

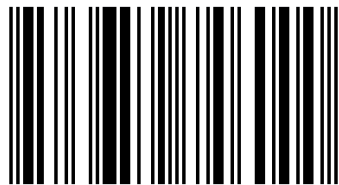
This short book summarizes the results of serological analysis of weak Rh D antigen done by the author as a project during her BSc degree in Mount Kenya University Kenya. Commercially acquired monoclonal antibodies and anti human globulin were used in this analysis. Both micro titre and tube methods were employed during the analysis. The blood samples were sampled from the remains of the donations from the Regional Blood Transfusion Center (RBTC) Nairobi. The report includes the stages that were involved in the determination of the presence and absence of weak Rh D antigen on the donor red cells. The report also presents the discussion, outcome, conclusion and recommendation of the study.



Rachel Nyaguthii Githiomi
Kennedy Muna Kuria

Prevalence of Weak Rh D in the Blood Donors of RBTC Nairobi-Kenya

Rachel Githiomi is a medical laboratory scientific officer working at Kenya National Blood Transfusion Service and heads the technical service, a registered member of African society for blood transfusion, Kenya Medical Laboratory technician and Technologist board, a researcher, trainer and a mentor. This is her first edition of Weak Rh D prevalence



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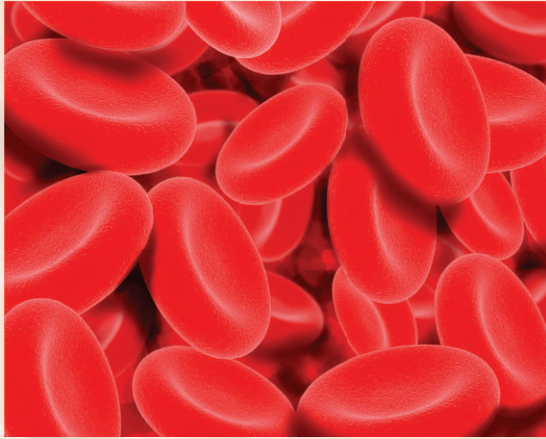
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Prevalence of Weak Rh D Phenotype in the Blood Donor Population of Nairobi Regional Blood Transfusion Centre – Kenya



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Keywords

Weak RhD antigen, D^u Test, Microtitre, Anti Human Globulin (AHG), Monoclonal anti-D

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Abstract

The weak D phenotype (D^v) is a weakened form of the Rh D antigen that in routine Rh D typing will not react directly with potent monoclonal anti-RhD serum but will require addition of anti-globulin to show the presence of D antigen. This weak form of Rh D antigen determines if an individual is rhesus positive or negative. It was described in 1944 by Wiener and was formally referred as D^v. In 1946 Stratton termed this form D as weak expression of the Rh D antigen. Weak Rh D red cells have the D antigen but are fewer per cell than normal Rh D positive cells.

The number of Rh D antigen sites on the Rh (D)-positive red blood cell is normally in the range of 9900 to 33000. The weak D phenotype appears to be a quantitative variation in the number of D antigen sites on the red blood cell (i.e. 110 to 9000) per red blood cell. This makes it very crucial to correctly identify this weakened form of D antigen in the blood donor pool to ensure recipient safety. Individuals who express weak D cannot make anti-D when exposed to a normal D antigen but if weak D red cells were not identified and donated blood from donors and labelled as weak D positive and be transfused to D- negative patients, the patients might be immunized to produce anti-D. As recipients, patients with the weak D phenotype should be considered to be Rh (D)-negative, and thus receive only Rh (D)-negative red cells (Daniel, 2010). This weak form of D antigen is associated with amino acid substitutions in the membrane.

The frequency of the weak D phenotype in whites is approximately 0.3% (3 in 1000). This frequency will vary depending with method and reagents used, plus racial mix tested. It has also been established that the frequency of weak D among Blacks is higher than in Whites.

The purpose of this study was to determine the point prevalence of weak RhD antigen in donated blood at Regional Blood Transfusion Centre Nairobi (RBTC Nairobi). Blood donor samples from Voluntary non- remunerated donors who had consented to donate blood during the study period was collected in 6mls Ethylene Diamine Tetra-chloral acetic Acid (ETDA) tubes. 384 samples were sampled and were delivered to the National Blood grouping laboratory. Out of 384 blood samples typed 26 failed to agglutinate in the microtitre and tube methods. The 26 samples were subjected to antihuman globulin test. 8 of the samples reacted with AHG (D_v test) while the remaining 18 samples failed to show any agglutination or haemolysis.

The results elucidated that the point prevalence of weak Rh D antigen at RBTC Nairobi is 2.1% (8/384x100). This frequency is high compared with the Caucasians which is between 0.2 to 0.3 %; and Africans have already indicated prevalence of weak RhD in blacks rating is higher than in Whites.



List of Acronyms and Abbreviations

- D^u** – Weakened form of Rh D antigen
- DCT** – Direct coomb Test
- G/MLS** – Grams per deciliters
- HB** – Haemoglobin
- HBV** – Hepatitis B virus
- HCV** – Hepatitis C virus
- HDFN** – Haemolytic Disease of the Fetus and the New Born
- HIV** – Human Immunodeficiency Virus
- HTR** – Haemolytic Transfusion Reaction
- KNBTS** – Kenya National Blood Transfusion Service
- KG** – Kilogram
- RBTC** – Regional Blood Transfusion Service



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1.0 Background

1.1 Introduction

The weak D phenotype (D^u) is a weakened form of the D antigen that in routine Rh D typing will not react directly with potent monoclonal anti-RhD serum but will require addition of antiglobulin to show the presence of D antigen. This weak form of D antigen was described in 1944 by Wiener and was formally referred as D^u . In 1946 Stratton termed this form as weak expression of the D antigen. Weak D red cells have the D antigen but are fewer per cell than normal Rh D positive cells. The number of D antigen sites on the Rh (D)-positive red blood cell is normally in the range of 9900 to 33000. The weak D phenotype appears to be a quantitative variation in the number of D antigen sites on the red blood cell (i.e. 110 to 9000) per red blood cell. The frequency of the weak D phenotype in whites is approximately 0.3% (3 in 1000). This frequency will vary depending with method and reagents used, plus racial mix tested. It has also been established that the frequency of weak D among Blacks is higher than in Whites. (Agre PC, *et al*; 2002).

Individuals who express weak D cannot make anti-D when exposed to a normal complete D antigen but if weak D red cells were not correctly identified donated blood from donors and labelled as weak D positive and be transfused to D- negative patients, the patients might be immunized to produce anti-D. As recipients, patients with the weak D phenotype should be considered to be Rh (D)-negative, and thus receive only Rh (D)-negative red cells (Daniel, 2010). This weak form of D antigen is associated with amino acid substitutions in the membrane spanning or cytosolic domains of the RhD protein and are not exposed to the outside of the membrane. The frequency of the weak D phenotype in Caucasians is approximately 0.3 percent (3 in 1000). However, the frequency varies with the method used, the reagent used, and the racial mix tested, since the frequency of weak D among Blacks is higher than in Whites.

1.2 Statement of problem

In Kenya, 400,000 units of blood are required each year yet only less than half of these are collected (Kenya National Blood Transfusion, 2010). All the donated blood is serologically analyzed for RhD antigens. This is only the blood units collected by the Kenya National Blood Transfusion. Due to the issues of immunization associated with D antigen it is very important that all the donors Rh D antigen status is determined to ensure that the blood does not cause adverse events in the recipients and also the donors some them who are mothers and aspiring mothers will not be given antibody D (Rhogam) un necessarily.

1.3 Justification of the study

The RhD antigens of the Rh blood group system particularly D is known to be the most immunogenic second to ABO antigens. The Rh D antigens are among the clinically significant antigens because they cause severe to fatal hemolytic transfusions reactions (HTR) and hemolytic diseases of the fetus and the new born (HDFN) which may occur in D- individuals when exposed to D antigens following transfusion or pregnancy.

Kenya is yet to determine this prevalence weak D phenotype in the donor population thus the reason for undertaking this study.

1.4. Hypothesis

1.4.1 Null hypothesis- H_1 :

Weak D antigen in the Blood donor population of Nairobi Regional Blood Transfusion Centre is not prevalent.

1.4.1 Alternative Hypothesis- H_2 :

Weak D antigen in the Blood donor population of Nairobi Regional Blood Transfusion Centre is prevalent.

