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Preface

This Immunohaematology Lecture Note is prepared to meet the needs of Medical Laboratory professionals and Blood Bank personnel for a material that comprise the theories and laboratory techniques concerning blood transfusion service. The Lecture Note is also important for health professionals in other disciplines as a reference related to blood transfusion therapy. In addition, this material alleviates the problems that have been faced due to shortage of material on the subject matter as it considers the actual level in most Blood Bank laboratories in Ethiopia. It further solves the problem of scarcity of books for the instructors.

Acknowledgments

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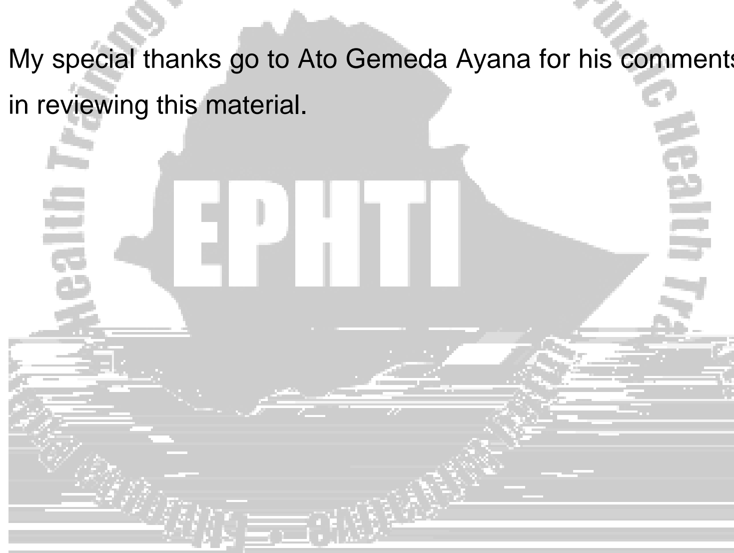

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Abbreviations

ACD - Acid citrate dextrose

AHG - Anti human globulin

AIDS - Acquired immuno deficiency syndrome

AIHA - Autoimmune hemolytic anemia

Ab - Antibody

Ag - Antigen

ATP - Adenosine triphosphate

CPD - Citrate phosphate dextrose

CPDA - Citrate phosphate dextrose adenine

DAT - Direct antiglobuline test

2,3, DPG 2,3 diphosphoglycerate

EDTA - Ethyldiamine tetra acetic acid

HCT - Hematocrit

Hgb - Hemoglobin

HDN - Hemolytic disease of new born

HIV - Human immuno virus

Ig - Immunologlobulin

IAT - Indirect antiglobulin test

KB - Kleihaner- Betke

Lab - Laboratory

MW - Molecular weight

NRBC - Nucleated red blood cell

PCV - Packed cell volume

QAP - Quality assurance programme

RBC - Red blood cell
Rpm - revolution per minute
Rh - Rhesus
RT - Room temperature
Sp.gr - Specific gravity

CHAPTER ONE

INTRODUCTION TO IMMUNOHAEMATOLOGY

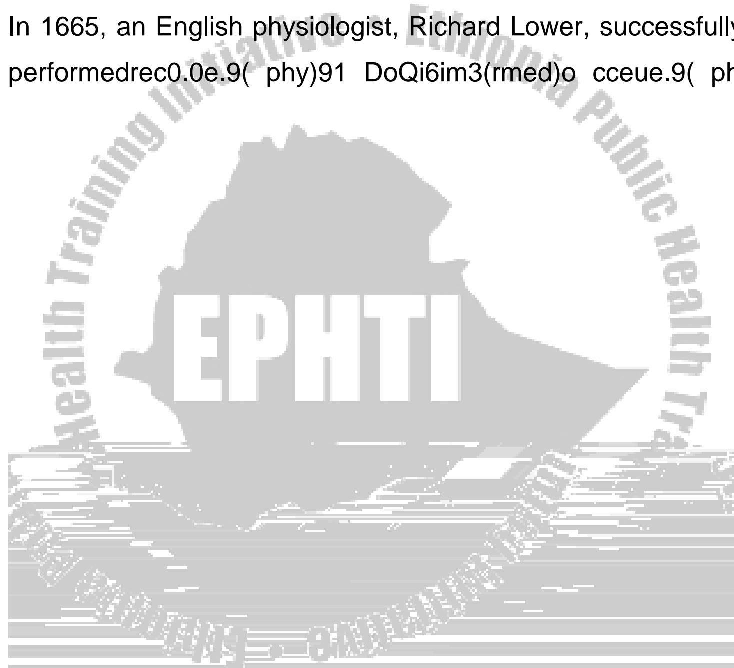
Learning Objectives

At the conclusion of the chapter, the student should be able to:

- Explain a brief history of the science of Immunohaematology
- Discuss the patterns of inheritance of A and B genes
- Describe the synthesis of H, A and B antigens
- Name the specific transferase for the A, B & H genes
- State the genotype of individuals with the Bombay phenotype
- State the characteristic genotype of secretor and non-secretor
- Identify the product or products found in the saliva of persons of variou

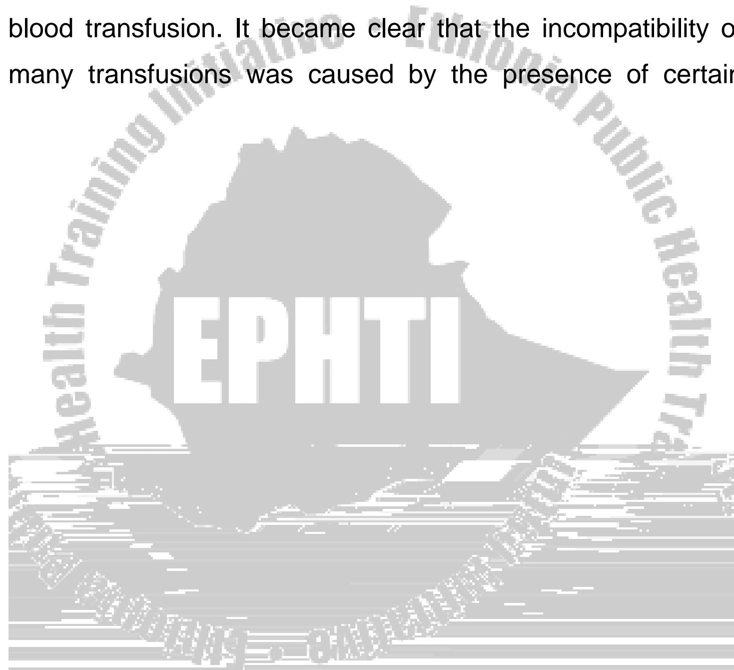
techniques related to modern transfusion therapy. Efforts to save human lives by transfusing blood have been recorded for several centuries. The era of blood transfusion, however, really began when William Harvey described the circulation of blood in 1616.

In 1665, an English physiologist, Richard Lower, successfully performedrec0.0e.9(phy)91 DoQi6im3(rmed)o cceue.9(phy)91 tao-phy5.4ue.9(TJ0r)8.5( )]TJ0 -



same species of animal) seemed to work about half the time but mostly the result was death.

Blood transfusions continued to produce unpredictable results, until Karl Landsteiner discovered the ABO blood groups in 1900, which introduced the immunological era of blood transfusion. It became clear that the incompatibility of many transfusions was caused by the presence of certain



chromosome is divided in to many small units called genes, which are important as they contain the different physical characteristics, which can be inherited including those of the blood groups.

Allomorphic genes (Alleles): Each gene has it own place called its locus along the length of the chromosome. However, a certain inherited characteristic can be represented by a group of genes, and the place or locus can be occupied by only one of these genes. Such genes are called alleles or allomorphic genes.

For example, every one belongs to one or other of the following blood groups: group A, group B, group O or group AB. Therefore, there are three allelomorphic genes which make up the ABO Blood group system such as gene A, gene B, and gene O. Only one of these alleles can occupy the special place or locus along the chromosomes for this blood group characteristic.

Body cells and mitosis: When body cells multiply they do so by producing identical new cells with 46 chromosomes. This process is called mitosis.

Sex cells and meiosis: When sex cells are formed either male or female the pairs of chromosomes do not multiply but

simply separate so that each of the new cells formed contains only 23 chromosomes not 46 as in the body cells. This process is called meiosis.

However, during fortification when the egg and sperm unite, the fertilized ovum receives 23 chromosomes from each sex cell half of these from the male and half from the female and thus will contain 46 chromosomes which again arrange them selves in pairs in the nucleus.

For example, a child who inherits gene A from its father and also gene A from its mother would be homozygous, where as a child who inherits gene A from its father and gene B from its mother would be heterozygous.

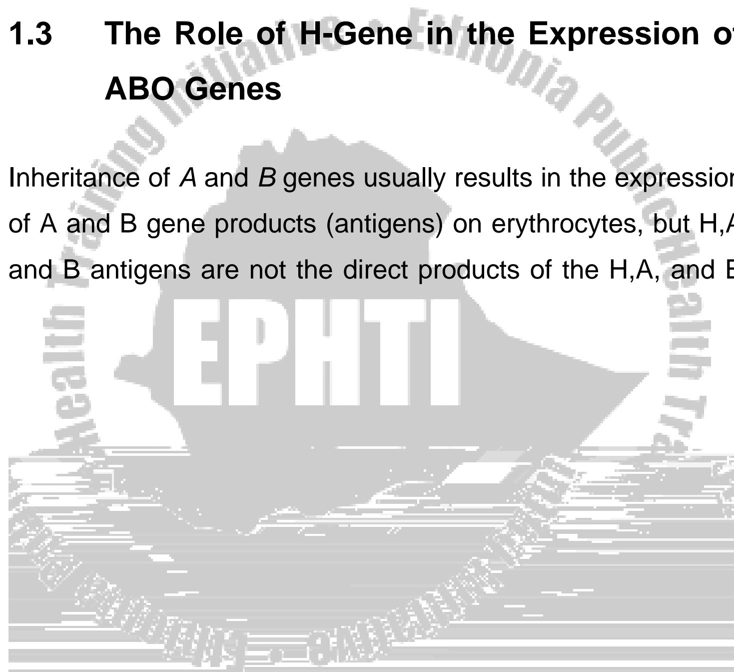
Dominant and recessive genes: A dominant gene will always show itself if it is present but a recessive gene will only show itself if there is no dominant one, that is if both genes are recessive.

For example, in the ABO blood group system the gene A and B are dominant over gene O. Thus if a child receives from its parents gene A and O it will belong to group A. In the same way if a child receives from its parents genes B and O it will belong to group B only if it receives gene O from both its parents will it belong to group O.

Genotype and phenotype: The genetic composition from a particular inherited characteristic is called the phenotype and the way this can be seen is called phenotype. Thus if a person is group A (phenotype) his phenotype could be either AA or AO.

1.3 The Role of H-Gene in the Expression of ABO Genes

Inheritance of *A* and *B* genes usually results in the expression of A and B gene products (antigens) on erythrocytes, but H,A and B antigens are not the direct products of the H,A, and B



- As predicted in Fig 1.1 the H gene (HH/Hh) encodes for an enzyme, which converts the precursor substance in red cells in to H substance (H antigen).
- A and B genes encode specific transferase enzymes which convert H substance in to A and B red cell antigens. Some H substance remains unconverted (the H substance is partly converted).
- O gene encodes for an inactive enzyme, which results in no conversion of the substance in-group O red cells. This indicates group O individual contains the greatest concentration of H antigen.
- Persons who do not inherit H gene (very rare hh genotype) are unable to produce H substance and therefore even when A and B genes are inherited, A & B antigens can not be formed . This rare group is referred to as O_h (Bombay group).

H Substance

Precursor Substance

Fig 1.1 ABO Genetic pathway

1.4 Secretors and Non-Secretors

The term secretor and non-secretor only refer to the presence or absence of water- soluble ABH antigen substances in body fluids (saliva, semen, urine, sweat, tears, etc). Every individual contains alcohol soluble antigens in body tissues and on the red cells, whether secretor or non-secretor, but secretors, in addition to this, possess the water soluble (glycoprotein) form of antigen, which appears in most body fluids.

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Review Questions

1. Briefly out line the historical background of blood transfusion.
2. What was the reason for the failure of attempted intra and inter species blood transfusions (relate this with the discovery of blood group by Karl Landsteiner).
3. Define the following terms:
   A. Chromosome
   B. Gene
   C. Dominant gene
   D. Phenotype
   E. Secretors
4. Explain why group O individuals contain the greatest concentration of H antigen.

CHAPTER TWO

PRINCIPLES OF ANTIGENS AND ANTIBODIES

Learning Objectives

At the conclusion of the chapter, the student should be able to:

- Define an antigen
- Explain the basic essential for antigenic substances
- Define an antibody
- List the classes of immunoglobulin
- Compare the characteristics of IgG, IgM and IgA
- Contrast between the natural and immune antibodies
- Explain the non- red cell- immune antibodies

2.1 Antigens

An antigen can be defined as any substance which, when introduced in to an individual who himself lacks the substance, stimulates the production of an antibody, and which, when mixed with the antibody, reacts with it in some observable way.

Foreign substances, such as erythrocytes, can be immunogenic or antigenic (capable of provoking an immune response) if their membrane contains a number of areas recognized as foreign. These are called antigenic determinants or epitopes.

The immunogenicity of a substance (relative ability of a substance to stimulate, the production of antibodies when introduced in to a subject lacking the substance) is influenced by a number of characteristics:

Foreignness: The substance should present, at least in part, a configuration that is unfamiliar to the organism. The greater the degree the antigenic determinant is recognized as non-self by an individual's immune system, the more antigenic it is.

Molecular weight: The antigen molecule must have a sufficiently high molecular weight. The larger the molecule, the greater is its likelihood of possessing unfamiliar antigenic determinant on its surface, and hence the better the molecule functions as an antigen.

Molecules with a molecular weight of less than 5000 fail to act as antigen, with 14,000 are poor antigens unless conjugated with adjuvant and with 40,000 or more are good antigens. High MW molecules of 500,000 or more are the best antigens.

However, physical size of the molecule is not a controlling factor. Since dextran (a carbohydrate) with a MW of 100,000 is not antigenic.

Structural stability: Structural stability is essential characteristic; structurally instable molecules are poor antigens, eg. Gelatin.

Structural complexity: The more complex an antigen is, the more effective it will be complex proteins are better antigens than large repeating polymers such as lipids, carbohydrates, and nucleic acid, which are relatively poor antigens.

Route of administration: In general, intravenous (in to the vein) and intraperitoneal (into the peritoneal cavity) routes offer a stronger stimulus than subcutaneous (beneath the skin) or intramuscular (in to the muscle) routes.

2.2 Antibodies

Antibodies are serum proteins produced in response to stimulation by a foreign antigen that is capable of reacting specifically with that antigen in an observable way. Five major immunoglobulin (Ig) classes exist; which are called IgG, IgA, IgM, IgD and IgE, with heavy chains gamma ( ) alpha ( ), mu (μ) delta(δ) , and epsilon( ) respectively. Each is unique and

possesses its own characteristic. Blood group antibodies are almost exclusively IgG, IgM and IgA.

Characteristics of immunoglobulin

IgG:

- Is the predominant immunoglobulin in normal serum, accounting for about 85% of the total immunoglobulin
- Is the only immunoglobulin to be transferred from mother to fetus, through the placenta, a fact that explains its role in the etiology of hemolytic disease of the new born (HDN)
- Is the smallest antibody which has a MW of 150,000
- Is capable of binding complement
- Is predominantly produced during the secondary immune response.

Sub classes of IgG: within the major immunoglobulin classes are variants known as sub classes. Four sub classes of IgG have been recognized on the basis of structural and serological differences and are known as IgG_1, IgG_2, IgG_3 and IgG_4. They also have different characteristics as shown in Table 2.1.

Table 2.1. IgG subtype characteristics

IgA:

- Ig A with a MW of 160,000 constitutes 10 to 15 % of the total circulatory immunoglobulin pool.
- Is the predominant immunoglobulin in secretions such as, tears, saliva, colostrum, breast milk, and intestinal secretions.
- Does not fix complement and is not transported across the human placenta.

2.2.1 Types of Antibodies

Based on their development, blood group antibodies are classified into Natural and Immune antibodies.

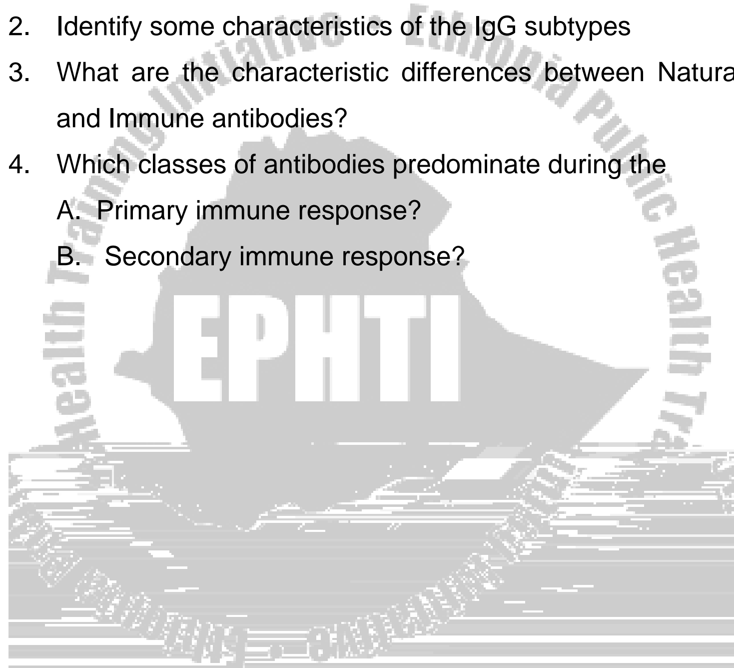
Natural antibodies: are red cell antibodies in the serum of an individual that are not provoked by previous red cell sensitization. But, it is believed that these antibodies must be the result of some kind of outside stimulus and the term naturally occurring gives an inaccurate connotation, so they are called non- red cell or non- red cell immune antibodies.

Characteristics

- Exhibit optimum in vitro agglutination when the antigen bearing erythrocytes are suspended in physiologic saline (0.85%) sodium chloride, sometimes referred to as complete antibodies.

Review Questions

1. Define:
   A. Antigen
   B. Antibody
   C. Immunogenicity
2. Identify some characteristics of the IgG subtypes
3. What are the characteristic differences between Natural and Immune antibodies?
4. Which classes of antibodies predominate during the
   A. Primary immune response?
   B. Secondary immune response?



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3.1 The Discovery of ABO Blood Group

In the 1900, a German Scientist Karl Landsteiner established the existence of the first known blood group system, the ABO system. Classification of the blood group was based on his observation of the agglutination reaction between an antigen on erythrocytes and antibodies present in the serum of individuals directed against these antigens. Where no agglutination had occurred, either the antigen or the antibody was missing from the mixture.

Landsteiner recognized the presence of two separate antigens, the A & B antigens. The antibody that reacted with the A antigens was known as anti A, and the antibody that reacted with the B antigen was known as anti B. Based on the antigen present on the red cells, he proposed three separate groups A, B & O. Shortly hereafter, von Decastello and Sturli identified a fourth blood group AB, by demonstrating agglutination of individuals red cells with both anti-A and anti-B.

3.2 Inheritance of the ABO Groups

In 1908, Epstein and Ottenberg suggested that the ABO blood groups were inherited characters. In 1924 Bernstein postulated the existence of three allelic genes. According to

the theory of Bernstein the characters A,B and O are inherited by means of three allelic genes, also called A,B and O . He also proposed that an individual inherited two genes, one from each parent, and that these genes determine which ABO antigen would be present on a person's erythrocytes. The O gene is considered to be silent (amorphic) since it does not appear to control the development of an antigen on the red cell. Every individual has two chromosomes each carrying either A, B or O, one from each parent, thus the possible ABO genotypes are AA, AO, BB, BO, AB and OO. ABO typing divides the population in to the four groups, group A, B, O and, AB, where the phenotype and the genotype are both AB (heterozygous), see Table 3.1.

Table 3.1 The ABO phenotypes and their corresponding genotypes

Phenotypes	**Genotypes**
A	AA
	AO
B	BB
	BO
O	OO
AB	AB

To illustrate the mode of inheritance, a particular mating, that in which a group A male mates with a group B female, is

considered. The group A male may be of genotype AA or AO and similarly the group B female may be of the genotype BB or BO; therefore within this one mating four possibilities exist, namely (a) AA with BB, (b) AA with BO, (c) AO with BB and (d) AO with BO, see Table 3.2.

- This mating can result in children of all four ABO groups or phenotypes although it is only in mating AO with BO that children of all four ABO groups can occur in the same family.
- This mating also shows that a knowledge of the groups of relatives will sometimes disclose the genotype of group A

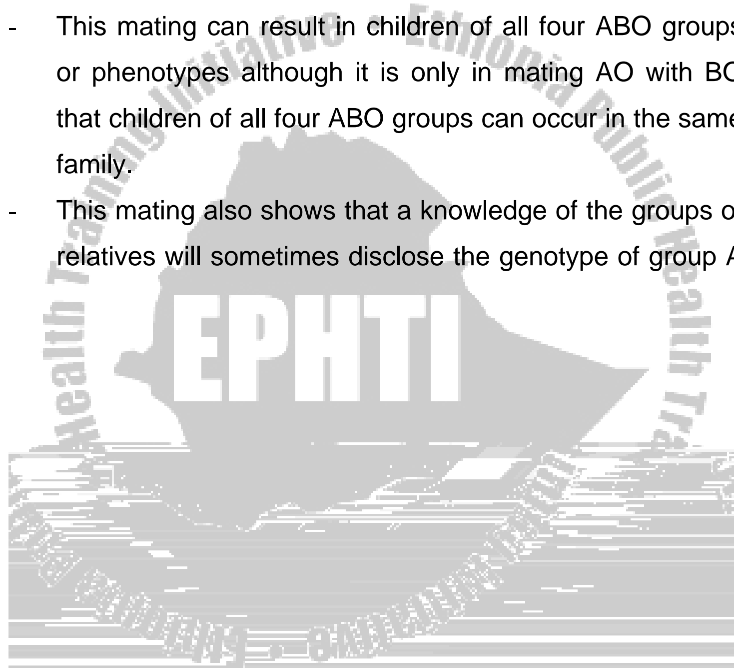

Table 3.2 The ABO mating with possible genotype and phenotype of children.

Mating		Children	
Phenotypes	**Genotypes**	**Genotypes**	**Phenotypes**
AxA	(1)AAxAA	(1)AA	A and O
	(2)AAxAO	(2)AA and AO	
	(3) AOxAO	(3)AA,AO and OO	
AxB	(1)AAxBB	(1)AB	A,B AB, and O
	(2)AAxBO	(2)AB and AO	
	(3)AOxBB	(3)AB and BO	
	(4)AOxBO	(4)AB,BO, AO, and OO	
AxAB	(1)AAxAB		

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A_1 and A_2 sub groups. Thompson's four-allele theory encompassed the four allelic genes, A_1, A_2, B and O. This four allelic genes give rise to six phenotypes: A_1, A_2, B, O, A_1B and A_2B and because each individual inherits one chromosome from each parent, two genes are inherited for each characteristic and these four allelic gene give rise to ten possible genotypes (table 3.3).

Table 3.3 ABO phenotypes and genotypes, including A_1 and A_2

Phenotypes	Genotypes
A_1	A_1A_1
	A_1A_2
	A_1O
A_2	A_2A_2
	A_2O
B	BB
	BO
A_1B	A_1B(or A_1B/O)
A_2B	A_2B(orA_2B/O)
O	OO

In group AB, the *A* gene is normally carried on one chromosome and the *B* gene on the other, each being co-dominant, although rare families have been described in

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This follows that taking all A_1xB mating together, all six phenotypes can occur. However, the finding of, for instance, a

Table 3.5 Frequency of ABO blood groups in different population

Examples	A%	B%	AB%	O%
Asian	28	27	5	40
African	26	21	4	49
Nepalese	33	27	12	28
Caucasian	40	11	4	45
Ethiopians (Blood donors)	31	23	6	40

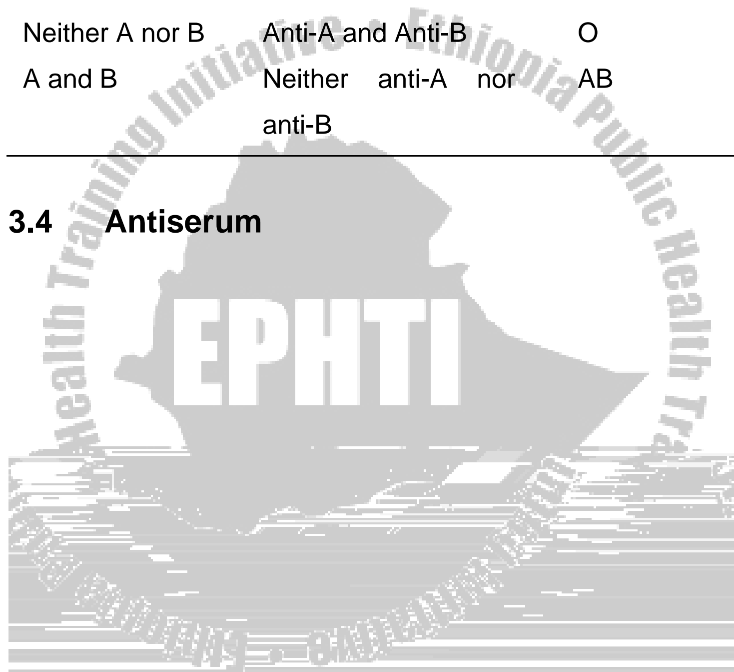
Whenever an antigen A and, or B is absent on the red cells, the corresponding antibody is found in the serum (Table 3.6)

- Individuals who possess the A antigen on their red cells possess anti- B in their serum.
- Individuals who possess the B antigen on their red cells possess anti A in their serum.
- Individuals who possess neither A nor B antigen have both anti A and anti- B in their serum.
- Individuals with both A and B antigens have neither anti A nor anti B in their serum.

Table 3.6. Classification of the ABO blood groups

Antigen on Red Cells	Antibodies in Serum	Blood Group
A	Anti-B	A
B	Anti-A	B
Neither A nor B	Anti-A and Anti-B	O
A and B	Neither anti-A nor anti-B	AB

3.4 Antiserum



Sources of antisera

- Animal inoculation in which animals are deliberately inoculated by known antigen and the resulting serum containing known antibody is standardized for use as antiserum.
- Serum is collected from an individual who has been synthesized to the antigen through transfusion, pregnancy or injection.
- Serum collected from known blood groups

Antisera requirements: Antiserum must meet certain requirements to be acceptable for use. In using antisera the manufacturer's instruction should always be followed. The antiserum has two be **specific:** does not cross react, and only reacts with its own corresponding antigen, **avid**: the ability to agglutinate red cells quickly and strongly, **stable:** maintains it specificity and avidity till the expiry date. It should also be clear, as turbidity may indicate bacterial contamination and free of precipitate and particles. It should be labeled and stored properly.

3.5 Manifestation and Interpretation of Antigen- Antibody reactions

The observable reactions resulting from the combination of a red cell antigen with its corresponding antibody are

agglutination and/ or haemolysis. Agglutination is the widely observed phenomenon in blood grouping.

Agglutination: is the clumping of particles with antigens on their surface, such as erythrocytes by antibody molecules that form bridges between the antigenic determinants. When antigens are situated on the red cell membrane, mixture with their specific antibodies causes clumping or agglutination of the red cells.

An agglutination in which the cells are red cells synonymously called hemagglutination. In hemagglutination the antigen is referred to as agglutinogen and the antibody is referred to as agglutinin.

The agglutination of red cells takes place in two stages. In the first stage- sensitization, antibodies present in the serum become attached to the corresponding antigen on the red cell surface. A red cell, which has thus coated by antibodies is said to be sensitized. In the second stage, the physical agglutination or clumping of the sensitized red cells takes place, which is caused by an antibody attaching to antigen on more than one red cell producing a net or lattice that holds the cells together. The cells form aggregates, which if large enough, are visible to the naked eye. There are also degrees

of agglutination which can not be seen without the aid of a microscope.

The strength of an agglutination reaction can be indicated by the following grading system (Fig. 3.1 a-f), as recommended by the American Association of Blood Banks.

(4+) one solid aggregate;
With no free cells
clear supernatant
Fig. 3.1a

(3+) several large aggregates;
Few free cells
Clear supernatant
Fig 3.1b

(2+) Medium sized aggregate
Some free cells
Clear supernatant
Fig 3.1 c

3.6 Techniques:

Determination of ABO grouping is important in pretrarsfusion studies of patients and donors as well as in cases of obstetric patients. There are different technique to determine ABO grouping in the laboratory: slide, test tube & microplate. In each technique results are interpreted based on the presence or absence of agglutination reaction. Agglutination reaction is interpreted as a positive (+) test result and indicates, based on the method used, the presence of specific antigen on erythrocytes or antibody in the serum of an individual. No agglutination reaction produces a negative (-) test indicating the absence of specific antigens on erythrocytes or antibody in the serum of an individual.

3.6.1 Rules for Practical Work

- Perform all tests according to the manufacturer's direction
- Always label tubes and slides fully and clearly.
- Do not perform tests at temperature higher than room temperature.
- Reagent antisera should be tested daily with erythrocytes if known antigenicity. This eliminates the need to run individual controls each time the reagents are used.
- Do not rely on colored dyes to identify reagent antisera.
- Always add serum before adding cells.

- Perform observations of agglutination against a well – lighted background, and record results immediately after observation.
- Use an optical aid to examine reactions that appear to the naked eye to be negative.

3.6.2 The Right Conditions for RBCs to Agglutinate

The correct conditions must exist for an antibody to react with its corresponding red cell antigen to produce sensitization and agglutination of the red cells, or hemolysis. The following factors affect the agglutination of RBCs:

Antibody size: normally, the forces of mutual repulsion keep the red cells approximately 25 nanometer apart. The maximum span of IgG molecules is 14 nanometer that they could only attach the antigens, coating or sensitizing the red cells and agglutination can not be effected in saline media. On the other hand, IgM molecules are bigger and because of their pentameric arrangement can bridge a wider gap and overcome the repulsive forces, causing cells to agglutinate directly in saline.

pH: the optimum PH for routine laboratory testing is 7.0. Reactions are inhibited when the PH is too acid or too alkaline.

Temperature: The optimum temperature for an antigen-antibody reaction differs for different antibodies. Most IgG antibodies react best at warm temperature(37^0C) while IgM antibodies, cold reacting antibodies react best at room temperature and coldest temperature(4 to 22^0C).

Ionic strength: lowering the ionic strength of the medium increases the rate of agglutination of antibody with antigen. Low ionic strength saline (LISS) containing 0.2% NaCl in 7% glucose is used for this purpose rather than normal saline.

Antibody type: Antibodies differ in their ability to agglutinate. IgM antibodies, referred to as complete antibodies, are more efficient than IgG or IgA antibodies in exhibiting in vitro agglutination when the antigen - bearing erythrocytes are suspended in physiologic saline.

Number of antigen sites: Many IgG antibodies of the Rh system fail to agglutinate red cells suspended in saline, however IgG antibodies of the ABO system (anti-A & anti-B) agglutinate these red cells, because there are many A&B

antigen sites (100 times more than the number of Rh sites) than the D site on the cell membrane of erythrocytes.

Centrifugation: centrifugation at high speed attempts to over come the problem of distance in sensitized cells by physically forcing the cells together.

Enzyme treatment: treatment with a weak proteolytic enzymes (eg. Trypsin, ficin, bromelin, papain) removes surface sialic acid residue- by which red cells exert surface negative charge, thereby reducing the net negative charge of the cells, thus lowering the zeta potential, and allowing the cells to come together for chemical linking by specific antibody molecules. However, enzyme treatment has got a disadvantage in that it destroys some blood group antigens.

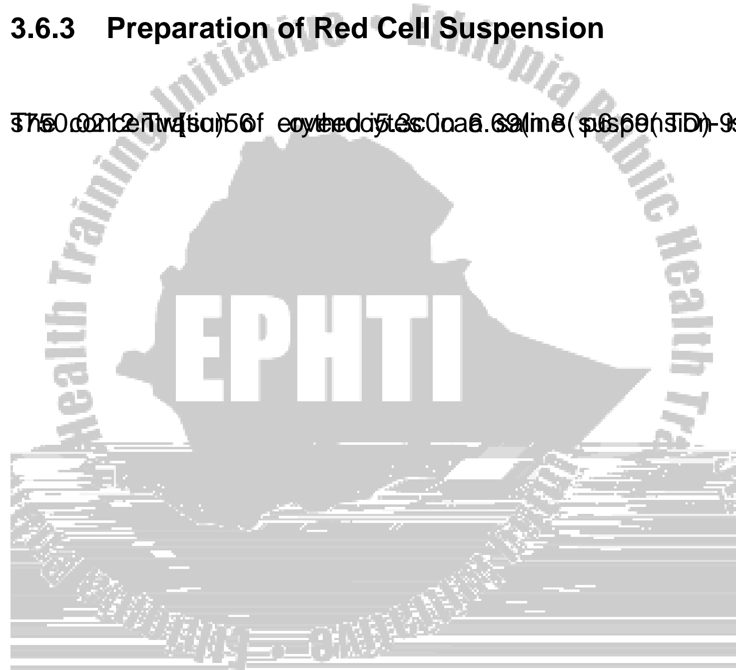
Colloidal media: certain anti-D sera especially some IgG antibodies of the Rh system would agglutinate Rh positive erythrocytes suspended in colloid (bovine albumin) if the zeta potential is carefully adjusted by the addition of the colloid.

Ratio of antibody to antigen: There must be an optimum ratio of antibody to antigen sites for agglutination of red cells to occur. In prozone phenomena (antibody excess), a surplus of antigens combining site which are not bound to antigenic determinants exist, producing false- negative reactions. These

can be over come by serially diluting the anti body containing serum. It is also important to ensure that the red cell suspension used in agglutination test must not be too week or too strong, as heavy suspension might mask the presence of a weak antibody.

3.6.3 Preparation of Red Cell Suspension

The concentration of erythrocytes in a saline suspension is CeTJdi4526 Tens98256 Tw02 0 .5(ght



3. Aspirate or decant the supernatant saline.
4. Repeat (steps 2 and 3) until the supernatant saline is clear
5. Pipette 10 ml of saline in to another clean test tube.
6. Add 0.2 ml of the packed cell button to the 10 ml of saline
7. Cover the tube until time of use. Immediately before use, mix the suspension by inverting the tube several times until the cells are in suspension.

3.6.4 The ABO Blood Grouping

The ABO blood groups (A, B, AB, &O) represent the antigen expressed on the erythrocytes of each group, and whenever an antigen (A and / or B) is absent on the red cells, the corresponding antibody is found in the serum. Based on the above facts, an individual's unknown ABO blood group is performed by two methods in the laboratory: Forward (Direct) and Reverse (Indirect) grouping. Reverse grouping is a cross check for forward typing. Both tests should be done side by side and the results of both methods should agree.

3.6.4.1 The Direct ABO Blood Grouping

The direct blood grouping also called cell grouping employs known reagent anti sera to identify the antigen present or their

absence on an individual's red cell.It can be performed by the slide or test tube method.

Slide method

1. Make a ceramic ring on the slide.
2. Label one ring as anti- A and the other ring as anti-B
3. Add anti- serum to the ring labeled anti-A
4. Add anti-B serum to the ring labeled anti-B
5. Add 10% unknown cell suspension to both rings
6. Mix using a separate applicator stick.
7. Observe the reaction within 2 minutes by rotating the slide back and forth
8. Interpreter the results: Look at Table 3.7

Test tube method:

1. Take two tubes, label one tube 'anti- A' and the second ' anti -B'
2. Add one drop of anti- A serum to the tube labeled 'anti-A' and one drop of anti- B to the tube labeled anti- B'
3. Put one drop of the 2-5% cell suspension to both tubes
4. Mix the antiserum and cells by gently tapping the base of each tube with the finger or by gently shaking
5. Leave the tubes at RT for 5- minutes. Centrifuge at low speed (2200-2800 rpm)for 30 seconds

6. Read the results by tapping gently the base of each tube looking for agglutination or haemolysis against a well-lighted white background.
7. Interpret the results as presented on Table 3.7.

Table 3.7 Reactions of patient Erythrocytes and known Antisera

RED CELLS TESTED WITH		**BLOOD GROUP INTERPRETATION**
ANTI- A	ANTI- B	
Positive	Negative	A
Negative	Positive	B
Positive	Positive	AB
Negative	Negative	O

3.6.4.2 The Indirect ABO Blood Grouping

The indirect blood grouping, also called serum grouping employs red cells possessing known antigen to see the type of antibodies (anti A & -B) present, or absence of these antibodies in serum. It usually is performed by test tube method alone. Slide reverse grouping is not reliable as serum antibodies agglutinate most cell samples when centrifuged, and use of test tube enhances the agglutinated reaction.

Test tube method

1. Take two tubes, label one tube A- Cells' and the second 'B cells'
2. Put one drop of the serum to be tested each tube.
3. Add one drop of 2-5% A cells to the tube labeled 'A cells' and one drop of 2-5% B cells to the tube labeled 'B cells'.
4. Mix the contents of the tubes.
5. Leave the tubes at RT for 5- minutes. Centrifuge at low speed (2200-2800 rpm) for 30 seconds.
6. Read the results by tapping gently the base of each tube looking for agglutination or haemolysis against a well-lighted white background.
7. Interpretation of results: look at table 3.8

Table 3.8 Reactions of patient serum and reagent erythrocytes

SERUM TESTED WITH		BLOOD GROUP INTERPRETATION
A cell	**B cell**	
Negative	Positive	A
Positive	Negative	B

3.6.5 Anomalous Results in ABO Testing

Technical errors and various clinical conditions can contribute to a discrepancy between erythrocyte and serum results in ABO grouping. Most ABO discrepancy's however, are technical in nature, and can be resolved by careful repeating of the test procedure. These include: contaminated reagents or dirty glass ware, over centrifugation, incorrect serum: cell ratio, under centrifugation or incorrect incubation temperature, failure to add test specimen or reagents, and the like. If carefully controlled repeat testing yields the same agglutination patterns, the variation can be assigned to one of the following four categories.

1. Missing or weak reacting antibodies

Age: testing of infants who have not begun to produce their own antibodies, or who possess antibodies that have been passively acquired from the mother, or during testing of elderly persons whose antibody levels have declined.

Hypogamaglobulininemia:

immunosuppressive drugs, and following bone marrow transplantation.

Resolution: Enhancing reaction in reverse grouping by incubating of patients serum with the red cells at room temperature for 15 min or incubation at 16^0C or 4^0C for 15 min.

2. Missing weak antigens

Sub groups of A or B antigens: The A or B antigens may be weakly expressed because of an unusual genotype (i.e, sub groups of A&B).

Disease: In some conditions like acute leukemias, the red cell antigens in the ABO system may be greatly depressed that they give weak reactions.
Blood group specific substances: in conditions like ovarian cyst & carcinomas, blood group specific substance may be of such high concentration is that anti-A & and – B are neutralized when unwashed cells are used.

Acquired B antigen: effect of bacterial enzymes & absorption of bacterial polysaccharide on to the red cells of group A or O patients results in B specificity which involve weak B antigen reaction in the forward grouping.

Additives to sera: acriflavin, the yellow dye used in some commercial anti B reagents, can produce false agglutination in some persons, which results from antibodies against acriflavin in the serum combining with the dye and attaching to the erythrocytes of the individual.

Mixtures of blood: Mixture of cell types in recently transfused patients or recipients of bone marrow transplants can produce unexpected reactions in forward typing.

Resolution:

- Investigating the possibility of sub groups of A&B
- Investigating the diagnosis
- Washing the patient's red cells in saline to eliminate the problem with blood group specific substances.
- Acidifying the anti- B reagent to PH 6.0 to rule out acquired B and then determining secretor status
- Washing the patient's cells three times and then regrouping if dye is suspected as the problem or using reagents that do not contain dye.

3. Additional antibody

Autoantibody: cold autoantibodies can cause spontaneous agglutination of the A and B cells used in reverse grouping. Patients with warm autoimmune hemolytic anemia may have

red cells coated with sufficient antibody to promote spontaneous agglutination.

Anti A_1: A_2 & A_2 B individuals may produce naturally occurring anti-A_1, which cause discrepant ABO typing.

Irregular antibodies: Irregular antibodies in some other blood group system may be present that react with antigens on the A or B cells used in reverse grouping.

Resolution:

- Washing the patient red cells in warm (37^0C) saline to establish cold autoantibodies as the cause.
- Treating cells with chloroquire diphosphate to eliminate bound antibodies if warm autoantibody is suspected.
- Identifying the irregular antibody, and using A & B cells, which are negative for the corresponding antigen.

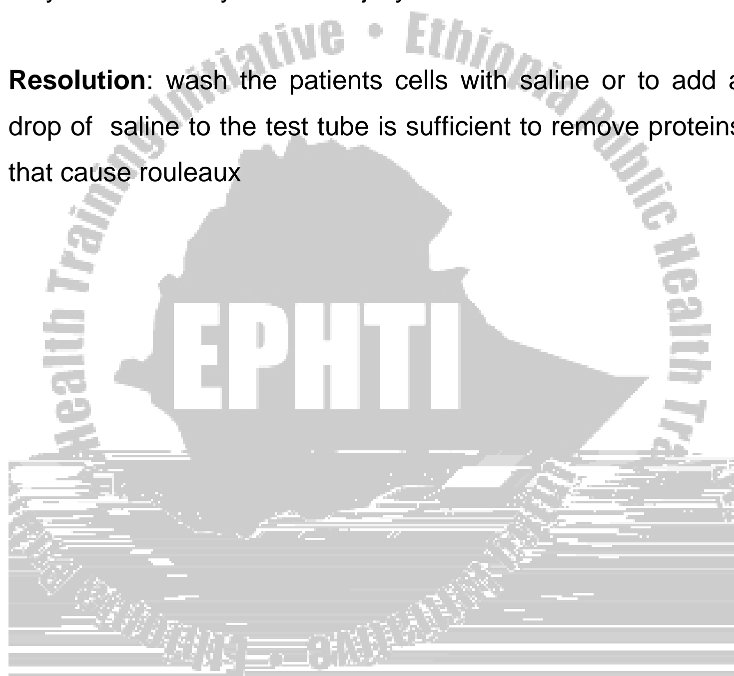
4. Plasma Abnormalities

Increased gamma globulin: elevated levels of globulin from certain disease states such as multiple myeloma result in rouleaux formation.

Abnormal proteins: Abnormal proteins, altered proportions of globulins, and high concentration of fibirogen may cause rouleaux formation, which could be mistaken for agglutination.

Wharton's jelly: when cord blood is used, reverse grouping may be affected by wharton's jelly which causes rouleaux.

Resolution: wash the patients cells with saline or to add a drop of saline to the test tube is sufficient to remove proteins that cause rouleaux



Review Questions

1 Briefly discuss on the discovery of the ABO blood group system
2. Classify the ABO blood group system based on the antigen and antibodies present in an individual
3. Give a description for grade of agglutination reaction as recommended by the America blood bank society.
4. List conditions that influence agglutination of red cells
5. Describe how to prepare a 10 ml volume of 5% red cell suspension
6. Discuss how to perform direct & indirect method of ABO blood typing
7. Discuss conditions that lead to anomalous results in ABO testing

CHAPTER FOUR

THE Rh-Hr BLOOD GROUP SYSTEM

Learning Objectives

At the conclusion of the chapter the student should be able to:

- Describe the historical background of the Rh system.
- Compare the genetic inheritance and nomenclature of the Rh antigens in Wiener and Fisher- Race theories
- List the most common Rh antigens including their characteristics
- Discuss on the common variants of the D antigen (D^u), including the clinical significance.
- Discuss the chai 946an of t(the)5.7(Lisri)an of t(sD ad (clini)an of t((al)6(i(gnifi)an an)5.7n of t

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immunized guinea pigs and rabbits with blood from the Macacus rhesus monkey, and the antiserum obtained agglutinated not only the red cells of the rhesus monkey but also 85% of humans. They realized that this serum which they called anti-Rh was about detecting an unknown human blood group antigen which, independent of all other blood groups discovered before that time. They used it to type as Rh positive those donors whose red cells were agglutinated by the new antibody and as Rh negative to those whose red cells were not so agglutinated.

This discovery followed the detection of an antibody by Levine & Stetson in 1939. This antibody occurred in the serum of a woman delivered a stillborn fetus, who suffered a hemolytic reaction to her husband's ABO compatible blood transfused shortly after delivery. The antibody was found to agglutinate approximately 80% of randomly selected ABO cof an s.1651530 -lar 3e3 appolutinay 487.2

not only could an Rh negative mother become immunized to an Rh positive fetus in utero but also that the antibody could then traverse the placenta and give rise to erythroblastosis fetalis (HDN).

Later work demonstrated that the animal or rabbit anti-Rhesus and human anti-Rh are not the same, and were not detecting the same antigen but the system had already named the human antibody as anti-Rh. The animal anti-hesus was detecting another antigen possessed by Rh positive & Rh negative persons but in much greater amount in Rh positives. Therefore the animal antibody was renamed anti- LW after Landsteiner & Wiener who discovered it, and the human antibody retained the title anti Rh.

4.2. Nomenclature & Genetic Theories

Fisher- Rase Nomenclature

The Fisher- Race theory states that there are three closely linked loci, each with one of the set of allelic gene (D& d, C & c, E &e) and these three genes are inherited as a complex. These three loci are believed to be so closely linked that crossing over occurs only very rarely.

Complex Rh genes control the Rh antigens, these genes are C, D, E, c, d & e. The Rh antigens are therefore named C, D, E, c, d &e. The antigen d (and anti- d) do not exist, the symbol

“d” is used to express the absence of D.The Rh gene complex possesses closely linked genes (antigens) which could be assembled in eight different ways: CDe, cDE, cde, cDe, cdE, Cde, CDE & CdE. Because of the strong antigenic characters

Table 4.2 Comparison of Fisher- Race & Wiener nomenclature

Fisher- Race	Wiener	
CDe	Rh_1	$(rh^{'}, Rho, hr^{''})$
c DE	Rh_2	$(hr^{'}, Rho, rh^{''})$
c de	rh	$(hr^{''}, hr^{''})$
Cde	rh^1	$(rh^{'}, hr^{''})$
c dE	$Rh^{''}$	$(hr^{'}, rh^{''})$
CdE	rh^y	$(rh^{'}, rh^{''})$
CDE	Rh^z	$(rh^{'}, Rho, rh^{''})$
c De	Rho	$(hr^{'}, Rho, hr^{''})$

The Rh gene that determine the Rh antigens are inherited as a single gene (wiener) or gene complex (Fisher- Race) from each parent. According to Fisher – Race, three pairs of allelic genes on the same chromosome (haplotype) will determine the production or non- production of D with C or c, E or e. The inheritance of the Rh genes through haplotype gene is shown in Fig 4.1

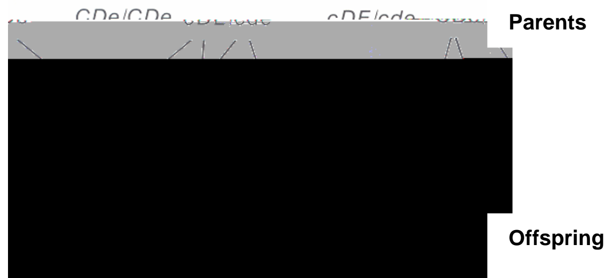


Fig 4.1 Rh Inheritance with Fisher Race nomenclature

4.3 The antigens of the Rh-Hr blood group system

The Rh antigens can be demonstrated on fetal red cells as early as 38 days after conception, and are well developed at birth. There are five rhesus antigens, D, C, c, E &e which are only expressed on red cells. They are not found in body fluids (like saliva, amniotic fluid) and not detected on leucocytes or platelets. The ‘d’ gene is not expressed and there is no ’d’ antigen, it only implies the absence of ’D’. Individuals who lack any of these antigens may be stimulated to produce the corresponding antibodies (anti-D, anti- C, ant -c, anti-E, anti-e) by transfusion or pregnancy.

Antigen D, having antigen site between 110,000 and 202,000 per erythrocyte, is the most important of the rhesus antigens

medically, because it is highly antigenic than the other Rhesus antigens.

A person is grouped as Rhesus (Rh) positive or negative based on the presence or absence of antigen D:

- Rh positive: a person who inherits gene D and the red cell express antigen D.
- Rh negative: a person who does not inherit gene D and the red cells do not express antigen D

For transfusion purpose, Rh positive blood can be given to Rh positive individuals and Rh negative blood can be given to both Rh^+ & Rh^- individuals. Never give Rh^+ blood to Rh^- individuals especially to women of child bearing age.

4.4 Variants of antigen

Weak antigen D (D^u)

Weak forms of antigen D where the number of D sites on the red cells is reduced. Such weak D cells react less strongly than red cells with normal numbers of D receptors. There are two grades of D^u : High grade D^u red cells, which are agglutinated by certain anti-D sera and lower grade D^u red cells, which are agglutinated only by the Indirect Antiglobulin (IAG)test.

In case of blood transfusion, donors with D^u + red cells are regarded as Rh+ because, a severe hemolytic transfusion reaction may result from the transfusion of D^u + red cell to a recipient whose serum contains anti D. As a recipient individuals with D^u + red cells regarded as Rh negative, because of the risk of provoking the formation anti-D in a D^u + subject through the transfusion of D+ blood.

In addition, Du + red cells are clinically important in that, they may be destroyed at a higher rate by anti-D, and a D^u infant can suffer from HDN if the mother possesses anti –D.

- As a donor individuals with D^u positive antigen regarded as Rh positive.
- As a recipient individuals with D^u positive antigen regarded as Rh negative.

4.5. Rhesus Antibodies

The common Rh antibodies are anti –E, anti -e, anti -C,anti-c

Rh antibodies generally develop from 2 to 6 months after the initial immunization by red cells. Their production is consistent

naturally occurring Rh antibodies are not found in the serum of persons lacking the corresponding Rh antigens. Therefore ‘reverse grouping’ cannot be done in Rh blood group system.

3. Place a drop of Rh control (albumin or other control medium) on another labeled slide.
4. Add one drop of a 2-5% cell suspension to each tube
5. Mix well and centrifuge at 2200-2800 rpm for 60 seconds
6. Gently re suspend the cell button and look for agglutination and grade the results (a reaction of any grade is interpreted as Rh positive) a smooth suspension of cells must be observed in the control.
7. Collect a weakly positive ($^{\pm}$) and negative sample to perform the D^u test.

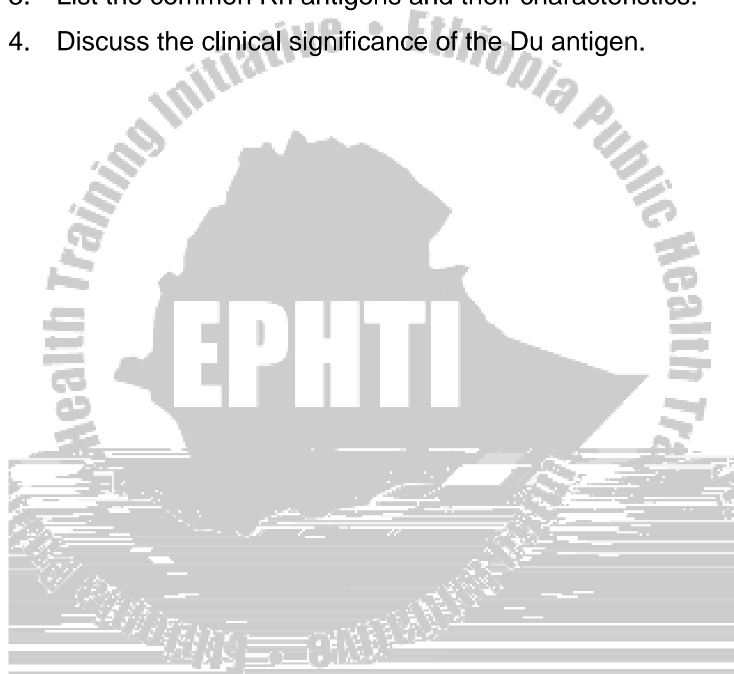
4.6.4 Du Typing Using Indirect Anti- Globulin Test (IAT)

1. Use the intial Rh D typing tube and control in procedure 4.6.3 and incubate the Rh . Negative or weakly reactive ($^{\pm}$) samples and the control at 37^0C for 30 minutes.
2. Wash cells in both test and control tube 3-4 times with normal saline.
3. Add one drop of the poly specific anti- human globulin (coombs) to each tube and mix well.
4. Centrifuge at 2200-2800 rpm for 10 second.
5. Gently re suspended the cell button and observe for agglutination

6. Interpretation: the positive result is agglutination in the tube containing anti–D and the control is negative. A negative result is absence of agglutination in both the test & control.

Review Questions

1. Discuss the history of the development of the Rh system
2. Contrast the Fisher- Race & Wiener's genetic theories on the Rh antigens.
3. List the common Rh antigens and their characteristics.
4. Discuss the clinical significance of the Du antigen.



CHAPTER FIVE

THE ANTI- GLOBULIN TEST
(COOMB'S TEST)

Learning objective

At the conclusion of the chapter, the student should be able to:

- Describe the purpose of the antihuman globulin (AHG) test.
- Name and contrast the Anti-human globulin (coomb's) reagents.
- Know the principle and carryout the AHG procedure.

THE ANTI- GLOBULIN TEST is introduced in to clinical medicine by Coomb's in 1945. It is a sensitive technique in the detection of incomplete antibodies, anti bodies that can sensitize but which fail to agglutinate red cells suspended in saline at room temperature, mainly IgG. These antibodies are agglutinated by the anti- IgG in antiglobulin serum through the linking of the IgG molecules on neighboring red cells,as shown in Fig 5.1.

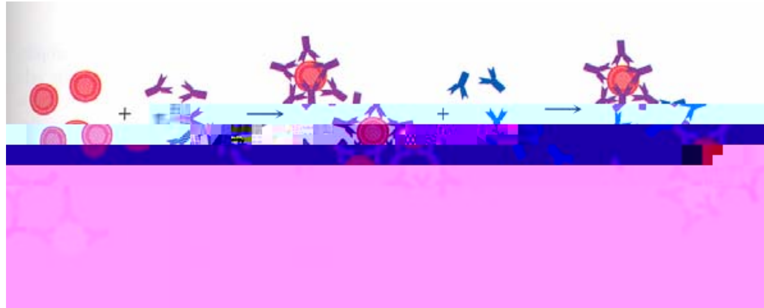
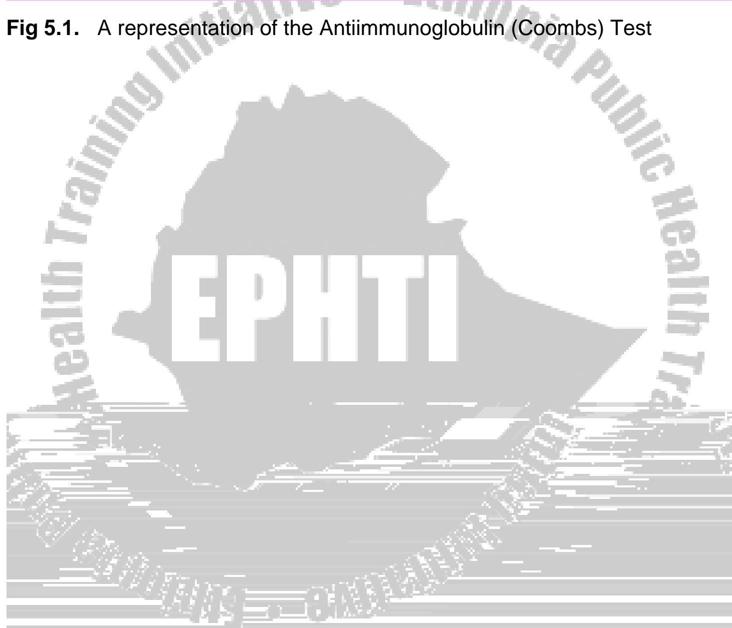


Fig 5.1. A representation of the Antiimmunoglobulin (Coombs) Test



Broad spectrum (polyspecific):

Procedure

1. Place one drop of 5% saline red cell suspension in a test tube.

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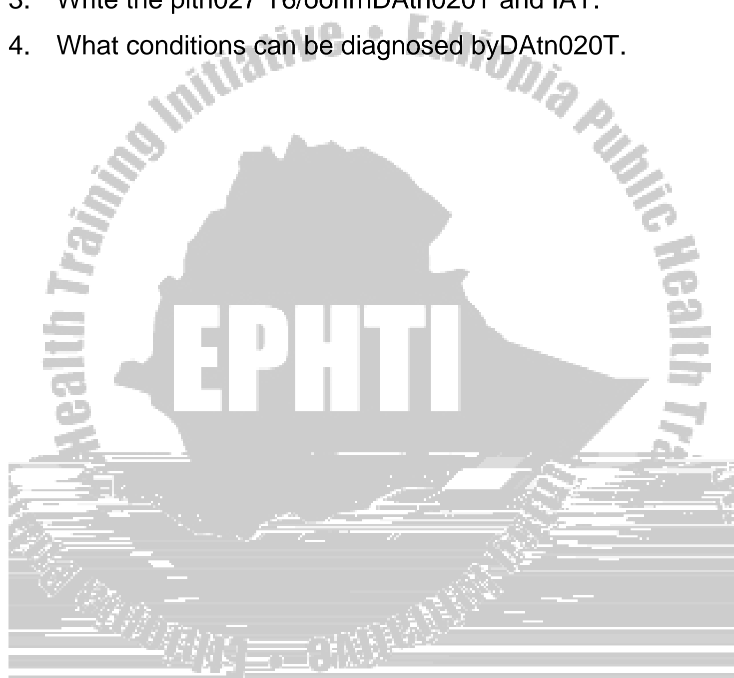
Principle:

Review Questions

1. What is the purpose of the AHG test?
2. How can the AHG reagent make agglutination of sensitized erythrocytes?
3. Write the pitn027 T6/oonrnDAtn020T and IAT.
4. What conditions can be diagnosed byDAtn020T.



CHAPTER SIX

HAEMOLYTIC DISEASES

Learning Objective

At the conclusion of this chapter the student should be able to:

- Understand the cause of and the laboratory method to demonstrate Auto Immune Hemolytic Anemia (AIHA).
- Briefly explain the cause and consequences of hemolytic disease of the newborn (HDN).
- Name the major immunoglobulin class and sub classes responsible for HDN.
- Describe the laboratory diagnosis & treatment of HDN caused by ABO and Rh incompatibility.
- Compare the relationship of HDN caused by ABO incompatibility to HDN caused by Rh incompatibility.
- Describe the prenatal treatment that is significant in HDN caused by antiD.

classified into four groups namely: warm- reactive autoantibodies, cold – reactive auto antibodies paroxysmal cold hemoglobinuria and drug induced hemolysis

through the placenta by active transport mechanism, coat the fetal erythrocytes and cause damage to them.

Every blood group antibody that can occur as IgG can cause HDN. It is only IgG immunoglobulin that is capable of passing the placental barrier and which is found in cord blood in a concentration equivalent to that found in maternal blood. IgM agglutinin though produced in response to fetal red cells in utero, plays no part in the cause of HDN, and are either present in much lower concentration in the new born than the mother or entirely absent.

Fetal hematopoietic tissue (liver, spleen and bone marrow) respond to hemolysis by increased production of RBCs , predominantly NRBCs . Increased destruction of red cells leads the fetus to develop anemia and jaundice from the hemoglobin breakdown product, bilirubin. If this blirubin reaches excessive levels in the newborn or infants circulation it causes mental retardation or death.

6.2.1 HDN Due to Rh Blood Group Incompatibility

HDN due to anti- Rh(D) occurs when mother and infant are always incompatible with respect to the Rh factor: The mother Rh(D) negative, and the infant Rh (D) positive (inherited the D factor from the father). ABO incompatibility between the

mother and fetus reduces the chance of maternal immunization to the D Ag. This is probably because the fetal cells, which are incompatible with the maternal ABO antibodies are destroyed by existing ABO antibodies before they have a chance to act as an antigenic stimulus.

The first Rh- incompatible infant is usually unaffected because the number of fetal cells that cross the placenta during pregnancy (after 24 weeks gestation) is small and insufficient to cause IgG anti D production, unless a prior transfusion of D positive blood has been given.

During transplacental hemorrhage, the amount of fetal blood that enters the maternal circulation increases, and in 6 months time after delivery only 10% of these Rh negative women could produce detectable antibodies. The actual production of anti-D antibodies depends on the dosage and antigenecity of the D antigen, and the mother's ability to respond to these foreign antigens. About one third of mothers are non-responders, they fail to form anti- D despite intentional repeated injections of Rh (D) positive erythrocytes.

During a second pregnancy with a Rh positive fetus, small number of fetal cells cross the placenta (2^0 doses of antigen) stimulating the antibody to high concentration, mainly Ig G anti- D that passes in to the fetal circulation destroying fetal

red cells. The severity of the disease increases with each Rh positive pregnancy. IgG anti-D is found predominantly in sub classes IgG_1 & IgG_3 and these subclasses possess properties

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6.2.3 Assessment of HDN

Prenatal

Some investigations are carried out on blood of the mother to identify women at risk of having a child affected with HDN. It is recommended that all pregnant women at their first attendance at a clinic need to have ABO grouping, Rh typing for D & D^u, alloantibody screening test and amniocentesis.

Postnatal

After birth different laboratory procedures are helpful in determining the presence and assessing the severity of HDN.

1. ABO & Rh Typing: the ABO group of the infant is based on forward (cell) grouping as anti- A and anti B agglutinins do not develop until a few months after birth.

- ABO grouping most commonly reveals the mother to be group O and the baby to be group A or possible group B.
- Rh typing shows the baby to be D or D^U positive and the mother D or D^U negative

2. DAT on cord or infants blood:

- In ABO HDN, DAT is usually negative or weakly positive ,

3. Antibody elution test of cord blood: done if DAT is positive, may reveal the presence of immune anti- A or anti-B in ABO HDN and anti-D in Rh HDN.
4. Hgb level of cord blood: may be slightly to severely decreased
5. Serum bilirubin level on cord serum: may exceed the normal values of cord total serum bilirubin of 1 to 3 mg/ml.
6. Peripheral blood smears on cord blood: blood smear evaluation shows anemia with RBC morphology abnormalities: hypochromia, microspherocytosis with the demonstration of reticulocytes and immature nucleated RBCs.
7. Kleihauer- Betke acid elution test: is a test to be performed for quantitating the extent of fetal maternal hemorrhage (number of fetal cells in the maternal circulation). It is an indicator for treatment of the mother with anti-D immunoglobulin, more importantly used to determine the size of dose to be given.

Reagents: 80% ethanol

Solution A: 0.75 haematoxylin in 96% ethanol

Solution B : 2.4 g $FeCl_3$ & 2 ml of 25% HCl in 100 ml distilled water

Elution solution: 2 parts of A mixed with 1 part of B & 9 part of 80% ethanol

Counter stain: 0.1% erythrosin or 0.5% aqueous eosin.

Method: Mother's whole blood is diluted with an equal quantity of saline and used for making films. The slides are

Dose: The usual recommended dose (contained in one vial) is about 300 µg which is believed to offer protection against a fetomaternal hemorrhage of 30 ml (15 ml packed cells) or less.

If a massive fetomaternal hemorrhage has occurred, the volume of the hemorrhege must be determined to calculate the number of vials of Rh (D) immune globulin to administer. Calculated as follows:

Volume of fetomaternal hemorrhage = Percentage of fetal cells (seen in the acid elution stain) x*50

No of vials of Rh IG = $\frac{\text{volume of fetomaternal hemorrhagex*2}}{30}$

NB: Factor of *50 = 5000 ml (estimated maternal volume) x 1/100%

*2 is a common factor, because the actual fetomaternal hemorrhage may be twice the estimate.

Example:

- KB reported as 1.2% fetal cells
- 1.2 x 50= 60ml fetomaternal hemorrhage
- $\frac{60}{30}$ x2 = 4 vials

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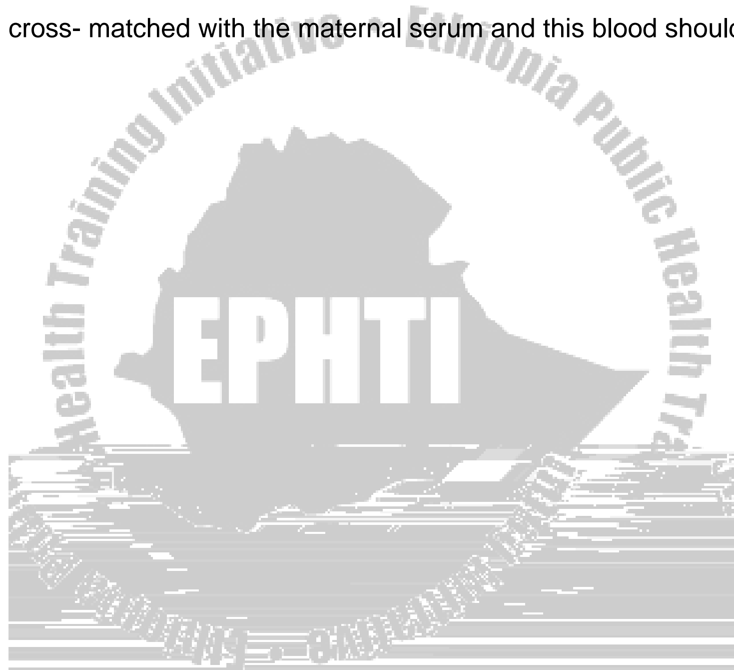
simultaneously the infant is provided with compatible donor red cells. To give exchange transfusion for an infant clinical & laboratory findings must be considered. Cord Hgb (<10g/dl) and raised serum bilirubin are strong indicator for treatment.

For compatible exchange transfusion, donor's blood should be cross- matched with the maternal serum and this blood should



CHAPTER SEVEN

THE CROSS- MATCH (COMPATIBILITY TESTING)

Learning Objective

At the conclusion of this chapter, the student should be able to:

- Understand the cross match and its primary purpose.
- Explain the constituents of the major and minor cross match.
- Select appropriate blood for cross match.
- Describe the types of antibodies that can be encountered at various phases of a cross match.

7.1. Purpose of Cross-Match

The cross- match (compatibilit

the ABO and Rh group of the blood to be transfused is compatible with patient's ABO and Rh group and by detecting most unexpected (irregular) antibodies in the patient's serum that will react with the donor's red cells causing their destruction or reducing their normal survival.

However, a cross match will not prevent immunization of the patient, and will not guarantee normal survival of transfused erythrocytes or detect all unexpected antibodies in a patient's serum.

7.2. Types of Cross Match

There are two types of cross- matches major & minor curse match.

Major cross- match: includes mixing recipient's serum with the donor's red cells. It is much more critical for assuring safe transfusion than the minor compatibility test. It is called major because the antibody with the recipient's serum is most likely to destroy the donor's red cells and that is why it is called major cross match.

Minor cross match:6.5(p6fsrnrT5n01 Tc0a)6.9(o)-ra1ra0 Tc0e0.8(st(u))0.00019g**[**Minor o2 1 Tt)3

recipient's blood, so it causes relatively less problem and so called minor cross match.

7.3 Selection of Blood for Cross Match

Generally, when whole blood is to be transfused, the blood selected for cross- match should be of the same ABO and Rh (D) group as that of the recipient. However, Rh positive recipients may receive either Rh positive or Rh negative blood.

Table 7.1 Selection of ABO blood for cross-match

Group of patient	***Choice of blood***			
	1st	**2nd**	**3rd**	**4th**
Group A	**Gp A**	**Gp O**	**-**	**-**
Group B	**Gp B**	**Gp O**	**-**	**-**
Group O	**Gp O**	**-**	**-**	**-**
Group AB	**Gp AB**	**GP A***	**Gp B**	**Gp O**
*** Group A is the second choice of blood because anti-B in Gp A blood is likely to be weaker than anti-A in Gp B blood.**				

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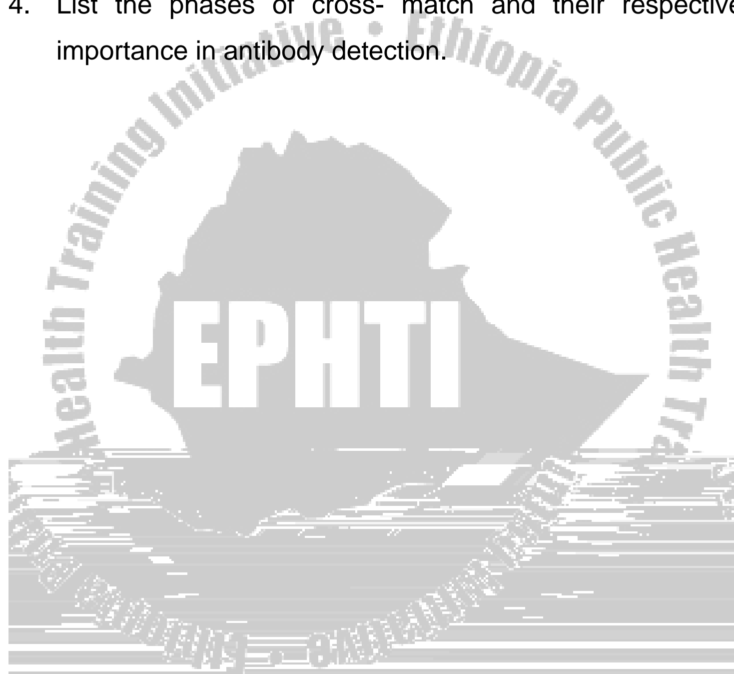
Note: potentiators such as a drop of 22% albumin may be added at this phase to increase the sensitivity of the test.

6. Centrifuge at 3400 rpm for 15 seconds and examine for agglutination or hemolysis. If there is no hemolysis or agglutination proceed with the next phase.
7. Wash the contents of the tube 3-4 times with normal saline.
8. After the last wash, decant all saline and add two drops of AHG reagent and mix.
9. Centrifuge at 3400 rpm for 15 seconds.
10. Gently re suspend the cells button and examine macroscopically and microscopically for agglutination or hemolysis .

Enzyme cross match can be performed by using different enzymes: bromelin, ficin, papain & trypsin. Two methods are available to carry out enzyme cross mactch - One stage & two stage methods. The one-stege technique involves enzyme, patient's serum and donor's red cell incubated together. The two-stage technique involves red cells pretreated with enzyme and then tested with the patient's serum.

Review Questions

1. What is cross match?
2. What is the purpose of cross-match?
3. List the types of cross-match with their constituents.
4. List the phases of cross- match and their respective importance in antibody detection.



CHAPTER EIGHT

THE DONATION OF BLOD

Learning objective:

At the end of this chapter the student should be able to:

- Discuss the medical and physical requirements that would exclude an allogeneic donor
- Describe the proper procedure for collecting blood from donors
- Name the commonly used anticoagulants for donated blood and their respective approved maximum storage time
- Name the common blood components with their storage temperature and shelf life
- Explain the possible donor reactions

8.1 Selection of blood donors

A blood transfusion service aims to prepare safe blood from a safe donor to a recipient who needs blood. The medical person who screens donors should identify conditions which

can harm both the donor who gives his blood on one hand and the recipient who receives blood and blood products on the other hand. Therefore, to ensure the well being of both donors and patients the screening person should understand the requirements that make a donor acceptable and not acceptable to donate blood.

8.1.1 Selection Criteria:

Age:

If between 17-65 years acceptable.

If less than 17 years after guardian's consent or depending on the local law

If more than 65 years after consulting a medical doctor.

Hemoglobin:

Females should not be less than 12.5 g/dl (PCV 38%)

Males should not be less than 13.5 g/dl (PCV 41%)

In both sexes Hgb above 19g% (Hct above 57%) are not acceptable.

Pulse, Blood pressure & Temperature:

Pulse between 60-100 per minute acceptable.

Systolic pressure between 90 and 180 mmHg acceptable

Diastolic pressure between 50 and 100 mmHg acceptable

A donor's temperature must not exceed 37.5^{o}C.

Weight:

If between 45-50 kgs can donate 350 ml of blood

If above 50 kg can donate 450 ml of blood

- Obese donors who are unable to climb the coach are not acceptable.
- If weight is very low compared to the height of the donor do not accept.
- Donors with unexplained weight loss of a significant degree (more than kg) are not acceptable to donate.

If a prospective donor weighs less than 50 kg, a lesser amount of blood may be collected, and the amount of anticoagulant in the collecting bag must be reduced proportionally, calculated as follows:

$$\text{Volume of blood to draw} = \frac{\text{Donor's weight in kg}}{50} \times 450 \text{ ml}$$

$$\text{Amount of anticoagulant to remove from a 450 ml bag} = 63\text{ml} - \frac{\text{Donors weight}}{50 \text{ kg}} \times 63 \text{ m}$$

Pregnancy:

pregnant women excluded from donating for 1 year after the conclusion of their pregnancy.

Medication:

In general, medications taken by a donor are not harmful to a recipient. Deferral of a donor because of drug depends on the

nature of the disease for which the drug was ordered. Consult medical doctor for donor's on long term treatment.

Illness: prospective donors with disease of the heart, liver, lungs, or individuals with a history of cancer, or those with bleeding problems should be excluded subject to evaluation by a physician.

- Donors who have had leukemia must be permanently deferred.
- Donors with previous history of tuberculosis are acceptable after completion of therapy and if no longer active.

Infectious diseases: A donor must be free from infectious diseases that can be transmitted by blood such as hepatitis, HIV & malaria.

- Recipients of blood or blood products known to be possible sources of hepatitis and donors having had close contact with an individual with viral hepatitis must be deferred for 1 year.
- Persons at high risk for acquiring or transmitting AIDS should not donate blood.
- Donors who have a history of malaria, or were previously resident in an endemic area, should be deferred for 3

years after becoming symptomatic or after leaving the endemic area.

Previous donation: If a person has donated blood, an interval of at least four months for men and six months for women is required before the next donation.

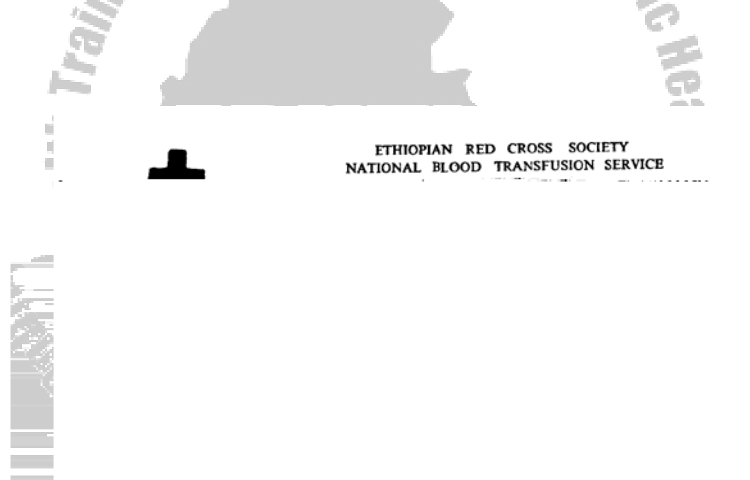
Surgery: If the surgery is minor (such as tooth extraction) a donor is excluded until healing is complete and full activity has been resumed.

Vaccinations: Persons recently immunized with toxoids and killed viral, bacterial and rickettsial vaccines (such as for anthrax, cholera, diphtheria, influenza, polio, tetanus, typhoid, typhus) are acceptable, if they are symptom free and a febrile.

- After small pox vaccination, a donor is acceptable when the scab has fallen off, or 2 weeks after an immune reaction.
- A donor who has received an attenuated live virus vaccine such as mumps or yellow fever is deferred for 2 weeks after the last immunization.
- If rabies vaccination has been given following a bite by a rabid animal, the donor must be deferred for 1 year after the bite.

8.2 Collection of Blood

Trained personnel must collect donation of blood. Basic information from the donor: date of donation, full name, address, sex, age and the ABO & Rh blood group including the prospective donor's medical history must be obtained and signed by the phlebotomist who perform the procedure. A form similar to Figure 8.1, which is taken from Ethiopian Red Cross Society, is the basic guideline for donor registration and the medical history interview.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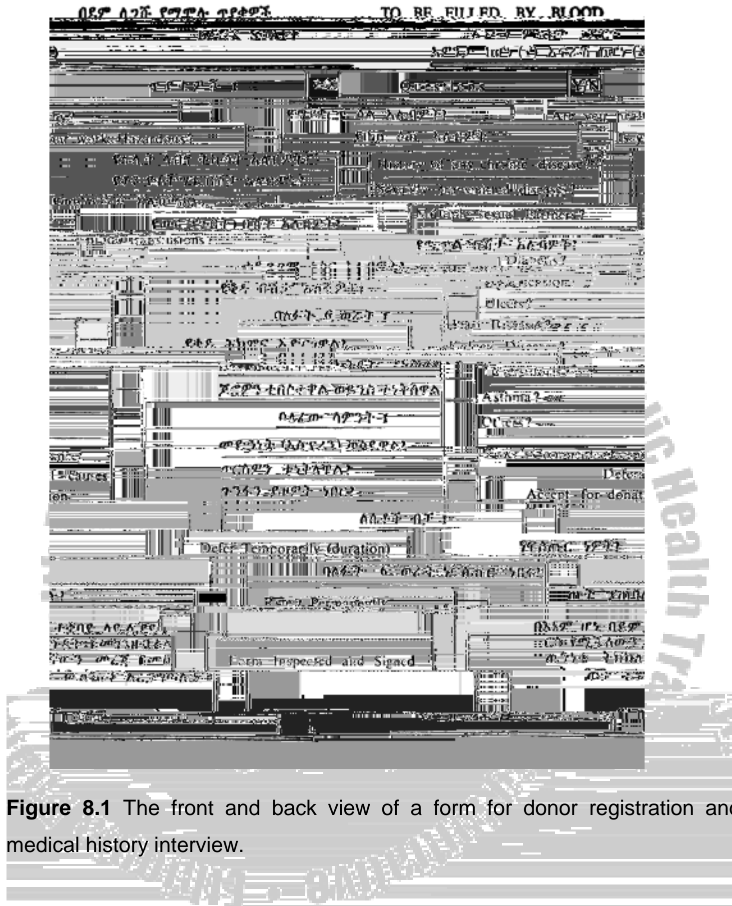


TO BE FILLED BY BLOOD

Asthma?

Defer Temporarily (duration)

Form Inspected and Signed

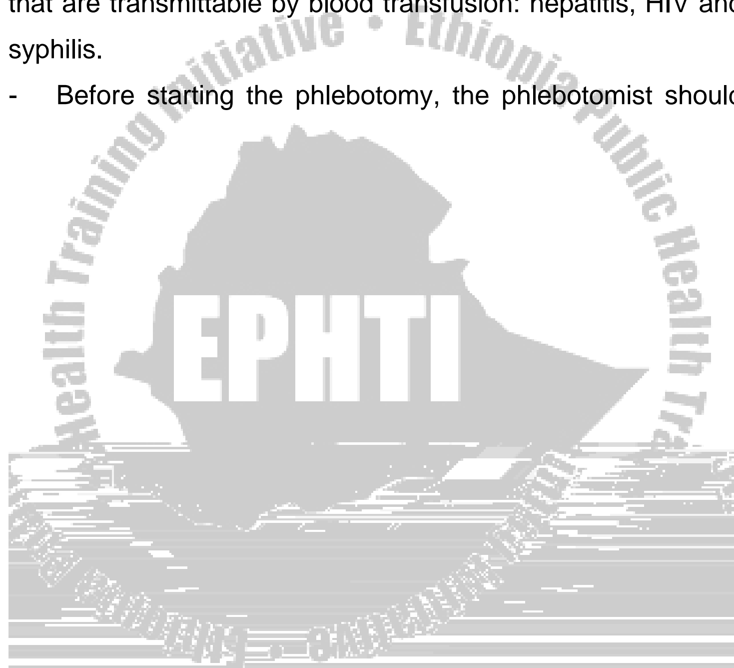
Figure 8.1 The front and back view of a form for donor registration and medical history interview.

Patient identification also is an important step in blood collection. Forms accompanying blood samples from the recipient must contain sufficient information: full name, identification number of patient, sex, age, clinical diagnosis and the like for identification of the recipient.

8.2.2 Macro Sampling

Macro sampling is the collection of large volume of blood from the veins by venipuncture. In Blood Bank laboratory, this blood is usually collected for transfusion purpose from volunteer blood donors, and for serologic tests of diseases that are transmittable by blood transfusion: hepatitis, HIV and syphilis.

- Before starting the phlebotomy, the phlebotomist should



2. Apply the tourniquet, and ask the patient to make a fist (sometimes a roll of gauze is placed is the patient's hand). This usually makes the veins more prominent. Using the left index finger, palpate for an appropriate vein. The ideal

the venipuncture immediately. A clean venepuncture will guarantee a full, clot free unit

5. Open the hemostat clamp and check that the blood flow is adequate. Carefully tape the tubing to hold the needle in place and cover the venipuncture site with a sterile gauze pad. Have the donor squeeze a rubber ball or other soft object every 10 to 12 seconds during collection.
6. Keep the donor under observation throughout the phlebotomy. A person should never be left unattended during or immediately after donation.
7. Mix the unit of blood periodically (every 30 seconds). Time limits for collecting a unit are not fixed, so long as the blood flow is continuous However, it usually takes 8-10 minutes. A unit containing 450-495 mL should weigh 425-520 g plus the weight of the container with its anticoagulant.
8. Remove the tourniquet & hold a sterile gauze lightly over the venipuncture site and remove the needle from the donor's arm. Apply pressure on the gauze. Have the donor raise the arm (elbow straight) and hold the gauze firmly over the phlebotomy site with the opposite hand.
9. Strip the donor tubing from the end of the tube towards the bag as completely as possible in order to mix well with the anti coagulant. Invert the donor unit and allow the line to refill. Then strip again. Seal the tubing attached to bag into segments suitable for subsequent tests with either a

heat sealer or metal clips.(see fig 8.3 for appropriately collected donor blood).

10. Place blood at appropriate temperature.

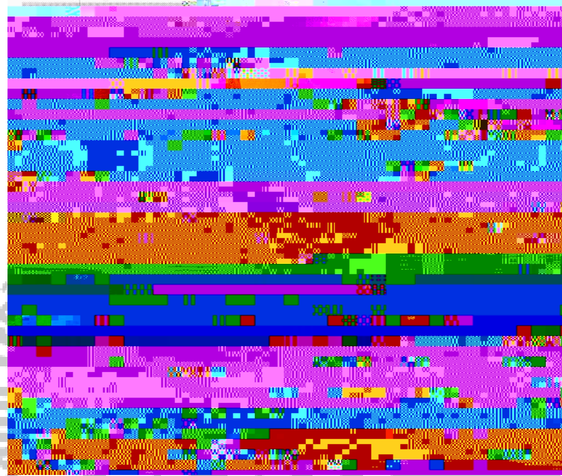


Fig. 8.3 Donor blood bag with segmented tubing

Note: immediately after collection, blood must be placed in storage at a temperature between 1°C & 6°C.However, if the blood is to be used as a source of components, up to 8 hours may elapse before storage.

8.3 The Anticoagulants and Storage of Blood and Blood Products

Anticoagulant is a substance that prevents the clotting of blood some anticoagulants contain preservatives that provide proper nutrients for metabolism in the red cell during storage. Anticoagulants maintain red blood cells hemoglobin function and viability and the biochemical balance of certain elements: glucose, ATP, 2,3 diphosphoglycerate (2,3DPG) and PH, so that the red cells will maintain the means of delivering oxygen to the tissues of the recipient.

Anticoagulants and/or anticoagulant preservation for whole blood and red cell concentrate storage include: ACD(acid-citrate- dextrose),CPD(citrate- phosphate dextrose).CPD-A_1 and CPD-A_2(citrate phospate dextrose adenine) and heparine.

1. ACD

- Acts as an anticoagulant by binding Ca
- Composition: Trisodium citrate- binds Ca

  Citric acid:- maintains P^H

  Dextrose: acts as a nutrient & preservative
- To prevent the clotting of 100 ml of blood 15 ml ACD is required.
- Shelf life: 75% survival after 21 days of storage.

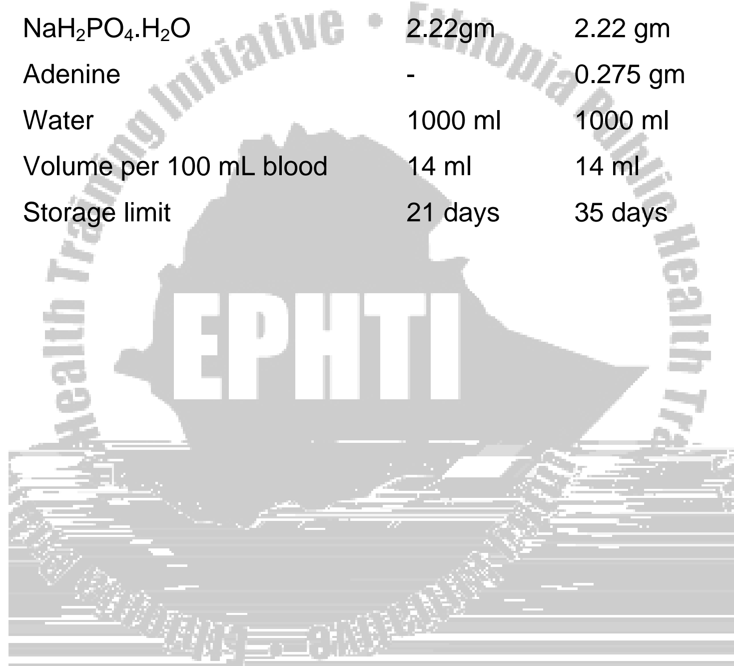
2. CPD

- Acts by binding Ca.
- Composition: In addition to the composition of ACD, CPD contains sodium phosphate, which maintains ATP levels in the red cells.
- Advantages of CPD over ACD.
  - Contains less acid.
  - Gives less hemolysis.
  - Smaller leak of K from the red cells.
  - Prolonged post- transfusion survival of red cells.
- To prevent the clotting of 100 ml blood 14 ml of CPD is required.
- Shelf life: survival of red cells in CPD 24hrs post transfusion is 80 to 85% after 21 days

The composition of two anticoagulant preservative solutions(CPD and CPDA1) is presented comparison purpose in Table 8.1

Table 8.1 Two anticoagulant preservative solutions in general use

	CPD	**CPDA-1**
Na_3 citrate	26.3gm	26.3 gm
Citric acid	3.27gm	3.27 gm
Dextrose	25.2 gm	31.9 gm
$NaH_2PO_4.H_2O$	2.22gm	2.22 gm
Adenine	-	0.275 gm
Water	1000 ml	1000 ml
Volume per 100 mL blood	14 ml	14 ml
Storage limit	21 days	35 days



collected, processed and stored under conditions, which maximize its storage capacity.

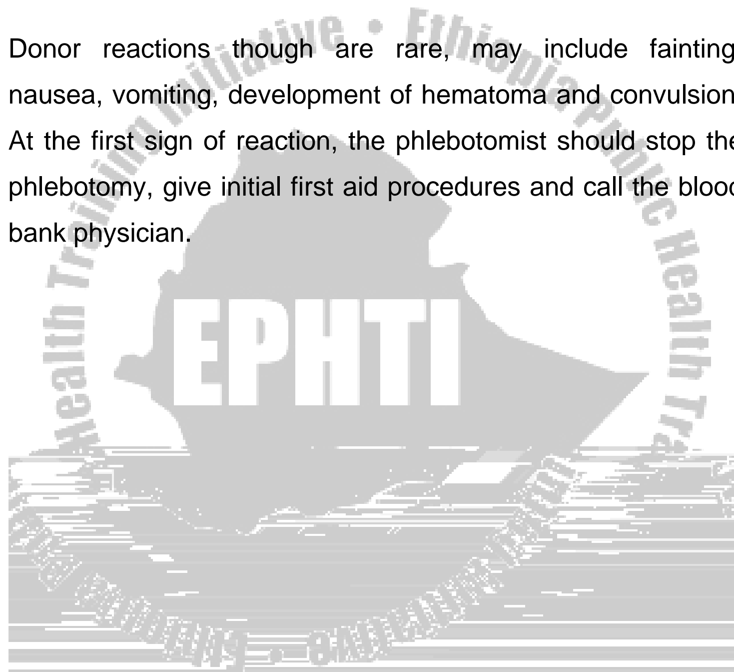
Fig.8.4 Blood component preparation

These components can effectively meet patient transfusion needs while keeping the risk of transfusion to a minimum. By using a single unit one can treat anemia with the packed cells,

platelate deficiency with platelate preparations, clotting factor and other plasma deficiencies with plasma preparation.

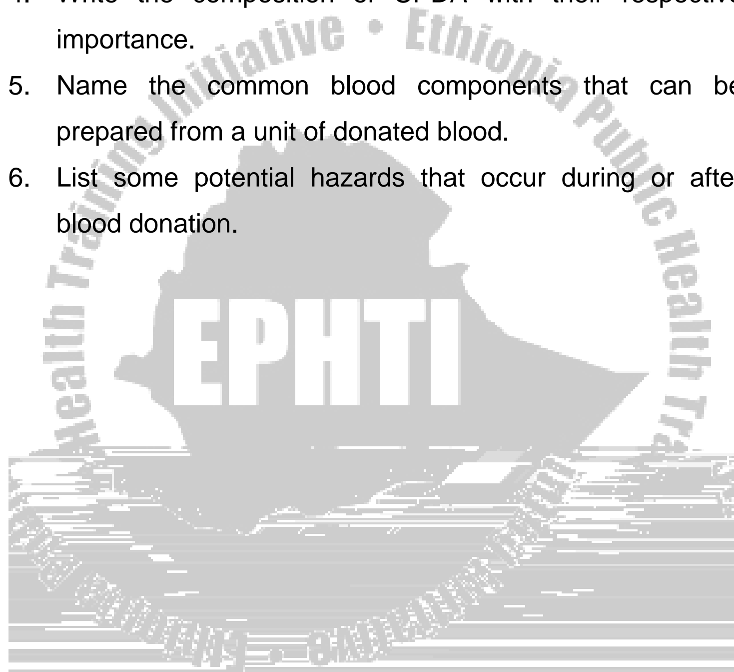
8.4 Potential Hazards During & after Blood Collection

Donor reactions though are rare, may include fainting, nausea, vomiting, development of hematoma and convulsion. At the first sign of reaction, the phlebotomist should stop the phlebotomy, give initial first aid procedures and call the blood bank physician.



Review Questions

1. List some clinical conditions that exclude a donor.
2. What are the steps in performing a venipuncture?
3. Where is the most common venipuncture site?
4. Write the composition of CPDA with their respective importance.
5. Name the common blood components that can be prepared from a unit of donated blood.
6. List some potential hazards that occur during or after blood donation.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CHAPTER NINE

THE TRANSFUSION REACTION

Learning ObjectiveS

At the conclusion of this chapter the student should be able to:

- Define the term "transfusion reaction"
- Classify transfusion reaction
- Carryout laboratory tests during transfusion reaction

9.1 Types of Transfusion Reaction

Any unfavorable response by a patient that occurs as a result of the transfusion of blood or blood products is termed as the transfusion reaction. Transfusion reactions can be divided into hemolytic and non-hemolytic types. Hemolytic reactions may be defined as the occurrence of abnormal destruction of red cells of either the donor or recipient followins up0 -19(i)-1(iodp0 -1-15.0063 0 TD86 TD0.0007.105

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Febrile reactions are the most prevalent type of immediate

pregnancy. The antibody is too weak to be detected in routine cross-match, but becomes detectable 3 to 7 days after transfusion, eg. Antibodies of the Rh system & kidd system.

9.2 Laboratory Tests to be done When Transfusion Reaction Occurs

Review Questions

1. What is a transfusion reaction?
2. On what basis do the transfusion reaction classified?
3. List laboratory investigations to be carried out when incompatible transfused reactions are suspected?

CHAPTER TEN

BASIC QUALITY ASSURANCE PROGRAM IN BLOOD BANKING

Learning objectives:

At the conclusion of the chapter the students should be able to:

- Understand the purpose of quality assurance program (QAP)
- Understand the areas to be focused in QAP
- Describe how to evaluate the quality of reagents, equipment and personnel

Quality Assurance is employed in the blood bank to support error- free performance to ensure the highest quality of patient care. Important factors in a routine quality assurance program include evaluation of reagents, equipment, and personnel qualification.

Quality control of reagents: commercial reagents in blood bank such as ABO and Rh antisera, Red blood cell products and Anti human globulin (AHG) reagent must meet the required specificity and potency. Each reagent on each day of

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Review Questions

1. What is the purpose of quality assurance program in Blood Banking.
2. List the areas to be focused in QAP in Blood Banking
3. How do you evaluate the competency of Blood Bank personnel?

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subsequent exposure to a previously encountered and recognized foreign antigen. An anamnestic response is characterized by rapid production of IgG antibodies.

Atypical antibody An antibody that occurs as an irregular feature of the serum.

Autologous donation Donation of blood for one's self. Autologous donation may take the form of predeposit or autotransfusion, for example, intraoperative autotransfusion, hemodilution, or postoperative auto transfusion.

Avidity (of an antiserum) A measure of the ability and speed with which an antiserum agglutinates red cells as a property of the combining constrant (K)

Bombay phenotype The failure of an individual to express inherited A or B genes because of the lack of at least one H gene and the subsequent lack of the resulting H precursor substance.

Bromelin A proteolytic enzyme prepared from the pineapple Ananas sativus.

Co-dominant genes Tow or more allelic genes, each capable of expressing in single dose.

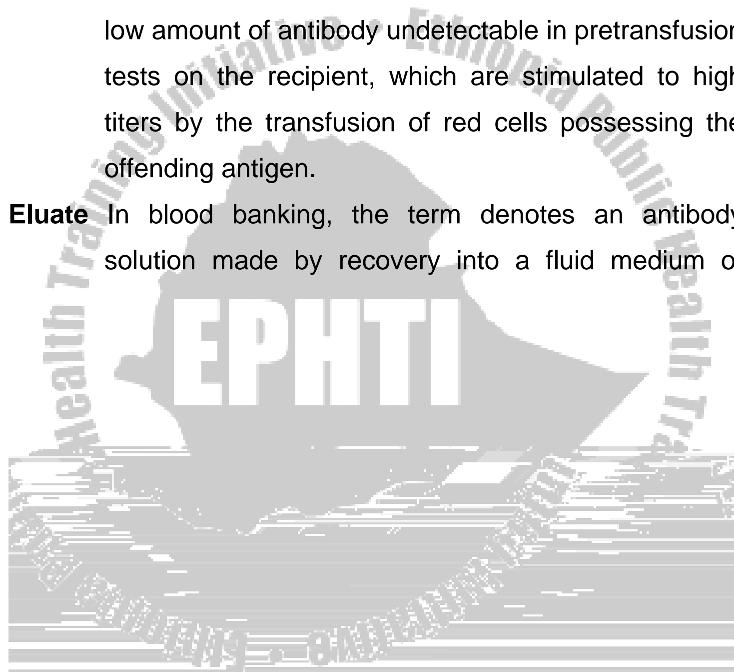
Compatibility test A series of procedures used to give an indication of blood group compatibility between the donor and the recipient and to detect irregular antibodies in the recipient's serum.

Coombs’test: The older term for the antiglobulin test.

Cord blood Blood taken from the umbilical vein or the umbilical cord of a newborn

Delayed hemolytic transfusion reaction A rapid increase in antibody concentration and destruction of transfused red cells a few days after transfusion usually due to low amount of antibody undetectable in pretransfusion tests on the recipient, which are stimulated to high titers by the transfusion of red cells possessing the offending antigen.

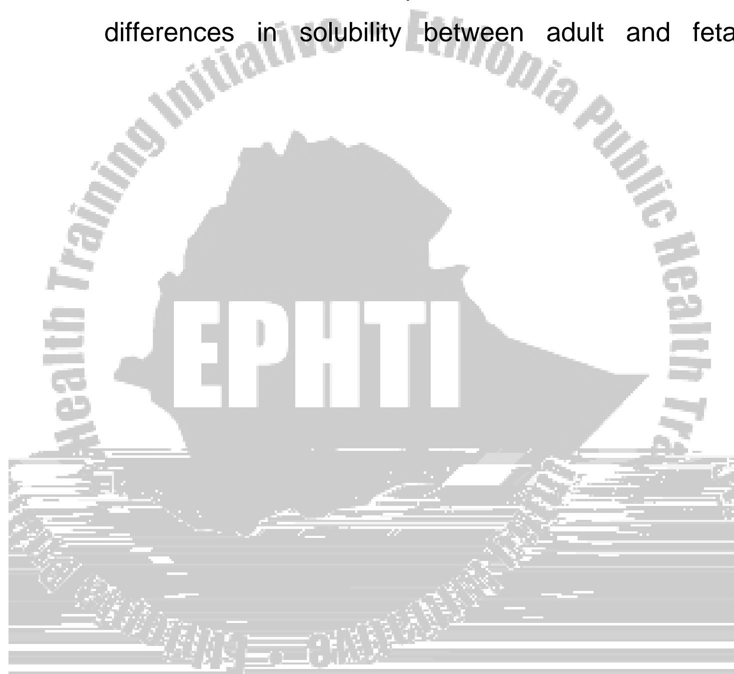
Eluate In blood banking, the term denotes an antibody solution made by recovery into a fluid medium of



Incomplete antibody Any antibody that sensitizes red cell suspended in saline but fails to agglutinate them.

Inheritance The acquisition of characteristics by transmission of chromosomes and genes from ancestor to descendant.

Kleinhauer- Betke test. A procedure based on the differences in solubility between adult and fetal



commonly seen as an acute transient condition secondary to viral infection.

Phenotype. The detectable or expressed characteristics of genes.

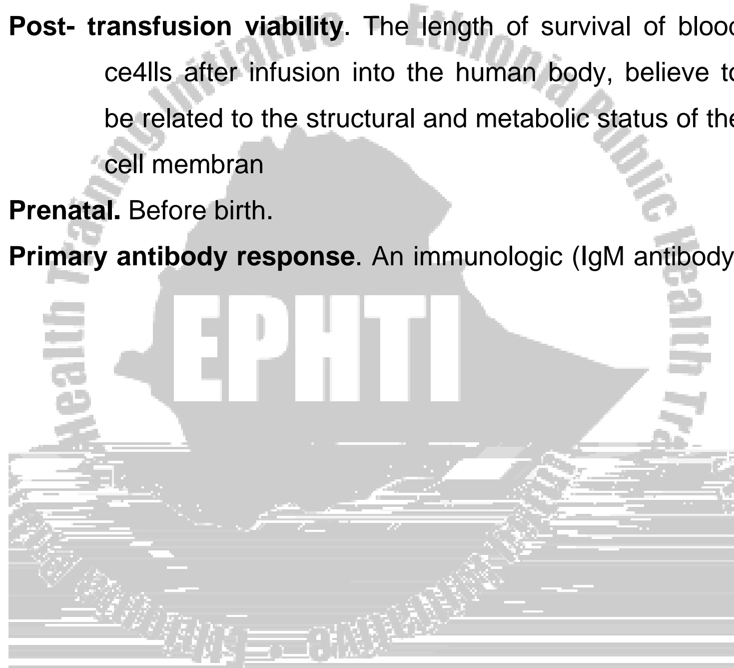
Postpartum. After birth.

Postnatal subsequent to birth

Post- transfusion viability. The length of survival of blood ce4lls after infusion into the human body, believe to be related to the structural and metabolic status of the cell membran

Prenatal. Before birth.

Primary antibody response. An immunologic (IgM antibody) h3u18 -1.aom 7mmu re memce4l9(o



Rouleaux. Pseudoagglutination or the false cluming of erythrocytes when the cells are suspended in their own serum. This phenomenon resembles agglutination and is due to the presence of an abnormal protein in the serum, plasma expanders, such as dextran, or wharton's jelly from cord blood samples.

Specificity. The complementary relationship between the binding sites of anibodies directed against determinants of a similar- type antigen.

Sensitization(of red cells) The specific attachment of antibody to its antigenic receptors on red cells without agglutination or lysis.

Sialic acid Any of a family of amino sugars containing nine or more carbon atoms that are nitrogen- and oxygen-substituted acylderivatives of neuraminic acid. It is a component of lipids, polysaccharides, mucoproteins and it is the main substance removed from the red cells by enzyme treatment.

Species- Specific Antigens restricted to members of a particular species.

Subgroups subdivisions of antigens; often weakened forms.

Specificity. The complementary relationship between the binding sites of anibodies directed against determinants of a similar- type antigen.

Transferase enzyme. A type of enzyme that catalyzes the transfer of a monosaccharide molecule from a donor substrate to the precusrsor substance. This type of biochemical activity is related to the development of A,B, and H antigens

Transplacental hemorrhage. The entrance of fetal blood cells into the maternal circulation.

Universal donor. A minomer often used for group O Rh negative blood.

Universal recipient. A general term used to refer to a group AB patient.

WAIHA. Warm autoimmune hemolytic anemia. This form of autoimmune anemia is associated with antibodies reactive at warm temperatures.

Wharton's jelly A mucoid connective tissue that makes up the matrix of the umbilical cord

Zeta potenitial The difference in electrostatic potential between the net charge at the cell membrane and the charge at the surface of shear.

Bibliography

1. Benjamini E, Coico R, sunshine G. **Immunology A short Course**, 4th ed. Wiley Liss,2000.
2. Boorman Kathleen E, Dodd Barbara E, Lincolon P.J. **Blood Group Serology**, 6th ed. Churchill Livingstone,UK, 1988.
3. Bryant Neville J **An Introduction to Immunohematology**, 3rd ed. Philedelphia, WB saunders, 1994.
4. Cheesbrough Monica. **District Laboratory practice in Tropical countries**, Part 2. Cambridge, UK, 2000.
5. Estridge BH, Reynolds AP, Walters NJ. **Basic Medical Laboratory Techniques**, 4th ed. Delmar, 2000.
6. Linne JJ, Ringsrud KM. **Clinical Laboratory science: The Basics and Routine Techniques**, 4th ed. Mosby, 1999.
7. Louise Mary Turgeon. **Fundamentals of Immunohematology, Theory and Technique**. 2nd ed. Williams and Wilkins, New York, 1995.
8. Mc Clelland DBL. **Hand book of transfusion medicine**, 3rd ed. The stationery office, London, 2001.
9. Tibebu M, **The Blood Bank Manual**, Ethiopian Red cross society, National Blood Transfusion Service, Addis Ababa, 1998.