"STUDY OF DISTRIBUTION OF PRINCIPAL Rh BOOD GROUP ANTIGENS AND THEIR PHENOTYPE IN BLOOD DONORS"

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LIST OF ABBREVIATIONS

Abbreviation	Full Form
Rh	Rhesus
ISBT	The International Society of Blood Transfusion
RBC	Red blood cells
AABB	the American Association of Blood Banks
cDNA	Complementary DNA
CAT	Column Agglutination Technology
ELISA	enzyme-linked immunosorbent assay
HDFN	Hemolytic disease of the fetus and newborn
PCR	Polymerase Chain Reaction
TACO	Transfusion-associated circulatory overload
TRALI	Transfusion-related acute lung injury
TAD	Transfusion-associated dyspnea
ICT	Indirect Coombs Test
EDTA	Ethylenediamine tetra acetic acid
IgM	Immunoglobulin M
AHG	anti-human globulin

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ABSTRACT

INTRODUCTION:

Blood transfusions are vital in healthcare, relying on the ABO and Rh blood group systems. Rh antigens are complex, with D being the most important. Rh negative individuals receiving Rh positive blood may develop antibodies causing adverse reactions. Blood group information is crucial for inventory management, legal matters, and genetics research. ABO and RhD are pivotal for pre-transfusion testing to prevent severe reactions, especially in patients receiving multiple transfusions.

OBJECTIVES:

To study the distribution of Rh antigens D, C, c, E, e among the donors attending blood centre of a tertiary health centre and to determine the prevalent phenotype based on the frequency of Rh antigens.

MATERIALS AND METHODS:

A prospective cross-sectional study was conducted at the blood center of our institute consisting of 382 donors. Samples were collected for traditional blood grouping and Rh typing. Conventional tube method was utilized for Rh D typing and to detect major Rh antigens by using specific monoclonal antisera.

RESULTS:

Among Rh antigens, e was the most common antigen, followed by D, C, c. DCCee was the most common phenotype, and the least common phenotype were DcCee, dCcee, dccEe, dcceE. In Rh

positive donors, most common phenotype was found to be DCCee and in Rh negative donors most common phenotype was found to be dccee.

CONCLUSION:

In Blood transfusion, accurate population-based Rh antigen frequency data are crucial for clinical purposes. Before transfusion, antigenic phenotyping and antibody screening and identification are advised for multi-transfused patients and multipara women to avoid blood transfusion reactions.

KEY WORDS: Alloimmunization, Transfusion, Blood group, Phenotype, Rh system

"STUDY OF DISTRIBUTION OF PRINCIPAL Rh BOOD GROUP ANTIGENS AND THEIR PHENOTYPE IN BLOOD DONORS"

1. INTRODUCTION

The goal of the blood transfusion service is to guarantee blood safety in order to prevent the spread of infections linked to transfusion and the emergence of transfusion responses. Blood transfusion services are vital in healthcare, relying on the ABO and Rh blood group systems.[1]

The identification of the ABO blood type in 1901 and the Rh blood groups in 1939–1940 marked the beginning of the concept of "safe blood." Transfusions and transplants mostly still rely on the ABO blood group system. The Rh blood group system is the second most significant blood group system after the ABO system.[2]

The 43 blood group systems recognized by the ISBT comprise 340 antigens, the most therapeutically significant of which are ABO, Rh, Kell, Duffy, Kidd, MNS, and Diego. Regarding delayed hemolytic transfusion responses, the most frequently implicated antibodies targeted antigens are D, K, E, C, c, Fya, Dia, S, and Jk [3].

The Rh blood group system is clinically significant in blood transfusion services because Rh-negative individuals who receive Rh-positive blood transfusions may generate anti-D IgG antibodies that may subsequently result in hemolytic illness of the fetus and infant as well as hemolytic transfusion reaction.[4]

Rh system is one of the most complicated blood group systems because Rh (D) antigens are more immunogenic than all other red cell antigens. Complete D, D Mosaic, Partial D, and weak D kinds are all included in the D antigen. Because certain people who express a mutant Rh D allele may develop anti-D alloimmunization, the other D antigens are also essential.[5]

Three sets of allele genes, namely D/d, C/c, and E/e, combine to create the antigen of the Rh system, which is responsible for creating the antigens D, C, c, E, and e on the surface of RBCs. Since there is no "d" antigen on RBC, the "d" gene is considered ambiguous. Although there are more than 50 antigens in the Rh system, most clinically essential antibodies are directed against D, C, c, E, and e, which is why these five key antigens are the most frequently recognised and significant in blood transfusion systems.[4]

Till date, four nomenclatures have been proposed thus far to describe the Rh system's heredity, antigenicity, and antibodies, and they are

Fisher-Race (CDE) Nomenclature (1946)

The Rh system's phenotype is determined by the presence or lack of the D, C, c, E, and e antigens. There are eight potential gene set combinations, according to Fisher & Race: DCe, Dce, DcE, DCE, dCe, dce, dcE, & dCE. The five main Rh system antigens are represented by this nomenclature.[6]

Wiener (Rh-Hr) Nomenclature (1939)

Weiner uses complicated and less widely used words. Rh0, Rh1, Rh2, Rhz, rh, rh1, rh2, and rhy are the symbols assigned to genes in this hypothesis, while Rh0, rh', rh," hr', and hr are the labels assigned to antigens.[7]

Rosenfield Numeric Nomenclature (1960)

Rosenfield et al.[8] proposed the alphanumerical terminology for Rh system antigen, and this system simply demonstrates the presence or absence of antigen on red cells. For five significant antigens, symbols are assigned as D→Rh1, C →Rh2, E →Rh3, c→Rh4, and e→Rh5.[8]

International Society of Blood Transfusion (ISBT) (2004)

The ISBT committee assigned a numerical terminology. Specific antigens have been assigned six digit numbers; the blood group system is represented by the first three digits, and the antigen specificity is represented by the final three.[9]

For the blood bank to effectively maintain its inventory, blood group information on donors and patients is crucial. Blood group antigens can be reasonably used to resolve medico-legal concerns like challenged parentage and their usefulness in blood transfusion practices and pregnancy management. Blood type information is also helpful in directing population genetic research for uncommon variants that are difficult to detect by mass-scale molecular techniques.[10]

Regarding compatibility testing to reduce the occurrence of any severe transfusion reactions, blood transfusion services seek to assure the availability of sufficient and safe blood. The most crucial blood group systems for pre-transfusion testing are ABO and RhD. Alloimmunisation may occur following the transfusion of ABO-compatible blood with unidentified phenotypes for clinically important antigens, particularly in patients who have received many transfusions. Therefore, we could assess the frequencies of these phenotypes and create a donor data library of red blood cell (RBC) antigens using information on red-cell antigen phenotypic frequencies in frequent voluntary blood donors. Also, it would be advantageous to immediately give compatible antigen-negative blood to alloimmunized patients and stop alloimmunization in patients who have received many transfusions.[11]

When providing blood transfusion services to alloimmunized patients, the ability to identify clinically significant antibodies in the patient's serum and match them to antigen-negative blood from the donor's database is made possible by knowledge of data on frequencies of antigens of the Rh blood group system in the local donor population.[2]

Hence the study was conducted to find the distribution Rh antigens in donors attending our hospital blood centre and also to identify the most prevalent phenotype based on the frequency of Rh antigens.

2. AIM AND OBJECTIVES

- To study the distribution of Rh antigens D, C, c, E, e among the donors attending our blood centre.
- To know the most prevalent phenotype based on the frequency of Rh antigen

3. REVIEW OF LITERATURE

3.1 History of blood transfusion and blood grouping

There are two distinct periods in the history of blood transfusions. First, the history before the ABO blood type system was discovered, and then the history following its discovery.[12-13]

Transfusion history prior to the ABO blood group systems

1628: William Harvey discovered blood circulation.[14]

1665: Richard, an English doctor, successfully transfused blood in a dog.[15]

1667: A successful transfusion from lamb to human was independently reported by Richard Lower in England and Jeon Baplesti Denis in France. Animal-to-human transfusion was outlawed within ten years due to adverse effects.[16]

1818: To treat postpartum hemorrhage, British doctor James Blundell administered the first human-to-human blood transfusion to a patient. He conducted ten transfusions between 1825 and 1830 and found several tools for safe transfusions. Additionally, he offered several logical proofs for the same.[17]

1840: At London's St. George's School, Samuel Armstrong Lane successfully treated hemophilia with a whole blood transfusion under the guidance of Dr. Blundell.[18]

History of blood transfusion after the discovery of the ABO blood group system.

1900: The discovery of the first three human blood groups A, B, and C by physician Karl Landsteiner led to a revolution in blood transfusion. Later, the blood type "C" was altered to "O." He was awarded the 1930 Nobel Prize in Medicine for the ABO blood type system.[19]

1902: Alfred Decastello and Adnano Sturli, the colleagues of Karl Landsteiner, added the AB blood group to the ABO blood group system.[20]

1907: Hekton proposed that cross-matching blood between donors and recipients could increase transfusion safety by exclude incompatible combinations.[21]

1908: The French scientist Xuyeon Alexis Carrel won the Nobel Prize in 1912 for his invention of direct vein-to-vein transfusion, which uses gravity to transfer blood from donor to recipient and prevent clotting during storage. This technique is essential for a successful organ donation.[22]

1908: Moreschi described the anti-globulin reaction.[13]

1912: Roger Lee showed that patients of any blood type could safely receive group O blood, and patients in group AB could receive blood from any group. The phrases “universal donor” and “universal recipient” were created.[23]

1914: To enable extended blood preservation, sodium citrate, a long-term anticoagulant, was created.[24]

1916: Citrate glucose solution developed by Francis Rous and J.R. Turner allows blood to be stored for several days following collection.[25]

1927: Landsteiner and Levine discovered the MNS and P blood group system.[26]

1932: The 1st blood bank was set up in a hospital in Leningrad, Russia.[27]

1939: The Rh blood group system was discovered by RE Stetson, Philip Levine, Karl Landsteiner, and Alex Wiener. Soon after its discovery, it and the ABO blood type system became important in the field of transfusion medicine.[28]

1940: Cold ethanol fractionation, which separates plasma into constituents and products, including albumin, gamma-globulin, and fibrinogen, was created by Edwin Cohn.[29]

1940: The first blood container was created by John Elliott; the Red Cross uses this vacuum bottle for plasma fractions.[30]

1940: P Beeson described transfusion-transmitted hepatitis.[31]

1950: Audrey Smith described freezing red blood cells with glycerol myo-protection.[32]

1950: WP Murphy Junior and Carl Walter. Introduced the blood-collecting plastic bag.[33]

1953: The invention of the chilled centrifuge accelerated the treatment of blood components.[34]

1958: The first edition of the standard for blood transfusion services was released by the American Association of Blood Banking (AABB).[35]

1959: The molecular structure of haemoglobin, a molecule that carries oxygen and gives red blood cells their colour, was described by Max Perutz of Cambridge University.[36]

In addition to the recognised blood groups **(Table 1)**, roughly 20 public antigens and 60 specific or familial antigens (Private Antigens) have been reported.[37]

Table 1: Various blood groups, year of report, discoverer/s [37]

Blood Group	Year	Reporter(s)
ABO – System	1901	Landsteiner K
M/N – System	1927	Landsteiner K, Levine P
P – System	1927	Landsteiner K, Levine P
Secretor / Non – (ss)	1932	Schiff F, Sasaki H
Factor Q	1935	Imamuras S
Rhesus (Rh)	1940/41	Landsteiner K, Wiener A
Lutheran (Lu)	1945	Callenders S, Race RR, Paykoc Z
Lewis (Le)	1946	Mourant AE
Kell (K)	1946	Coombs RRA, Mourant AE, Race RR
Factor S/s	1947	Walsh RJ, Montgomery C
Duffy (FY)	1950	Cutbush M, Mollison PL
Kidd (Jk)	1951	Race RR et al.
Diago (Di)	1955	Levine P et al.
Yt System	1956	Eaton BR et al.
Auberger (AU)	1961	Salmon C et al.
Xg	1962	Mann JD et al.
Dombrock (Do)	1965	Swanson J et al.

3.2 Discovery of the ABO blood group system (1901)

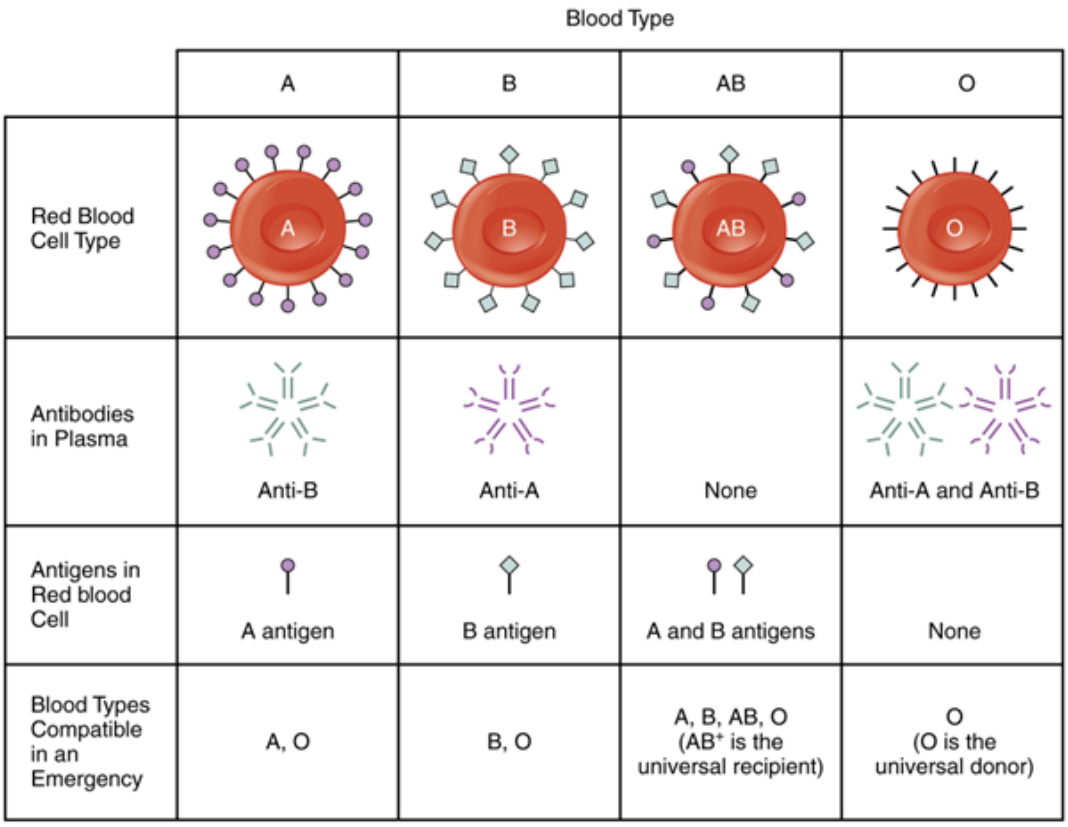
An important turning point in medical science was reached with the discovery of the ABO blood type system, which established the groundwork for contemporary hematology and transformed in the area of transfusion medicine. Karl Landsteiner conducted an extensive study in the early 20th century that led to this discovery. When Landsteiner identified the primary human blood groups A, B, O, and AB in 1900, his most well-known and significant work got underway.[38]

Blood transfusions were unsafe before blood types were discovered. Although the causes were unknown, doctors were aware that transfusions occasionally resulted in deadly reactions. It was widely held that all human blood was basically the same. Therefore, transfusion failures were frequently ascribed to patient weakness or technical mistakes rather than being a fundamental biological incompatibility. In a number of tests, Landsteiner combined blood samples from various subjects and tracked the outcomes. He postulated that the reactions resulted from variations in each person's blood. Landsteiner's main finding was that blood from several people occasionally clumped together when combined; this is referred to as agglutination.[37]

He observed that this agglutination followed a predictable pattern depending on any specific blood samples utilized. Landsteiner suggested that different types of human blood may be distinguished based on his tests. The agglutination is caused by contact between blood and blood serum containing the antibodies, as he found in 1901. From these data, he derived three different blood groups—A, B, and O. The RBCs of the A blood group contain the A antigen, while the plasma contains anti-B antibodies. RBCs with the B antigen and plasma with anti-A antibodies are characteristics of the B blood group. On the other hand, the O blood group has both anti-A and anti-B antibodies in the plasma and no A or B antigens on RBCs. Identifying these groups made it

clear why specific transfusions were successful, and others were not: Agglutinations could be caused by incompatible blood types.[39]

Table 2: ABO blood group system, showing the relationships between blood type, antigens, antibodies, and compatibility for transfusions.[39]



3.3 Inheritance of ABO blood group[40]

Table 3: ABO blood groups inheritance pattern[40]

Parental Phenotype		B	
	Parental Genotype	*B*	*O*
A	*A*	**AB**(*AB*)	**A**(*AO*)
	O	**B**(*BO*)	**O**(*OO*)

Parental Phenotype - *Parental Genotype* - Offspring **Phenotype** (*Genotype*)

Parental Phenotype: The blood type (A or B) of the parents.