1.5. Objectives

1.5.1 Broad objective.

To determine the point prevalence of weak D antigen in donated blood at RBTC Nairobi

1.5.2 Specific objectives

To establish the distribution of weak D antigen in donated blood at RBTC Nairobi in relation to age

To determine Weak D antigen in donated blood at RBTC Nairobi in relation to gender

Research questions

1. What is the point prevalence of weak D antigens in donated blood at RBTC Nairobi?
2. What is the distribution of weak D antigens in relation to age?
3. What is the distribution of weak D antigens in relation to gender?

1.6 Significance of the study

The study will reveal the point prevalence of RH D weak antigens Rh D negative in donated blood in Nairobi which can be associated with the whole country basing on the fact that Nairobi is a cosmopolitan county compared with the other 46 counties in Kenya. This will enable KNBTS in implementation of blood safety strategies aimed at reducing RhD antigen immunization to the recipients also to these donors. The study will also form the basis for further studies and interventions. In such situations, the Rh blood group should be determined by extended serological analysis to ensure better and safer transfusion practices and antenatal/postnatal care.

1.7 Delimitation of the study:

The study will focus on dependent variable point prevalence of weak Rh D antigens and two independent variables age and gender in donated blood of Nairobi RBTC donor population.

1.7.1 Limitation of the study:

Some of the expected challenges include time constrain, lack of extended D typing antisera to enable in further identification of weak Rh D antigen forms.

1.8 Assumption of the study:

That the calculated number of donor samples will be representative of the donor population in Nairobi, that the serological findings will be valid and that all the instruments will be in good storage conditions and their integrity will not be interfered with. It is also assumed that the reagents will be working including the control cells.



Literature Review

2.1 Blood Groups

Blood groups (antigens) are inherited characters on the surface of red cells and are detected serologically by a specific alloantibody. Blood groups systems consist of one or more antigens which are governed by a single gene locus or by homologous genes. (Daniels 2002). Blood groups are proteins, glycoproteins or glycolipids in the red cell membrane surface with several functions such as transporters, anchors, enzymes and receptors. They were discovered in 1901 by Karl Landsteiner when he noticed that plasma from some individuals' agglutinated red cells from others but did not agglutinated others. He named the blood groups as; A, B and O. Afterwards, Decastello and Sturli added a fourth group AB (Landsteiner, 1901, Daniels, 2002).

The Rh blood group system was described in 1939 by Levine and Stetson while investigating a haemolytic transfusion reaction (HTR) in a woman who had given birth to a stillborn baby and had reacted to a blood transfusion from her husband.(Scott 2004,Levine, and Stetson, 1939). In the field of transfusion medicine the antibodies of the ABO and Rh blood group systems are the most clinically significance because they are capable of causing haemolytic transfusion reactions (HTRs) and haemolytic disease of the fetus and the newborn (HDFN), (Daniels *et al*, 2010).In 2010 at the International Society of Blood Transfusion, the committee on Terminology for Red Cell surface Antigens reported 328 human blood groups of which 284 are contained within the 30 blood groups systems. (Story. J.R, *et al*; 2011). Before 1950's human blood groups were known as pattern of inheritance which could only be detected serologically.

From 1950's with the assistance of biochemical analysis, the structure of carbohydrate and protein antigen was discovered, which helped in revealing the functions of some blood group antigens. Some blood group antigens are now known to have some specific functions such as membrane transporters seen in Diego (anion transporter), Kidd (urea transporter), and the Colton (water channel), receptors expressed by Duffy, complement regulatory glycoproteins seen in Cromer and Knops, enzymatic function as observed Yt and as part of the glycocalyx seen in MN antigen. Functions of the Rh proteins and the Rh-associated glycoproteins RhAG proteins is not yet fully understood but many assumptions have been made due to their structural characteristics of membrane transporters suggesting their involvement in ammonium transport or in maintaining the symmetry of the phospholipid in the red cell membrane. It has also been suggested that the Rh proteins are involved in CO₂ and O₂ transportation (Daniel, 2002, Daniels, *et, al* 2010). All these assumptions remain to be confirmed.

Most of the 30 defined blood groups systems exhibit the null phenotype which is associated with lack of specific blood group antigen expression on the red cell membranes. The null phenotype is detected serologically by absence of agglutination with specific antisera. Molecular analysis has revealed that the null phenotypes is caused by mutations including; deletions, missense mutations, frameshift mutations, introns splice-site and promoter mutations which cause lack of antigen expression on the surface of the red cell. The null phenotypes are rare but clinically significant.

Individuals with null phenotypes are at risk of immunisation due to transfusion of incompatible blood or pregnancy via fetal maternal haemorrhage.

Variation of antigen expression has also been reported which is caused by nucleotide duplications and hybrid gene as seen in weak or/and partial RhD (Daniels, *et al*,2010).

2.2 The Rh Blood Group

The Rh blood group system was discovered in 1939 by Levine and Stetson while investigating the serum of a mother who had given birth to a stillborn and developed a haemolytic transfusion reaction with blood transfusion from her husband. Her serum agglutinated her husband's red cells and those of 80% of ABO compatible donors. [Scott ML 2004]. Unfortunately, they did not name the newly discovered antibody. In 1940, Landsteiner and Wiener produced an antibody by introducing rhesus monkey red cells into rabbits. The rabbit serum agglutinated Rhesus monkey red cells and also 85% of human red cell; the antibody was named anti-Rh. In 1941, Levine and Stetson's human antibody was shown to be similar to the anti-Rh antibody but was later demonstrated that the two antibodies (human and animal) were different and the human antibody was renamed as anti-D of the Rh blood group system. (Scott ML, 2004, Daniels, 2002)

2.3 The Rh antigens

The Rh blood group system is the most complex human blood groups system with 50 defined blood group antigens expressed only on the red cells membranes (Daniels, 2010).

The D antigen is highly immunogenic making it the most clinically significant antigen in the Rh system. 85% of D negative individuals will make anti-D if transfused with 200ml of D positive blood; anti-D is readily produced in D negative mothers with a D positive fetus leading to risks of HDFN. HDFN is caused by the maternal IgG anti-D antibodies produced by the Rh D negative mother against an RhD positive fetus.

The alloanti-D acquired via transfusion and pregnancy can cause major HTR and/or HDFN when they cross the placenta and attach to the foetal red cells marking them for destruction (Wagner, *et al*, 2010). Knowing the genetic background of the Rh blood group system has led to the determination of the Rh protein structure within the red cell membrane. The Rh



antigens are situated on two proteins RhD and RhCE present on the red cell membrane. The Rh proteins span the red cell membrane 12 times with 6 extracellular and 5 intracellular loops with N-and C terminals located within the cytoplasm. As shown in figure1.

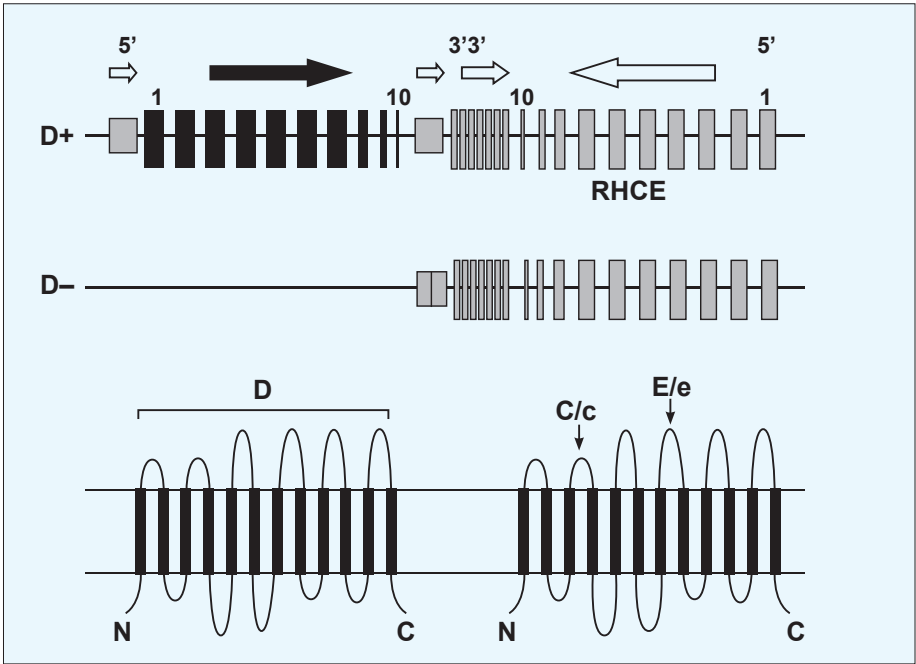


Fig 1: The diagrammatic representation of the D and CcEe polypeptide which span the red cell membrane 12 times. Fig adapted from (Daniels 2005)

