TROPICAL COLLEGE OF MEDICINE

DEPARTMENT OF MEDICAL LABORATORY TECHNOLOGY

MODULE

ON

Performing immuno hematological tests

MODULE CODE: HLT MLS4 M08 10 11

FOR

Medical Laboratory Services Level IV

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Preface

This Learning Module on performing immuno hematological tests has been designed for level III Medical Laboratory Technicians based on the current curriculum.

The Learning Module comprises the following chapters: Introduction, Blood group genetics, Blood donation and processing, Performing immune hematological test, and The transfusion reaction.

This module aims to provide the learners with the knowledge, skills and right attitudes to exercise good laboratory practice and effective participation in performing immuno hematological tests.

The Learning Module comprises of four Learning Outcomes. Assessment method and criteria, as well the list of References books are included in the module.

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LEARNING MODULE	Logo of TVET Provider
TVET-PROGRAMME TITLE: **medical laboratory service Level IV**	
MODULE TITLE: P**erforming immuno hematological tests**	
MODULE CODE: **HLT MLS4 M08 10 11**	
NOMINAL DURATION: **120 Hours**	
MODULE DESCRIPTION: This module aims to provide the learners with the knowledge, skills and right attitudes to perform routine tests and procedures that are part of the requirements of pre- and post-blood transfusion practice. The unit also covers tests and procedures that are indicated in laboratory investigations in obstetric and perinatal medicine, in suspected haemolysis and haemolytic episodes and in other clinical circumstances.	

LEARNING OUTCOMES

1. Explain Immunohematological principles to resolve potential problems according to the guidelines
2. Discuss the different types of blood groups, associated antigens and antibodies that are important to prevent transfusion reaction using the immunohematological principles as a guideline.
3. Describe blood components and derivatives used for transfusion according to existing references
4. Collect samples for Immunohematological analysis following SOP
5. Perform Immunohematological tests for transfusion purposes according to a preset requirement
6. Apply OHS and safe working practices to maintain own health, health of others & environment following OHS guidelines.
7. Exercise good laboratory practice with effective participation in quality improvement using pre-set procedures.

LEARNING METHODS

- Lecture
- Group discussion
- Demonstration
- Practical exercise
- Project work
- Co-operative training

MODULE ASSESSMENT

- **Assessment Methods**
- Practical assessment by direct observation of tasks
- Written exam/test on underpinning knowledge
- questioning or interview on underpinning knowledge
- project-related conditions (real or simulated) and require evidence of process

- **Assessment Criteria**
  - Able to perform immune hematological test
  - Able to select blood donors
  - Capable of collecting blood for transfusion
  - Able to follow OHS
  - Able to achieve passing mark in written exam

REFERENCE BOOKS

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CHAPTER ONE

INTRODUCTION TO IMMUNOHEMATOLOGY

Immuno hematology:

- is more commonly known as **"blood banking"** or **"transfusion medicine"**
  - is a science deals with the immunologic reactions (antigen-antibody reactions) involving blood components.
  - Is the area of laboratory medicine dealing with the general procedures involved in collecting, preparing, storing (preservation) and transfusing blood.
- deals with concepts and clinical techniques related to modern transfusion therapy.

Application of immuno hematology:

- ☞ safe blood transfusion
- ☞ pathogenesis, treatment and prevention (such as HDN)
- ☞ Human genetics, Parenity testing & Criminology (e.g Forencing)

1.1 Historical perspective of blood transfusion:

1st phase:

- ❖ Ancient people knew that blood loss may cause death but they don't know Immuno hematology even if they know that blood transfusion is important.
- ❖ They used to give patients blood from lamb orally, which then be digested.

2nd phase (Era of blood transfusion):

- ❖ The era of blood transfusion began when William Harvey described the circulation of blood in 1616.
- ❖ They transfuse blood from human to human but the following factors and anti-factors affect their work:
  - Factors => RBCs (A, B, O…)
  - Anti-factors => Plasma/ serum (anti A, anti B, anti A&B…)
- ❖ These factors and anti-factors cause hemolytic reaction after transfusion.
- ❖ In 1818, James Blundell of England successfully transfused human blood to women suffering from hemorrhage at childbirth.
- ❖ Such species-specific transfusions seemed to work sometimes but mostly the result was death.

3rd phase (the modern scientists approach/ immune hematology era):

- Karl Landsteiner
  - discovered the ABO blood groups in 1900,
  - introduced the immunological era of blood transfusion.
- It became clear that the incompatibility of many transfusions was caused by the presence of certain factors on red (blood) cells now known as antigens.
- Two main postulates were drawn:
  - Each species of animal or human have certain factors on the red cells that are unique to that species, and
  - Each species have some common and some uncommon factors to each other.
- This landmark event initiated the era of science based transfusion therapy and was the foundation of immunohematology as a science.

1.2 Blood Group Genetics

- Deals with the way in which the different blood groups are inherited from parents to offspring.

Chromosomes and Genes:

- The nucleus of each human body cell contains 46 small thread-like structures called **chromosomes**, arranged in 23 pairs.
- The length of each chromosome is divided into many small units called **genes.**
- Genes code for different inherited physical characteristics, including blood groups.

Allomorphic genes (Alleles), and Polymorphism

- Each gene has its own locus, along the length of the chromosome.
- Certain inherited characteristic can be represented by a group of genes, and the locus can be occupied by only one of these genes.
- Such genes are called **alleles** or **allomorphic genes**.
- A, B & O are alleles of the gene blood group system.
- **Mitosis**: While body cells (autosomes) multiply they do so by producing identical new cells with 46 chromosomes.

- **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.

❖ During fertilization when the egg and sperm unite the fertilizer 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 arrange themselves in pairs in the nucleus.

Genotype versus phenotype:

Phenotype

- Physical expression of inherited traits,
- Determined by reacting red cells with known antisera

Genotype

- Actual genes inherited from each parent
- Can only be inferred from the phenotype .
- Family studies are required to determine the actual genotype .

Phenotype	Genotype
A	AA, AO
B	BB, BO
AB	AB
O	OO

Table 1.1. The ABO phenotypes and their corresponding genotypes

Punnet square

- Illustrates the probabilities of phenotypes from known or inferred genotypes.
- Visually portrays the potential offspring`s genotypes or the probable genotypes of the parents .

  - Two group A parents can have a group O child.
  - The parents of an AB child can be A, B or AB, but not group O.

	A	**O**
A	AA	AO
O	AO	OO

Table1.2. Punnet squares showing ABO inheritance

Dominant and recessive genes:

- **Dominant**
  - a gene which can be expressed over others (show itself if it is present)
- **Recessive**
  - a gene which is maxed if dominant gene is present (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.

- **Co-dominant**
  - The alleles are expressed equally (e.g AB)

❖ In most cases blood group antigens are inherited with co dominant expression.
❖ The product of each allele can be identified when inherited as a co dominant trait.

Homozygosity & Hetrozygosity:

Homozygous-

- Genotype is made up of identical genes, such as AA, BB, or OO,

Heterozygous.

- Genotype is made up of different alleles from each parent, such as AO, AB, or BO,

Mendelian principle (law of independent assortment):

Blood group antigens inherited on different chromosomes, are expressed separately and discretely.

"The inheritance of ABO is not affected by Rh" or "ABO and Rh are independently to the offspring."

Normally A and B are inherited independently on different chromosomes but exceptionally A & B are present and inherited on the same chromosome due to gene mutation and this is called **Cis-AB condition**.

If genes are inherited on opposite chromosome, it is said to be **Trans.**
Trans interaction may weaken the expression of one of the antigens encoded by the genes,
For example:

- The C and D genes of Rh system are inherited on different genetic loci .
- When C is inherited in trans to D, it will weaken the D antigen expression on the red blood cell.

Blood group O => silent blood group because it cannot be expressed
=> can receive blood only from O because A & B have anti A & anti B.
=> is a universal donor
Blood group AB => is a universal receptor because it has A & B antigens but no anti A & anti B.

Law of inheritance of ABO:
"Offspring do not have A or B blood type unless received from their parent."
"AB parents can't produce O offspring except Cis –type.

i.e, Father (AB/O) → **Offspring O**
Mother (AB/O) →

Q. Describe Chromosomal assignment of genes in blood group system?

Q. Describe the following terms:

- Linkage & haplotypes
- Silent genes (Amorphic genes) & Null phenotypes
- Suppressor/ regulator genes

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 genes, respectively. Each gene codes for the production of a specific transferase enzyme (Table 1.3), which catalyzes the transfer of a monosaccharide

molecule from a donor substance to the precursor substance, and enable us to convert the basic precursor substance to the particular blood group substance.

Gene	**Enzyme**
H	L- fucosyl transferase
A	3 N-acetyl- D- galactosaminyl transferase
B	3-D- galactosyl transferase
O	None

Table 1.3 ABH Genes and Their Enzymatic Products

- 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 cannot be formed. This rare group is referred to as **Oh (Bombay group).**
    - **Bombay phenotype (Oh):**
        - Have no H-gene (hh)
        - No ABO & H-antigen
        - Have anti-A, anti-B & anti-H
        - Safe (compatable) blood donors are another Bombay individuals

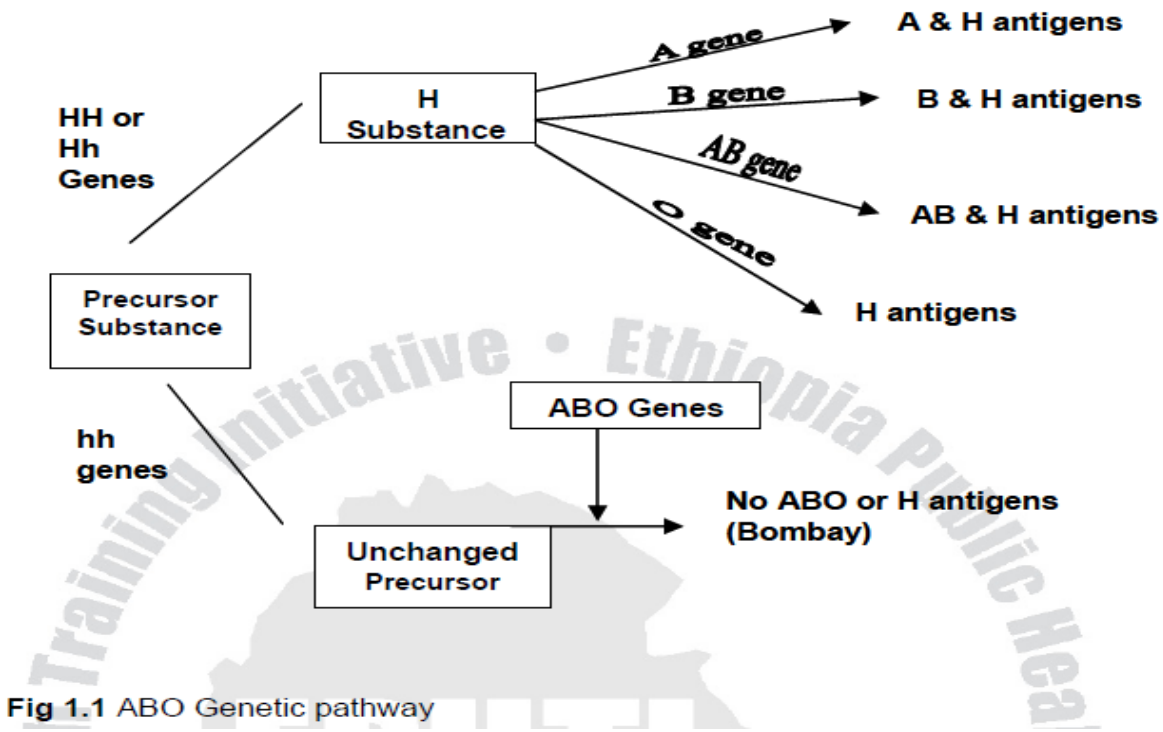


Fig 1.1 ABO Genetic pathway

Inheritance of Rh:

- There are about 35 factors expressing Rh.
- Mostly expressed include: D, C, E, d, e, c
- There are 3 alleles that are present on one chromosome in different locus; these are: Dd, Cc, Ee
- D is the most important allele to indicate Rh because it has 60 sites and it max C and E alleles.
- DD = the most important
- Dd
- dd = indicate that there is no D allele
- The correct order is C D E
    - C D E => Rh+ } because they contain D factor
    - C D e => Rh+ }
    - c d E => Rh- because it do not contain D factor

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.
Majority of the population secrete water- soluble substances in saliva and most other body fluids that have the same specificity as the antigens on their red cells.

- **Secretors =>** have water soluble antigens & alcohol soluble antigens
- **Non-secretors =>** doesn't have water soluble antigens but have alcohol soluble antigens

Saliva is mostly used as a reference for identifying secretors and non-secretors.
The production of A, B & H antigens in saliva is controlled by a **secretor gene**, which is inherited independently of the ABO and H genes.
The relevant gene is called **Se**, and its allele which amorphic is **se.** At least one Se gene (genotype **SeSe** or **Sese**) is essential for the expression of the ABH antigens in secretors. Individual who are homozygous for se (**sese**) do not secrete H,A, or B antigens regardless of the presence of H,A or B genes.

- The secretor gene controls only the presence or absence of the H-substance (i.e, the L- fucosyltransferase) in body secretions. It doesnot affect the presence of H-subs on erythrocytes. The presence of A&B transferases on erythrocytes is not controlled by the secretor gene.

The Se gene does not affect the formation of A,B or H antigens on the red cells or in hematopoietic tissue, which are alcohol soluble and which are not present in body secretions.
Oh (Bombay) individuals do not secrete A, B or H substance, even when the Se gene is present.

1.3 Blood group antigens:

Antigen is 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.

A unique set of red blood cell Ag is determined through genetic inheritance.

These antigens protrude from the surface of the RBC in three dimensional configurations.
As a result, they are accessible to Ab molecules for agglutination reaction.
In biochemical terms these antigens may take the form of:

- proteins,
- Glycoprotein,
- Glycolipids

Some of the red blood cell antigens are more immunogenic than the others

Example: The D antigen within the Rh group system.
RBCs of the donor will elicit the recipients' immune response because RBCs of the donor contains A and B antigens.

1.4 Blood group 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. 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, it has 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:. Four sub classes of IgG on the basis of structural and serological differences and are known as IgG_1, IgG_2, IgG_3 and IgG_4.

IgM:

- Accounts for about 10% of the immunoglobulin pool, with a concentration of about 1.0 g/l in normal serum.
- Is the predominant antibody produced in a primary immune response
- Is structurally composed of five basic subunit (pentameric), and has the largest MW of 900,000.

Because of its large size IgM cannot pass the placental barrier to the fetus
- Is complement binding.

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.

1.4.1 Type of antibodies

Based on their development, blood group antibodies are classified into two:

A. 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 in modern use.

- Example: Antibodies in **ABO** blood group system

Characteristics:

- Exhibit optimum in vitro agglutination reaction with antigen bearing erythrocytes suspended in physiologic saline (0.85%) sodium chloride, sometimes referred to as **complete antibodies**. This is because they have wide antigen binding site (example IgM).
- Give optimum reaction at room temperature or lower, and they are also called **cold agglutinins**.
- *These antibodies do not generally react above 37^{o}C that is at body temperature, for this reason most of these do not generally give rise to transfusion reactions.*
- These antibodies are of high molecular weight that they can't cross the placental barrier, eg. IgM.

B. Immune antibodies: are antibodies evoked by previous antigenic stimulation either by transfusion, transplantation or pregnancy,i.e. as a result of immunization by red cells.

- Example: Antibodies in **Rh** blood group system

Characteristics:

- Do not exhibit visible agglutination of saline- suspended erythrocytes but in albumin medium and called **incomplete antibodies.**
- React optimally at a temperature of *37^{o}C*, and are so called **warm agglutinins.**
- *These antibodies obviously have more serious transfusion implications than the naturally occurring ones.*
- These antibodies are so small that they can cross the placental barrier, e.g. IgG

Landsteiner's principle says:

"*An individual produce ABO antibody when corresponding Ag is lacking*."

- A-cell produce anti-B because it lacks B antigen.
- B-cell produce anti-A because it lacks A antigen.

This principle is used in the production of natural antibodies.

CHAPTER TWO

THE ABO BLOOD GROUP SYSTEM

2.1 The discovery of ABO blood group system:

- It was the first blood group system discovered by **Karl Landsteiner** in 1990.
- Karl Landsteiner:
  - the beginning of modern blood banking and transfusion medicine.
  - described the blood groups as A, B and O.
  - Stated rule - **Land steiner's rule:**
    "Normal, healthy individuals possess ABO antibodies to the ABO Antigens lacking on their RBCs."
  - Identify ABO BGs based on the agglutination reaction between RBC & serum.
    - **A** = agglutination reaction between anti-A antiserum and RBCs.
    - **B** = >> >> anti-B antiserum and RBCs
    - **O** = no agglutination reaction
- Von Decastello and Sturli:
  - Discover the way that anti-A antiserum and anti-B antiserum both react with RBC which indicate blood group **AB**.

Blood group	Ag on RBC	Ab in plasma
A	A antigen	anti-B
B	B antigen	anti-A
O	Neither A nor B antigen	anti-A & anti-B
AB	A & B antigen	Neither anti-A nor anti-B

Inheritance of the ABO Groups:

- 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).

- For example if 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.
  - 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 or group B individuals, eg. the finding of a group O child in an AxB mating demonstrates the presence of the O gene in both parents, and it follows that any A or B children from this particular mating are heterozygous , i.e. AO or BO.

Mating		**Children**	
Phenotypes	**Genotypes**	**Genotypes**	**Phenotypes**
AxA	(1)AAxAA (2)AAxAO (3) AOxAO	(1)AA (2)AA and AO (3)AA,AO and OO	A and O
AxB	(1)AAxBB (2)AAxBO (3)AOxBB (4)AOxBO	(1)AB (2)AB and AO (3)AB and BO (4)AB,BO, AO, and OO	A,B AB, and O
AxAB	(1)AAxAB (2)AOxAB	(1)AA and AB (2)AB, AO,BO and OO	A,B and AB
AxO	(1)AAxOO (2)AOxOO	(1)AO (2)AO and OO	A and O
BxB	(1)BBxBB (2)BBxBO (3)BOxBO	(1)BB (2)BB and BO (3)BB,BO, and BO	B and O
BxAB	(1)BBxAB (2)BOxAB	(1)AB and BB (2)AB,BB, AO, and BO	A,B, and AB
BxO	(1)BBxOO (2)BOxOO	(1)BO (2)BO and OO	B and O
ABxAB	(1)ABxAB	(1)AA,AB and BB	A,B, and AB
ABxO	(1)ABxOO	(1)AO and BO	A and B
OxO	(1)OOxOO	(1)OO	O

Table 2.2 The ABO mating with possible genotype and phenotype of children.

- In1930 Thompson proposed a four allele theory of inheritance based on the discovery that A antigen could be divided in to **A_1** and **A_2 sub groups.**
- Thompson's four-allele theory encompassed the four allelic genes, **A_1, A_2, B** and **O**. These 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 2.3).

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(or A_2B/O)
O	OO

Table 2.3 ABO phenotypes and genotypes, including A1 and A2

- 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 which both A and B have been shown to be inherited from one parent, this condition is called **Cis- AB** .
- In serological testing, individuals of this type have a weaker B antigen and possess some kind of anti- B in the serum.

Mating possible Phenotypes	Mating possible Genotypes	Possible phenotypes of children
A_1 x B	A_1A_1xBB	A_1B
	A_1A_1xBO	A_1B,A_1
	A_1OxBB	A_1B,B
	A_1OxBO	A_1,B,A_1B,O
	A_1A_2xBB	A_1B,A_2B
	A1A2xBO	A1,A2,A1B,A2B

Table 2.4 the six possible genotype mating included in the one phenotype mating A_1 x B together with the phenotypes which can be found among the offspring of each mating.

- Sometimes by studying the phenotypes of the children it is possible to say which genotype the parents belong. For example, it can be seen that for the matings A_1xB, A_2 and A_2 B children never occur in the same family as B or O children.
- This follows that taking all A_1xB mating together, all six phenotypes can occur. However, the finding of, for instance, a group O child in a family where other children are A_2 and A_2 B would not be possible if they all had the same parents.

Q. Discuss more about ABO subgroups??

Q. What is acquired B phenomena??

2.2 Antigens of the ABO blood group system:

- Are A, B and H-antigens.
- ABO BGS antigens are widely distributed and located on:
  - ☞ red blood cells
  - ☞ lymphocytes
  - ☞ platelets
  - ☞ tissue cells
  - ☞ bone marrow, and
  - ☞ organs such as the kidneys.

- Soluble forms of the antigens can be:

  - synthesized and secreted by tissue cells
  - found in association with cellular membranes and
  - in all body fluids except CSF
  - are glycoproteins

- ABO system Ags, which are intrinsic to the RBC membrane

  - exist as either Glycoprotein or Glycolipid molecules,
  - begin to develop & are detectable in the 6th week of fetal life in utero.
  - are weaker on cord blood and result in weaker ABO phenotype reaction.
  - develop slowly and reach the full expression of adult levels at approximately 2 to 4 year of age (average ≥3 years of age).
  - Antigen determining sites are 50% less of the adult in children <3 years (neonates).

Development of the A and B Ags:

- The gene for A and B Ags are located on Chr.9.
- Three major alleles on ABO locus: A, B, and O.

- A-allele codes for N-acetylgalactosaminyl transferase, which transfers N- acetyl galactosamine (immunodominant sugar) to H-Ags

- The B-allele codes for D-galactosyl transferase which transfers D-galactose (Immunodominant sugar) to the H-antigen.

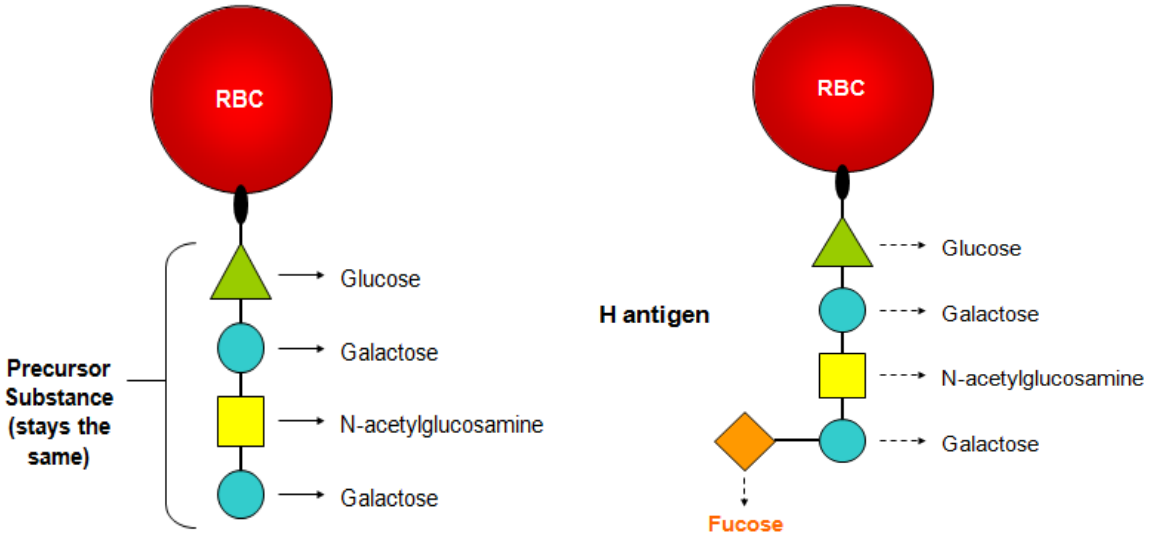
- The O allele is considered non functional, since the resulting gene product is an enzymatically inactive protein.

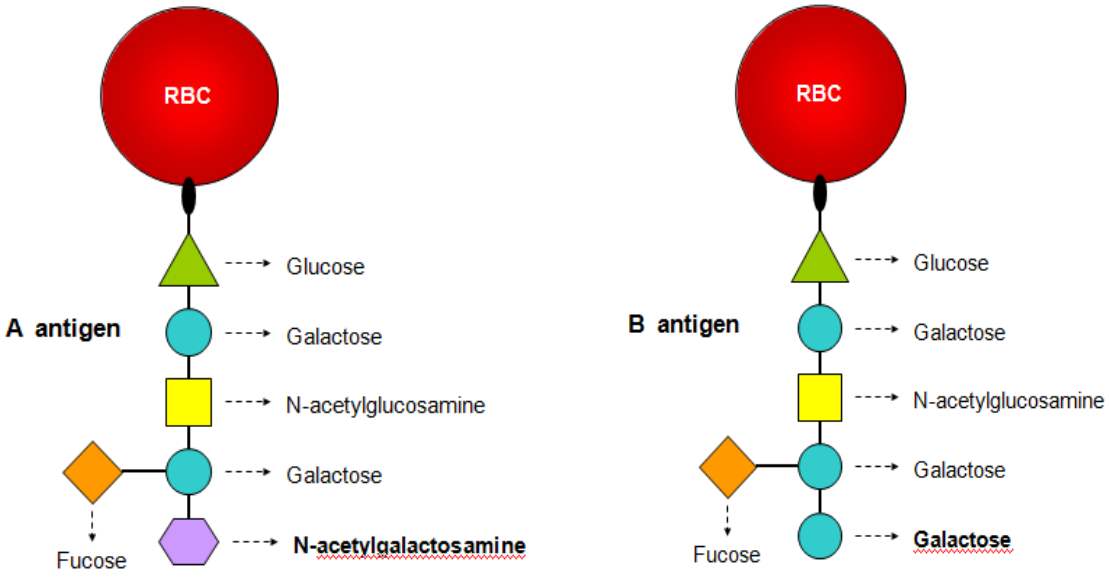
- Group O RBCs carry no A or B Ags but are rich in unconverted H Ags.
- Adult group O RBCs possess the greatest concentration of H Ags per RBC.

- Other ABO phenotypes have fewer copies of H Ags, since the H Ags is the acceptor molecule for the A and B enzymes.
- Group A_1B phenotype possesses the lowest number of unconverted H sites.

Concentration of H-antigen on the surface of RBCs:

O > A_2 > B > A_2B > A_1 > A_1B





2.3 Bombay and para-bombay groups:

Bombay phenotype (hh) individuals:

- do not inherit the H gene and do not possess the enzyme H transferase
- are unable to produce H substance.
- Can inherit the A and B genes, as they do not make the H substance the A and B gene is not expressed and so typed as group “O” unlike group O individuals they lack H.
- Cause - mutation in the H gene on chromosome 19 that causes a non functional H glycosyl transferase to be produced.

Para Bombay:

- Do not have H antigen but possess trace amounts of A or B antigen depending on the ABO gene on chromosome 9.
  - Small amount of H antigen is produced and almost completely converted to A or B antigen if these enzymes are present.
- These individuals are termed Ah or Bh respectively.
- Cause - production of very weakly acting H glycosyl transferase.

2.4 Antibodies of the ABO blood group system:

- Present in individuals naturally with no known exposure to blood or blood products (without any antigenic stimulation)
- Are not detected in the serum of new born until 3 to 6 months of age.
- With advanced age the ABO titers tend to decrease

- Immune Abs have greater transfusion implication than natural Abs. e.g HDN

1st view (Environmentalist theory):

- ❖ A-cells produce anti-B antibody sensitized by external factors which is similar to A & B antigen.
- ❖ B-cells produce anti-A antibody sensitized by external factors which is similar to A & B antigen.

- This theory was experimentally supported by experiment on Chikens that develop similar, naturally occurring Isoagglutinins.
- But in 'germ free' environment these Chickens failed to develop naturally occurring Isoagglutinins.

2nd view:

- Ab production is genetically determined.
- For instance, A_1 phenotype **=>** has 3 genotypes (A_1A_1, A_1A_2, A_1O)
  - ☞ These 3 genotypes produce different **anti-B** antibody**.**
- Rather than saying natural Ab, they are called **NRCS antibodies** (Non Red Cell Stimulated Abs).

General characteristics of Human anti-A and anti-B:

- Anti- A and anti- B are primarily IgM but it might be IgM & small amount IgG mixture.
- Sera from group O and B individuals contain anti- A antibodies.
- anti- A can be separated into: anti- A and Anti- A_1.
- Common anti- A can react with A_1 and A_2 antigens.
- anti- A_1 antibody is specific for the A_1 antigen and does not agglutinate A_2 RBCs.

A_1 phenotype:

- ➢ Is encoded by the A_1 gene
- ➢ exists in approximately 80% of group A individuals
- ➢ is more antigenic, strong and dominant than A_2
- ➢ A_1 gene effectively acts on the H antigens in the production of A Antigen.

A_2 phenotype:

- ➢ encoded by the A_2 gene,
- ➢ Constitutes approximately 20% of group A individuals.
- ➢ Has much H-antigen because it has small enzyme and so produce small H-substance than that of A_1

❖ Both A_1 and A_2 RBCs agglutinate with commercially available anti- A reagents.

2.5 Anti-serum:

- An antiserum is a purified, diluted and standardized solution containing known antibody, which is used to know the presence or absence of antigen on cells and to phenotype once blood group.
- Antiserum is named on the basis of the antibody it contains:
  - ☞ Anti- A antiserum which contains anti- A antibody
  - ☞ Anti- B antiserum which contains anti- B antibody
  - ☞ Anti- AB antiserum, which contain both anti A and B antibodies.
  - ☞ Anti –D antiserum which contains anti- D antibody

Sources of anti-sera:

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

Basic criteria for antisera:

a.) Specificity: Ab should be produced to the corresponding Ag to prevent cross reaction.

b.) **Avidity:** Ab should give quick and clear reaction

c.) Sterility: should be sterile

d.) Stability: should be stable/ not deteriorate (maintain specificity & avidity) till its 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**.
- It should be preserved with **1% sodium azide**, have a marked expiration date, and should be stored at **4^0C**. The manufacturer directions must be followed carefully.

2.6 Manifestation and Interpretation of Antigen- Antibody reactions:

- In blood banking there are two observable reactions resulting from the combination of a red cell antigen with its corresponding antibody that manifest positive reaction:
    - Hemagglutination (most commonly used)
    - Hemolysis

Hemagglutination: is the clumping of RBCs with antigens on their surface 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.

- 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.

A. Sensitization - first stage: 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.

B- Lattice formation – the second phase: is the establishment of cross links between sensitized RBCs and Abs resulting in clumping. There is breakage of zeta potential. 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.

Hemagglutination can be graded by aggregate as follows:

4+ - One solid aggregate;
- With no free cells (all RBCs react)
- Clear supernatant

3+ - Several large aggregates;
- Few free cells
- Clear supernatant

2+ - Several medium sized aggregate
- Some free cells
- Almost clear supernatant

1+ - Much small aggregates
- Many free cells

(Negative) - No aggregates,
- Red blood cell all intact.

Hemolysis: is the break down or rupture of the red cell membrane by specific antibody (hemolysin) through the activation of complement with the release of hemoglobin, and the librated hemoglobin can easily be observed staining the supernatant fluid red pink.

ABO Blood Grouping:

- Determination of ABO grouping is important in pre-transfusion studies of patients and donors as well as in cases of obstetric patients.
- There are different techniques to determine ABO grouping in the laboratory: slide & test tube. 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.

Rules for Practical Lab Work:

- Perform all tests according to the manufacturer's direction
- Run controls at least once per day
- Always label tubes and slides fully and clearly.
- Do not perform tests at temperature higher than room temperature.
- Do not rely on colored dyes to identify reagent antisera.
- Always add serum before adding cells.
- Tubes should be shaked well & inspected against well lighted background.
- Use optical aid if the reaction is not clearly visible by naked eye.

Methods of Blood Grouping:

1. Direct method/ forward grouping/ cell typing/ front typing:

- Use known reagent anti sera to identify the antigen present or their absence on an individual's RBCs.
- Is mostly accomplished and routinely done.

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 2.5

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 room T^{o} 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 2.5

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

Table 2.5 Reactions of patient Erythrocytes and known Antisera

2. Indirect grouping/ reverse grouping/ serum typing/ back typing:

- Use red cells possessing known antigen to determine the type of antibodies (anti A & -B) present, or absence of these antibodies in serum.
- It is also called confirmatory grouping; it used as a confirmatory test for direct grouping.
- 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 room T^{o} 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 2.6

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

Table 2.5 Reactions of patient serum and reagent erythrocytes

The Right Conditions for RBCs to Agglutinate:

Antibody size (suspending medium):

- 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 cannot 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**. While **IgG** molecules may use **bovine Albumin** that can break the zetha potential of RBCs causing them to agglutinate.

PH:

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

Temperature:

- IgM reacts at room temperature and cold temperatures (≤25^{o}C)
- IgG reacts at warm temperature (37^{o}C)

Centrifugation:

- Centrifugation at high speed attempts to overcome 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.

Ratio of antibody to antigen:

- There must be an optimum ratio of antibody to antigen sites for agglutination of red cells to occur. **Prozonal** (Ab excess) & **post zonal** (Ag excess) conditions should be avoided.
- 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 overcome 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.

Time:

- Too short incubation time results in weak agglutination
- Too long incubation time results in agglutination (false positive reaction) or dissociation (false negative reaction).

Preparation of Red Cell Suspension:

- The procedures include a red blood cell washing step to remove certain impurities; and when necessary you can use this formula to prepare different red cell concentrations.

$$\text{\% required} = \frac{\text{PCV} \times 100}{\text{Volume of suspension}}$$

Procedure: (e.g. preparation of a 2% red blood cell suspension of 10 ml volume)

1. Place 1 to 2ml of anticoagulated blood in a test tube
2. Fill the tube with saline (0.85%) and centrifuge
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 into another clean test tube
6. Add 0.2 ml of the packed cell button to the 10ml of saline
7. Cover the tube. Immediately before use, mix the suspension by inverting the tube several times until the cells are in suspension.

Discrepancies (Anomalous Results) in ABO testing:

1. Technical errors: are common sources of discrepancies. Includes:

- ☞ Clerical errors (identification, labeling & recording errors)
- ☞ Dirty glass wares & contaminated reagents
- ☞ Under & over centrifugation

- ☞ Incorrect incubation temperature
- ☞ Failure to add reagent or adding improper amount or using expired/ contaminated reagent
- ☞ Careless reading (not considering hemolysis as positive result)
- ☞ Using too heavy or too light RBC suspension
- ☞ Failure to follow the manufacturer's instruction

To overcome technical errors, do procedures repeatedly with correct testing protocols. But if discrepancy happens again, it may be due to patient abnormality.

2. Patient abnormalities:

I. **Missing or weak reacting antibodies**

- **Age:** - Infants have weak ABO Abs
  - Elders have decreased Ab level
- **Hypogamaglobulininemia:** - less amount of globulin (Ab) in plasma caused by conditions like lymphomas, leukemias, immunodeficiency disorders, use of 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 (25^oC) for 15 min
  - or at 16^0C or 4^0C for 15min.

II. **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 & 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.

III. **Additional antibodies**

- **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 and Anti-A_2:** A_2 & A_2B individuals may produce naturally occurring anti-A_1 similarly anti-A_2 may be produced by A_1B individuals, which cause discrepant ABO typing.
- **Irregular antibodies:** Irregular antibodies in some other blood group system may be present that react with antigens in ABO blood group system.
- **Cis-AB:** cis-AB individuals can produce anti-B but not anti-A
- **Polyagglutination:** occurs naturally when zetha potential which is maintained by sialic acid is disturbed

Resolution:

- ☞ Washing the patient red cells in warm (370C) 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.
- ☞ Lectin study – knowing the level of sialic acid which is genetically determined

IV. **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 giving false positive agglutination.

Resolution:

☞ wash the patients cells with saline to remove proteins that cause rouleaux

CHAPTER THREE

The RH-Hr Blood group system

3.1 Historical background of Rh-Hr blood group system:

- In 1940 **Landsteiner** & **Wiener** discovered a human blood factor, which they called Rhesus factor.

- They immunized guinea pigs and rabbits with blood from the Macacus rhesus monkey, and the antiserum obtained (**anti- Rh**) agglutinated not only the red cells of the rhesus monkey but also 85% of human.

    - ☞ **If donor red cells were agglutinated by antiserum => Rh+**
    - ☞ **If donor red cells were not agglutinated by antiserum => Rh-**

- This discovery followed the detection of an antibody occurred in the serum of a woman who delivered a stillborn fetus by **Levine** & **Stetson** in 1939.

- They also postulated that the antibody had arisen as the result of immunization of the mother by a fetal antigen which had been inherited from the father.

- In 1940, **Wiener** & **Peters** showed that the antibody (anti- Rh) could be found in the serum of individuals who had transfusion reactions following ABO group – compatible transfusions.

- In 1941, **Levine** & his co- workers showed that not only could 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 or Hemolytic Disease of the New born (HDN).

- Later work demonstrated that the animal or rabbit anti-Rhesus and human anti-Rh are not detecting the same antigen but the system had already named the human antibody anti-Rh.

- The animal anti-rhesus 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 and Wiener who discovered it, and the human antibody retained the title anti-Rh.

3.2 Nomenclature and Genetics theories:

1. 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 **one gene 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 (doesn't represent any gene, allele or antigen), 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.
- ☞ D is the most strong and dominant antigen. Because of the strong antigenic characters of D, all individuals who lack the D antigen are said to be **Rh negative** regardless of whether the C or E antigen or both are present.

2. Wiener Nomenclature:

- Wiener's theory states one gene instead of three closely linked ones, produces one complex antigen (called agglutinogen) which is made up of three factors found on the red cells.
- The antigen is complex but the gene is not complex rather the gene produce complex antigen.
- According to Wiener, eight alleles exist at the Rh gene locus: R^O, R^1 R^2, R^z, r, r^l r^{ll} and r^y.
- The difference between the wiener and Fisher- Race theories is the inheritance of the Rh system is on a single gene locus rather than 3 separate genes.

3. Rosen field Nomenclature:

- Use numbering system to name the genes.
  D = Rh_1 E = Rh_3 e = Rh_5
  C = Rh_2 c = Rh_4
- Add "**-**" sign on the numbers to indicate absence.

  e.g. CDE => Rh: +1, +2, +3, -4, -5

- It is easy to write but difficult to speak (oral communication), so it is ignored.
- But Fisher – Race Nomenclature is the best one.

Fisher- Rase	Wiener	Rosen field
D	Rh^o	Rh_1
C	rh'	Rh_2
E	rh''	Rh_3

c	hr'	Rh_4
e	hr''	Rh_5

Table 3.1 Comparison of Nomenclature of antigens of the Rh system

Example: CDe (Fisher - Rase) = rh', Rh^o, hr'' (Wiener)

cdE = hr', rh'' here we ignore d because it doesn't represent any gene

Fisher- Rase	**Wiener**	Shorthand for Wiener
CDe	Rh_1 (rh',Rho,hr '')	R_1
c DE	Rh_2 (hr',Rho,rh'')	R_2
c De	Rh_o (hr',Rho,hr'')	R_o
CDE	Rh_z (rh',Rho,rh'')	R_z
Cde	rh_1 (rh',hr'')	r'
c dE	rh'' (hr',rh'')	r''
c de	rh (hr'',hr'')	r
CdE	rh_y (rh',rh'')	r_y

Table 3.2 Comparison of Fisher- Race & Wiener nomenclature

- 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.

3.3 The antigens of the Rh-Hr blood group system:

- 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 Rh antigens can be demonstrated on fetal red cells at **38 days after conception**, and are well developed at birth.
- Order of antigenicity: **D > c > E > C > e**
- Rh-Hr antigens are totally proteins.
- 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.

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.

D antigen:

- Is the most immunogenic antigen in the Rh system (having antigen site between 110,000 and 202,000 per erythrocyte,).
- The C gene weakens the D expression if inherited on the opposite chromosome.
- Based on D expression:

D> R_2R_2 > R_1R_1 > R_1r or R_or>R_1r^1 or R_or^1

Weak antigen D (Du):

- 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 Du :
- **High grade Du** red cells, which are agglutinated by certain **anti-D** sera and
- **Lower grade Du** red cells, which are agglutinated only by the **Indirect Antiglobulin (IAG) test**.
- In case of blood transfusion, donors with Du + red cells are regarded as Rh+ because, a severe hemolytic transfusion reaction may result from the transfusion of Du + red cell to a recipient whose serum contains anti D. As a recipient individuals with Du + red cells regarded as Rh negative, because of the risk of provoking the formation anti-D in a Du + 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 Du infant can suffer from HDN if the mother possesses anti –D.
    - As a **donor** individuals with Du + antigen regarded as **Rh +**.

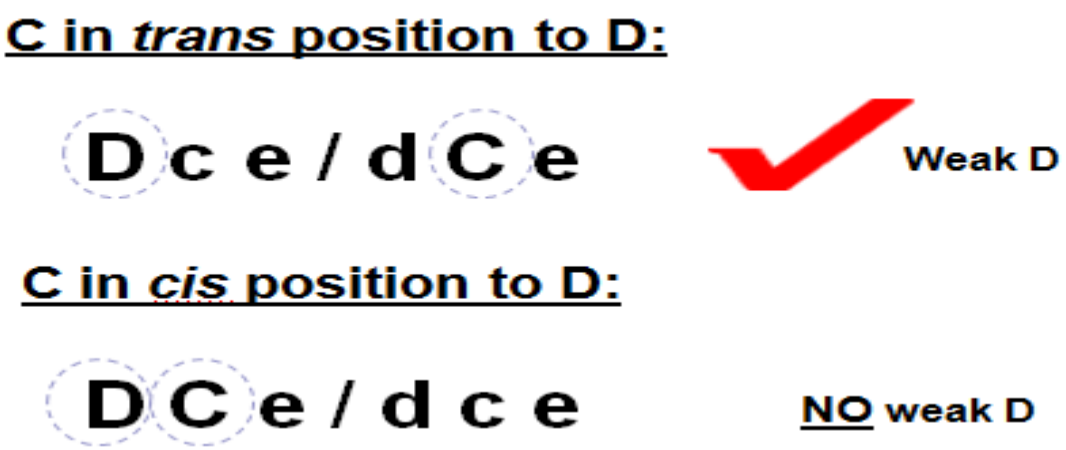
- As a **recipient** individuals with Du + antigen regarded as **Rh -**.
- Weaker D expression can result from several different genetic circumstances, such as:
  - Genetic
  - Position effect
  - Partial D (D-mosaics)

D Mosaic/Partial D

- If the patient is transfused with D positive red cells, they may develop an anti-D alloantibody to the part of the antigen (epitope) that is missing.
- It is ***qualitative***.

Position Effect

- Gene interaction effect.
- C allele is in trans position to D allele.
- Does not occur when C is in cis position.
- Steric hindrance causes the anti-D reagent to weakly attach (C Ags crowds the D antigen).



Weakened D (Genetic)

- Inherited weak D. More common in African Americans.
- These individuals may inherit the RHD gene that encodes for a weaker expression (Dce haplotype).
- Quantitative, meaning less is expressed.

3.4 The antibodies of Rh-Hr blood group system:

- The common Rh antibodies are **anti –E**, **anti-e**, **anti-C**, **anti-c** and **anti –D**.
- Rh antibodies occur in individuals who lack the corresponding antigens, and as a consequence of **transfusion** or **pregnancy** (i.e as a result of immunization by red cells) but **not in transplantation** b/c they are not present present in tissue cells; they are fixed in RBCs.
- Are not present in soluble form.
- The major antigen is D-antigen, so the major antibody is **anti-D.**
- Rh antibodies generally develop from **2 to 6 months** after the initial immunization by red cells.
- The predominant Rh antibodies are immunoglobulin class IgG, which most of them are IgG_1 or IgG_3 subclasses.
- More than 90% problems of Rh-Hr BGS are due to anti-D.
- Following transfusion of one or more units of Rh positive blood, 50 to 75% of D negative recipients develop anti- D; but 25 to 30% of D negative individuals are non responders, unable to produce anti-D inspite of repeated stimulation with Rh+ blood.
- Rh antibodies cause severe hemolytic transfusion reaction in a recipient if transfused with blood possessing the offending antigen.

- Rh antibodies being IgG, are capable of crossing the placenta and are associated with hemolytic disease of the new born (HDN).

The Rh- Hr Blood Grouping Technique:

- Direct slide and
- Direct tube

❖ Reverse grouping cannot be done due to the absence of naturally occurring Rh antibodies in the serum of persons lacking the corresponding Rh antigen.

Clinical significance:

- For safe blood transfusion
- Prevention of Hemolytic Disease of the Fetus and Newborn (HDFN)

Principle:

When an antigen is mixed with its corresponding antibody under the right conditions it causes **agglutination** or **haemolysis** of the red cells.

1. Slide Typing:

Specimen:

Washed RBC suspension (40-50%)

Equipments and reagents:

- Anti- D reagent
- Microscopic slide
- Microscope
- Applicator stick
- Albumin (Control)

Procedure:

- Place a drop of anti-D on a labeled slide.
- Place a drop of Rh control (albumin or other control medium) on another labeled slide.
- Add two drops of 40-50% Red cell suspension to each slide.
- Mix the mixture on each slide using an applicator stick, spreading the mixture evenly over on most of the slide.

Interpretation of the test result

- Agglutination of red cells ----------Rh positive.
- No agglutination of red cells------Rh negative.

A smooth suspension of cell must be observed in the control.
Note: Check negative reactions microscopically**.**

2. Modified Tube Test:

Specimen:

Washed RBC(2-5%)

Equipments and reagents:

- Anti- D reagent
- Test tube
- Centrifuge
- Microscope
- Albumin (Control)

Procedure:

1. Make a 2-5% red cell suspension.
2. Prepare two test tubes and mark “D” on the first tube and add two drops of anti-D
3. Mark “A” on the second tube and Place a drop of Rh control (albumin).
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 all weakly positive (+/-) and negative sample to perform the D^u test.

3. D^U Typing Using Indirect Anti- Globulin Test (IAT)

Specimen:

Washed RBC(2-5%)

Equipments and reagents:

- Anti- D serum (coombs’ reagent)
- Test tube
- Centrifuge
- Microscope
- Albumin (Control)
- AHG serum

1. Use the initial Rh D typing tube and control in the above procedure. Incubate the Rh negative or weakly reactive (+/_) samples and the control at 37^0c for 30 minutes.

2. Wash cells in both the test and the control tubes 3-4 times with normal saline to remove excess anti-D.
3. Add one drop of the poly specific anti-human globulin (coomb`s) to each tube and mix well.
4. Centrifuge at 2200-2800 rpm for 10 seconds.
5. Gently re suspend the cell button and look for agglutination.

Interpretation:

Positive -shows agglutination in the tube containing anti-D while the control is negative.
Negative-absence of agglutination in both the test and control tubes

CHAPTER FOUR

OTHER MINOR BLOOD GROUP SYSTEMS

- The minor BGS have applications in the case of transfusion, compatibility testing, paternity testing, HDN, forensic investigation.
- For instance, those people who have Duffy BGS resist malaria infection.

4.1.The kell Blood Group System

- Discovered in 1946
- Includes 21 high and low frequency antigens well developed at birth
- Kell (KEL) locus is found on chromosome 7 and has 4 sub locus – Kpa/Kpb, K/k, Jsa/Jsb, KELL11/KELL17
  - High frequency antigens – k, Kpb, Jsb, KELL11
  - Lower frequency antigens – K, Kpa , Jsa, KELL17
- The K antigen is a powerful immunogen
- Anti-K is:
  - ☞ the kell system antibody most commonly encountered in routine blood bank practice.
  - ☞ an immune mediated IgG
  - ☞ can cause both HDN and HTR

4.2. The Duffy blood group system

- discovered in 1950
- is a single locus system with two antigens, Fya and Fyb
- Possible phenotypes includes Fy (a+b-), Fy(a-b+) Fy(a+b+), Fy(a-b-)
  - The only rare phenotype is Fy (a-b-).
    - Protects from P.falciparum infection.
- Anti-Fya and anti-Fyb are the antibodies encountered in this Fy sytem with anti-Fya being seen much more commonly.
- These antibodies usually are IgG
- Even though rare antibodies of this system are associated with mild HDN and HTR

4.3. The Lutheran Blood Group system

- Identified in 1945
- Single locus (on chromosome 19) system with antigens Lua and Lub
- Antibodies (anti-Lua and anti-Lub) are IgG
  - Are the mild causes of HDN even not common.
  - They are not associated with HTR.

4.4. The Lewis blood group

- Identified in 1946
- Lele locus is on chromosome 19.
- Le^a and Le^b are the major antigens.
- Others include Le^c, Le^d, and Le^x (LE^3)

Difference of Lewis blood group from the others:

- Lewis antigens are not intrinsic to the RBC membrane produced during cell development rather the antigens are produced by tissue cells and are found in plasma & secretions.
- An individual's Lewis phenotype is not determined solely by genes at the **Lele** locus but also by the action of genes at the **Hh** and **Sese** loci.
- The amount of Lewis antigen expressed on the RBC (Lewis phenotype) varies according to the cell's ABO phenotype.

Lewis system phenotype & antigen expression:

- Phenotypes commonly seen in the Lewis blood group system are:
  - Le (a- b+)
  - Le (a + b-)
  - Le (a- b-)
  - and rarely Le (a+b+) ---- rare in adults, more frequent in young children.
- Lewis antigens are not well develop in fetus, but develop after birth.
  - New born => Le (a- b-)
  - 2-3 months => Le (a + b-)
  - 16-18 months => Le (a- b+)
  - Le (a+b+) may occure between ages of 2-3 months and 16-18 months

Genetics and biochemistry of Lewis BGS:

- Le^a is not affected by H and Se genes. That means, if there is at least one dominant Le gene, then Le^a will be expressed. i.e, LeLe or Le/le

- This is because Le gene produce transferase enzyme which attach sugar with the precursor ssniubstance and form Le^a.

- Le^b is affected by H and Se genes because the precursor substance should be changed to H-substance with the help of H-antigen.
- For Le^b to be formed there should be **Se gene** & **H-gene** in addition to **Le gene.**
- Le^a is small amount in secretions than Le^b , so on adsorption to RBCs, high amount of Le^b adsorbed than Le^a. Therefore, mostly Le^a is not expressed and appear as Le (a-b+) since Le^a is present in small amount; that is why Le (a+b+) is rare.

Genes Present	Lewis Phenotype	Antigen in Plasma/ Secretions	Lewis Antigen on Red Cells
Le sese	Le(a+b-)	Le^a	Le^a
Le Se	Le(a-b+)	Le^b	Le^a, Le^b
lele sese	Le(a-b-)	None	None
lele Se	Le(a-b-)	None	None

Table 4.1 Effect of Gene Interactions on Lewis Antigen Expression

Acquisition of Lewis Ags by RBCs

- The Lewis phenotype of RBC's
  - depends on the phenotype of the plasma in which they are suspended
  - can be changed by incubating the cells in plasma containing different Lewis active glycolipids.

Eg. If Le (a – b-) cells incubated with plasma containing Le^a or Le^b glycolipid,

- they adsorb the available antigen from the plasma and
- subsequently type as Le (a+ b-) or Le (a-b+)

Antibodies of the Lewis system

Two main types: **anti-Le^a** and **anti-Le^b**

- **Anti-Le^a**
  - Very common and naturally accruing
  - Produced almost exclusively by the **Le (a-b-)** phenotype
  - Usually IgM, react best at room temp and rarely at 37^0C, rarely IgG
  - Not associated with HDN because the antigen is not present on fetal RBCs
- **Anti Le^b**

- usually IgM, rarely IgG
- produced by **Le(a-b-)** individuals and rarely by **Le (a+b-)**

❖ Generally the Abs of Lewis BGS has no implication in HDN & HTR. The exceptional IgG Lewis Ab couldn't cause HDN because antigens of Lewis BGS are not intrinsic to RBCs, means the Lewis antigens are not produced in fetal life. So, evenif the mother produce the Abs and these Lewis Abs get into the fetus through placenta, they can't cause hemolysis (HDN) since they can't get Le antigens in the fetus.

4.5. The I Blood group system

- Discovered around 1956 by Winner and his associates
- The I Ag is found on almost all adults
- Is part of the precursor component of the oligosaccharide that forms the A, B and H Ags.
- Red cells that posses this Ag are labeled as I and those that lack as i.
- The anti-I antibodies are naturally occruing, of IgM type with their optimal temp at 4^0C
- The antibodies of the I blood group system are not associated with HDN and HTR

4.6. The P Blood group system

- Discovered in 1927
- The most common phenotype are P1 and P2 which are analogous to the A_1 and A_2 phenotypes seen in the ABO system.
- P1 individuals have two antigens on their RBCs: P1 and P.
- P2 individuals have only the P Ag and can produce anti-P1.
- Anti-P1 is frequently encountered antibody
  - It is naturally occurring
  - IgM, cold – reactive agglutinin, and does not react above RT
  - Not associated with HDN or HTR

4.7. The MNSs Blood group system

- Discovered in 1927 by Land Steiner and Levine
- There are two loci on chromo. 4 M/N and S/S.
- The Antigens are M, N, S and s
- Anti-M, Anti-N and Anti-S Anti-s are the major Abs of the system

Anti-M

- Naturally occurring reacting at RT or below
- May be either IgM or IgG
- Rarely causes HDN or HTR

Anti-N

- Usually weak cold-reactive,
- naturally occurring IgM Ab produced by individuals who are M+N- and who are positive for S or s
- Clinically not significant

Anti-S and Anti-s

- Usually present as immune Abs.
- Usually IgG but rare IgM types present
- Anti-M and anti-N are not associated with HDN or HTR but Anti-s and anti-s

4.8. The KIDD (JK) Blood group system

- was discovered in 1951
- is a single locus system with two antigens Jka and Jkb
- Phenotypes
  - Jk (a-b-)-------rare
  - Jk(a-b+)
  - Jk (a+b+)
  - JK (a+b-).
- The primary antibodies of the kidd system,
  - are anti-Jka and anti-JKb
  - are usually immune mediated (IgG)
  - mild HDN and delayed HTR

CHAPTER FIVE

THE DONATION OF BLOOD

5.1 Selection of blood donors

Aim:

- to prepare safe blood from a safe donor to give a recipient
  - ☞ by identifying conditions which could harm both the donor and the recipient

Selection Criteria:

Age:

- Between 17 – 65 years
- It may be beyond this age based on local law and dealing with the physician.

Hemoglobin:

- Females - not less than 12.5 g/dl (PCV 38 %)
- Males - not less than 13.5 g/dl (PCV 41%)
- In both sexes Hgb above 19g% (Hct above 57%) are not acceptable.

Pulse, Blood pressure and Temperature:

- **Pulse** - between 60 – 100 per minute.
- **B.P**
  - Systolic between 90 and 180 mmHg.
  - Diastolic between 50 and 100 mmHg.
- Temperature should not exceed 37^0C.

Weight:

- A person
  - between 45-50 kgs can donate 350 ml of blood.
  - above 50 kg can donate 450ml of blood.
- