No		Yes	4	8	16	64
116	28	Male	No		No	16	16	64	32
117	25	Male	No		Yes	64	64	128	128
118	24	Male	No		Yes	32	32	8	64
119	38	Female	No	No	Yes	32	16	64	32
120	38	Male	No		No	16	16	64	32
121	22	Male	No		Yes	32	32	128	32
122	27	Male	No		Yes	8	4	128	128
123	18	Male	No		Yes	128	32	128	2

124	45	Male	No		Yes	8	16	64	128
125	36	Male	No		Yes	8	16	128	128
126	30	Female	No	No	Yes	64	32	128	128
127	21	Female	No	Yes	Yes	64	128	128	128
128	31	Male	No		Yes	64	8	128	64
129	31	Male	No		Yes	8	4	16	32
130	27	Male	No		Yes	8	8	128	64
131	18	Male	No		Yes	8	32	32	32
132	39	Male	No		Yes	16	64	64	128
133	35	Male	No		Yes	8	4	32	64
134	40	Male	No		Yes	32	16	64	64
135	24	Male	No		Yes	16	64	32	8
136	48	Male	No		Yes	64	16	128	64
137	21	Male	No		No	128	16	128	128
138	23	Male	No		Yes	128	32	128	16
139	27	Male	No		Yes	64	32	8	4
140	25	Female	No	Yes	Yes	128	8	128	32
141	23	Male	No		Yes	128	8	128	128
142	40	Female	No	Yes	No	128	64	32	8
143	54	Female	No	Yes	Yes	16	16	128	16
144	19	Male	Yes		Yes	64	64	128	4
145	30	Male	No		Yes	64	8	0	64
146	22	Female	No	No	Yes	128	16	128	64
147	47	Male	No		Yes	32	8	128	64
148	21	Male	No		Yes	128	8	128	64
149	18	Male	No		Yes	128	16	8	32
150	19	Male	No		Yes	128	16	64	16
151	39	Male	No		Yes	128	8	128	32
152	22	Male	No		No	128	64	32	2
153	22	Male	No		Yes	64	32	128	64
154	21	Male	No		Yes	128	16	128	64
155	32	Male	No		Yes	16	8	16	128
156	27	Male	No		Yes	32	32	64	8
157	26	Male	No		Yes	32	8	32	16
158	36	Male	No		Yes	32	32	64	32
159	35	Female	No	No	Yes	128	16	64	128
160	24	Female	No	No	Yes	128	64	8	64
161	19	Female	No	No	Yes	128	8	128	32
162	38	Female	No	Yes	Yes	64	64	32	64
163	21	Male	No		Yes	64	8	64	32
164	45	Female	No	Yes	Yes	128	8	128	32
165	21	Male	No		Yes	32	64	32	16
166	48	Male	No		Yes	16	64	32	32
167	25	Male	No		No	128	16	32	16

168	20	Male	No		No	64	16	32	8
169	23	Male	No		Yes	128	64	32	128
170	19	Male	No		No	128	64	16	8
171	20	Male	No		No	16	64	32	64
172	26	Male	No		Yes	64	64	64	16
173	39	Male	No		Yes	64	64	64	16
174	21	Female	No	No	Yes	32	32	128	32
175	30	Male	No		Yes	8	16	16	32
176	34	Female	No	Yes	Yes	8	16	64	8
177	20	Female	No	No	Yes	64	128	64	32
178	36	Female	No	Yes	Yes	128	32	32	32
179	33	Male	No		Yes	16	32	64	16
180	39	Male	No		Yes	4	4	64	4
181	29	Male	No		Yes	64	32	8	8
182	18	Female	No	No	Yes	32	16	128	8
183	25	Female	No	No	Yes	32	64	64	16
184	28	Female	No	Yes	Yes	16	64	32	32
185	32	Male	No		Yes	8	16	16	16
186	45	Male	No		Yes	8	8	16	8
187	21	Male	No		Yes	32	32	16	16
188	32	Male	No		Yes	64	64	64	32
189	23	Male	No		No	128	128	64	32
190	19	Male	No		Yes	32	16	8	16
191	20	Male	No		Yes	64	32	64	16
192	30	Male	No		No	64	16	64	16
193	26	Male	No		Yes	32	16	128	64
194	40	Male	Yes		Yes	8	16	32	8
195	28	Male	No		Yes	64	64	64	64
196	33	Female	No	Yes	Yes	32	64	64	8
197	34	Male	No		Yes	4	32	16	32
198	18	Male	No		Yes	32	64	64	16
199	33	Male	No		Yes	64	16	32	16
200	29	Male	No		Yes	16	32	32	32
201	34	Male	No		Yes	16	64	128	32
202	24	Male	No		Yes	32	16	16	4
203	38	Male	No		Yes	32	64	32	32
204	37	Female	No	Yes	Yes	128	16	128	16
205	31	Male	No		Yes	16	32	64	32
206	33	Male	No		Yes	16	64	64	8
207	33	Male	No		Yes	32	16	32	8
208	21	Female	No	Yes	Yes	32	8	8	8
209	29	Male	No		No	32	16	64	32
210	22	Male	No		Yes	32	32	64	8
211	24	Male	No		No	32	64	128	32

212	24	Female	No	No	Yes	32	32	128	128
213	21	Male	No		Yes	16	32	128	32
214	26	Male	No		Yes	16	16	64	16
215	42	Male	No		Yes	64	32	64	64
216	34	Male	No		No	8	32	32	128
217	32	Male	No		Yes	128	128	8	8
218	25	Male	Yes		Yes	8	32	32	8
219	26	Male	No		Yes	8	8	32	8
220	22	Male	No		Yes	64	32	128	32
221	34	Male	No		Yes	32	64	32	64
222	55	Male	No		Yes	8	32	32	4
223	24	Male	No		Yes	32	8	16	32
224	26	Male	No		Yes	128	64	4	32
225	40	Male	No		Yes	128	32	4	4
226	25	Male	No		Yes	32	64	16	32
227	26	Male	No		Yes	32	32	16	2
228	30	Male	No		Yes	64	64	16	8
229	26	Male	No		Yes	32	32	32	16
230	58	Male	No		Yes	64	16	32	8
231	30	Male	No		Yes	32	32	32	8
232	25	Male	No		Yes	32	64	64	16
233	31	Male	No		Yes	8	8	16	16

Appendix IV: Informed Consent to Participants

TITLE OF STUDY: Prevalence and titre levels anti A and anti B antibodies among blood group O donors at Kenyatta National Hospital Blood Transfusion Unit, Kenya.

Introduction and study information

I'm HASHIM MUSA HAJI, a senior laboratory staff at KNH and a postgraduate student at Jomo Kenyatta University. I am conducting a study about antibodies A and B titres among blood group O donors at KNH.This study will help us understand the titre levels among blood donors. Am administering a questionnaire to all donors who present for donation at KNH. You have been selected because you have blood that might have these antibodies and your donor characteristics are also important in this study. Having these antibodies is normal and does not cause any problem to the donor. However, high titres of these antibodies in group O are associated with blood transfusion reactions if given to a recipient of a different blood group, since blood group O is considered a "universal donor" in many settings. Because the titre levels among these blood donors are not known in Kenya and KNH in particular, this study will help us shed light on this matter. A blood sample will be taken from the blood you donate. The specimen obtained will be tested at the laboratory.

Risks/benefits

During the sample collection procedure, there was no pain since blood will not be directly drawn from you, rather a portion (3ml) of the donated blood will be used. There will be no payment for participating in this study and neither will you incur any extra cost. The results of the study will help us in understanding levels of this antibodies and donor characteristic at KNH so that it can inform policy and improve safety in transfusion practices.

Procedure for specimen collection

At the end of the blood donation process, a sample of 3ml will be obtained from the blood you have donated. This process will not add any extra pain to you.