2.4 RhD polymorphism and its molecular basis

The Rh blood groups antigens are encoded by two closely linked genes RHD and RHCE located on the short arm of chromosome 1(1p36.11).The RHCE gene was the first to be described in 1990 followed by the discovery of RHD two years later (Daniel,2002). The RHD gene encode the RhD antigen and the RHCE gene encodes the two pairs of antithetical antigens Rh C/c and Rh E/e (Daniels et al; 2010).The two Rh genes (RHD AND RHCE) are almost similar in genomic arrangement; each having 417 amino acids in 10 exons arranged in opposite orientation on the chromosome that is tail –to –tail arrangement with the SMP1 gene between them. There is no evidence to indicate that the function of the SMP1 gene is connected to RH. Although the RhD and the RhCcEe proteins have 417 amino acids each, they differ by between 32-35 amino acids in their sequence. The two ends of the RHD have a DNA segment called the box and each box has 9.000 base pairs. D represents the presence of the D antigen in the red cell membrane but no antigen antithetical to D (“d”)



has been found. The D negative phenotype results from the absence of the RhD protein on the red cell membrane and is usually caused by complete deletion of the RhD gene between the two Rh boxes and is marked as “d” to indicate absence of RhD antigen. The Rh antigens are inherited as haplotypes and the most common Rh phenotype seen include: CDe, dce, cDE, cDe, cdE, Cde, CDE, and CdE also denoted as R¹, r, R², R⁰, rr⁺, r⁻, R^z, r^y. In these case “R” denotes the presence of the D antigen while “r” denotes the absence of D antigen as shown in table 1.

Table 1: Showing eight RhD haplotypes and their frequencies

Haplotype		Frequencies		
Fisher-Race	Weiner	English (%)	Nigerian	Chinese
Dce	R ¹	42	6	73
Dce	R	39	20	2
DcE	R ²	14	12	19
Dce	R ⁰	3	59	3
dcE	rr ⁺	1	Rare	Rare
dCe	r ⁻	1	3	2
DCE	R ^z	<1	Rare	<1
dCE	r ^y	Rare	Rare	<1

Table 1: Showing eight haplotypes and their frequencies in English, Nigerian, and Chinese populations. Adopted (Daniels 2010)

A similar gene to RHD and RHCE genes is the associated glycoproteins Rh AG which is encoded by the RHAG gene located on chromosome 6 at 6p12-p21. During erythropoiesis, RhAG appears on the erythroid precursors before the D, C, c, E and e antigens. RhAG is necessary for the expression of Rh antigens on the red cell membrane (Daniels, 2002).

2.5 D antigen Variants

The D antigen expression on the red cell varies greatly in all phenotypes, from the greatly enhanced expression seen in individuals with missing C, c, E, e phenotype due deletion (D-) to weak D or even to the extreme of Del antigen where the D antigen cannot be detected



by the routine serological methods but only by special methods such as adsorption and elution [Daniels, et al; 2010].

The Del Phenotype is common in the Far East especially among the Chinese and Japanese population where approximately 10% to 30% of D- individuals have the Del phenotype (Daniels 2002, Scott M.L; 2004). In Taiwan the most possible explanation of the Del phenotype is associated with a 1013-base pair (bp) deletion of RHD from introns 8 to introns 9 by compressing exon 9. [Daniels 2002]. Another explanation of Del phenotype could be due to an incomplete integration of the RhD protein in the red cell membrane (Flegel *et al*: 2007).

In the Caucasians population, the D negative phenotype is almost always caused by the deletion of the whole of RHD gene. In black Africans 66% of D negative phenotypes are associated with homozygosity or hemizyosity for a complete but inactive RHD gene known as the RHD pseudogene which is denoted as RHD ψ . This inactive gene is caused by a nonsense mutation at exon 6 and a 37 base pair duplication which introduces a premature stop codon in exon 4 producing a shortened RhD protein. Another abnormal gene associated with D negative phenotypes in Africans is the RHD-CE-D^s hybrid gene. This hybrid gene has exons for both the RHD and RHCE gene and produces no D protein (Daniel 2002).

Another D variant is the Rh null phenotype where the red cell membrane lacks all the Rh antigens. This is caused by deletion of the RHAG gene. (Daniel 2002, Avent and Reid, 2000). The D antigen is the most immunogenic of the Rh antigens and is also the most clinically significant in the field of transfusion and transplantation medicine.

The expression of D antigen is dependent on the RhD protein structure. Changes in the RHD gene cause changes in the structure of the Rh D proteins. Changes to the Rh protein may alter the epitopes on the red cell membrane.

Many variations in the Rh D phenotype are caused by a number of changes including, duplications, missense mutations, frameshift mutation, and inactivation of the gene. These changes are classified in to two categories of D phenotypes such as: quantitative (weak) D phenotype where the individual do not make anti-D and qualitative (partial) D phenotype where an individual is capable of making anti-D when exposed to the D antigen (Daniels, 2010, Avent and Reid, 2000). The frequency of D antigen in Caucasian is up to 88%, 95% in black Africans and almost 100% in the far East. (Daniels, 2005). Determination of weak D and partial D by serological methods involves use of commercial monoclonal antibodies which detect only one epitopes. An analysis of tests with many such antibodies against red cells expressing different D variants has led to the definition of 30 reaction patterns (Daniels 2010). Even with these many antibodies against the red cell antigens expressing different D variants showing reaction patterns, determination of RhD antigen can still be a challenge



due to the multiple conformational epitopes of the D antigen and also due to the numerous D typing methods in use today. It is now a fact that the D antigen is dependent on the RhD protein structure.

The number of D antigen sites on the weak D phenotype is between 100 and 4000 per red cell compared with the D antigen present on the Rh D positive red cells which range from 9900 to 33000 (Daniel,2002). Some partial D phenotypes such as DVI and DIVa have normal or more numbers of D sites per cell while others such as DVI types I and II have shown to be having very low D site density. Changes in RHD gene can alter the amino acid sequence in the RhD protein and this may affect the structure and the antigen expression in the red cell membrane, altering or creating new epitopes.

In weak D the whole D antigen is present but expressed weakly and thus these individuals cannot make anti-D when immunised with D antigen while in partial D some part of D antigen is missing and is associated with either RHD-CE-D hybrid gene or RHD point mutations. Individuals with partial D are capable of making an antibody to the epitopes they lack if exposed to D antigen. Formally, partial D was grouped into six categories D^{II} to D^{VII} based on their distinct reactivity with polyclonal anti-D sera from immunised partial patients. DVI is one of the most common and the most clinically significant partial D because it lacks most of the D epitopes compared to other D variants [Wagner 1999]. This partial D D^{VII} is associated with RHD and RHCE gene substitution of exons 4-6 of the RHD gene with exons 4-6 from the RHCE (Daniels 2005). In the transfusion practices, monoclonal anti -D reagents are used to classify the partial Ds based on the different reaction patterns. These different monoclonal reagents recognise different epitopes of the D antigen (Wagner, 1998, Daniels, 2010).

2.6 Anti-D Immunization

All the Rh antibodies are produced in response to red cell immunisation. Formation of the Rh antibodies can be due to blood transfusion or pregnancy. Rh antibodies are of IgG and react best at 37°C and thus considered as potential causes of HTR and HDFN.

In transfusion practices, routine serology typing is used for the detection of the Rh antibodies using two commercial monoclonal anti-D sera. It is very critical for transfusion practice to correctly detect and identify D variant phenotypes in donors to reduce the risk of alloimmunisation via blood and blood products. In prevention of anti-D alloimmunisation, D positive blood and blood products should not be transfused to D negative patients and also D positive blood and blood products should not be transfused in females of child bearing age who are Rh D negative to prevent risks of HDFN. In UK and other developed countries, prophylactic human anti-D immunoglobulin (IgG) was introduced to Rh D negative women during pregnancy and at delivery to prevent alloimmunisation and reduce risks of HDFN.



Methodology

3.1 Study Area

Nairobi Blood Transfusion Centre in Nairobi County; one of the one of the 20 blood transfusion centres of KNBTS. It is one of the first blood transfusion centres to be established in Kenya in the year 2000. Its main mandate is to collect blood, process, test group store, and distribute to the user hospitals under Nairobi county region and its environs.