Parental Genotype: The genetic makeup of the parents, which determines blood type.

- A parent with blood type **A** could have genotype **AA** or **AO**.
- A parent with blood type **B** could have genotype **BB** or **BO**.

Offspring Phenotype and Genotype:

- If one parent has genotype **A** and the other has **B**, the child could have **AB (AB)** blood type.
- If one parent has genotype **A** and the other has **O**, the child could have **A (AO)** blood type.
- If one parent has genotype **O** and the other has **B**, the child could have **B (BO)** blood type.
- If both parents have genotype **O**, the child will have **O (OO)** blood type.

3.4 Rh Blood Group System Nomenclature and Inheritance

The following four nomenclatures have been proposed to describe the Rh system's heredity, antigenicity, and antibodies.

1. Fisher & Race (1946)
2. Weiner (1939)
3. Rosenfield (1960)
4. International Society of Blood Transfusion (ISBT) (2004)

Fisher & Race (1946)[6]

Fisher proposed that the Rh system is controlled by three genes, each located at a separate locus on chromosome 1. These genes are **C - (c), D - (d), E - (e)**.

Each gene has two alleles, and they are inherited as a unit.

The Rh antigens are D, d, C, c, E, and e, according to Fisher & Race. A little d gene, which is silent gene, or a D gene absence could be the cause if no D antigen has been discovered. Therefore, the presence or lack of D, C, c, E, and e antigens determines the phenotype of the Rh system.

Rh genes, specifically D or d, C or c, and E or e are inherited from each parent, according to Fisher and Race. Each inherited gene matches an antigen found on RBCs because the Rh genes are co-dominant. The combination of a person's maternal and paternal haplotypes determines their genotype, and serological procedures can identify the antigen on their red blood cells to determine their associated phenotype.

There are eight potential gene set combinations, according to Fisher & Race: DCe, Dce, DcE, DCE, dCe, dce, dcE, and dCE. Although the Fisher & Race nomenclature is the simplest method for identifying the five main Rh system antigens, it has the disadvantage of leaving out many more recent Rh antigens.

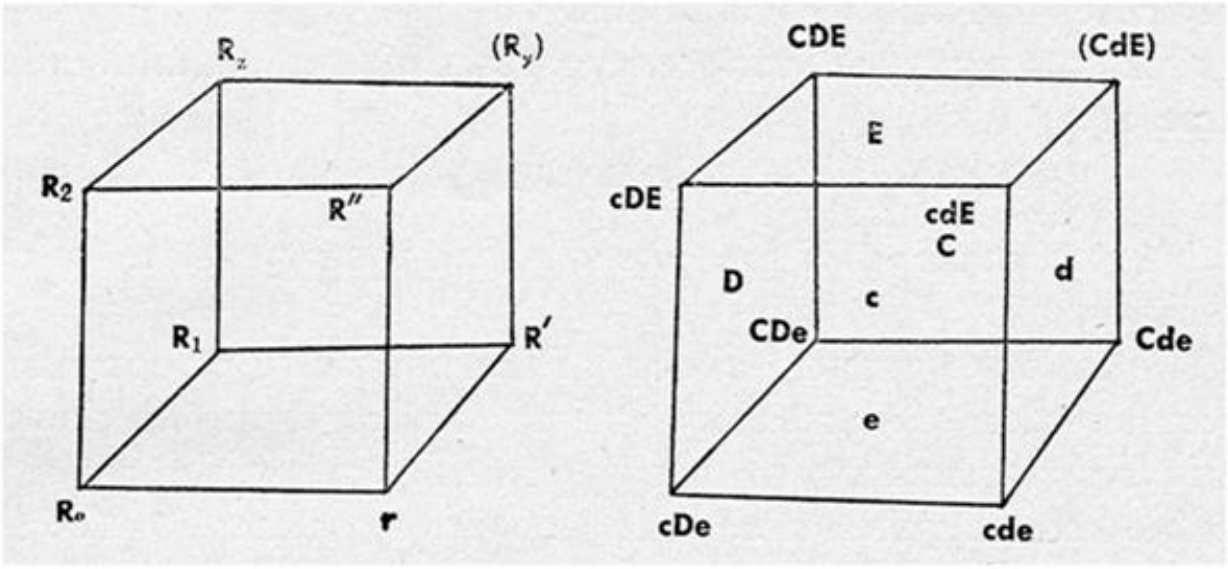


Figure 1. The two notations for the Rhesus antigens from Fisher

According to Fisher, "the eight heritable antigen complexes can be geometrically represented as the cube's corners, while the faces stand in for the six elementary antigens; each allomorphic pair of antigens is then a pair of opposite faces, and the three faces meeting in any point specify the antigens in each complex." (Figure 1)

Weiner (1939) [7]

The Rh-hr terminology was proposed by Weiner in order to explain how the Rh system is inherited. He states that there is a single locus with eight allelic genes on the short arm of chromosome 1.

In Weiner's theory, genes are designated as symbols. Rh^0 , Rh^1, Rh^2, Rh^z, Rh, rh', rh", rh^y

And antigens are labelled as: Rh^0- D, rh'- C, rh", -E, hr' -c, hr"-e

Weiner nomenclature can be used instead of Fisher & Race nomenclature, and vice versa. Although Weiner's nomenclature is less standard and more complex, certain blood banks use it, and it has historical significance.

Table 4: Rh-hr Terminology of Weiner[7]

Gene	Agglutinogen	Blood Factor	Shorthand Designating	Fisher & Race Antigen
Rh^0	Rh_0	Rhohr'hr"	R_0	Dce
Rh^1	Rh_1	Rhohr'hr"	R_1	DCe
Rh^2	Rh_2	Rhohr'hr"	R_2	DcE
Rh^z	Rh_Z	Rhohr'hr"	R_Z	DCE
rh	rh	hr'hr"	r	dce
rh'	rh'	hr'hr"	r'	dCe
rh"	rh"	hr'hr"	r"	dcE
rh^y	rh^y	hr'hr"	r^y	dCE

This table represents **Weiner's Rh-hr terminology**, which differs from **Fisher & Race's classification** by considering a **single gene with multiple alleles** rather than three separate genes.

Rosenfield (1960)[8]

The discovery of further antigens in the Rh system has made it more challenging to characterize the names and symbols of the newly found antigen using the existing (Fisher & Race & Weiner) terminology. Thus, the alpha number language for the Rh system antigen was developed by Rosenfield et al. in the 1960s.This method is not genetically based and simply detects the presence or lack of an antigen on a red cell. The symbols attributed to the five main antigens are as follows:

D -Rh1, C -Rh2, E -Rh3, c-Rh4 and e -Rh5

Antigen absence is indicated by a minus sign before the number, whereas presence is indicated by a plus (+) or no sign. Rh system antigens are numbered.

For example, if the four principal antigens on a red cell (D, C, E, and c) are present but e is lacking, the labelling Rh: 1,2,3,4, and -5 should be used.

International Society of Blood Transfusion (ISBT) (2004)[9]

The ISBT established a working committee to standardize red cell surface antigens terminology, resulting in a generally accepted blood group system. The committee used a six-digit number for specific antigens that are easily readable by humans and machines, in line with the genetic basis of blood groups. The first three numbers reflect the blood group system, while the latter three represent antigen specificity.

Table 5: The ISBT numerical terminology

No.	System Name	System Symbol	Gene Name(s)	Chromosomal Location	CD Numbers
001	ABO	ABO	ABO	9q34.2	CD235
002	MNS	MNS	GYPA, GYPB, GYPE	4q31.21	CD235
003	P	P1		22q11.2–qter	
004	Rh	RH	RHD, RHCE	1p36.11	CD240
005	Lutheran	LU	LU	19q13.32	CD239
006	Kell	KEL	KEL	7q34	CD238
007	Lewis	LE	FUT3	19p13.3	
008	Duffy	FY	FY	1q23.2	CD234
009	Kidd	JK	SLC14A1	18q12.3	CD233
010	Diego	DI	SLC4A1	17q21.31	
011	Yt	YT	ACHE	7q22.1	
012	Xg	XG	XG, MIC2	Xp22.33, Yp11.3	CD99
013	Scianna	SC	ERMAP	1p34.2	
014	Dombrock	DO	DO	12p12.3	
015	Colton	CO	AQP1	7p14.3	CD242
016	Landsteiner-Wiener	LW	ICAM4	19p13.2	
017	Chido/Rodgers	CH/RG	C4A, C4B	6p21.3	
018	H	H	FUT1	19q13.33	CD173
019	Kx	XK		Xp21.1	
020	Gerbich	GE	GYPC	2q14.3	CD236
021	Cromer	CROM	DAF	1q32.2	CD55
022	Knops	KN	CR1	1q32.2	CD35
023	Indian	IN	CD44	11p13	CD44
024	Ok	OK	BSG	19p13.3	CD147
025	Raph	RAP	CD151	11p15.5	CD151
026	John Milton Hagen	JMH	SEMA7A	15q24.1	CD108
027	I	I	GCNT2	6p24.2	
028	Globoside	GLOB	B3GALT3	3q26.1	
029	Gill	GIL	AQP3	9p13.3	

This table provides detailed information regarding various blood group systems, including their symbols, gene names, chromosomal locations and associated CD numbers

For example, the ABO blood group system is written as 001.

Rh blood group system is written as 004.

Rh D antigen should be written as 004001.

The previous blood group system's alphabetical names (e.g. Rh, Kell) were retained but converted to all capital letters (e.g. RH, KELL). D is now written as RH1 and C as RH2, with no space between the alphabet and the assigned number. Symbols for genes, alleles, and haplotypes are written in italic print.

3.5 Evolution of the Rh antigen

Rh blood group system is the second most important blood group system in clinical practice, after ABO. Karl Landsteiner and Wiener first discovered the Rh blood group system in 1940. In the experiment, Landsteiner and Wiener injected rhesus monkey RBCs into rabbits. The rabbits' immune system developed antibodies against the foreign cells. Surprisingly, testing these antibodies against human RBCs resulted in agglutination in around 85% of samples. This revealed the presence of a previously unknown antigen on the surface of human RBCs. The Rh factor, named after the rhesus monkey, is a novel antigen that was initially found. Individuals are classed as Rh-positive or Rh-negative based on the presence or absence of this antigen.[41]

In 1939, Levine and Stenson found an antibody in the serum of a mother who had delivered a stillborn baby. After receiving her husband's ABO-compatible blood, she experienced a hemolytic transfusion reaction. Levine et al. (1939) found that these antibodies had a comparable reaction pattern to Landsteiner & Weiner's anti-Rh antibody and were considered similar.[42]

The antigen was identified as RhD, and human alloantibodies as anti-RhD. Antithetical antigen C/c and antithetical antigen E/e were two of the four new Rh antigen types discovered in 1945. In

recognition of Landsteiner and Wiener, rhesus monkey heteroantibodies were designated as anti-LW antibodies and the antigen as LW antigens.[41]

3.6 Molecular basis of Rh antigens

The molecular bases of the Rh antigens have been studied substantially since the first characterization of Rh cDNAs. These include gene deletion (D-negative phenotype), conversion (C/c polymorphism), antithetical missense mutations (E/e), and other missense mutations (VS and V).[43]

The D antigen

In 1953, Wiener postulated that the Rh D antigen consists of four parts: RhA, RhB, RhC, and RhD. Individuals with partial D deficiency may develop anti-D antibodies that target the missing epitopes.[7] Tippett established seven D groups in the 1960s by examining Rh D-positive individuals" cells and serum for anti-D levels. Superscript Roman numbers I-VII were used to indicate the D categories, such as D III and D VI.[44]

Some categories were also subdivided. Monoclonal anti-D antibodies have helped identify additional incomplete D types. There is no systematic approach for naming partial D types. In 1993, a nine-epitope model was established for Rh D, followed by a 30-epitope model in 1995. Partial D types are often identified by their D category (or name if not in a category) and the epitopes they express or lack in the nine- or 30-epitope models.[43]

Table 6: Rh Phenotypes Based on Reactions of Antisera with Erythrocytes [7]

Anti-D	Anti-C	Anti-E	Anti-c	Anti-e	Wiener Phenotype	Phenotype
+	+	-	-	+	Rh_1r_1	DCeCe
+	+	-	-	+	Rh_1	DCeCe
+	+	+	-	+	Rh_1Rh_2	DCECe
+	-	-	-	+	Rh_0	DceCe
+	-	+	-	+	Rh_2r_h	DcECe
+	-	+	-	-	Rh_2	DcECE
+	-	+	+	+	Rh_2Rh_1	DcEce
+	-	+	+	-	Rh_2Rh_2	DcEcE
+	-	+	-	-	Rhz	DcECE
-	-	-	+	+	rh	dcece
-	+	-	+	+	rh'rh	dCece
-	-	+	+	+	rh"rh	dcEce
-	+	+	+	+	rh'rh"	dCEce

This table provides a structured view of **Rh phenotypes** based on **antisera reactions with erythrocytes**, using **Wiener nomenclature**.

3.7 Various methods of blood grouping

ABO and Rh group determination by both Forward (Direct) and Reverse (Indirect) grouping is essential for pre-transfusion investigations, including both donors and patients. A cross-check for forward typing is reverse grouping. The outcomes of both approaches should be consistent, and the tests should be conducted side by side.[45]

ABO and Rh blood groups can be determined in the lab using a variety of methods.

Slide Agglutination Method[45]

This is a rapid and simple method where a drop of blood is mixed with antisera on a glass slide to observe agglutination.

Procedure

1. Take three clean glass slides labelled **Anti-A**, **Anti-B**, and **Anti-D** (for Rh factor).
2. Place a drop of the antiserum (or antibody solution) on one side and a drop of normal saline on the other (for control).
3. Add a drop of antigen on both sides.
4. Gently mix each drop using separate sterile sticks or loops.
5. Rock the slide gently for 1–2 minutes and observe for agglutination (visible clumping) under adequate lighting.

Interpret results:

Check for agglutination:

1. **Clumping in Anti-A** = A group

2. **Clumping in Anti-B** = B group
3. **Clumping in both** = AB group
4. **No clumping** = O group
5. **Clumping in Anti-D** = Rh positive
6. **No clumping in Anti-D** = Rh negative

Result: Determine the ABO and Rh blood type based on agglutination patterns.

Advantages:

- Quick results (within 2 minutes).
- Requires minimal equipment.

Disadvantages:

- It is less accurate than tube and gel methods.
- Risk of false positives due to drying effects.

Tube Method (Gold Standard for Blood Grouping) [45]

Blood is mixed with antisera in a test tube and centrifuged to enhance agglutination.

Procedure:

1. Prepare 3 clean test tubes labeled **Anti-A**, **Anti-B**, and **Anti-D** (for Rh factor).
2. Add a drop of each corresponding antiserum into the labeled tubes.
3. Add a drop of the patient's blood (usually diluted in saline) to each tube.
4. Gently mix and tilt the tubes.
5. Spin for about 1 minute at 1000 rpm (to enhance reaction).

Check for agglutination:

a. **Clumping in Anti-A** = A group
b. **Clumping in Anti-B** = B group
c. **Clumping in both** = AB group
d. **No clumping** = O group
e. **Clumping in Anti-D** = Rh positive
f. **No clumping in Anti-D** = Rh negative

Result: Determine the ABO and Rh blood type based on agglutination patterns.

Advantages:

- It is more accurate than the slide method.
- Detects weak antigen-antibody reactions.

Disadvantages:

- Requires a centrifuge.
- More time-consuming.

Gel Card Method (Column Agglutination Technique - CAT) [46]

This method uses gel columns preloaded with these three (anti-A, anti-B, and anti-D) antibodies. Agglutinated cells are trapped in the gel, making the reaction easy to read.

Procedure:

1. Collect blood samples and prepare a red cell suspension.
2. Use the gel cards already denoted ABO, Rh, grouping — each micro-tube contains a gel matrix with specific antibodies.

3. Add a drop of the red cell suspension to each micro-tube.
4. Incubate the card (usually at 37-degree centigrade for 15–30 minutes).
6. The gel card is centrifuged at 1000 rpm for 10 minutes to separate agglutinated and free red cells.

Interpretation

Positive Reaction: Agglutinated red cells are trapped in the upper part of the gel.

Negative Reaction: Non-agglutinated red cells pass through and form a pellet at the bottom.

Weak Reaction: Some red cells remain in the gel but are not fully trapped at the top.

7. Check for agglutination:
    a. **Clumping in Anti-A** = A group
    b. **Clumping in Anti-B** = B group
    c. **Clumping in both** = AB group
    d. **No clumping** = O group
    e. **Clumping in Anti-D** = Rh positive
    f. **No clumping in Anti-D** = Rh negative

Result: Determine the ABO and Rh blood type based on agglutination patterns.

Advantages:

- Highly sensitive and automated.
- Reduces the risk of contamination and human error.

Disadvantages:

- Requires special gel cards and a centrifuge.
- It is more expensive than the slide and tube methods.

Microplate Method (ELISA-based Blood Typing) [47]

Blood typing is performed in a microplate where antibodies are immobilized, and an enzyme-linked system is used for detection.

Procedure

1. Add anti-A, anti-B, and anti-D sera into separate wells.
2. Place a small drop of diluted blood into each corresponding well.
3. Gently mix the blood and reagents.
4. Allow the plate to sit at room temperature for a few minutes.
5. Check for clumping (agglutination) in each well.

Interpretation:

1. **Positive reaction:** agglutination present.
2. **Negative reaction:** agglutination absent.

Advantages:

- Suitable for large-scale testing (e.g., blood banks).
- It can be automated for high-throughput screening.

Disadvantages:

- Requires specialized equipment.
- More complex than manual methods.

Flow Cytometry for Blood Grouping[48]

- Uses **fluorescent-labeled antibodies** that bind to red blood cell antigens.
- Cells pass through a laser, and fluorescence intensity is measured to determine antigen presence.

Advantages:

- Highly sensitive and specific.
- Can detect weak antigen expressions and subgroups.
- Applicable in complex serology cases, such as HDFN.

Disadvantages:

- Requires expensive flow cytometers.
- Needs skilled personnel for analysis.

Molecular (DNA-Based) Blood Typing[43]

Instead of serological methods, DNA sequencing and PCR techniques detect blood group genotypes at the molecular level.

Advantages:

- Detects weak or rare blood group variants.
- Useful for prenatal and forensic testing.

Disadvantages:

- It is expensive and requires skilled personnel.

- It is not widely available in routine clinical settings.

Table 7: Comparison of Various Blood Grouping Methods

Method	Accuracy	Time Required	Cost	Sensitivity	Application
Slide Agglutination Method	**Low** (risk of false positives)	**Fast (1-2 min)**	**Low**	Moderate	Emergency blood typing, field testing
Tube Method	**High** (Gold standard)	**Moderate (5-10 min)**	**Moderate**	High	Routine blood typing in hospitals & labs
Gel Card Method (Column Agglutination)	**Very High**	**Moderate (10-15 min)**	**High**	Very High	Automated blood banks, cross-matching
Microplate Method (ELISA-based)	**Very High**	**Slow (30-60 min)**	**High**	Very High	Large-scale screening (blood banks, research)
Flow Cytometry	**Extremely High**	**Moderate (30-45 min)**	**Very High**	Ultra-sensitive	Research, detecting weak blood group antigens, rare typing
Molecular (DNA-based) Typing	**Extremely High**	**Slow (Few hours to days)**	**Very High**	Ultra-sensitive	Rare blood group identification, forensic & prenatal testing

The above table compares different blood grouping methods based on **accuracy, time, cost, sensitivity, and application.**

To check ABO incompatible, we follow reverse grouping after forward grouping.[45]

Forward Grouping: Tests patient's RBCs with anti-A and anti-B sera to detect ABO antigens.

Reverse Grouping: Tests patient's serum with A and B red cells to detect ABO antibodies.

3.8 Crossmatching[49]

- **Major crossmatch**: A test that detects antibodies in the recipient's plasma that could interact with antigens on the donor's red blood cells.
- **Minor crossmatch**: A test to detect antibodies in the donor's plasma that could interact with antigens in the recipient's red blood cells.

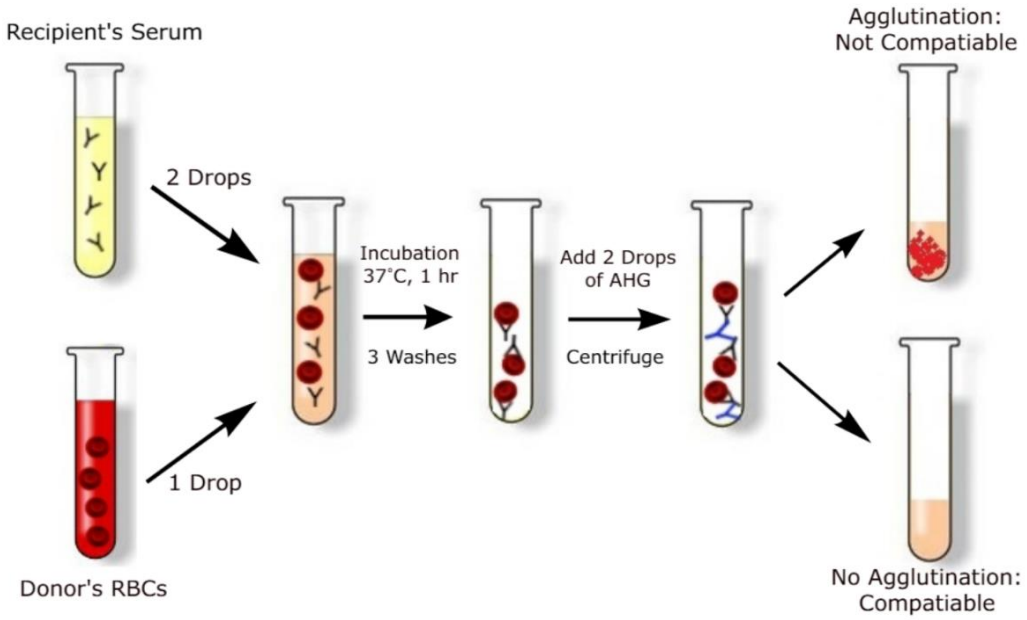


Figure 2. Stages of Major crossmatch[49]

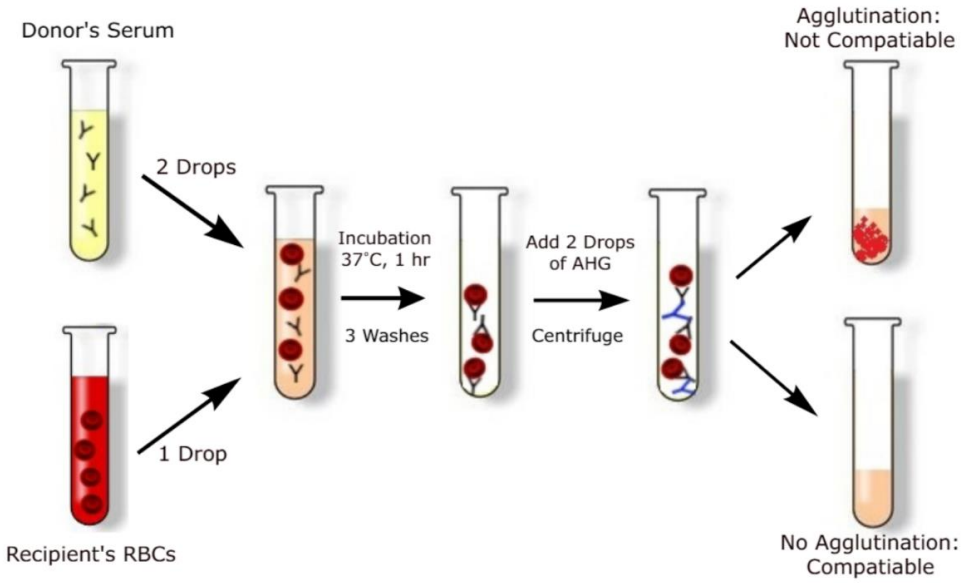


Figure 3. Stages of Minor crossmatch[49]

3.9 Blood Transfusion Reactions

Blood product transfusions can cause a variety of adverse reactions and consequences for recipients, such as acute immune hemolysis, acute septic reaction that causes hypotension, shock, and eventually death, or subclinical viral infection that may go undetected for decades.[50]

Several classification schemes are available to classify adverse events and outcomes in transfusion recipients. These include, among other things, classifications by pathogenesis (immune versus non-immune; infectious [referred to as transfusion-transmitted diseases] versus non-infectious [referred to as the non-infectious serious hazards of transfusion] and reaction type (allergic or febrile). Two categories will be used in the classification system for adverse events.[50,51]

1. Acute Transfusion Reaction
2. Delayed Transfusion Reaction

Acute Transfusion Reaction[51]

- Occurs **within 24 hours** of transfusion.
- Causes: Hemolysis (ABO incompatibility), allergic reactions, febrile reactions, bacterial contamination.
- Symptoms: Fever, chills, chest pain, hypotension, rash, dyspnea.

Delayed Transfusion Reaction[51]

- Occurs **after 24 hours to weeks** post-transfusion.
- Causes: Alloantibody response (against donor red cells), iron overload, infections.
- Symptoms: Jaundice, anemia, fever, mild hemolysis.

Table 8: Transfusion responses, severity[50]

Name	Temporal Relationship	Severity
Acute hemolytic	0–24 hours	Mild–severe
Anaphylactic	0–1 hour	Severe
Febrile	0–4 hours	Mild
Hypotensive	0–15 minutes	Mild–Moderate
Metabolic	0–4 hours	Mild–Moderate
Septic	0–6 hours	Mild–severe
TACO	0–6 hours	Mild–severe
TRALI	0–6 hours	Mild–severe
TAD	0–24 hours	Mild
Urticarial/allergic	0–4 hours	Mild–Moderate

This above table displays a list of transfusion responses, severity, and chronological relationships.

3.10 Various studies conducted regarding Rh phenotype

In a study by Rao C et al.[1] in 315 blood donors. Antigen e was shown to be the most prevalent Rh antigen system, whereas antigen E was found to be the least prevalent. They discovered that the DCCee phenotypes were common, whereas the dccEe phenotypes was the least frequent.

At the tertiary care hospital in North-western India, Prinja N et al.[2] examined the antigen frequencies of the ABO, Rh, and Kell blood groups in 8067 donors. The most common phenotype in their investigation was DCcee, whereas the least common phenotype was DCCe. Antigen E was the least common Rh antigen, whereas antigen E was the most common.

The distribution and frequency of the major Rh blood group antigens and their phenotypes in 315 blood donors who visited a blood bank in a tertiary care hospital in the Barpeta district of Assam were examined by Baruah D et al.[4] They found that D antigen was the most prevalent antigen and e antigen was the least prevalent.

The most common phenotype in North-Eastern India was DCCee, whereas dccee was the least common. ABO, Rhesus, and Kell Antigens, Alleles, and Haplotypes were examined in 1528 healthy West Bengali donors by Basu D et al.[10] They found that antigen D was the most prevalent antigen Rh system, while antigen E was the least prevalent; and that the CcDee phenotypic was more common than CCDEE.

In a study by Romphruk AV et al.[11] antigen e was the most prevalent antigen and antigen E was the least prevalent antigen among ethnic Thai blood donors from the northeast of Thailand. The study's authors found that the CCDee phenotype was the most prevalent, whereas CCDEE was the least prevalent.

Among 227 voluntary blood donors, Tariq F et al.[52] found that antigen c was the most common antigen Rh system in the Pakistani community and that antigen e was the least common. The most common phenotypic variant was DCCee, while ddCcee was the least common.

In a study by Gupta I et al.[53], antigen e was shown to be the most prevalent Rh antigen system, whereas antigen E was found to be the least prevalent. They discovered that the DCCee phenotypes were common, whereas the dccee, dccEe, and dccEE phenotypes were the least frequent.

Makroo et al.[54] conducted a study on the prevalence of the blood group antigens for the Rh, Duffy, Kell, Kidd, and MNSs in 3073 Indian blood donors. According to the study, there were statistical differences between the prevalence of the typed antigens among Indian blood donors and those in the Caucasian, Black, and Chinese populations. Still, these differences were statistically closer to those of Caucasians than those of the other racial groups.

The most frequent antigen in research by Gundrajukuppam et al.[55] on 1000 blood samples from donors was e, which was followed by D, C, c, and E. The most prevalent phenotype was DCe/DCe (R1R1), and the least prevalent trait was r'r.

4. Materials and Methods

4.1 Study Design

4.1.2 Study Type

This study was a prospective observational study designed to evaluate ABO grouping and Rh grouping (D, C, c, E, e) and their phenotype in blood donors.

4.1.3 Study Period:

The study was conducted from 1st April 2023 to 31st March 2024.

4.1.4 Sample Size Determination

The sample size for this study was calculated to ensure sufficient power to detect statistically significant differences in the most common phenotype and least common phenotype among donors who came to our blood centre.

With the anticipated Proportion of phenotype DCCee among blood donors at 45.7% [4], the study required a sample size of 382 patients with a 95% level of confidence and 5% absolute precision,

- $$n=\frac{z^2\ p*q}{d^2}$$

Z = Z statistic at α level of significance,

d2 = Absolute error,

P = Proportion rate,

q = 100-p

4.2 Patient Recruitment

4.2.1 Inclusion Criteria

- Blood donors who fulfilled the criteria of the Drugs and Cosmetics Act, 1940 and Regulations, 1945 were included in the study.

4.2.2 Exclusion Criteria

- Nil

4.2.3 Consent and Confidentiality

All participants were provided with detailed information regarding the study objectives, procedures, potential benefits, and risks. Written informed consent was obtained from each participant or their legal representative under institutional and ethical guidelines.

4.2.4 Ethical Clearance

Ethical approval for this study was obtained from the Institutional Ethics Committee/ BLDE (Deemed to be University) BLDE (DU)/IEC/931/2023-24.

4.3 Sample Collection

Four ml of blood was collected from each donor, two ml in a plain vial and 2ml in an EDTA anti-coagulant vial after cleaning the puncture site with spirit and iodine and taking all the aseptic precautions. ABO antisera vials which were being used for the testing was stored at 2-8 degree centigrade and was taken to room temperature before utilising it for testing.

4.4 Sample Processing

4.4.1 For ABO reverse grouping and indirect Coomb's test serum preparation

- The patient's blood sample was collected in a plain tube.
- The sample was clotted at room temperature for 20 to 25 minutes.
- It was centrifuged 1500 rpm for 10 minutes to separate the serum.
- The serum was carefully extracted using a pipette, ensuring no red blood cells were included.
- Finally, the serum was stored at 2^0-8^0C temperature until further testing.

4.4.2 Saline suspension preparation (5%)

5% cell suspension was used for

1. Forward ABO blood grouping.
2. Complete Rh typing by tube method.

Take 1ml of EDTA blood in a test tube and add equal amount of normal saline to the test tube. Now, the mixture was centrifuged at 3000 rpm for three minutes. Discard the supernatant. Repeat the procedure twice or more in the same manner. Wash red cells three times in isotonic saline, and then prepare the 5% saline suspension of RBCs by adding 95 drops of saline to the five drops of RBCs.

4.5 Procedures performed

1. ABO forward and reverse grouping was carried out by the tube method by utilizing commercially available anti-A, anti-B, anti-AB, anti-A1, anti-H antisera, and a 5% saline suspension of known A, B, and O pooled cells,
2. Rh typing was done using the tube method using anti-RhD (R0 & R1) antisera.

3. Other Rh antigens (different from antigen D), such as antigens C, E, c, and e, were detected using anti-C, anti-E, anti-c and anti-e monoclonal antibodies. These tests were conducted using the tube method.
4. Screening for weak D antibodies in the serum was done by indirect Coomb's test [ICT] by tube method

4.6 Test procedure

4.6.1 ABO Grouping by tube method Compared to the slide, the tube approach is favoured because it permits extended incubation without contamination or drying. It is simpler to quantify the antigen-antibody reaction intensity in a tube than on a slide, and centrifugation improves this process.