Participant right

Your decision to participate is entirely voluntary. You may withdraw from the study at any time without necessarily giving a reason for your withdrawal. Refusal to participate in this research will not affect the services you are entitled to in this health facility or other facilities.

Assurance of confidentiality of volunteer's identity

Records relating to your participation in the study and results will remain confidential and will only be available to the investigator. You will be given a consent form to sign. T**hank you for your time, please turn to the next page**

Appendix V: Consent Form

I……. hereby agree to participate in the study conducted by HASHIM MUSA. I agree for a sample to be taken for the test explained to me. I understand I will not suffer any extra discomfort or pain over and above what is required for usual donation of blood. I will not pay any extra cost for the laboratory investigations. I also understand that I may withdraw from the study at any time and my withdrawal will not in any way deny me any health benefits to which am entitled to.

Telephone Number …….....…Participant signature…….. Date……

Witness name ……Signature …….

Date……..

Signature of Principal investigator……..

Contact information

If you have any question now or in the future regarding your rights in this study, please contact the principal investigator HASHIM MUSA HAJI on 0721 842910 or the chairperson, KNH/UoN/ERC. P.O. BOX, 20723-00200 Nairobi. Telephone number 020-2725272

Appendix VI: English Questionnaire

Instruction: Please fill or tick where appropriate Serial no: .....

1. What is your Age? ……(years)

2. Gender? ☐ Male ☐ Female
3. What is your level of education

   No formal education ☐ Primary ☐ Secondary ☐

   College ☐ University ☐
4. Have you ever donated blood before?

   Yes ☐ No ☐

   If yes, how many times? …….
5. If you have ever donated blood, were you issued with a donor card?

   Yes ☐ No ☐ Not applicable ☐

   If not, why?

   [a] I was not told of donor card issuance ☐

   [b] I never came back to collect the donor card ☐

   [c] Donor cards were out of stock ☐
6. Have you ever been transfused blood?

   Yes ☐ No ☐
7. Currently, are you donating blood for some one in particular?

   Yes ☐ No ☐
8. When is the last time you donated blood? Month..... Year..... I don't recall ☐ Not Applicable ☐
9. In your last donation, were you donating blood for someone in particular?

   Yes ☐ No ☐ Not Applicable ☐
10. Do you know how many times a healthy donor like you can donate blood in a year?

    [a] Once ☐ [b] Twice ☐ [c] Thrice ☐

[d]Four times ☐ [e] More than four times ☐ [f] I don't know ☐

11. Do you intend to donate blood regularly?

    Yes ☐ No ☐

12. What challenges hinder you from donating regularly (Tick as many applicable)

    [a] Distance from donation site ☐

    [b] Fear about blood donation ☐

(i) Fear about needle prick ☐

(ii) Fear because of cultural beliefs ☐

(iii) Fear of developing anemia or loosing blood ☐

(iv) Other reasons.....

    [c] Lack of time ☐

    [d] There was no emergency requiring my donation ☐

    [e] I have never been asked to donate ☐

    [f] No challenge ☐

13. What is your occupational status?

    Unemployed ☐ Employed ☐ Student ☐

14. Do you know your blood group?

    Yes ☐ No ☐

    If yes, Which one is it?.....

15. Have you ever been pregnant?

    Yes ☐ No ☐ Not Applicable ☐

16. Do you take yogurt?

    Yes ☐ No ☐

    If yes how often?

    [a] Daily ☐ [b] Weekly ☐

    [c] Monthly ☐ [d]Once in a blue moon ☐

LABORATORY RESULTS

	Results
Blood Group type	
IgM anti A titre	
IgM anti B titre	
IgG anti A titre	
IgG anti B titre	
Haemoglobin level	

Appendix VII: Kiswahili Questionnaire

Maelezo : Tafali jaza au chagua jibu lilo sahihi. Nambari.....