3.2 The Study Design

Experimental study design will be used in which samples analysis will be done on selected blood donor samples.

3.3 Sampling Method

Systematic sampling method for donated blood samples was employed and every 23rd donated blood unit was selected as per the Fisher *et al* 2002, formula.

3.4 Study Population

The study population was Blood donors donating blood at Nairobi RBTC during the study period.

3.5 Selection Criteria

3.5.1 Inclusion Criteria: Blood donated at RBTC Nairobi by donors aged 16-65 years irrespective of sex during the study period, and consented for donations.

3.5.2 Exclusion Criteria: Those who did not qualify and did not consent for blood donations and those who donated at other RBTCs and Satellites.

3.6 Ethical Considerations

Helzinks ethical considerations of 2002 were used whose operational principle states: Research should be based on thorough knowledge of the scientific background (Article II), a careful assessment of risks and benefits (Article 16, 17), have a reasonable likelihood of benefit to the population studied (Article 19) and be conducted by a suitably trained investigators (Article 15) using approved protocols, subjected to independent ethical review and oversight by a property convened Committee (Article 13). Information regarding the

study should be publicly available (Article 16). Ethical publications extend to publication of the results and consideration of any potential conflict of interest (Article 27). Experimental investigations should always be compared against the best methods, but under certain circumstances a placebo or no treatment group may be utilized (Article 29). The interests of the subject after the study should be part of the overall ethical assessment, including assuring their access to best proven care (Article 30). Whenever possible unproven methods should be tested in the context of research where there is reasonable belief of possible benefit (Article 32)-2008: sixth revision, 59th meeting, Seoul.

The following was adhered to:

1. Research authorization was sought from Mount Kenya University ethical committee and The Director, Kenya National Blood Transfusion Services.
2. Codes were used for the identification of the blood donor samples instead of their name.
3. The outcome of the study will be shared between the subjects, staff and the researcher, and the organization for further action.

3.7 Sample size and sampling

3.7.1 Sample size determination

The fisher et al formula (Fisher *et, al*; 2002)

$N = \frac{Z^2 P (1-P)}{d^2}$ will be used for sample size based on the following assumptions

Where:

n = the desired sample size, (N= 384)

P = national prevalence of weak Rh D antigens 0.5%

d = the level of significant set = 5% (0.05)

z = standard, corresponding to 95% confidence; (1.96)

$N = (1.96^2 \times 0.5 (1-0.5)) / 0.05^2 = 384$ samples

3.8 Study variables

3.8.1 Independent variable: Weak Rh D antigen in donated blood.

3.8.2 Dependent variable: Age and gender in relation to Weak Rh D antigen



Test Procedure

4.1 Materials/Requirements

Blood donors, Blood bags, purple/lavender Vacutainer [EDTA], blood sample, and microtitre plates Centrifuges, Magnifying mirror, shaker, Test tubes, Micropipettes and Multichannel micropipettes, Gloves, Biohazard disposal container, analytical sodium chloride (NaCl) Sodium hypochlorite for decontamination, registers, pens waterbath, refrigerator, analytical weighing balance, water bath, Incubator, , glass slides disposable plastic pipettes, Microscope, Micro titer- plates and distiller.

4.2 Reagents

- Monoclonal antisera D
- Antihuman globulin [AHG] reagent
- Normal Saline
- Distilled water
- Control cells [O Rh D positive and negative cells]

4.3 Method/Technique

4.3.1 Serological agglutination method: was employed both microtitre and tube methods

4.3.2 Principle of the test: It is based on haemagglutination

4.3.3 Rh D typing Microtitre Procedure

- The microtitre plates were labelled appropriately with donor numbers
- 1 drop of monoclonal anti-D will was dispensed to all the wells
- I of drop of sample was added to respective wells
- The microtitre plates with contents were placed into the microtitre centrifuge and centrifuged at 2000rpm for 1 minutes
- The plate were shaken with the shaker for I minute
- The results were observed and interpreted with the aid of the magnifying mirror viewer

- Agglutination was interpreted as positive (RhD antigens present). Those that did not agglutinate were identified as Rh D negative and Rh D typing was repeated using the tube method.
- Control cells will be treated as test cells

4.3.4. Tube Method

- Non-reactive samples were identified
- 26 test tubes were labelled with donor's numbers of non-reactive blood
- 2 drops of blood samples were placed in respective tubes.
- 2 drops of monoclonal anti D were added into all the test tubes
- All tube contents were placed into the sample centrifuge.
- Centrifuged at 1000rpm for 1 minute.
- Results were read both macroscopically and microscopically.
- Results were interpreted as follows; agglutination as positive for Rh D antigens and absence of agglutination as Rh D antigen negative.

4.3.5 D^u Test [AHG or IAT test]

- All the 26 test tubes contents (Rh D negative) were incubated at 37°C water bath for 60 minutes.
- All test tubes contents were washed x3 with normal saline and in the last wash the supernatant was discarded and 2% saline cell suspension was prepared.
- A new set of 26 test tubes were labelled with respective donor numbers
- 2 drops of 2% saline cell suspension was placed into respective test tubes.
- 1 drop of Antihuman Globulin (AHG) was added and gently mixed
- All tubes contents were centrifuged at 1,000rpm for 1 minute
- Examined for agglutination or haemolysis both microscopically and macroscopically
- Results were recorded as agglutination or haemolysis D^u positive and absence as D^u negative.
- The test tubes that showed agglutination were grading and results recorded as D^u positive and this was interpreted as weak D antigens positive.



The D^u test is an indirect antiglobulin test that uses the patient's red cells and an IgG anti-D. An IgG anti-D must be used because antiglobulin serum contains anti-IgG. The D^u test, like other antigen typing done using an antiglobulin test, is controlled by doing a direct antihuman globulin test (DAT) on the test cells. Direct antihuman globulin test serves as an auto control which reveals whether the test cells are sensitized with antibody in vivo. If the DAT is positive, the D^u test is invalid, as it will be positive whether or not the patient is a weak D.



Data analysis and management

The Data was cleaned, coded, stored in a handbook and entered using MS-excel spread sheet then analyzed using the Statistical Package for Social Sciences (SPSS) Version 22. Presentation was done using pie charts, bar charts and tables.

5.1 Results

The 384 blood donor samples were phenotyped (grouped) using monoclonal anti- D reagents 358 agglutinated directly with the anti-D in the initial micro-titre typing. 26 samples did not react directly with anti- D and thus were retyped using the tube method. In the tube method there was no agglutination and thus an indirect antihuman globulin test the (D^u) was done. 8 of the 26 tubes showed agglutination when tested using antihuman globulin test as shown below

Table 2: Gender of the Blood donors

Gender	Frequency	Percent	Cumulative Percent
Male	236	61.5	61.5
Female	148	38.5	100.0
Total	384	100.0	

Table 4: Prevalence of weak Rh D antigen among blood donors

	Frequency	Percent	Cumulative Percent
Other type of donors	376	97.9	97.9
Weak Rh D antigen	8	2.1	100.0
Total	384	100.0	

Chart 1: Weak Rh D Antigen Prevalence

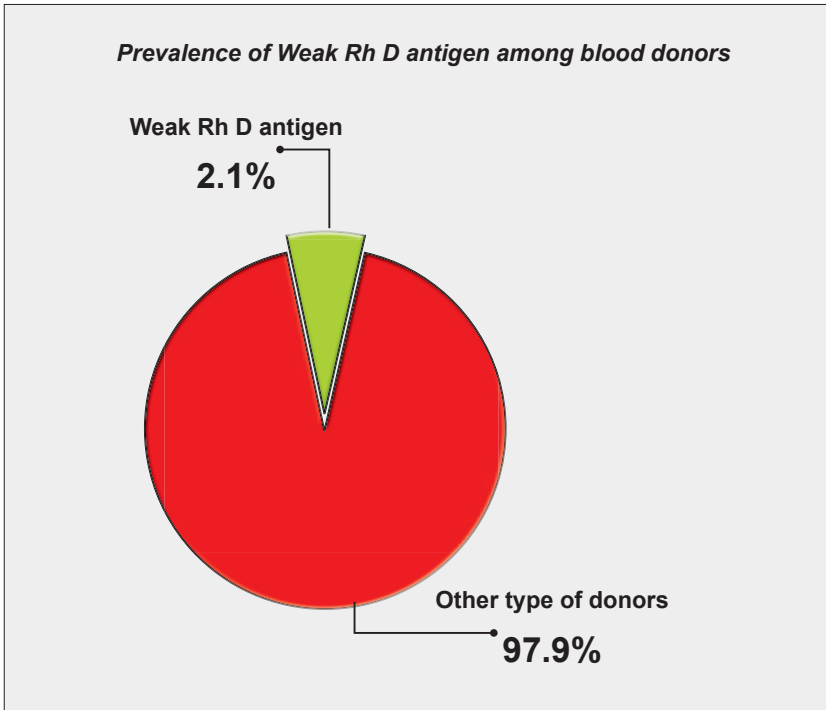


Table 5: Rh D antigen typing results (micro-titre plate method)

	Frequency	Percent	Cumulative Percent
Rh D antigen Positive	358	93.23	93.23
Rh D antigen Negative	26	6.80	100.00
Total	384	100.00	



Table 6: Rh D antigen typing results (tube method and D^u Test)

	Frequency	Percent	Cumulative Percent
Rh D antigen Negative	18	69.2	69.2
Weak Rh D antigen Positive	8	30.8	100.0
Total	26	100.0	

Table 7: Weak Rh D antigen Positive in relation to gender

Gender	Frequency	Percent	Cumulative Percent
Male	4	50	50
Female	4	50	100
Total	8	100	

Pie – chart 2: Weak Rh D in relation to gender

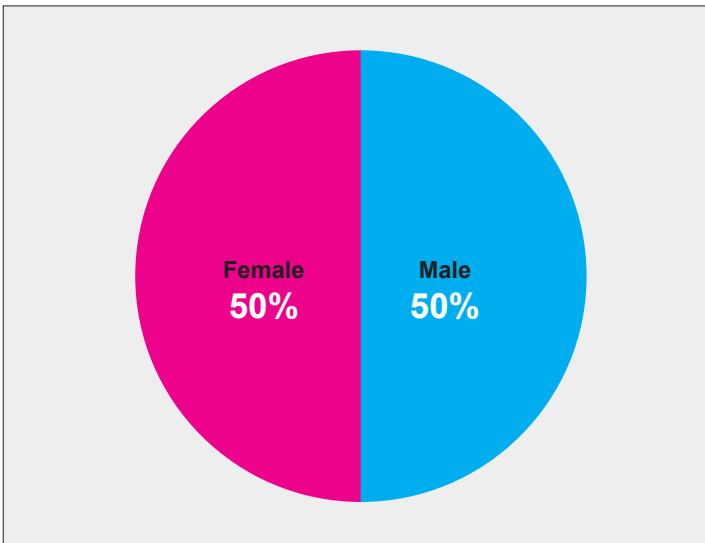


Table 8: Weak Rh D antigen Positive in relation to Age

Age	Frequency	Percent	Cumulative Percent
16	1	12.5	12.5
19	1	12.5	25
22	1	12.5	37.5
25	2	25	62.5
26	1	12.5	75
32	1	12.5	87.5
33	1	12.5	100
Total	8	100	

Bar-chart 3: Weak Rh D antigen Positive in relation to Age

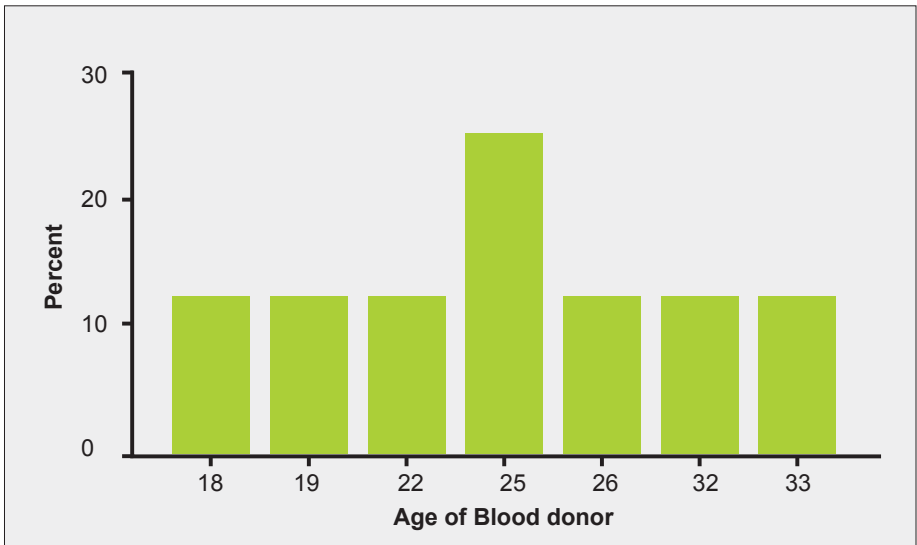


Table 9: Correlation between Age and Weak Rh D antigen

		Weak Rh D antigen	Age of Blood donor
Weak Rh D antigen	Pearson Correlation	.(a)	.(a)
	Sig. (2-tailed)	.	.
	N	8	8
Age of Blood donor	Pearson Correlation	.(a)	1
	Sig. (2-tailed)	.	.
	N	8	8

a Cannot be computed because the weak Rh D antigen is a constant variable

Table 10: Correlation between Gender and Weak Rh D antigen

		Gender of Blood donor	Weak Rh D antigen
Gender of Blood donor	Pearson Correlation	1	.(a)
	Sig. (2-tailed)	.	.
	N	8	8
Weak Rh D antigen	Pearson Correlation	.(a)	.(a)
	Sig. (2-tailed)	.	.
	N	8	8

a Cannot be computed because the weak Rh D antigen is a constant variable



Table 11: Weak Rh D antigen Chi-Square Test

	Observed N	Expected N	Residual
Weak Rh D antigen			
Positive	8	8.0	.0
Total	8(a)		

a = the weak Rh D antigen variable is constant. Chi-Square Test cannot be performed.

Discussion

Appropriate assignment of Weak Rh D status is of critical importance in modern transfusion medicine. This study investigated the status of blood donors who donated blood at RBTC Nairobi which has remained totally unexplored so far.

The prevalence of weak D antigens varies worldwide depending on the methods used and the race. In this study, 384 blood donor samples were serologically typed and 8 were found to exhibit weak Rh D antigen giving a prevalence of 2.1 %.

Rh D is very immunogenic with approximately 20-30 % of Rh D negative patients who receive large volume of blood units of D antigen positive making anti- D (Daniels *et al*; 2007). This indicates that the implications of these findings could be far reaching in transfusion medicine.

Units of blood from weak D positive donors can pose a risk of allo-immunizing in Rh D negative transfusion recipients, transfusion reactions and haemolytic disease of the fetus and the new born for Rh D negative mothers carrying Rh D positive fetus (Wagner *et al*; 2005, Daniels, 2002).

Serological analysis of Rh D antigens have been known since 1964. Today there are over 200 D variants recognised (C .Opoku *et al*; 2008) though in most cases they can be differentiated serologically, others do not directly react with the commercially available D antisera thus requiring extended typing and molecular analysis.

The results of 2.1 % prevalence are lower compared to those of Ghana 6.45% (C .Opoku *et al*; 2008). Although these samples tested as RhD negative in the first and second rounds of serological typing with anti-D, they exhibited a weak reactivity with indirect antiglobulin test (IAG) or indirect coombs test.



Comparing the study outcome with other documented data from other countries, it is evident that there are major variations in different populations as shown by the 6.45% of Ghana as quoted by C .Opoku *et, al;* 2008, 0.2-1% in the Caucasians (Daniels 2010), 0.01% prevalence of the Indian population (R.N. Makroo *et, al;* 2010) and the 0.14% prevalence in the Albania population (Xhetani.M. Seferi I *et.al;* 2014). This shows that the weak D prevalence is different in different countries and population or races which make it even more important for all countries to establish the Weak RD antigens prevalence.

However the 2.1% weak Rh D prevalence highlights the distribution of this type of blood group in RBTC Nairobi which is comparable with other documented literature from different countries.



Conclusion

- The study revealed that the point prevalence of weak Rh D antigen in RBTC Nairobi donor population is 2.1%.
- That the results outcome was not influenced by age or by gender

Recommendation

While this test results looks ideal, there is need to carry out this type of analysis in all the Kenya National Blood Transfusion Service sites.

Molecular and extended typing should be introduced to enhance determination of this type of Weak D antigen.

This antihuman globulin test should be recommended to all patients sample at the point of care who type negative with monoclonal anti-D.



References

1. Agre PC, et al; (2002). A proposal to standardize terminology for weak D antigen. *Transfusion*. 1992 Jan; 32(1):86-7.
2. Argall CL, Ball JM, Trentelman E. Presence of anti-D in the serum of a D^u patient. *J lab Clin Med* 1953; 41: 895-898. 03/03/2015
3. Avent ND, and Reid E.M. The Rh blood group system: a review. *Blood* 2000; 95: 375-387 03/03/2015
4. Connie WM. Rh complexities: serology and DNA genotyping. *Transfusion*, 2007; 47: 17s-22s. 03/03/2015
Cid J, Lozano M, Fernandez Aviles F, *et al.* Anti-D alloimmunisation after D-mismatched allogeneic hematopoietic stem cell transplantation in patients with hematologic diseases. *Transfusion* 2006; 46:169.
5. Contreras M, *et al*; (1987) de Silva M, Teesdale P, Mollison PL. The effect of naturally occurring Rh antibodies on the survival of serologically incompatible red cells. *Br J Haematol* 1987; 65:475.
6. Daniels G: (2013) Variants of Rh D current testing and clinical consequences. *British Journal of haematology*, 2013, 161, 461-470
7. Daniels G: *Human blood groups*. 2nd edn. Oxford: Blackwell Science Ltd, 2002
8. Daniels G. and Brommiliow I. *Essential Guide to Blood Groups*. 2nd end Willey-Blackwell Ltd, 2010
9. Daniels G, (2005). The molecular genetics of blood group polymorphism. *Transplant immunology* 2005; 14: 143-153
10. Daniels G, Finning K., Martin P, Summers J. Fetal RhD genotyping A more efficient use of anti-D immunoglobuline. *Transfusion Clinique et Biologie* 2007; 14: 5571
11. Daniels G. and Reid ME. Blood groups: the past 50 years. *Transfusion* 2010; 50(2): 281-9.
12. Denomme GA, Wagner FF, Fernades BJ; *et al*; (2005): Partial D, weak D types and novel RHD alleles among 33,864 multi-ethnic patients: implications for anti-D alloimmunisation and prevention. *Transfusion* 2005; 45(10): 1554-60.
13. Flegel WA and Wagner FF. Molecular biology of partial D and weak D: Implications for blood bank practice. *Clin Lab* 2002; 48(1-2): 53-9

14. Flegel W A: (2006).Molecular genetics of RH and its clinical application. *Transfusion: Clin Biol*: 2006; 13(1-2): 4-12.
15. Flegel W A: (2011).Molecular genetics and clinical application for RH. *Transfusion Apher Sci*: 2011; 44(1): 81-91.
16. Flegel W.A. (2007).The genetics of the Rhesus blood group system. Review in *Blood Transfusion*. 2007; 5(2): 50-57
17. Kormoczi GF. 106 Unusual RhD phenotypes - recent advances and clinical implications. ISH EAD 2007. S23-25. Hungary
18. Liu W. Et al, (1999). Molecular configuration of Rh D epitopes as defined by site directed mutagenesis and expression of mutant Rh constructs in K562 erythroleukaemia cells. *Blood* 1999; 94: 3986-96
19. Opoku-Okrah *et al*; (.2008). Detection of weak D (Du) phenotype ... 02/03/2015 *Journal of Science and Technology*, Vol. 28, No. 3, Dec., 2008 02/03/2015
20. Olovnikova N.I ,et al;(1992). Anti-Rho [D] antigen monoclonal antibodies
21. Serological characteristics of monoclonal **IgG1** antibodies.*Gematol Transfuziol*_1992.37 ;(9-10):5-9.
22. P i S A e K.A. M, F L i D R O V A. H. (1996). Results of serological testing of D variants and weak D antigens. *TCB*. 1996; 6:369-371.
23. Prasad MR, et al; (2006). Anti-D in Rh positive pregnancies. *Am J Obstet Gynecol* 2006; 195:1158.
24. Scott M L. The complexity of the Rh system. *Blood Transfusion*.2004; vol 87: s1: 58-62.
25. Scott ML: Section 1A: Rh serology co-ordinator's report. *Transfus Clin Biol* 2002; 9: 23-9
26. Wagner F.F, et al; (1999). Molecular basis of weak D phenotypes presented at the 25th congress of the International Society of Blood Transfusion. *Blood*: 1999; 93; 1: 385-395.
27. Wagner F.F, et al; (2000). Weak D alleles express distinct phenotype. *Blood*. 2000; 95: 2699-2708.
28. Wagner F.F, et al; (2002). DNB: a partial D with anti-D present in central Europe. *Blood*. 2002; Vol 100: 6; 2253-2256
29. *Blood Transfus.* 2014 Oct; 12(4): 565–569.Prepublished online 2014 Jun 12. doi: [10.2450/2014.0240-13](https://doi.org/10.2450/2014.0240-13)
30. R.N.Makroo, et,al;(2010). Weak D prevalence among the Indian Blood donors. “Asian Journal of Transfusion Science 2010: 4(2). 137-139



31. Transfusion. 2005 Oct; 45(10):1554-60. [Accessed 18/04/2015]
32. Xhetani.M. M. Seferi I. et, al; (2014). Distribution of Rhesus blood group antigens and Weak D alleles in population of Albania. Blood Transfusion. 2014; 12(4): 565-569.
33. <http://www.ualberta.ca/~pletendr/tm-modules/rh/70rh-weakd.html> ©1999 Division of Medical Laboratory Science University of Alberta opened on 21/03/2015
34. <http://bloodjournal.hematologylibrary.org/content/100/6/2253.full.pdf> Franz F. et al; (2002) DNB: a partial D with anti-D frequent in Central Europe [Accessed 08/02/2015]
35. www.ncbi.nlm.nih.gov/pubmed/16563832. Genome GA et al; (2005). Partial D, weak D types, and novel RHD alleles among 33,864 multiethnic patients: implications for anti-D alloimmunisation and prevention.
36. <http://www.ncbi.nlm.nih.gov/pmc/article/PMC4212038>. Merita Xhetan et al(2014) .Distribution of Rhesus blood group antigens and weak D alleles in the population of Albania Blood Transfus. 2014 Oct; 12(4): 565–569. Republished online 2014 Jun 12. doi: [10.2450/2014.0240-13](https://doi.org/10.2450/2014.0240-13) [[Accessed on 19/02/2015]
37. <http://ajcp.ascpjournals.org/content/134/3/438.full>. Dehua Wang. et al ; (2010). Prevalence of RhD Variants, Confirmed by Molecular Genotyping, in a Multiethnic Prenatal Population. DOI: 10.1309/AJCP SXN9HQ4DELJE (19/04/2015)



Appendices

1.1 Kenya National Blood Transfusion Service Donor Questionnaire (KNBTS)

This questionnaire consists of two parts; kindly answer all the questions by ticking in the appropriate box or filling in the spaces provided.

Clinic Venue

Clinic Code:

Donor Number

Donor Registration Form (Donors please complete this section below)

Surname

Names

National ID Number:

Date of Birth: / / Sex: F/M

Age group:

16-20 []

21-25 []

26-30 []

31-35 []

36-40 []

41-45 []

46-50 []

51-55 []

56-60 []

61-65 []



Contact Details:

Postal Address (where you would like to receive your correspondence Code

Home phone number: Cell phone number:

Email:

Occupation:

When did you last donate Blood?

Blood Group:

Health Questionnaire

Instructions: Circle the appropriate answer

- 1. Are you feeling well and in good health today? Yes/No
- 2. Have you eaten in the last 6 hours? Yes/No
- 3. Have you ever fainted? Yes/No

In the past 6 months have you: Yes/No

- 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: Yes/No

- 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 Yes/No

Please specify



Section C

Risk Assessment Questionnaire

Kindly answer the following questions with utmost honesty. Your answers will be treated in a confidential manner.

Please mark(x) on 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 eg 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 you 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



Client 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 do / do not give consent for my blood sample to be used for a study on Hepatitis B. (Circle where applicable)

Signature:

Date:

For Official Use:

Weight (kg)	Hb >12.5g/dl	BP	Pulse

Donor is Accepted	
Yes	No

Low Volume	> 1 Venepuncture	Haematoma	Faint		
			Mild	Moderate	Severe

Time Needle In	00h00	Time Needle Out	00h00

Report:

Name of Interviewer:

Date:

1.2: Age of the Blood donors

Age	Frequency	Percent	Cumulative Percent
16	9	2.3	2.3
17	37	9.6	12.0
18	54	14.1	26.0
19	21	5.5	31.5
20	25	6.5	38.0
21	17	4.4	42.4
22	21	5.5	47.9
23	10	2.6	50.5
24	28	7.3	57.8
25	14	3.6	61.5
26	13	3.4	64.8
27	13	3.4	68.2
28	18	4.7	72.9
29	10	2.6	75.5
30	11	2.9	78.4
31	5	1.3	79.7
32	11	2.9	82.6
33	7	1.8	84.4
34	10	2.6	87.0
35	7	1.8	88.8
36	3	0.8	89.6
37	5	1.3	90.9
38	3	0.8	91.7



Age	Frequency	Percent	Cumulative Percent
39	5	1.3	93.0
40	4	1.0	94.0
41	4	1.0	95.1
42	2	0.5	95.6
43	2	0.5	96.1
44	4	1.0	97.1
45	1	0.3	97.4
46	1	0.3	97.7
47	1	0.3	97.9
48	3	0.8	98.7
51	1	0.3	99.0
53	1	0.3	99.2
55	1	0.3	99.5
58	1	0.3	99.7
61	1	0.3	100.0
Total	384	100.0	



1.3. Anti-D Manual Insert

EUROCLONE Anti-D (Rho) (IgM+IgG)

MONOCLONAL BLOOD TYPING ANTIBODIES FOR SLIDE AND TUBE TESTS

SUMMARY

Monoclonal antibodies are derived from hybridoma cell lines, created by fusing mouse antibody producing B lymphocytes with mouse myeloma cells or are derived from a human B cell line through EBV transformation. Each hybridoma cell line produces homogenous antibodies of only one immunoglobulin class, which are identical in their chemical structure and immunological activity.

Human red blood cells are classified as Rh positive or Rh negative depending on the presence or absence of D antigen on them. Approximately 85% of the caucasian population is Rh positive. The D⁺ phenotype is a variant of the D antigen and is recognised by performing Antiglobulin test.

About 60% of the D⁻s may react with EUROCLONE Anti-D (IgM+IgG) in slide tests and about 90% may be detected by the tube technique.

REAGENT

EUROCLONE Anti-D (IgM+IgG) is a ready to use reagent, prepared from supernatants of cell cultures with antibody producing B lymphocytes obtained through EBV transformation and is a blend of monoclonal antibodies of immunoglobulin classes IgM and IgG. These antibodies are a mixture of several monoclonal antibodies of the same specificity but having the capability of recognising different epitopes of the human red cell antigen D (Rho).

EUROCLONE Anti-D is a blend of IgM and IgG class of monoclonals, a characteristic which accords versatility to the reagent. It gives an avid saline reacting slide test reagent the capability of detecting D⁺ in the Anti Human Globulin Phase.

Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity, avidity and titre.

REAGENT STORAGE AND STABILITY

1. Store the reagent at 2-8°C. **DO NOT FREEZE.**
2. The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label.

PRINCIPLE

Human red blood cell possessing D antigen will agglutinate in the presence of antibody directed towards the antigen. Agglutination of red blood cells with EUROCLONE Anti-D (IgM+IgG) reagent is a positive test result and indicates the presence of D antigen. No agglutination with the reagent is a negative test result and indicates absence of D antigen. All negative test results should be further tested for D⁻ by performing the D⁻ test procedure, as described later.

NOTE

In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.

EUROCLONE Anti-D (IgM+IgG) reagent is not from human source, hence contamination due to HBsAg and HIV is practically excluded.

The reagent contains sodium azide 0.1% as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.

Extreme turbidity may indicate microbial contamination or denaturation of protein due to thermal damage. Such reagents should be discarded.

SAMPLE COLLECTION AND STORAGE

No special preparation of the patient is required prior to sample collection by approved techniques. Samples should be stored at 2-8°C if not tested immediately. Do not use haemolysed samples.

Anticoagulated blood using various anticoagulants should be tested within the below mentioned time period:

EDTA or Heparin	: 2 days
Sodium citrate / Sodium oxalate	: 14 days
ACD or CPD	: 28 days

ADDITIONAL MATERIAL REQUIRED FOR SLIDE AND TUBE TESTS

Glass slides (50 x 75 mm), Test tubes (10 x 75 mm), Pasteur pipettes, Isotonic saline, Centrifuge, Timer, Mixing sticks, Anti Human Globulin (Coombs) reagent.

EME

TEST PROCEDURE

Bring reagent and to room temperature before testing.

Slide Test

1. Place one drop of EUROCLONE Anti-D (IgM + IgG) reagent on a clean glass slide.
2. Pipette one equal drop of whole blood on the slide.
3. Mix well with a mixing stick uniformly over an area of approximately 2.5 cm².
4. Rock the slide gently, back and forth.
5. Observe for agglutination macroscopically at two minutes.

Tube Test

1. Prepare a 5% suspension of the red cells to be tested in isotonic saline.
2. Place one drop of EUROCLONE Anti-D (IgM + IgG) reagent into labelled test tubes.
3. Pipette into the test tube, one drop of the 5% cell suspension and mix well.
4. Centrifuge for one minute at 1000 rpm (125 g) or 20 seconds at 3400 rpm (1000 g).
5. Gently resuspend the cell button observing for agglutination macroscopically.

D^o Test Procedure

1. Prepare a 5% suspension of the red cells to be tested in isotonic saline.
2. Place one drop of EUROCLONE Anti-D (IgM + IgG) reagent into a labelled test tube.
3. Add to the test tube one drop of the cell suspension, mix well and incubate at 37°C for 15 minutes.
4. Wash the contents of the tube thoroughly, at least three times, with isotonic saline and decant completely after the last wash.
5. Add two drops of Anti Human Globulin reagent and mix well.
6. Centrifuge for 1 minute at 1000 rpm (125 g) or 20 seconds at 3400 rpm (1000 g).
7. Very gently, resuspend the cell button and observe for agglutination macroscopically.

INTERPRETATION OF RESULTS

Slide and Tube Tests

- (a) Agglutination is a positive test result and indicates presence of D antigen. Do not interpret peripheral drying or fibrin strands as agglutination. No agglutination is a negative test result and indicates absence of D antigen.
- (b) Cord Cells, heavily sensitized with Anti-D, may give false negative immediate spin test result.

D^o Test Procedure

- (a) Agglutination indicates the presence of D^o antigen. No agglutination indicates the absence of D^o antigen.
- (b) Mixed field agglutination in D^o test on red cells from a recently delivered woman may indicate a mixture of maternal Rh negative and fetal Rh positive blood.
- (c) Red cells demonstrating a positive direct antiglobulin test cannot be accurately tested for D^o antigen.

REMARKS

As undercentrifugation and overcentrifugation could lead to erroneous results, it is recommended that, each laboratory calibrate its own equipment and the time required of achieving the desired results.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

1. Kohler C. & Milstein C. (1975), Continuous cultures of fused cells secreting antibody of predefined specificity., Nature, 256, 495-497.
2. Lee H.H., Rouger P, Germain C., Muller A & Salmon C. (1983). The production and standardisation of monoclonal antibodies as AB blood group typing reagents. Symposium of International Association of Biological Standardisation on Monoclonal antibodies.
3. Race R. & Sanger R., Blood Groups in Man, 6th Ed., Blackwell Oxford, 1975.
4. Technical Manual, American Association of Blood Banks, ninth Edition, 1985, 127-153.
5. Data on file: Euro Medi Equip Ltd., U.K.

EME-15/0407/AE/VER-1

EUROMEDI EQUIP LTD.

48, WEIBECK ROAD, WEST HARROW,
MIDDX, HA2 ORW, U.K.

EME

1.4. Anti-Antihuman globulin manual insert

EUROCLONE ANTI HUMAN GLOBULIN REAGENT FOR DIRECT AND INDIRECT ANTIGLOBULIN TESTS

SUMMARY

Rh antibodies are of two types, namely the complete and the incomplete, whereas the complete antibodies agglutinate red cells in saline medium, the incomplete type of antibody sensitizes red cells without agglutination. Usually IgM class of antibodies and IgG, and IgG, type of IgG class of antibodies fix complement. Cell lysis, *in vivo*, is mediated through the complement system and the complement component C₃ is further acted upon to produce C₃d. Auto cold antibodies as well as some blood group specific antibodies may belong to any of the three immunoglobulin class, i.e. IgA, IgM and IgG. Thus it is imperative that a truly polyvalent Antihuman globulin reagent is standardized to detect non IgG types of antibodies in addition to IgG, C₃b and C₃d.