Principle

A' and/or 'B' antigens on the surface of red blood cells and the concurrent presence of anti-A and/or anti-B antibodies in serum (whatever antigen lacks from the cells) are what characterize the ABO grouping. "Human red blood cells possessing 'A' and 'B' antigen will agglutinate in the presence of antibody directed towards the corresponding antigen," according to forward typing and reverse typing, which identified the erythrocyte antigens as ANTI-A, ANTI-B, or both

Procedure

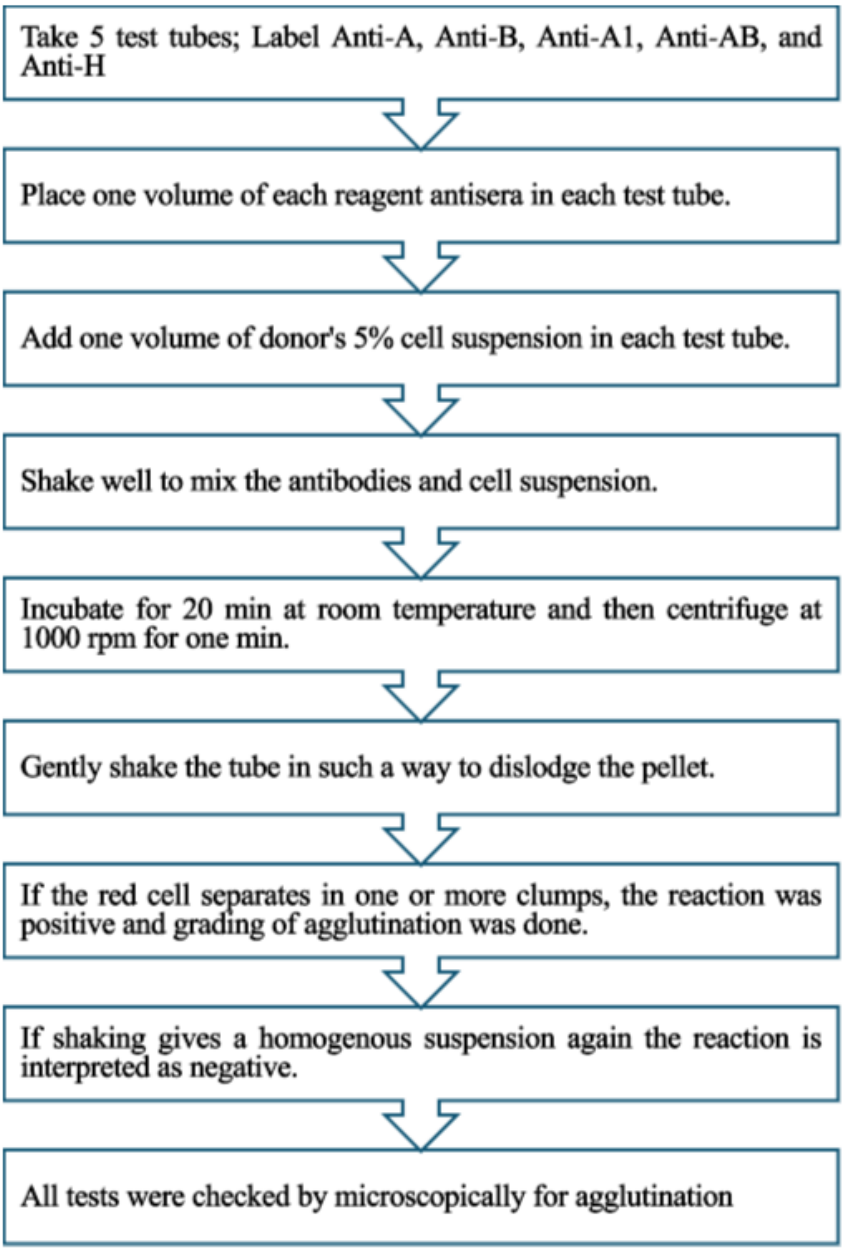
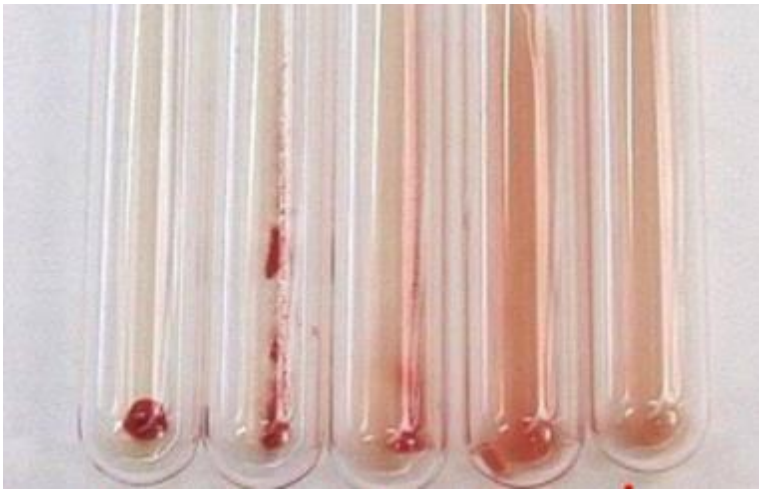

Take 5 test tubes; Label Anti-A, Anti-B, Anti-A1, Anti-AB, and Anti-H
Place one volume of each reagent antisera in each test tube.
Add one volume of donor's 5% cell suspension in each test tube.
Shake well to mix the antibodies and cell suspension.
Incubate for 20 min at room temperature and then centrifuge at 1000 rpm for one min.
Gently shake the tube in such a way to dislodge the pellet.
If the red cell separates in one or more clumps, the reaction was positive and grading of agglutination was done.
If shaking gives a homogenous suspension again the reaction is interpreted as negative.
All tests were checked by microscopically for agglutination

Table 9: Interpretation agglutination reactions

Strength	Symbol	Description
Very Strong	4 (+++++)	One complete mass of agglutination was readily visible on the slide before microscopic examination.
Strong	3 (+++)	Large separate masses of agglutination are readily visible on the slide before microscopic examination.
Moderate	2 (++)	Smaller agglutinates are still readily visible on the slide before microscopic examination.
Weak	1 (+)	A granular appearance, just visible on the slide microscopically. Shows big clumps of more than 20 cells.
Negative	-	All cells are free and evenly distributed.
Mixed Field	Mixed	Agglutinates in a field of free cells.



4 3 2 1 -ve

Figure 4. Grading of agglutination

Interpretation

Forward Grouping using different **anti-sera (Anti-A, Anti-B, Anti-A1, Anti-AB, and Anti-H):**

Table 10: Blood Group Identification Based on Serological Reactions with Antisera

Blood Group	Anti-A	Anti-B	Anti-A1	Anti-AB	Anti-H
A1	+	-	+	+	-
A2	+	-	-	+	+
B	-	+	-	+	-
A1B	+	+	+	+	-
A2B	+	+	-	+	+
O	-	-	-	-	+
Bombay (Oh)	-	-	-	-	-

- **+ (Positive):** Agglutination indicates the presence of the antigen.
- **- (Negative):** No agglutination means the antigen is absent.
- **A1 vs. A2:** The **A1 blood group** reacts with **Anti-A1**, while **A2 does not**.
- **Anti-H:** Strong reaction with **O and A2** groups.

  No reaction with **A1, B, AB, or Bombay** (since Bombay lacks the H antigen).

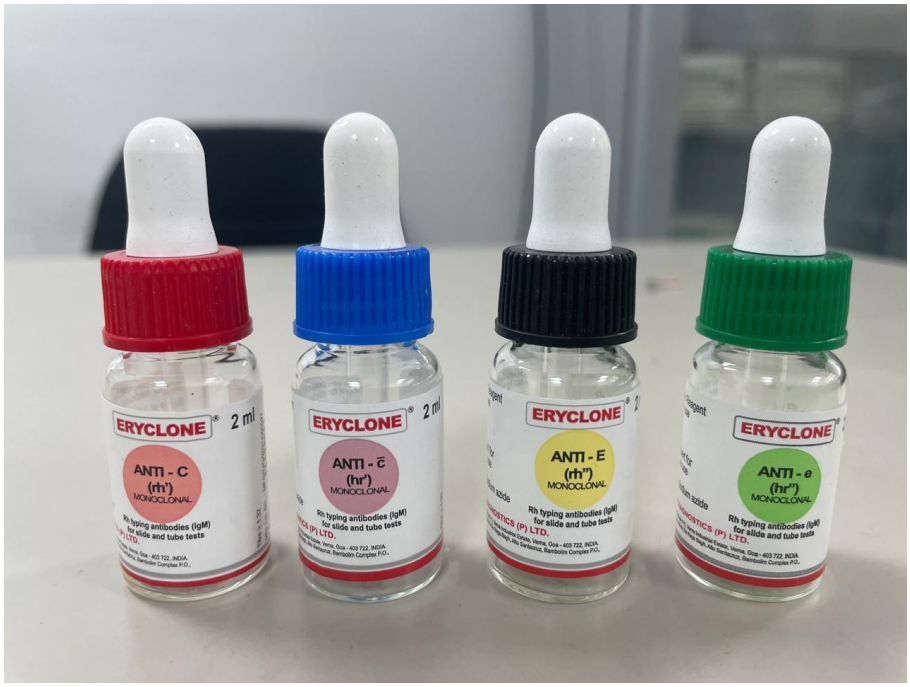


Figure 5. Photograph of antigen reagent serum

4.6.2 Rh Grouping by Tube Method

The anti-D Rh1 (IgM) monoclonal antibodies are produced using hybridoma technology, the

In-vitro culture supernatant of hybrids created by cellular fusion. The following characteristics of anti-D Rh1 (IgM) monoclonal antibodies are

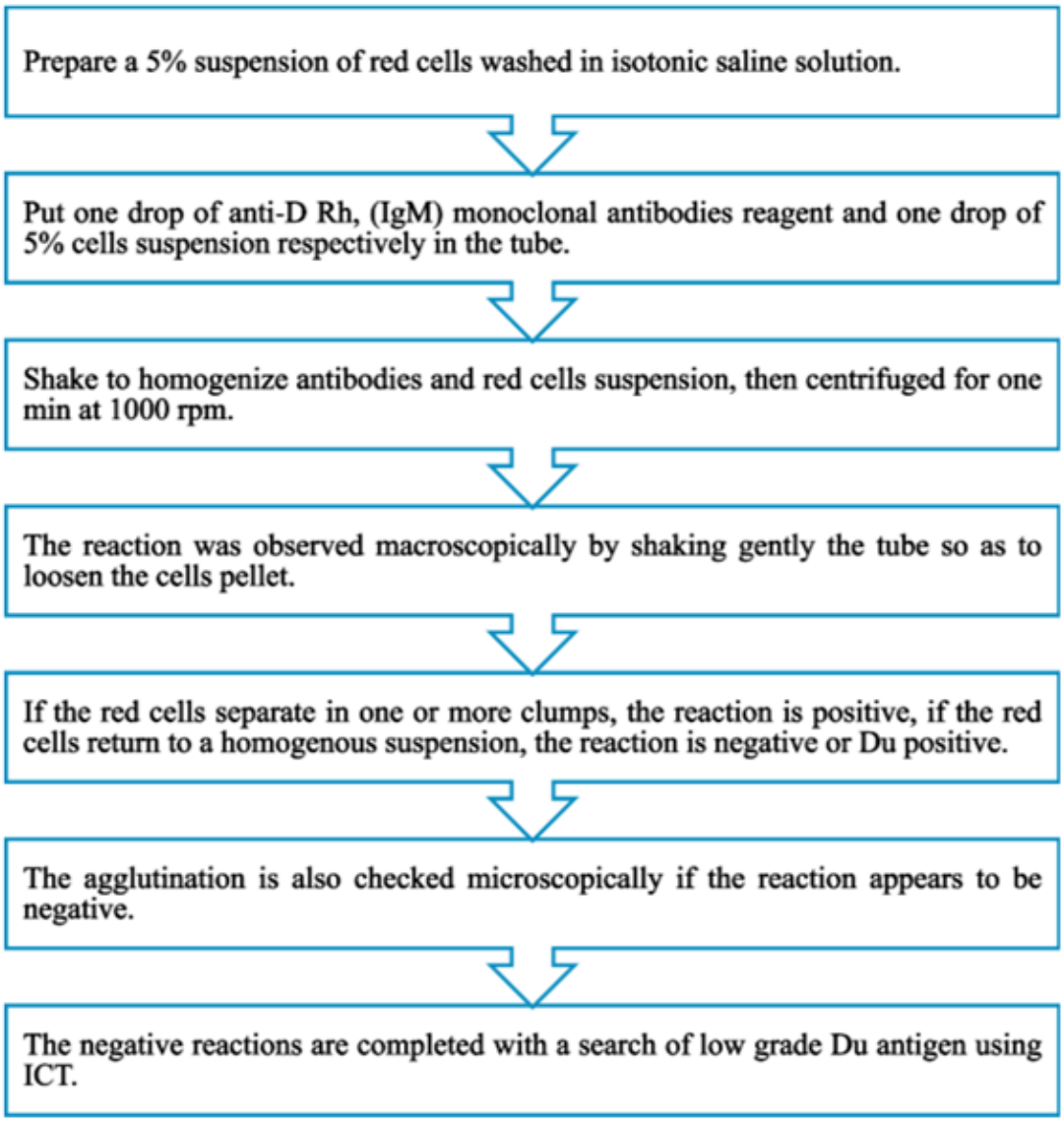
1. It clumps in a saline solution.
2. Room temperature active.
3. Suitable for use in tubes and on glass slides.

Principle

"Human red blood cells possessing D antigen will agglutinate in the presence of corresponding antibody". When red blood cells agglutinate with anti-D Rh (IgM) monoclonal antibodies, D-antigen is present, resulting in a Rh-positive test. Both D antigen and high-grade Du antigen (a

weaker form of antigen D) can be detected by it. By utilizing anti-D (IgG) and anti-human globulin serum (Coomb's reagent), the cells were then checked for the presence of low-grade Du antigen if no agglutination was achieved with anti-D Rh1 (IgM) monoclonal antibodies.

Procedure



In ABO grouping and Rh typing, false positive and false negative findings were carefully avoided by adopting the following techniques

1. Rechecking the identification of specimens.

2. Cell-to-reagent ratio
3. Identification of hemolysis
4. Proper storage of reagents
5. Fibrin clots
6. Proper incubation, i.e. not over/under incubation of cells and reagents.
7. Proper centrifugation avoiding over/under centrifugation
8. Acquired antibodies, i.e. Weak d variant antibodies.

4.6.3 Test for Anti-C, Anti-c, Anti-E, Anti-e (IgM)

Monoclonal Rh/HR typing antibodies C, c, E, and we are the other frequent Rh antigens. Compared to D antigens, these are less immunogenic. The expression for immunogenicity is D>c>E>C>e. The letters C and c, E and e represent two opposing pairs of antigens.

Anti C, Anti C, Anti E, and Anti E tests are helpful for

1. Determining an individual's phenotypic and likely Rh genotype.
2. To select donors who have received transfusions or pregnancy-related vaccinations against the C, c, E, or e antigen.
3. In HDN prediction and pre-transfusion testing.

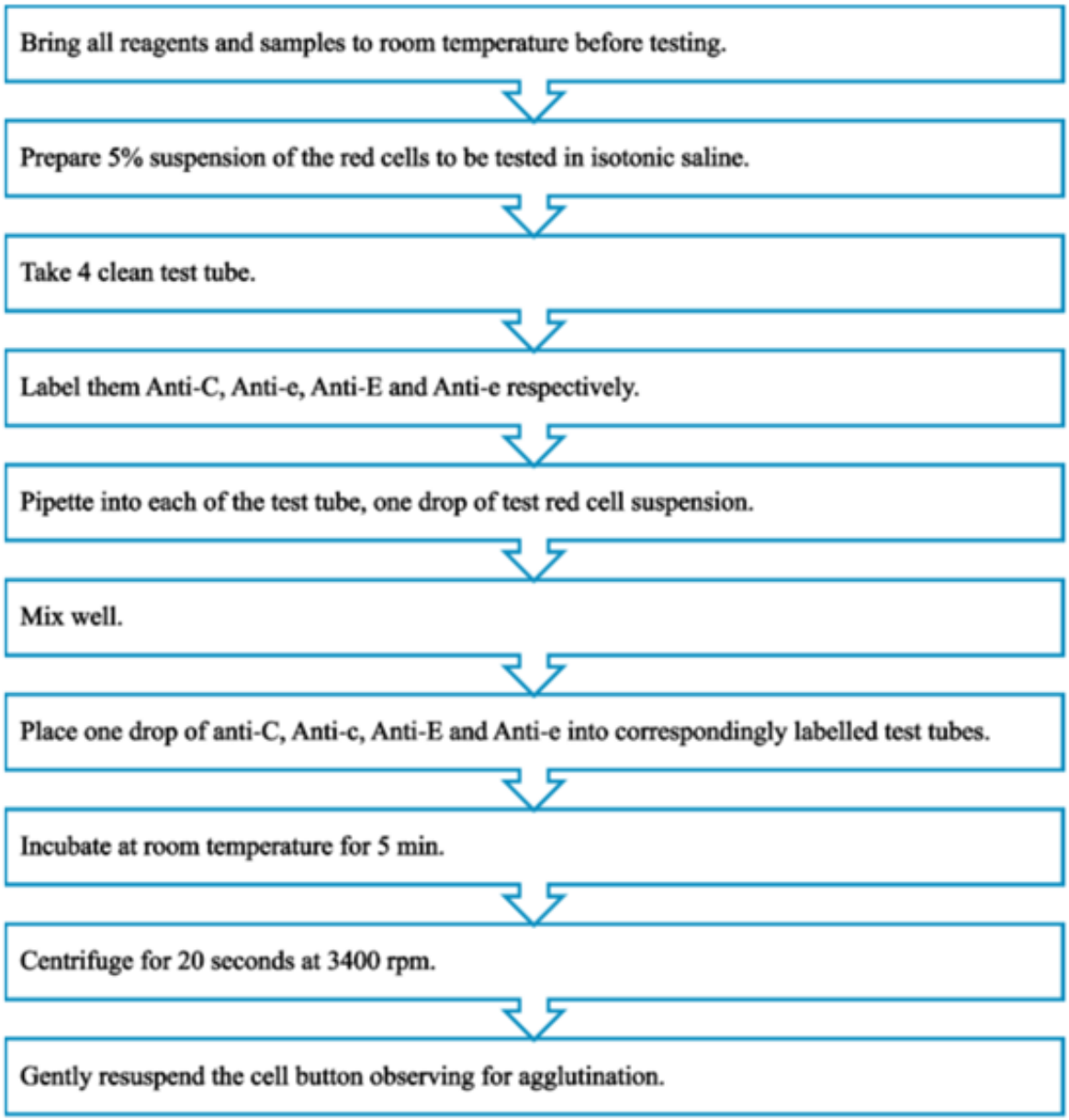
The anti-C, anti-C, anti-E, and anti-E reagents are ready to use and were made from the supernatant of the corresponding human cell cultures.

Principle

Human red blood cells that contain the C, c, E, and e antigens will agglutinate in the presence of the antigen-specific antibody. A positive test result showing the relevant antigen was obtained when red blood cells were agglutinated with anti-C, anti-c, anti-E, and anti-e reagents. The

negative test result indicated the absence of the corresponding antigen, which was the absence of agglutination of red blood cells with anti-C, anti-c, anti-E, and anti-e reagents.

Procedure



Interpretation of test result

- Agglutination indicated a positive test result and the presence of the C, c, E, or e antigen.

- No agglutination was a negative test result, showing that the C, c, E, or e antigen was absent.
- Each test batch was conducted in parallel with a negative control (red blood cells devoid of the corresponding antigen) and a positive control (heterozygous red blood cells) known to possess the C, c, E, or e antigens.
- Tubes with negative reactions were centrifuged, and the results were repeated after 5 minutes to check for the presence of weak antigens.
- Under centrifugation was avoided to prevent incorrect results.

4.6.4 Test for weak D^u variant of antibodies by indirect Coomb's test

Coomb's reagent, which is an anti-human serum, is a combination of murine monoclonal anti-C3d (BRIC-8) and polyclonal anti-IgG (rabbit/goat).

All subtypes of gamma globulins (IgG1, IgG2, IgG3, and IgG4) are reactive with anti-IgG, while anti-C3d reacts with the complement component of C3d.

The C4 segment of the complement does not react with the reagent. As a result, we exclusively used SPAN's Coomb's reagent, which uses an optimized combination of anti-human IgG and anti-C3d.

Principle

Coomb's sera, an antihuman globulin (AHG) reagent, will bind to the IgG antibodies on red blood cells coated with incomplete antibody (IgG) or C3 component of complement in vitro, causing the cells to clump together.

Sample collection

1. The sample was collected in a plain vial for ICT.

2. The serum was obtained from freshly clotted blood.
3. The test was done within 48 hours because complement activity is diminished in sera stored at room temperature for more than 48 hours.
4. If delayed testing was done, the serum sample was stored at 2-8^{0}C.
5. Plasma from anticoagulated samples was not used because it lacks complement components.

Procedure

Prepare test erythrocytes by taking 5-7 drops of appropriate blood in a test tube.

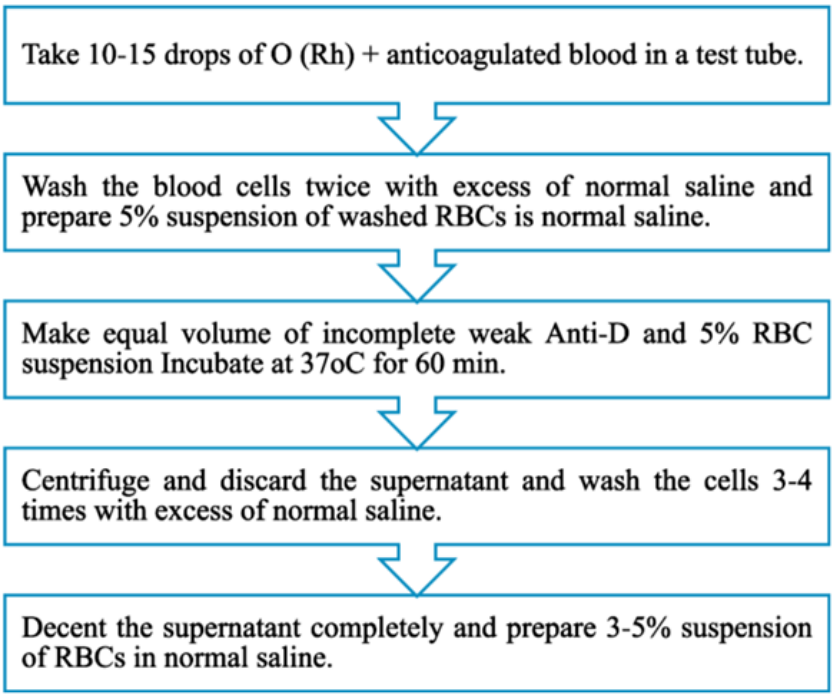
1. Wash red blood cells (RBCs) 3–4 times with saline to remove any extra proteins.
2. Make a mix of washed RBCs with saline (3–5% concentration).
3. Put 2 drops of the patient's serum into a test tube.
4. Add 2 drops of the RBC suspension into the same test tube.
5. Heat the tube at 37°C for 1 hour, then spin it in a machine (centrifuge) for 1 minute.
6. Remove the supernatant and wash the RBCs 3–4 times with saline.
7. Add 1 drop of Coombs reagent, let it sit for 15–30 minutes, then spin again for 1 minute. Check if clumping happens — look closely with your eyes and a microscope.

Results - If you see clumping, the test is **positive** (meaning Du antibodies are present). If no clumping happens, go to the next step to double-check the result.

Confirm with control cells - Add 2 drops of control cells, spin for 1 minute.

Final check - If the control cells clump, the test worked correctly. If not, the test might need to be repeated.

Preparation of Coomb's control



ICT is a diagnostic procedure used for

- Ab screening and identification
- Compatibility testing
- Red cell phenotype

4.7 Statistical Analysis

- Using the Statistical Package for the Social Sciences, the data was analysed statistically after being entered into a Microsoft Excel sheet (Version 20).
- The results were shown as a mean, standard deviation, median, interquartile range, frequency, percentages, and diagrams.

5. Results

Rh antigen typing was done on 382 voluntary blood donors during the study period.

Each category's frequencies (%) were calculated to perform descriptive statistics for the categorical variables.

The donors' ages varied from 18 years to 57 years. The mean age of the patients was 27.33 years. The most common age group was < 20 years.

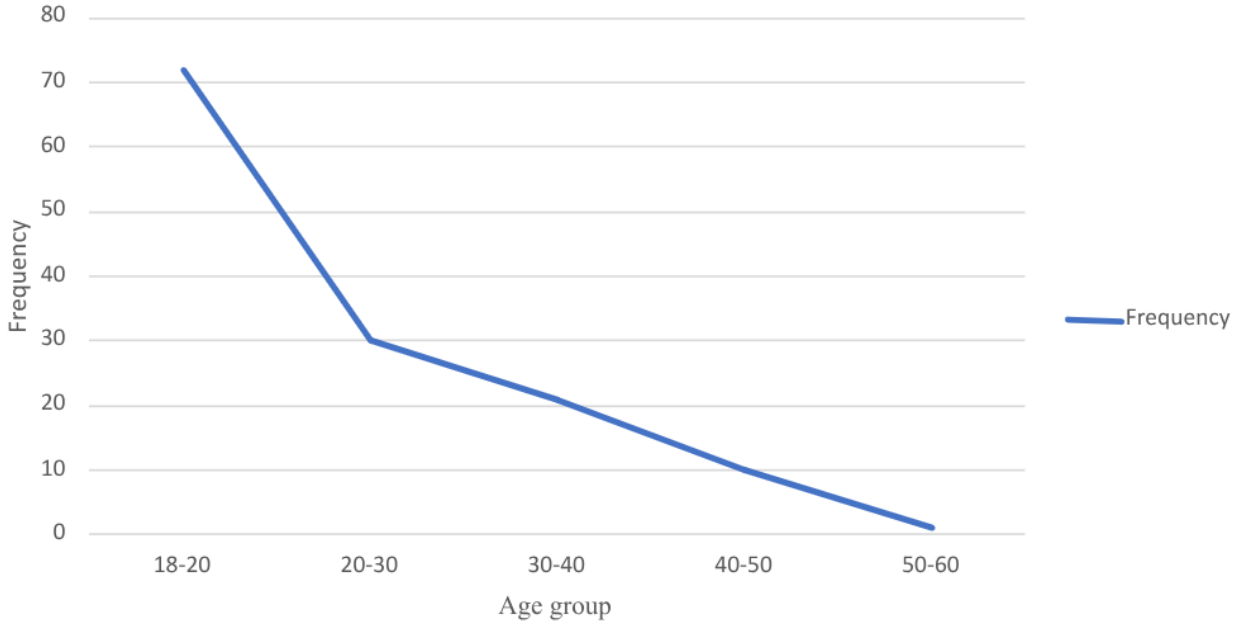


Figure 6. Line graph of frequency distribution across different age groups

Total 382 donors were tested, out of which male donors were 329 (86.10%) and Female donors were 53 (13.90%). **[Figure 7]**

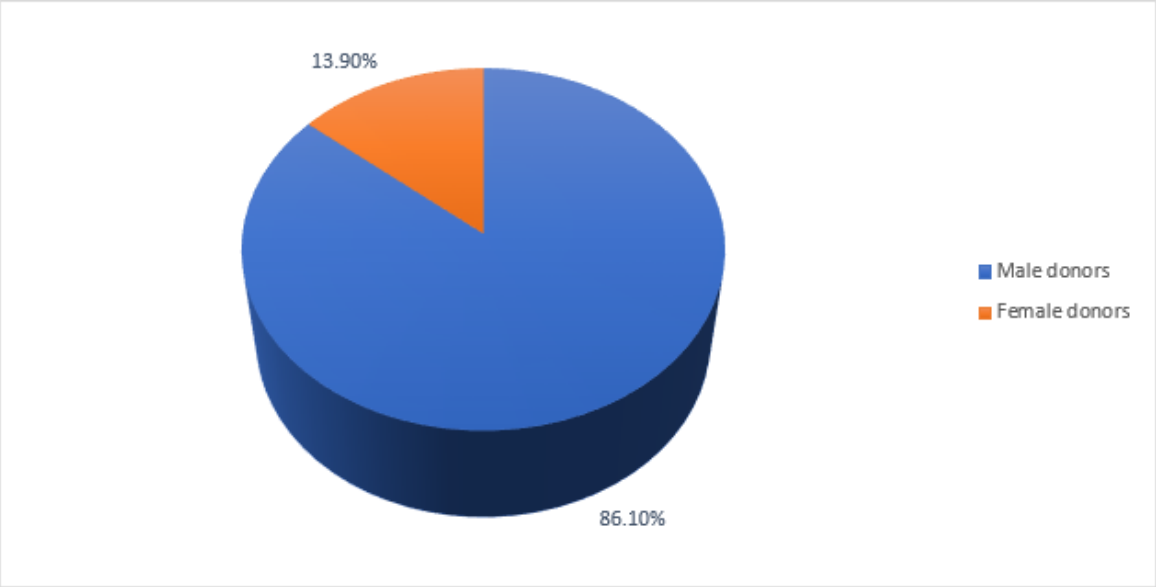


Figure 7. Pie Chart - Proportion of Male and Female Donors

Table 11: Distribution of ABO Blood Groups Among Participants

Blood Group	Number of Participants
A	109 (28.53%)
B	111 (29.06%)
O	128 (33.51%)
AB	34 (8.90%)

Nearly 128 donors (33.51%) belonged to the O blood group, followed by 111 donors (29.06%) in the B group, 109 donors (28.53%) in the A group, and 34 donors (8.90%) in the AB group. **[Table 11] [Figure 8]**

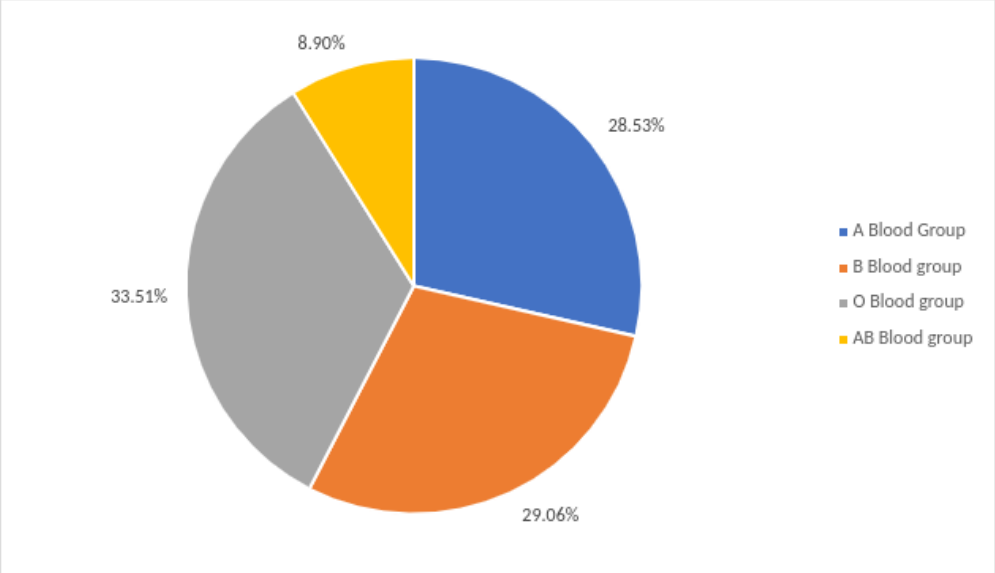


Figure 8. Pie Chart - Percentage Distribution of Blood Groups Among Participants

The incidence of Rh-positive donors was 350 (91.6%), while 32 donors (8.4%) belonged to the Rh-negative group. **[Figure 9]**

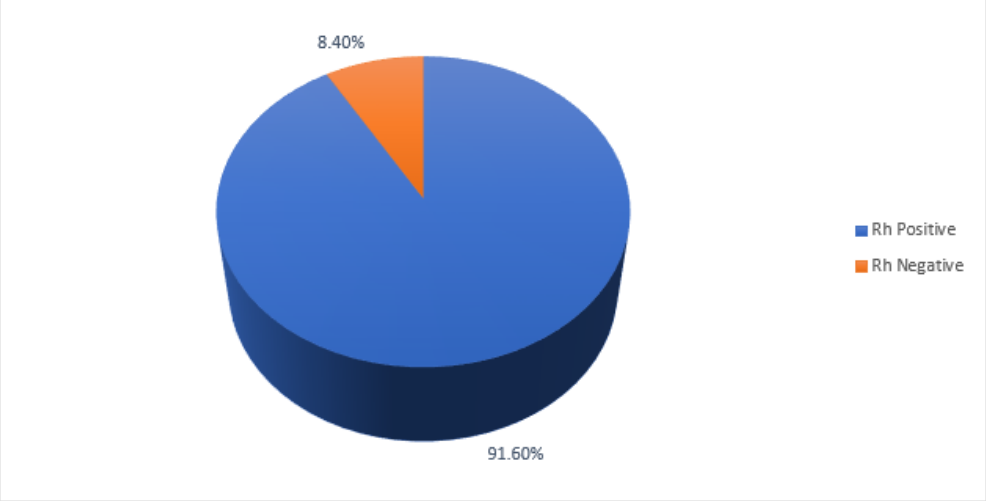


Figure 9. Pie Chart - Distribution of Rh-Positive and Rh-Negative Donors

Blood Group	A (109)	B (111)	O (128)	AB (34)
D positive (350)	96 (27.43%)	101 (28.86%)	122 (34.86%)	31 (8.86%)
D negative (32)	13 (40.63%)	10 (31.25%)	06 (18.75%)	03 (9.38%)

Table 12. Coexistence of D Antigen and ABO Blood Group of Donors

D positive antigen was present mostly in O blood group donors, followed by B > A > AB blood group donors **[Figure 10]**.

D negative antigen was present mostly in A blood group donors, followed by B > O > AB blood group donors **[Figure 11]**.

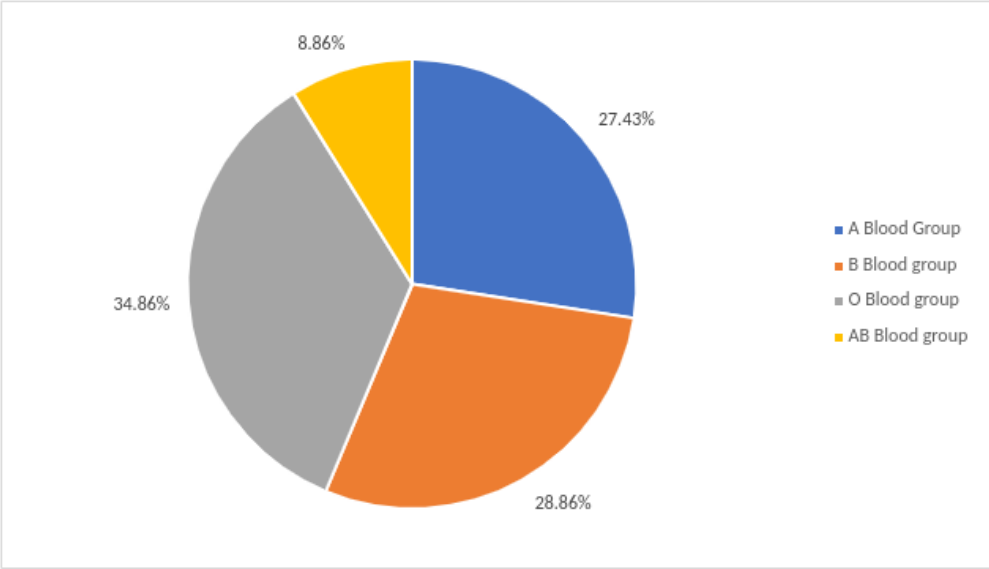


Figure 10. Pie Chart - Coexistence of D positive antigen and ABO Blood Group of Donors

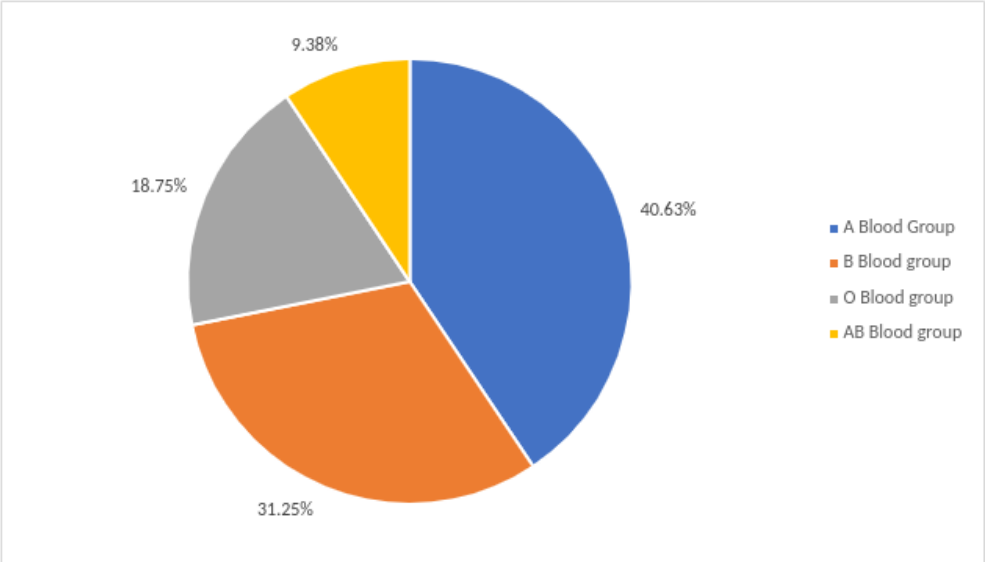


Figure 11. Pie Chart - Coexistence of D negative antigen and ABO Blood Group of Donors

5.1 The Distribution of Rh Antigens

Frequency of major Rh antigens, i.e. e (370 donors), D (350 donors), C (329 donors), c (200 donors) & E (50 donors) were 96.9%, 92.3%, 86.1%, 52.4% and 13.1% respectively. [**Figure 12]**

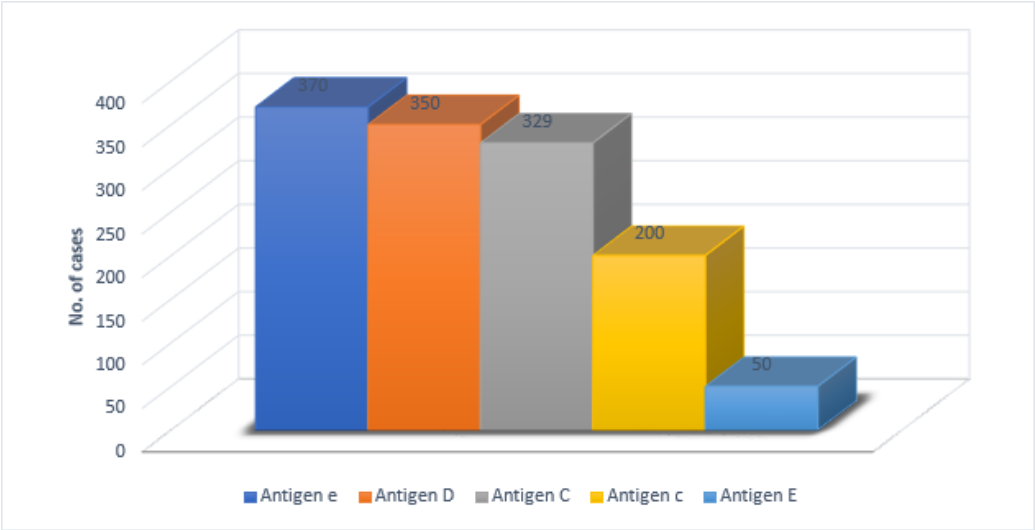


Figure 12. Bar Chart - The Distribution of Antigens (D, C, E, c, e) Among Donors

5.2 Frequency of Rh Phenotypes

Table 13. Rh Phenotype Distribution of Rh-Positive donors

Phenotype	Total Donors	Percentage
DCCee	**177**	**46.30%**
DCcee	121	31.70%
DCcEe	25	6.50%
DccEe	11	2.90%
DccEE	7	1.80%
DCCEe	4	1.00%
Dccee	3	0.80%
DCcEE	2	0.50%

Table 14. Rh Phenotype Distribution of Rh- Negative donors

Phenotype	Total Cases	Percentage
dccee	**28**	**7.30%**
dCcee	1	0.30%
dccEe	1	0.30%
dcCee	1	0.30%
dcceE	1	0.30%

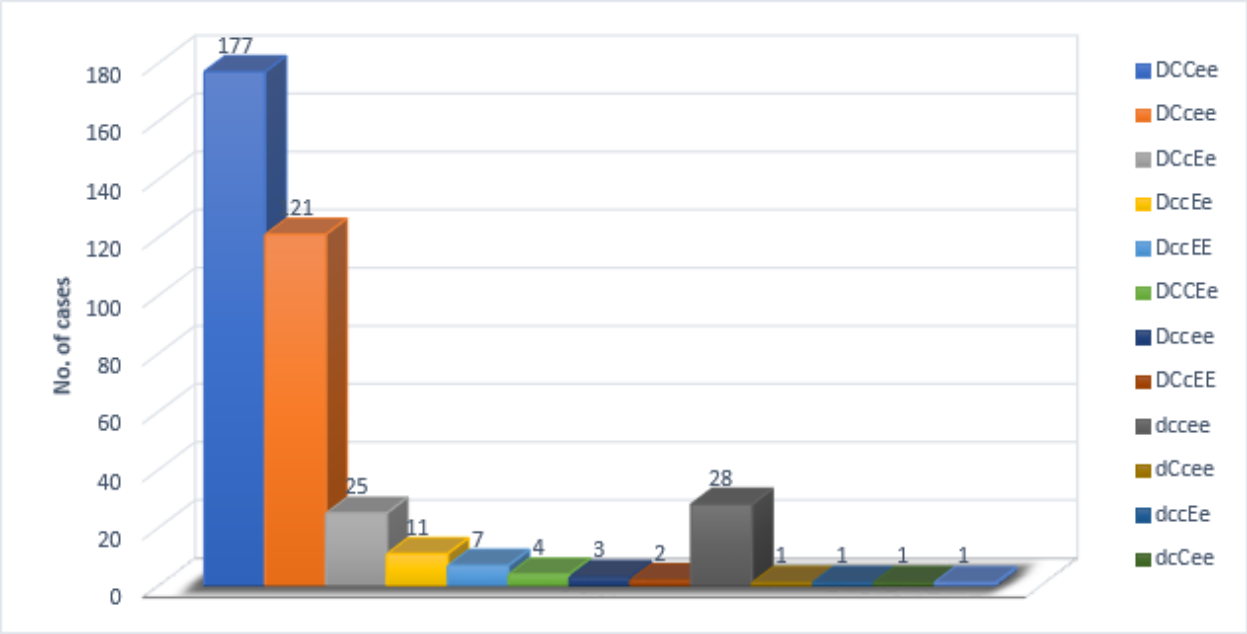


Figure 13. Bar Chart - Frequency of Rh Phenotypes Among Donors

Out of 18 possible phenotype combinations, nine belonged to Rh D positive, and nine belonged to Rh D negative group. In order of frequency, the common phenotypes were DCCee: 177 donors (46.30%), DCcee: 121 donors (31.70%), DCcEe: 25 donors (6.5%), DccEe: 11 donors (2.9%), DccEE: 7 donors (1.8%), DCCEe: 4 donors (1%), Dcceee: 3 donors (0.8%), DCcEE: 2 donors (0.5%), dccee: 28 donors (7.3%), dCcee: 1 donor (0.3%), dccEe: 1 donor (0.3%), dcceE: 0.3% 1 donors (0.3%), dcCee: 1 donor (0.3%).

The most common phenotype in Rh D positive donors was DCCee (46.30%) while in Rh D negative donors, it was dccee (7.30%).

6. Discussion

There are different factors responsible for alloimmunisation in multiple transfused cases. Differences in RBC surface antigenic profile between blood donors and recipients are among them. Antibody specificity also depends upon age, sex, number, and time interval between transfusions. Partial D is a variant of the RhD antigen where some parts are missing or altered. In pregnancy, a mother with partial D may produce anti-D antibodies if her baby inherits a fully D-positive blood type. These antibodies can cross the placenta, attacking the baby's red blood cells, causing HDFN — leading to jaundice, anemia, or more severe complications. Though Rh-negative mothers receive Rh immunoglobulin to prevent this, partial D cases need special antibody screening since they may produce anti-D despite appearing Rh-positive. Early detection through molecular testing helps manage and prevent HDFN.[2]

In our study, the age of donors varied from 18 years to 57 years, with a mean age of 27.33 years, and the male to female ratio was 6:1. In studies from various parts of India, like by Prinja N et al. [2], Baruah D et al.[4], and Basu D et al.[10], blood donors belonged to the young age group. The primary strength of every society is its youth, which is why many blood donation drives are held in educational institutions. Additionally, younger people are more fit and alert, which results in fewer deferrals. Accordingly, they are the age group that donates blood most frequently.

In our study, the commonest blood group was 'O', noted in 128 (33.51 %) donors, followed by blood groups 'B', 'A', and 'AB'. Blood group 'O' was found to be the most common in the study conducted by Rao C et al.[1], Tariq F et al.[52] and Gundrajukuppam et al.[55]

Blood group B was the most common documented by Prinja N et al.[2], Baruah D et al.[4] and Gupta I et al.[53] This may be because of geographical variation.

While most studies reported Rh antigen positivity in the 85–95% range, a study conducted in Assam by Baruah D et al.[4] reported 99.05% Rh antigen positivity. In our case, the incidence of Rh antigen-positive cases was 91.6% (350), and Rh antigen-negative cases were 8.4% (32). The prevalence of Rh D antigen varies widely worldwide, ranging from 70% to 99%. Tariq F et al.[52] from Pakistan documented 90.5% Rh antigen positive.

The most common Rh antigen is D, while other Rh antigens are C, c, E, & e. These are less immunogenic than D antigen. The immunogenicity is expressed as D>c>E>C>e. C and c, E & e represent two opposing pairs of antigens.

Among the Rh blood group system, the most frequent antigen in our study was found to be "e" closely followed by "D." This is similar to studies from other parts of India, such as Rao C et al. [1], Prinja N et al.[2], Gupta I et al.[53] Gundrajukuppamet al.[55] Pahuja S et al.[56], while in the studies conducted by Baruah D et al.[4] and Basu D et al.[10] the most common antigen was "D". Study done by Tariq F et al.[52] detected anti-c in 33.5% > anti-E in 25.1% > anti-C in 19.4% > anti-D 9.7% > anti-e 2.2%. In his study anti-c showed highest frequency and anti-e showed least frequency.

The least common antigen in our study was found to be "E", which is similar to the study by Rao C et al.[1], Prinja N et al.[2], Gupta I et al.[53], Gundrajukuppam et al.[55] and Pahuja S et al.[56] The phenotype was computed for the study subjects depending on the expression of different Rh antigens. Among Rh antigen-positive donors, the most common phenotype was DCCee, with a frequency of 177 (46.3%) in our study. The current study's findings were consistent with research conducted in different parts of India by authors such as Rao C et al.[1], Prinja N et al.[2], Baruah D et

al.[4], Basu Det al.[10], Gupta I et al.[53], Gundrajukuppam et al.[55] and Pahuja S et al.[56] In studies that were conducted abroad; Romphruk AV et al.[11] from Thailand, Tariq F et al.[52] from Pakistan, Owaidah AY et al.[57] from Saudi Araba, Yu Y et al.[58] from China, Reid ME et al.[59] from South Africa had same common phenotype DCCee.

Among Rh antigen negatives, the most common phenotype was dccee, with a 28 (7.3%) frequency in our study. The current study's findings were consistent with research conducted by various Indian authors such as Rao C et al.[1], Prinja N et al.[2], Baruah D et al.[4], Basu D et al.[10], Gupta I et al.[53], Gundrajukuppam et al.[55] and Pahuja S et al.[56] In studies that were conducted abroad, Romphruk AV et al.[11] from Thailand, Tariq F et al.[52] from Pakistan, Owaidah AY et al. [57] from Saudi Araba, Yu Y et al.[58] from China, Reid ME et al.[59] from South Africa had the same common phenotype dccee.

Variations in the distribution of Rh phenotypes among various groups most likely cause the observed variations in the frequency of alloimmunisation. Knowledge of the phenotype in a particular population may help formulate population-specific transfusion guidelines. The factors responsible for alloimmunisationation are complex, with the RBC antigenic difference between the blood donor and the recipient being one among them.

When undertaking Rh D antigen typing of patients and selecting blood donors, consideration must be given to the qualitative and quantitative variations in the expression of Rh D antigen. When D antigen typing of Rh-D antigen negative women is done, women must receive anti-D prophylaxis if the baby has a weak form of D. It is often necessary to assess phenotype by selecting blood for transfusion to a patient with Rh antibody, evaluate the likely effect on the fetus of a women Rh antibody and when performing family studies.

Table. 15 Summary of various Indian studies on Rh antigens and phenotypes

Sl No.	Study Conducted by	Most Common Rh Antigen	Least Common Rh Antigen	Most Common Phenotype	Least Common Phenotype
1	Rao C et al.(381) [1]	e	E	DCCee	DCCEe
2	Prinja N et al. (8067) [2]	e	E	DCCee	DCCee
3	Baruah D et al. (315) [4]	D	E	DCCee	dccee
4	Basu D et al. (1528) [10]	D	E	DCCee	dccEe
5	Gupta I et al. (500) [53]	e	E	DCCee	dCCEE,dccee
6	Makroo et al. (3073) [54]	e	E	DCCee	DccEe
7	Gundrajukuppam et al.(1000) [55]	e	E	DCCee	dCCee
8	Pahuja S et al.(2000) [56]	e	E	DCCee	DCCEe
9	**Current Study**	**e**	**E**	**DCCee**	**dCcee,dccEe DcCee,dcceE**

Table 16. Summary of various foreign studies on Rh antigens and phenotypes

Sl No.	Study Conducted by	Most common Rh Antigen	Least Common Rh Antigen	Most common Phenotype	Least Common Phenotype
1	Romphruk AV et al. (13567) [11]	e	E	DCCee	DCCEE
2	Tariq F et al. (227) [52]	c	e	DCCee	dCcee
3	Owaidah AY et al. (100) [57]	e	E	DCcee	DccEE
4	Yu Y et al et al. (1412) [58]	D	E	DCCee	DccEe
5	Reid ME et al. (309) [59]	D	E	Dccee	DccEE
6	**Current Study**	e	E	DCCee	**dCcee,dccEe DcCee,dcceE**

7. Summary:

- The present study was a prospective study conducted from 1st April 2023 to 31st March 2024, evaluating the distribution of Rh antigens (D, C, c, E, e) and their phenotypes among blood donors at our blood center.
- A total of 382 voluntary blood donors were included in the study, and Rh antigen typing was performed.
- The study aimed to determine the most prevalent Rh phenotype based on the frequency of Rh antigens.

- Data was analyzed using descriptive statistics to determine antigenic frequencies and phenotype distribution.
- Rh antigen positivity was found in 91.60% of donors, while 8.40% were Rh-negative.
- Among the Rh antigens, the most frequently occurring antigen was 'e' (96.9%), followed by D (92.3%) and C (86.1%), whereas E antigen was the least common (13.1%).
- The most common phenotype among Rh-positive donors was DCCee (46.3%), followed by DCcee (31.7%), while dccee (7.3%) was the most prevalent phenotype among Rh-negative donors.
- No cases of phenotypes such as DCCEE, dCCEE, dCcEe, dCCEe, and dCcEE were observed in our study.
- Our findings are similar to various studies conducted in India and abroad, which report a high prevalence of the DCCee phenotype among Rh-positive individuals and dccee as the predominant phenotype among Rh-negative individuals.
- These findings are significant for transfusion medicine, as understanding Rh antigen distribution aids in preventing alloimmunization, particularly in multi-transfused patients.
- The study also highlights the importance of phenotype-based blood donor registries to ensure better compatibility for patients requiring frequent transfusions.
- Further large-scale studies are needed to explore the regional and ethnic variations in Rh antigen distribution and their clinical implications in transfusion practices.

8. Conclusion

In the present study the incidence of Rh-positive antigens was 91.60%, in which e antigen was the most common and E antigen was the least common. In this study, the most common phenotype was DCCee.

Population-based frequency data of Rh has a vital role in transfusion practice, especially in situations where antigen-negative donor units are required and can assist in determining the number of blood units to be cross-matched to find a compatible donor. These irregular allo-antibodies can cause immunogenic hemolytic reactions following the subsequent transfusions. Therefore, it is essential to properly match the Rh antigens before issuing the blood. However, extensive studies need to be done in this field to make treatment decisions more scientific, population-specific, and cost-effective.

9. References:

1. Rao C, Shruthi S, Srilekha A. Frequency of Rh antigen and phenotypes in voluntary blood donors. Med J DY Patil Vidyapeeth 2024;17:S134-9.
2. Prinja N, Narain R. ABO, Rh, and Kell blood group antigen frequencies in blood donors at the tertiary care hospital of Northwestern India. Asian J Transfus Sci. 2020;14(2):179–84.
3. Soares SDS, Aquino JR, Petrolli F, de Oliveira TB, Almeida S, Fiegenbaum M. Frequencies of genetic variants of the Rh, Kell, Duffy, Kidd, MNS and Diego systems of northwest Rio Grande do Sul, Brazil. Hematol Transfus Cell Ther. 2023 Jul-Sep;45(3):317-323.
4. Baruah D, Devi G, Musfique J, Bharali A, Dutta UC. Distribution and frequency of principal Rh blood group antigens (D, C, c, E, and e) and their phenotypes in the blood donors attending blood bank in a tertiary care hospital in Barpeta district of Assam. Asian J Transfus Sci. 2022;16(2):167–74.
5. M. R. Miranda, T. D. Dos Santos, and L. Castilho, “Systematic RHD genotyping in Brazilians reveals a high frequency of partial D in transfused patients serologically typed as weak D,” Transfusion and Apheresis Science, vol. 60, no. 6, p. 103235, Aug. 2021
6. Edwards AW. R. A. Fisher's 1943 unravelling of the Rhesus blood-group system. Genetics. 2007 Feb;175(2):471-6.
7. WIENER AS. Heredity and nomenclature of the Rh-Hr blood types. Bull World Health Organ. 1950;3(2):265-78.
8. Rosenfield RE, Allen FH Jr, Rubinstein P. Genetic model for the Rh blood-group system. Proc Natl Acad Sci U S A. 1973 May;70(5):1303-7.

9. Daniels GL, Fletcher A, Garratty G, Henry S, Jørgensen J, Judd WJ, Levene C, Lomas-Francis C, Moulds JJ, Moulds JM, Moulds M, Overbeeke M, Reid ME, Rouger P, Scott M, Sistonen P, Smart E, Tani Y, Wendel S, Zelinski T; International Society of Blood Transfusion. Blood group terminology 2004: from the International Society of Blood Transfusion committee on terminology for red cell surface antigens. Vox Sang. 2004 Nov;87(4):304-16.
10. Basu D, Datta SS, Montemayor C, Bhattacharya P, Mukherjee K, Flegel WA. ABO, Rhesus, and Kell Antigens, Alleles, and Haplotypes in West Bengal, India. Transfus Med Hemother. 2018 Jan;45(1):62-66.
11. Romphruk AV, Butryojantho C, Jirasakonpat B, Junta N, Srichai S, Puapairoj C, et al. Phenotype frequencies of Rh (C, c, E, e), M, Mia and Kidd blood group systems among ethnic Thai blood donors from the north-east of Thailand. Int J Immunogenet. 2019;46(3):160–5.
12. Learoyd P. The history of blood transfusion prior to the 20th century--part 1. Transfus Med. 2012 Oct;22(5):308-14.
13. Giangrande, P. L. The history of blood transfusion: Historical Review. British Journal of Haematology, 2000; 110(4), 758–767.
14. Harvey W. Exercitatio Anatomica de Motu Cordis et Sanguinis in Animalibus [An Anatomical Exercise on the Motion of the Heart and Blood in Animals]. Frankfurt: William Fitzer; 1628.
15. Lower R. Tractatus de Corde [A Treatise on the Heart]. Oxford: Sheldonian Theatre; 1669.
16. Denis JB. A letter concerning a new way of curing sundry diseases by transfusion of blood. Philosophical Transactions of the Royal Society of London. 1667;2:617–23.

17. Blundell J. Observations on transfusion of blood. Lancet. 1829;1(1):321–4.
18. Lane SA, Blundell J. Transfusion of blood in the treatment of haemophilia. Lancet. 1840;1:185–7.
19. Landsteiner K. Zur Kenntnis der antifermentativen, lytischen und agglutinierenden Wirkungen des Blutserums und der Lymphe. Zentralbl Bakteriol. 1900;27:357–62.
20. Decastello A, Sturli A. Ueber die Isoagglutinine im Serum gesunder und kranker Menschen. Munch Med Wochenschr. 1902;49:1090–5.
21. Hektoen L. The results of the blood transfusion experiments. JAMA. 1907;49(24):2018–20.
22. Carrel A. The transplantation of veins and organs. Am J Med Sci. 1908;136(5):811–23.
23. Lee R. Studies on universal blood donors and recipients. J Exp Med. 1912;16(1):77–82.
24. Hustin A. Note sur un nouveau procédé de transfusion. Bull Acad R Med Belg. 1914;78:450–63.
25. Rous F, Turner JR. The preservation of living red blood cells in vitro. J Exp Med. 1916;23(2):219–37.
26. Bogdanov N. Organization of the first blood bank. Sovetskaya Meditsina. 1932;8:3–8.
27. Stetson RE, Levine P. An unusual case of intra-group agglutination. J Clin Invest. 1939;18(6):647–53.
28. Landsteiner K, Wiener AS. An agglutinable factor in human blood recognized by immune sera for rhesus blood. Proc Soc Exp Biol Med. 1940;43(1):223–4.
29. Cohn EJ. A system for the separation of the components of human blood: J Am Chem Soc. 1940;62(12):3396–400.
30. Elliott J. A vacuum bottle for blood fraction storage. Am J Clin Pathol. 1940;10(2):87–92.

31. Beeson PB. Jaundice occurring one to four months after transfusion of blood or plasma. JAMA. 1940;114(8):1103–9.
32. Smith AU. The use of glycerol for the freezing and thawing of red blood cells. Biochem J. 1950;46(5):564–70.
33. Walter C, Murphy WP Jr. Use of plastic bags for the collection and storage of blood. Surgery. 1950;28(1):123–9.
34. Loutit JF, Mollison PL. The use of a refrigerated centrifuge in blood component therapy. Br Med J. 1953;2(4840):724–7.
35. American Association of Blood Banks. Standards for blood banks and transfusion services. 1st ed. Washington, DC: AABB Press; 1958.
36. Perutz MF. Structure of haemoglobin. Nature. 1959;183(4671):293–5.
37. Farhud DD, Zarif Yeganeh M. A brief history of human blood groups. Iran J Public Health. 2013;42(1):1-6. Epub 2013 Jan 1.
38. American Society of Hematology." Milestones in Transfusion Medicine,2008,
39. Cowan H: The discovery of human blood groups: Karl Otto Landsteiner . Br J Card Nurs. 2017, 12:290-2.
40. Barry, Carey L. “Immunohematology and Transfusion Medicine.” Physician Assistant Clinics, vol. 4, no. 3, 2019, pp. 591–607.
41. Mohd Noor NH, Siti Asmaa MJ. Karl Landsteiner (1868-1943): A Versatile Blood Scientist. Cureus. 2024 Sep 7;16(9):e68903.
42. Levine P, Stetson RE. Landmark article July 8, 1939. An unusual case of intra-group agglutination. By Philip Levine and Rufus E Stetson. JAMA. 1984 Mar 9;251(10):1316-7.

43. Neil D. Avent, Marion E. Reid; The Rh blood group system: a review. *Blood* 2000; 95 (2): 375–387.

44. TIPPETT P, SANGER R. Observations on subdivisions of the Rh antigen D. Vox Sang. 1962;7:9-13.

45. Usman, Mukhtar J., et al. "Comparative Study of ABO and RH Blood Grouping by Slide, Test Tube and Gel Card Methods." International Journal of Contemporary Pathology, vol. 2, no. 2, 2016, p. 23,

46. Lapierre Y, Rigal D, Adam J, Josef D, Meyer F, Greber S, Drot C. The gel test: a new way to detect red cell antigen-antibody reactions. Transfusion. 1990 Feb;30(2):109-13.

47. Yip SP. Sequence variation at the human ABO locus. Ann Hum Genet. 2002 Jan;66(Pt 1):1-27.

48. Otsu M, Tanabe Y, Iwakiri A, Arima K, Uchiyama A, Yamamoto M, Ohtani S, Endo H, Komoto M, Miyazaki K. A report on a modified protocol for flow cytometry-based assessment of blood group erythrocyte antigens potentially suitable for analysis of weak ABO subgroups. Transfusion. 2023 Mar;63(3):463-469.

49. Harmening DM. *Modern Blood Banking and Transfusion Practices*. 7th ed. Philadelphia: F.A. Davis Company; 2018.

50. Babic AM, Hillyer CD. Overview of adverse events and outcomes following transfusion. In: Shaz BH, Hillyer CD, Roshal M, Abrams CS, editors. *Transfusion Medicine and Hemostasis.* 2nd ed. Elsevier; 2013. p. 383-387.

51. Ackfeld T, Schmutz T, Guechi Y, Le Terrier C. Blood Transfusion Reactions-A Comprehensive Review of the Literature including a Swiss Perspective. J Clin Med. 2022 May 19;11(10):2859.

52. Tariq F, Ashfaq J, Ahmed R, Fatima N, Ahmed Y, Borhany M. The frequency of Rh phenotype and its probable genotype. Cureus. 2022;14(6):e25775

53. Gupta I, Kumar R, Bharadawaj A, Raturi G. Prevalence of Rh phenotype in voluntary blood donors of Uttarkhand. J Dent Med Sci. 2018;17:37–41.

54. Makroo RN, Bhatia A, Gupta R, Phillip J. Prevalence of Rh, Duffy, Kell, Kidd & MNSs blood group antigens in the Indian blood donor population. Indian J Med Res. 2013 Mar;137(3):521-6.

55. Gundrajukuppam DK, Vijaya SB, Rajendran A, Sarella JD. Prevalence of Principal Rh Blood Group Antigens in Blood Donors at the Blood Bank of a Tertiary Care Hospital in Southern India. J Clin Diagn Res. 2016 May;10(5):EC07-10.

56. Pahuja S, Jain S, Nain M, Goel R, Sehgal S, Jain M. Assessment of rhesus and kell blood group antigens, phenotypes, and their allelic frequencies in North Indian blood donors. Asian J Transfus Sci. 2020 Jul-Dec;14(2):137-141.

57. Owaidah AY, Naffaa NM, Alumran A, Alzahrani F. Phenotype Frequencies of Major Blood Group Systems (Rh, Kell, Kidd, Duffy, MNS, P, Lewis, and Lutheran) Among Blood Donors in the Eastern Region of Saudi Arabia. J Blood Med. 2020 Feb 13;11:59-65.

58. Yu Y, Ma C, Sun X, Guan X, Zhang X, Saldanha J, Chen L, Wang D. Frequencies of red blood cell major blood group antigens and phenotypes in the Chinese Han population from Mainland China. Int J Immunogenet. 2016 Aug;43(4):226-35.

59. Reid ME, Lomas-Francis C, Olsson ML. The Blood Group Antigen Facts Book. 3rd ed. New York: Academic Press; 2012. doi:10.1016/ C2011-0-69689-9

ANNEXURE- I

Institutional Ethical Clearance Certificate

BLDE
(DEEMED TO BE UNIVERSITY)
Declared as Deemed to be University u/s 3 of UGC Act, 1956
Accredited with 'A' Grade by NAAC (Cycle-2)
The Constituent College
SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

BLDE (DU)/IEC/ 931/2023-24 10/4/2023

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this University met on **Saturday, 18th March, 2023 at 11.30 a.m. in the CAL Laboratory, Dept. of Pharmacology,** scrutinized the Synopsis/ Research Projects of Post Graduate Student / Under Graduate Student /Faculty members of this University /Ph.D. Student College from ethical clearance point of view. After scrutiny, the following original/ corrected and revised version synopsis of the thesis/ research projects has been accorded ethical clearance.

TITLE: "STUDY OF DISTRIBUTION OF PRINCIPAL Rh BLOOD GROUP ANTIGENS & THEIR PHENOTYPE IN BLOOD DONORS".

NAME OF THE STUDENT/PRINCIPAL INVESTIGATOR: DR. SIDHARTHA SANKAR RAJ

NAME OF THE GUIDE: DR.VIJAYALAXMI PATIL , ASSOCIATE PROFESSOR, DEPT. OF PATHOLOGY.

Dr. Santoshkumar Jeevangi
Chairperson
IEC, BLDE (DU),
VIJAYAPURA

Chairman,
Institutional Ethical Committee,
BLDE (Deemed to be University)
Vijayapura

Dr.Akram A. Naikwadi
Member Secretary
IEC, BLDE (DU),
VIJAYAPURA

MEMBER SECRETARY
Institutional Ethics Committee
BLDE (Deemed to be University)
Vijayapura-586103. Karnataka

Following documents were placed before Ethical Committee for Scrutinization.

- Copy of Synopsis/Research Projects
- Copy of inform consent form
- Any other relevant document

Smt. Bangaramma Sajjan Campus, B. M. Patil Road (Sholapur Road), Vijayapura - 586103, Karnataka, India.
BLDE (DU): Phone: +918352-262770, Fax: +918352-263303, Website: www.bldedu.ac.in, E-mail:office@bldedu.ac.in
College: Phone: +918352-262770, Fax: +918352-263019, E-mail: bmpmc.principal@bldedu.ac.in

ANNEXURE II

B.L.D.E(DEEMED TO UNIVERSITY) SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTER, VIJAYAPURA-586103

INFORMED CONSENT FOR PARTICIPATION IN DISSERTATION/RESEARCH

I, the undersigned, _____, S/O D/O W/O _____, aged ____years, ordinarily resident of _____ do hereby state/declare that Dr. Sidhartha Sankar Raj of Shri B. M. Patil Medical College and Hospital has examined me thoroughly on _____ and it has been explained to me in my language that I am suffering from a disease (condition). Further Doctor informed me that he/she is conducting dissertation/research titled **"STUDY OF DISTRIBUTION OF PRINCIPAL Rh BOOD GROUP ANTIGENS AND THEIR PHENOTYPE IN BLOOD DONORS"** under the guidance of Dr. Vijayalaxmi S. Patil requesting my participation in the study.

Further Doctor has informed me that my participation in this study will help in the evaluation of the results of the study which is a useful reference for the treatment of other similar cases soon, and I may be benefited in getting relieved from suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept secret and not assessed by a person other than me or my legal hirer except for academic purposes. The Doctor did inform me that though my participation is purely voluntary, based on the information given by me, I can ask for any clarification during treatment/study related to diagnosis, the procedure of treatment, the result of treatment, or prognosis. At the same time, , I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged. After understanding the nature of the dissertation or research, diagnosis made, and mode of treatment, I the undersigned Shri/Smt _____ under my fully conscious state of mind agree to participate in the said research/dissertation.

Signature of the Patient

Signature of the Doctor

Witness

1)

2)

B.L.D.E (DEEMED TO BE UNIVERSITY) ಶ್ರೀ ಬಿ.ಎಂ.ಪಟ್ಟೀಲ್ ಮೆಡಿಕಲ್ ಕಾಲೇಜು, ಆಸ್ಪತ್ರೆ ಮತ್ತು ಸಂಶೋಧನಾ ಕೇಂದ್ರ, ವಿಜಯಪುರ-586103

ಪ್ರಬಂಧ/ಸಂಶೋಧನೆಯಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ಮಾಹಿತಿ ಪಡೆದ ಸಮ್ಮತಿ

ನಾನು, ಕೆಳಗಿನವರು_____ ಸಹಿಯಿಟ್ಟವರು, ಮಗ/ಮಗಳು/ಪತ್ನಿಯ _____ ವಯಸ್ಸು _____ವರ್ಷಗಳು, ಸಾಮಾನ್ಯವಾಗಿ ನಿವಾಸಿಸುವ ಸ್ಥಳದ ಹೆಸರು_____, ಇಲ್ಲಿ ಹೇಳಿದ್ದೇನೆ/ಘೋಷಿಸುತ್ತೇನೆ ಡಾಕ್ಟರ್ ಹೆಸರು Dr. Sidhartha Sankar Raj ಅವರು ಆಸ್ಪತ್ರೆ ಹೆಸರು Shri B.M. Patil Medical College ಅವರು ನನ್ನನ್ನು ಪೂರ್ಣವಾಗಿ ಪರೀಕ್ಷಿಸಿದರು ದಿನಾಂಕದಲ್ಲಿ_____ ಸ್ಥಳ ಹೆಸರು_____ ಮತ್ತು ನನಗೆ ನನ್ನ ಭಾಷೆಯಲ್ಲಿ ವಿವರಿಸಲಾಗಿದೆ ನಾನು ಒಂದು ರೋಗ (ಸ್ಥಿತಿ) ಅನುಭವಿಸುತ್ತಿದ್ದೇನೆ. ಮುಂದುವರಿದು ಡಾಕ್ಟರ್ ನನಗೆ ತಿಳಿಸಿದ್ದಾರೆ ಅವರು ಒಂದು ಪದ್ಧತಿ/ಸಂಶೋಧನೆ ನಡೆಸುತ್ತಿದ್ದಾರೆ ಶೀರ್ಷಿಕೆಯುಳ್ಳ **"STUDY OF DISTRIBUTION OF PRINCIPAL Rh BOOD GROUP ANTIGENS AND THEIR PHENOTYPE IN BLOOD DONORS"** ಡಾಕ್ಟರ್ Dr. Vijayalaxmi S. Patil ಮಾರ್ಗದರ್ಶನದಲ್ಲಿ ನನ್ನ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಯನ್ನು ಕೇಳಿದ್ದಾರೆ ಅಧ್ಯಯನದಲ್ಲಿ.

ಡಾಕ್ಟರ್ ನನಗೆ ಇದನ್ನು ಕೂಡಾ ತಿಳಿಸಿದ್ದಾರೆ ಈ ಕ್ರಮದ ನಡುವಲ್ಲಿ ಪ್ರತಿಕೂಲ ಫಲಿತಾಂಶಗಳನ್ನು ಎದುರಿಸಬಹುದು. ಮೇಲೆ ಹೇಳಿದ ಪ್ರಕಟಣೆಗಳಲ್ಲಿ, ಅಧಿಕಾಂಶವು ಚಿಕಿತ್ಸಿಸಬಹುದಾದರೂ ಅದನ್ನು ನಿರೀಕ್ಷಿಸಲಾಗುತ್ತಿಲ್ಲ ಆದ್ದರಿಂದ ನನ್ನ ಸ್ಥಿತಿಯ ಹಿರಿದಾಗುವ ಅವಕಾಶವಿದೆ ಮತ್ತು ಅಪರೂಪದ ಸಂದರ್ಭಗಳಲ್ಲಿ ಅದು ಮರಣಕಾರಕವಾಗಿ ಪರಿಣಮಿಸಬಹುದು ಹೊಂದಿದ ರೋಗನಿರ್ಧಾರ ಮತ್ತು ಯಥಾಶಕ್ತಿ ಚಿಕಿತ್ಸೆ ಮಾಡಲು ಹೊಂದಿದರೂ, ಮುಂದುವರಿದು ಡಾಕ್ಟರ್ ನನಗೆ ತಿಳಿಸಿದ್ದಾರೆ ನನ್ನ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆ ಈ ಅಧ್ಯಯನದ ಫಲಿತಾಂಶಗಳ ಮೌಲ್ಯಮಾಪನದಲ್ಲಿ ಸಹಾಯಕವಾಗುತ್ತದೆ ಇತರ ಸಮಾನ ಪ್ರಕರಣಗಳ ಚಿಕಿತ್ಸೆಗೆ ಉಪಯುಕ್ತ ಉಲ್ಲೇಖವಾಗಿದೆ, ಮತ್ತು ನಾನು ಅನುಭವಿಸುವ ರೋಗದಿಂದ ವಿಮುಕ್ತಿ ಅಥವಾ ಗುಣಮುಖಗೊಳ್ಳುವಲ್ಲಿ ನನಗೆ ಪ್ರಯೋಜನವಾಗಬಹುದು.

ಡಾಕ್ಟರ್ ನನಗೆ ಇದನ್ನು ಕೂಡಾ ತಿಳಿಸಿದ್ದಾರೆ ನನ್ನಿಂದ ನೀಡಿದ ಮಾಹಿತಿ, ಮಾಡಿದ ಪರಿಶೀಲನೆಗಳು / ಫೋಟೋಗ್ರಾಫ್‌ಗಳು / ವೀಡಿಯೋ ಗ್ರಾಫ್‌ಗಳು ನನ್ನ ಮೇಲೆ ತೆಗೆದುಕೊಳ್ಳಲಾಗುವ ಅನ್ವೇಷಕರು ರಹಸ್ಯವಾಗಿ ಇಡುವರು ಮತ್ತು ನಾನು ಅಥವಾ ನನಗೆ ಕಾನೂನು ದೃಷ್ಟಿಯಲ್ಲಿ ಸಂಬಂಧಿತರನ್ನು ಹೊರತುಪಡಿಸಿ ಇತರ ವ್ಯಕ್ತಿಯಿಂದ ಮೌಲ್ಯಮಾಪನ ಮಾಡಲಾಗುವುದಿಲ್ಲ. ಡಾಕ್ಟರ್ ನನಗೆ ತಿಳಿಸಿದ್ದಾರೆ ನನ್ನ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆ ಶುದ್ಧವಾಗಿ ಸ್ವೇಚ್ಛಾಯಿತ, ನನ್ನಿಂದ ನೀಡಿದ ಮಾಹಿತಿಯ ಆಧಾರದ ಮೇಲೆ, ಚಿಕಿತ್ಸೆ / ಅಧ್ಯಯನದ ಸಂಬಂಧದಲ್ಲಿ ರೋಗನಿರ್ಧಾರ, ಚಿಕಿತ್ಸೆಯ ವಿಧಾನ, ಚಿಕಿತ್ಸೆಯ ಫಲಿತಾಂಶ ಅಥವ ಭವಿಷ್ಯದ ಪ್ರವೃತ್ತಿಗಳು ಬಗ್ಗೆ ಯಾವುದೇ ಸ್ಪಷ್ಟತೆ ಕೇಳಬಹುದು. ಅದೇ ಸಮಯದಲ್ಲಿ ನನಗೆ ತಿಳಿಸಲಾಗಿದೆ ನಾನು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನನ್ನ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಯನ್ನು ನಿಲ್ಲಿಸಬಹುದು ನಾನು ಬಯಸಿದರೆ ಅಥವಾ ಅನ್ವೇಷಕರು ಅಧ್ಯಯನದಿಂದ ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ನನ್ನನ್ನು ನಿಲ್ಲಿಸಬಹುದು.

ಪ್ರಬಂಧ ಅಥವಾ ಸಂಶೋಧನೆಯ ಸ್ವಭಾವ, ಮಾಡಿದ ರೋಗನಿರ್ಧಾರ ಮತ್ತು ಚಿಕಿತ್ಸೆಯ ವಿಧಾನವನ್ನು ಅರ್ಥಮಾಡಿಕೊಂಡು, ನಾನು ಕೆಳಗಿನ ಶ್ರೀ / ಶ್ರೀಮತಿ_____ ನನ್ನ ಪೂರ್ಣವಾದ ಪ್ರಜ್ಞೆಯ ಸ್ಥಿತಿಯಲ್ಲಿ ಹೇಳಿದ ಸಂಶೋಧನೆ / ಪ್ರಬಂಧದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ಒಪ್ಪುತ್ತೇನೆ.

ರೋಗಿಯ ಸಹಿ ಡಾಕ್ಟರನ ಸಹಿ

ಸಾಕ್ಷಿಗಳು

1)
2)

ANNEXURE- lll

PROFORMA

NAME:

OP/IP No:

AGE: SEX:

Address for communication:

A) Have you donated previously?

B) If yes, how many occasions:

C) Your blood group:

1. Did you have discomfort during/after donation?

2. Do you feel well today? Yes/no

3. Did you have something to eat in the last 4 hrs?

4. Did you sleep well last night? Yes/no

5. Have you any reason to believe that you may be infected by either Hepatitis, Malaria, HIV/Aids and or/ Venaral disease?

6. In the last 6 months have you had any history of following: Unexplained weight loss? Repeated Diarrhoea

7. In the last 6 months have you had any history of following?

Tattooing Ear Piercing

8. Do you suffer from or have suffered from any of the following diseases?

Heart Disease Lung Disease Tuberculosis Epilepsy

Abnormal Bleeding Tendency Jaundice Fainting Spells Typhoid (Last 1 Yrs.)

9. Are you taking or have taken any of these in the past 72 Hrs?

Antibiotics Aspirin Vaccinations Dog bite/Rabies

Vaccine (1 Yrs.) Swollen glands Dental extraction kidney disease

Malaria	Hepatitis B/C	Allergic Disease	Sexual Trans. Disease
Alcohol	Steroid's	Diabetes	Cancer/Malignancy

Continuous low-grade fever

When last Time of last meal:

OCCUPATION

DOB

ABO Blood Group:

Rh blood group:

KEY TO MASTER CHART

Sl No.	Description
Sl No.	Serial Number
M	Male
F	Female
+VE	Positive
-VE	Negative

MASTER CHART

Blood Grouping And Sub Grouping									
Sl. No	Age	Sex	Blood Group	Reaction with Antigen					Phenotype
				D	C	E	c	e	
1	21	M	A+ve	+	+	-	+	+	DCcee
2	19	M	O+ve	+	+	-	-	+	DCCee
3	18	M	AB +ve	+	+	-	-	+	DCCee
4	46	M	A+ve	+	+	-	-	+	DCCee
5	19	M	AB +ve	+	+	-	-	+	DCCee
6	18	M	A+ve	+	+	-	-	+	DCCee
7	18	M	A+ve	+	+	-	+	+	DCcee
8	18	F	A+ve	+	+	-	+	+	DCcee
9	42	M	O+ve	+	+	-	-	+	DCCee
10	19	F	AB +ve	+	+	-	-	+	DCCee
11	18	F	B+ve	+	+	-	-	+	DCCee
12	18	F	O+ve	+	+	-	-	+	DCCee
13	18	F	O+ve	+	+	-	-	+	DCCee
14	18	F	O+ve	+	-	+	+	+	DCCee
15	19	M	B+ve	+	+	-	-	+	DCCee
16	21	M	AB +ve	+	+	-	+	+	DCCee
17	19	M	A+ve	+	+	-	+	+	DCCee
18	18	M	O+ve	+	+	-	-	+	DCCee
19	19	F	A+ve	+	+	+	+	+	DCCee
20	24	M	B+ve	+	+	-	-	+	DCCee
21	48	M	O+ve	+	+	-	+	+	DCCee
22	35	M	A-ve	-	-	-	+	+	DCCee
23	24	M	AB +ve	+	+	+	+	+	DCCee
24	39	M	B+ve	+	+	-	-	+	DCCee
25	32	M	A+ve	+	+	-	-	+	DCCee
26	32	M	B+ve	+	+	-	-	+	DCCee

27	22	M	AB +ve	+	-	+	+	+	DCCee
28	36	M	AB +ve	+	+	-	-	+	DCCee
29	36	M	A+ve	+	+	-	+	+	DCCee
30	30	M	B+ve	+	+	-	-	+	DCCee
31	25	M	A+ve	+	+	+	+	+	DCCee
32	23	F	A+ve	+	+	-	-	+	DCCee
33	24	M	B+ve	+	+	-	+	+	DCcee
34	38	M	AB-ve	-	-	-	+	+	dccee
35	34	M	B+ve	+	+	-	+	+	DCcee
36	36	M	A+ve	+	+	-	+	+	DCcee
37	39	M	O+ve	+	+	-	+	+	DCcee
38	32	M	B+ve	+	+	-	+	+	DCcee
39	31	M	A+ve	+	+	-	-	+	DCCee
40	19	M	AB+ve	+	+	-	-	+	DCCee
41	27	M	A+ve	+	+	-	-	+	DCCee
42	34	M	O+ve	+	+	-	+	+	DCcee
43	28	M	O+ve	+	+	-	-	+	DCCee
44	28	M	B+ve	+	-	+	+	+	DccEe
45	34	M	A-ve	-	-	-	+	+	dccee
46	35	M	A+ve	+	+	-	-	+	DCCee
47	46	M	O-ve	-	-	-	+	+	dccee
48	23	M	B+ve	+	+	-	-	+	DCCee
49	35	M	B-ve	-	-	-	-	+	dcCee
50	31	M	A+ve	+	+	-	-	+	DCCee
51	33	M	B-ve	-	-	-	+	+	dccee
52	38	M	A+ve	+	+	-	-	+	DCCee
53	34	M	O+ve	+	+	-	+	+	DCcee
54	35	M	O+ve	+	+	-	+	+	DCcee
55	35	M	A+ve	+	+	+	+	+	DCcEe
56	20	M	A+ve	+	+	-	+	+	DCcee
57	30	M	B+ve	+	+	-	-	+	DCCee

58	33	M	B+ve	+	+	-	+	+	DCcee
59	32	M	O+ve	+	+	-	+	+	DCcee
60	48	M	O+ve	+	+	+	+	+	DCcEe
61	24	M	B+ve	+	+	-	+	+	DCcee
62	38	M	A+ve	+	+	-	-	+	DCCee
63	42	M	B+ve	+	+	-	+	+	DCcee
64	30	M	B+ve	+	+	-	+	+	DCcee
65	37	M	O+ve	+	-	-	+	+	Dccee
66	20	M	B+ve	+	+	-	-	+	DCCee
67	42	M	O+ve	+	+	-	+	+	DCcee
68	21	M	O+ve	+	+	-	-	+	DCCee
69	23	M	O+ve	+	+	-	-	+	DCCee
70	22	M	O+ve	+	+	-	+	+	DCcee
71	42	M	B+ve	+	+	-	-	+	DCCee
72	44	M	B+ve	+	+	-	+	+	DCcee
73	32	M	A+ve	+	+	-	-	+	DCCee
74	29	M	B+ve	+	+	-	+	+	DCcee
75	26	M	A+ve	+	+	-	+	+	DCcee
76	37	M	A+ve	+	+	-	+	+	DCcee
77	29	M	O+ve	+	+	-	+	+	DCcee
78	28	F	O+ve	+	+	-	+	+	DCcee
79	19	M	A+ve	+	+	-	-	+	DCCee
80	31	M	O+ve	+	+	-	-	+	DCCee
81	49	M	O+ve	+	+	-	-	+	DCCee
82	22	M	O+ve	+	+	-	+	+	DCcee
83	46	M	B+ve	+	+	-	-	+	DCCee
84	34	M	O+ve	+	+	-	-	+	DCCee
85	35	M	O+ve	+	+	-	-	+	DCCee
86	40	M	O+ve	+	+	-	-	+	DCCee
87	28	M	A+ve	+	+	-	+	+	DCcee
88	22	F	AB+ve	+	+	-	+	+	DCcee

89	31	F	A+ve	+	+	-	-	+	DCCee
90	38	M	O+ve	+	-	+	+	+	DccEe
91	31	M	A-ve	-	-	-	+	+	dccee
92	37	M	O-ve	-	-	-	+	+	dccee
93	34	M	B+ve	+	+	-	+	+	DCcee
94	37	M	A+ve	+	+	+	+	+	DCcEe
95	29	M	B+ve	+	+	+	+	+	DCcEe
96	40	M	A+ve	+	+	+	+	+	DCcEe
97	21	F	A+ve	+	+	-	-	+	DCCee
98	18	M	B+ve	+	+	-	-	+	DCCee
99	18	M	O+ve	+	+	-	+	+	DCcee
100	21	F	O+ve	+	+	-	+	+	DCcee
101	20	M	A+ve	+	-	+	+	+	DccEe
102	20	M	O+ve	+	+	-	-	+	DCCee
103	26	F	A+ve	+	+	-	+	+	DCcee
104	19	M	AB-ve	-	-	-	+	+	dccee
105	19	M	A+ve	+	+	+	+	+	DCcEe
106	42	M	A+ve	+	+	-	+	+	DCcee
107	35	M	O+ve	+	+	-	+	+	DCcee
108	20	M	B-ve	-	-	-	+	+	dccee
109	18	F	B+ve	+	+	-	-	+	DCCee
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114	18	M	B+ve	+	+	-	+	+	DCcee
115	18	M	AB+ve	+	+	-	+	+	DCcee
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117	18	M	O+ve	+	+	-	+	+	DCcee
118	18	M	B+ve	+	+	-	-	+	DCCee
119	22	M	O+ve	+	+	-	-	+	DCCee

120	23	M	O+ve	+	+	-	-	+	DCCee
121	36	M	O+ve	+	+	+	+	+	DCcEe
122	19	M	B-ve	-	-	-	+	+	dccee
123	21	M	B-ve	-	-	-	+	+	dccee
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133	22	M	B-ve	-	-	-	+	+	dccee
134	21	F	O+ve	+	+	-	+	+	DCcee
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141	21	M	O+ve	+	+	-	+	+	DCcee
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143	54	M	B+ve	+	+	-	-	+	DCCee
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150	18	M	O+ve	+	+	-	-	+	DCCee

151	18	M	B+ve	+	+	-	-	+	DCCee
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366	31	M	A+ve	+	+	-	+	+	DCcee
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379	25	M	O+ve	+	+	-	-	+	DCCee
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381	25	M	O+ve	+	+	-	-	+	DCCee
382	21	M	O+ve	+	+	-	-	+	DCCee

Siddharth

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