1. Umri wako? ……. .....(Miaka)
2. Kizazi? Mwanaume ☐ Mwanamke ☐
3. Kiwango cha elimu yako? Sijawahi kuenda shule ☐ Shule ya msingi ☐
   Shule ya upili ☐ Chuo ☐ Chuo kikuu ☐
4. Je, Umewahi kutolea mtu damu hapo awali? Ndio ☐ La ☐
   Kama jibu ni ndio, mara ngapi?
5. Kama umeshawahi kutoa damu, ulipewa kadi ya kutoa damu? Ndio ☐ La ☐ Haini hu☐
   Kama jibu ni la, taja sababu
   [a]Sikuelezewa juu ya kupewa kadi ☐
   [b] Sikurudi kuchukuwa kadi ☐
   [c] Kadi hazikuwepo ☐
6. Je, Umewahi kuongezwa damu? Ndio ☐ La ☐
7. Kwa muda huu, je unatolea damu mtu maalum? Ndio ☐ La ☐
8. **N**i lini mara ya mwisho ulipotoa damu? Mwezi.....mwaka..... Hainihusu☐
9. Mara yako ya mwisho kutoa damu, je ilikuwa ni ya mtu maalum?
   N☐ La ☐ Hainihusu ☐
10. Je unajua mtu aliyo na afya kamili anawezatoa damu mara ngapi kwa mwaka?
    a. Mara mmoja ☐
    b. Mara mbili ☐
    c. Mara tatu ☐
    d. Mara nne ☐
    e. Zaidi ya mara nne☐
    f. Sielewi ☐
11. Je, una nia ya kutoa damu mara kwa mara? Ndio ☐ La ☐
12. Je, ni changamoto zipi zinikuzuia kutoa damu mara kwa mara?
    [a]Umbali wa sehemu ya kutolea ☐
    [b]Uwoga wa hali ya kutoa damu. ☐
    (i) Uwoga wa uchungu wa sindano ☐

(ii) Uwoga wa mila ☐

(iii) Uwoga wa kupatikana na upungufu wa damu ☐

(iv) Sababu zingine.....

[c]Ukosefu wa muda ☐

[d**]**Haijatokea dharura inayonihitaji kutoa damu. ☐

[e**] S**ijawahi ulizwa kutoa damu ☐

[f**]** Hakuna changamoto ☐

13. Je, unajihusisha na shughuli gani?

Mfanyikazi☐ Bila ajira ☐ Mwanafunzi ☐

14. Je, unajua kikundi au aina ya damu yako?

Ndio ☐ La ☐ Sina uhakika ☐

Kama jibu ni ndio, ni aina ipi?

15. Je, umeshawahi pata ujauzito?

Ndio ☐ La ☐ Hainihusu ☐

16. Je, watumia maziwa ya mtindi ‘yoghurt’? Ndio ☐ La ☐

Ikiwa jibu ni ndio, eleza mara ngapi?

[a] Kila siku ☐

[b**]** Mara mmoja kwa wiki ☐

[c**]** Mara mmoja kwa mwezi ☐

[c**]** Baada ya muda mrefu ☐

Majibu ya Maabara

	Matokeo
Aina ya damu	
IgM anti A	
IgM anti B	
IgG anti A	
IgG anti B	
HB	

Appendix VIII: Swahili Consenting Information

Kiraufu cha Utafiti: ‘’Prevalence and titre levels of anti A and anti B antibodies among blood group O donors at Kenyatta National Hospital Blood Transfusion Unit, Kenya’’.

Maelezo yai dhini

Jina langu Ni HASHIM MUSA, mfanyikazi hospitali ya Kenyatta na mwanafunzi wa shahada ya uzamili katika chuo kikuu cha Jomo Kenyatta. Ninafanya utafiti kuhusu kiwango cha ‘antibody ya A na B’ katika damu ya aina ya O. Utafiti huu utatusaidia kuchunguza kiwango cha ‘antibody Ana B’katika damu yako ili kutuwezesha kuhudumia wagonjwa wanaohitaji damu kwa ujuzi wa juu zaidi. Wewe umechaguliwa kwa sababu una damu ambayo inaweza kuwa na hizi‘antibodies’.Kuwanahii‘antibody ‘nikawaida na haidhuru mwenyewe ila kiwango cha juu cha hizi ‘antibodies’ katika damu ya O inaaminika kwamba inaweza kudhuru mwenye kuchangiwa au kupewa damu ikiwa sio wa aina ya damu hiyo. Sampuli ya mililita 3 itachukuliwa na mimi ama daktari mwingine baada ya kukubali kushiriki. Damu itatolewa kwa ile damu uliyotoa bila uchungu Zaidi yakadri ya iliyo ya kawaida. Hii sampuli itapimwa kwenye maabara.

Hatari/ Faida

Hakutakuwa na malipo yoyote kwa kuhusika katika utafiti huu, pia hautalipa malipo yeyote. Utafiti huu utatusaidia kuchunguza kiwango cha ‘antibody a na B’ katika damu yako ilikutuwezesha kuhudumia wangonjwa wanaohitaji damu Kwa ujuzi wa juu Na pia kuelewa aina za wanaotoa damu kusaidia wagonjwa

Utaratibu wa kuchukuwa sampuli

Kiasi cha mililita 3 itatolewa kutoka ile damu uliyotoa

Haki za mshiriki

Kushiriki katika utafiti huu ni kwa kujitolea na ukiamua kutoshiriki, hautanyimwa huduma ambazo ungepata kwa kawaida katika hospitali hii. Hakuna adhabu kwa kutoshiriki utafiti huu.

Muda wa ushauri.

Itakuchukua dakika zako tano kujaza maswali Na kisha utatia sahihi baada ya kuelewa.

Hakikisho ya siri ya utambulisho wamshiriki

Rekodi ya kushiriki kwako katika utafiti huu zitabaki siri. Utapewa fomu ya idhini utie sahihi.

Appendix IX: Fomu ya Idhini

Mimi ……Ninakubali kushiriki katika utafiti unaofanywa Na HASHIM MUSA HAJI. Nakubali sampuli itolewe kwa damu yangu Kama nilivyoelezewa. Ninaelewa sitapata maumivu Zaidi ya ilivyokawaida. Sitalipa malipo yoyote kwa utafiti huu. Pia naelewa kuwa naweza kujiondoa kushiriki kwenye utafiti huu wakati wowote bila hofu ya adhabu yoyote

Nambari ya simu…….....

Sahihi ya mshiriki ……Tarehe…….....

Jina la Shahidi ……

Sahihi ya shahidi……..Tarehe…….

Sahihi ya mtafiti mkuu (ama mwakilishi wake) …….…….

Mawasiliano

Ukiwa na swali lolote wakati huu ama baadaye kuhusu haki zako kama mshiriki, kuhusu utafiti huu, tafadhali wasilianana mtafiti mkuu HASHIM MUSA HAJI nambariyasimu 0721842910 ama mwenyekiti wa kamati ya maadili ya utafiti ya:

Hospital Kuu ya Kenyata/Chuo Kikuu cha Nairobi kupitia:

S.L.P. 20723-00200 Nairobi. Nambari ya Simu 020-2725272

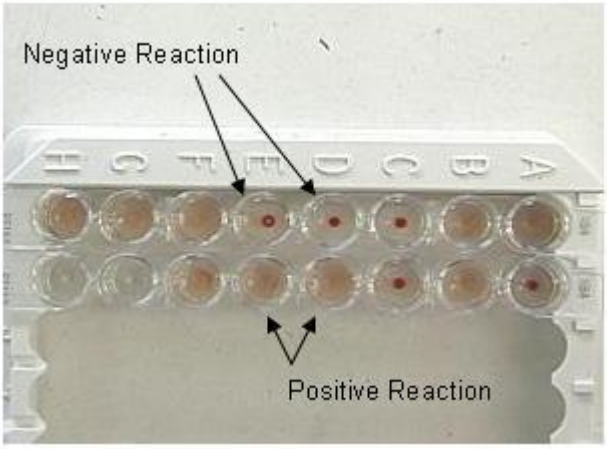
Appendix X: Detailed Methodology of Testing

IMMUCORGAMMA. GALILEO NEO

Principle:

GALILEO NEO is automated immunohematology analyzer that uses hema-agglutination and capture technique using solid phase medium. Hemagglutination is in IgM assay where red cell antigen-antibody are reacted and end point image is detected by camera and strength of titre interpreted by the machine. Capture technology is for IgG assay, and is a process where RBC antigens are immobilized on microtitre wells. Patient serum, plasma is added to each well along with a low ionic strength solution (LISS). The wells are incubated at 37°C, and washing is then required to remove unbound substance. Indicator cells (cells coated with anti-IgG) are added. Indicator cells react with the antibody/antigen complex that have adhered and form a diffused pattern. This is a positive reaction indicating an antibody is present. If there no reaction, indicator cells will form a pellet at the bottom of the well. This is a negative reaction indicating no clinically significant antibodies are present. Detection is achieved through an inbuilt camera.

The image below illustrates negative and positive reactions for capture assay.



Basic component of Galileo neo machine include;

- Loading rack
- Robotic arm
- Barcode reader
- Centrifuge
- Incubator
- CCD camera

Daily Immucor Gamma Galileo Neo Start up Maintenance and use procedure

1. Fill System Liquid (1:10 dilution, 2 bottles of System Liquid concentrate plus 9L distilled water)

2. Empty waste container

3. Log on (enter name and password into login window)

4. Shut Down Computer (use door in upper right corner) 

5. Turn off Neo instrument (power switch on right side of instrument)

6. Clean instrument (using 70% Isopropanol)

 Clean probes (lift probes carefully and move above loading bay area)

 Clean loading bays

 Clean left and right probe wash station

 Clean surfaces if dirty

7. Turn on PC and switch on Neo instrument

8. Log on as soon as login window appears. Icon will look like 

9. Initialize will occur automatically

10. Click to Maintenance. Icon was like this 

□ Select Clean Instrument (was done while instrument was turned off)

□ Press Continue

□ Select Check Pipettor Reference position

□ Press start and press Check Reference

□ Check reference position for left and right pipettor using a card

□ Press close after position was checked

□ Press continue

□ Select Pipettor Self Check

□ Press start, read instruction and continue

□ Mark PCheck in resource overview window

□ Load Pipettor Self Check Plate (red plate) into loading tower

□ Wait being scanned and press start

□ Select Reagent ABD Reagent QC

□ Press start, read instruction and continue

□ Mark QC_AB0D

□ Check reagents visually and load controls, diluents (record volume) and reagents (pressing appropriate bottom above red!)

□ If new reagent needs to be loaded -> remove air bubbles using pipette attached to lid; add one stir ball to fresh opened red cell reagents!

□ Return to Resource Overview

 Press Plates -> Press Scan Plates -> enter QC and Date -> load a new microplate in loading tower -> click on the previously entered QC name -> Press Select Assays -> Select QC_AB0DI -> Press Done -> Press Start

Running the test steps/procedure

Click on Start assistant  (Running man)

 Load Samples

 Order tests from the download Requests –Choose ABO Titration

 Load Resources/Reagents

 Press Assay on assay name -> check about missing resources (marked with red!) and load if necessary, using appropriate bottom above red! ->load plates via plate loading tower (barcode was scanned by instrument)

 Press Start Bottom

12. Loading of Capture Plates

 Select Capture Assay from Resource Overview -> Press Plates > mark appropriate assay name -> Press Strip Selection ->Deselect unused strips on capture plates > Press Done > Click on all plates (highlight in blue) in Resource Overview to start > Press Start

13. Look for completed plates under results, look at the images, approve and record or import the results. The machine will report the highest titre established

14. Finish Program after completion

 Remove all reagents, samples and plates

 Fill System Liquid (1:10 dilution, 2 bottles of System Liquid concentrate plus 9L distilled water)

 Empty waste container

 Log Off from machine

Reference: Immucor Gamma. Galileo Neo Operation Manual

Appendix XI: KNBTS Questionnaire

NATIONAL BLOOD TRANSFUSION SERVICE

Donation Number

Clinic Venue ----------Clinic Code: ---------- Donor Number ---------

DONOR REGISTRATION FORM (Donors please complete this section below)

Surname: _____Other Names: _____ _

Student Number/ National ID Number: _____ Date of Birth: / /

Gender: F/M

Marital Status:	Single	Married	Divorced/Separated	Widowed

Contact Details: Postal Address (where you would like to receive your correspondence)

Code----------

Home phone number: ---------- Cell phone number: ----------

Email: ----------

Level of education: None/ Primary/ Secondary/ Tertiary Occupation: ……

When did you last donate Blood? …… Blood Group: ……

HEALTH QUESTIONNAIRE Circle the appropriate answer

1. Are you feeling well and in good health today?	Yes/No
. Have you eaten in the last 6 hours?	Yes/No
3. Have you ever fainted?	Yes/No
In the past 6 months have you:	
4. Been ill, received any treatment or any medication?	Yes/No
5. Had any injections or vaccinations (immunizations)?	Yes/No
6. Female Donors: Have you been pregnant or breast feeding?	Yes/No
In the past 12 months have you:	
7. Received a blood transfusion or any blood products?	Yes/No
Do you have or have you ever had:	
8. Any problems with your heart or lungs e.g. asthma?	Yes/No
9. A bleeding condition or a blood disease?	Yes/No

10. Any type of cancer?	Yes/No
11. Diabetes, epilepsy or TB?	Yes/No
12. Any other long-term illness Please Specify	Yes/No

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RISK ASSESSMENT QUESTIONNAIRE

The lives of patients who receive your blood are totally dependent on your honesty & frankness in answering the questions below. Your answers will be treated in a confidential manner.

Circle the appropriate answer

In the past 12 months have you:
1. Received or given money, goods or favours in exchange for sexual activities? Yes/No
2. Had sexual activity with a person whose background you do not know? Yes/No
3. Been raped or sodomized? Yes/No
4. Had a stab wound or had an accidental needle stick injury e.g. injection needle? Yes/No

5. Had any tattooing or body piercing e.g. ear piercing? Yes/No
6. Had a sexually transmitted disease (STD)? Yes/No
7. Live with or had sexual contact with someone with yellow eyes or yellow skin? Yes/No
8. Had sexual activity with anyone besides your regular sex partner? Yes/No
Have you ever:
9. Had yellow eyes or yellow skin? Yes/No
10. Injected yourself or been injected, besides in a health facility? Yes/No
11. Used non medical drugs such as Marijuana, Cocaine etc? Yes/No
12. Have you or your partner been tested for HIV? Yes/No
13. Do you consider your blood safe to transfuse to a patient? Yes/No

DECLARATION

I declare that the information I have given above is correct.

I understand that my blood will be tested for HIV, Hepatitis B & C, and Syphilis and the results of my tests may be obtained from the National Blood Transfusion Service.

I understand that the Kenya National Blood Transfusion Service may use any communication medium(s) to send me important information. Such medium(s) shall include but not limited to e-mail, post office, mobile telephone and/or fixed telephone. I hereby give consent to KNBTS to use the contact details provided in this form to communicate to me as the need may be.

Signature: ---------- Date: ----------

For Official Use:

Weight (kg)	Hb>12.5g/dl	BP	Pulse	Donor is Accepted	
				Yes	No

Low Volume	1 Venepuncture	Hematoma	Faint		
			Mild	Moderate	Severe

Time Needle In		Time Needle Out	

Report:

Name of Interviewer: Date:

FRM CLN-01 REVISION 00 EFEECTIVE

DATE: JAN 2012