In the direct antiglobulin tests, Anti human globulin reagent is used to demonstrate antibodies adsorbed to the red blood cells *in vivo*.

In the indirect antiglobulin tests, Anti human globulin reagent is used to detect antibodies adsorbed to the red blood cells *in vitro*.

Anti human globulin reagent is useful for compatibility testing, antibody detection, antibody identification, umbilical cord red blood testing and detection of the D' variant of the human red blood cell antigen D (Rho).

REAGENT

EUROCLONE Anti human globulin is a ready to use blend of highly purified immunoglobulins raised in sheep through specific immunisation. It contains Anti IgG, Anti IgM, Anti IgA and antibodies to human complement components C₃b. EUROCLONE Anti human globulin also contains IgM class monoclonal Anti-C₃d antibody to impart the necessary sensitivity to the reagent.

The individual titres of EUROCLONE Anti human globulin reagent components are as follows:

Anti IgG = 1:512, Anti C₃b = 1:32, Anti C₃d = 1:16.

Each batch of reagent undergoes rigorous quality control at various stages of manufacture for its specificity, avidity and titre.

REAGENT STORAGE AND STABILITY

a) Store the reagent at 2-8°C. DO NOT FREEZE.

b) The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label.

PRINCIPLE

Normal human red blood cells, in the presence of antibody directed towards the antigen they possess, may fail to agglutinate and become sensitized. This may be due to the particular nature of the antigen and antibody involved. EUROCLONE Anti human globulin reagent would react with red cells sensitized with gamma globulins or components of human complements involved and cause agglutination of the red blood cells.

NOTE

(1) *In vitro* diagnostic reagent for laboratory and professional use only. Not for medicinal use. (2) The reagent contains sodium azide 0.1% as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water. (3) Extreme turbidity may indicate microbial contamination or denaturation of protein due to thermal damage. Such reagent should be discarded. (4) EUROCLONE reagents are not from human sources, hence contamination due to HBsAg and HIV is practically excluded.

SAMPLE COLLECTION AND STORAGE

No special preparation of the patient is required prior to sample collection by approved techniques. Do not use haemolysed samples.

For Direct Antiglobulin Test: Blood drawn into EDTA is preferred but oxalated, citrated or clotted whole blood may be used. The blood sample should be tested as soon as possible after collection and should not be stored.

For Indirect Antiglobulin Test: Serum, not more than 48 hours old, should be used. Donor units may be tested upto the end of their dating.

PREPARATION OF COOMBS CONTROL CELLS

(1) Dilute Anti-D reagent of IgG type 1:50 in isotonic saline. (2) Prepare a 5% suspension of group 'O' positive cells in isotonic saline. (3) Mix equal volumes of diluted Anti-D reagent of IgG class (as in 1 above) and 5% suspension of 'O' Rh positive cells (as in 2 above) and incubate at 37°C for 15 minutes. (4) Decant and wash thoroughly with tubes full of isotonic saline at least thrice. (5) Resuspend in isotonic saline to make a 5% suspension of coombs control cells.

ADDITIONAL MATERIAL REQUIRED

For Direct Antiglobulin Test: Test tubes (10 x 75 mm), Pasteur pipettes, Centrifuge, Isotonic saline, Coombs control cells, Optical aid.

For Indirect Antiglobulin Test and Compatibility Test: Test tubes (10 x 75 mm), Pasteur pipettes, EUROCLONE Bovine serum albumin, Centrifuge, Incubator (37°C), Isotonic saline, Coombs control cells, Optical aid.

PROCEDURE

Bring reagent to room temperature before testing.

Direct Antiglobulin Test

(1) Prepare a 5% suspension of the red cells to be tested in isotonic saline. (2) Pipette one drop of the cell suspension into a test tube. (3) Fill the tube with fresh isotonic saline and centrifuge for 30 seconds at 3400 rpm (1000g). (4) Decant and repeat this washing atleast thrice. (5) Add two drops of EUROCLONE Anti human globulin reagent and mix well. (6) Centrifuge for one minute at 1000 rpm (125 g) or for 20 seconds at 3400 rpm (1000 g). (7) Very gently, resuspend the cell button observing for agglutination macroscopically. (8) To all negative antiglobulin tests add one drop of coombs control cells and observe for agglutination.

EME

Indirect Antiglobulin Test

MAJOR CROSS MATCH PROCEDURE

Initial Phase

(1) Label two test tubes as A (for albumin) and B (for saline), depending upon the number of donors to be cross matched, as many pairs of such labelled tubes would be required. (2) Prepare a 5% suspension of the red cells to be tested in isotonic saline. (3) Pipette two drops of recipient serum in both the labelled test tubes. (4) Pipette one drop of donor red cells in both the labelled test tubes and mix well. (5) Only to the albumin tube (A) add two drops of EUROCLONE Bovine serum albumin reagent and mix well. (6) Centrifuge both the tubes for one minute at 1000 rpm (125g) or for 20 seconds at 3400 rpm (1000 g). (7) First observe for haemolysis. Resuspend the cell button and observe for agglutination macroscopically. (8) Proceed to incubation phase.

Incubation Phase

(1) Incubate the saline tube at room temperature and the albumin tube at 37°C for fifteen minutes. (2) First observe for haemolysis. Resuspend the cell button and observe for agglutination macroscopically. (3) Proceed to the antiglobulin phase.

Antiglobulin Phase

(1) Only the albumin tubes (A) are tested in the antiglobulin phase. (2) Wash the mixture of red blood cells and serum thoroughly with isotonic saline for minimum of three times. Decant completely after the last wash. (3) Place two drops of EUROCLONE Anti human globulin reagent into the test tubes containing the sedimented cells and mix well. (4) Centrifuge for one minute at 1000 rpm (125 g) or for 20 seconds at 3400 rpm (1000 g). (5) Very gently, resuspend the cell button and observe for agglutination macroscopically.

INTERPRETATION OF RESULTS

Direct Antiglobulin Test

Agglutination of the red blood cells is a positive test result and indicates the presence of human IgG or components of complement on the red blood cells.

No agglutination is a negative test result and indicates the absence of human IgG or components of complement on the red blood cells.

Indirect Antiglobulin Test

In all phases of the compatibility test, if no agglutination or haemolysis is observed then the patient and the donor may be considered compatible. If haemolysis or agglutination at any point till the completion of the antiglobulin phase is observed, the patient and the donor are considered incompatible.

REMARKS

(1) If plasma is used in the indirect antiglobulin test, the complement dependent antibodies may not be detected due to the absence of calcium. (2) To all negative test results, after the antiglobulin test phase, one drop of Coombs control cells should be added. If Coombs control cells do not agglutinate then the compatibility test must be repeated. (3) In the indirect antiglobulin test procedure an auto control tube (individual's cells in his own serum) should be run. (4) Red blood cells showing a positive direct antiglobulin test cannot be used for the indirect antiglobulin test. (5) It is recommended that Anti-IgG activity of the Anti human globulin reagent be tested from time to time using Coombs control cells as a positive control. (6) Contaminated Bovine serum albumin, saline or glassware may inactivate Anti human globulin reagent. (7) Use of various drugs and certain diseases (such as megaloblastic anaemia) are known to be associated with a positive direct antiglobulin test. (8) Cord cells obtained from a newborn exhibiting haemolytic disease of the newborn, especially due to ABO incompatibility may give false negative results. (9) EUROCLONE Anti human globulin reagent does not contain Anti-C, and is free from Anti-T. (10) As undercentrifugation or overcentrifugation could lead to erroneous results, it is recommended that each laboratory calibrate its own equipments and the time required for achieving the desired results.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

(1) Kohler C, & Milstein C. (1975), Continuous cultures of fused cells secreting antibody of predefined specificity, Nature, 256, 495-497. (2) Lee H. H., Rouger P., Germain C., Muller A & Salmon C. (1983), The production and standardisation of monoclonal antibodies as AB blood group typing reagents, Symposium of International Association of Biological Standardisation of monoclonal antibodies. (3) Race R. & Sanger R., Blood Groups in Man, 6th Ed., Blackwell, Oxford 1975. (4) Technical Manual American Association of Blood Bank, 9th Ed., 1985, 127-153. (5) Data on file: Euromedi Equip Ltd., U.K.

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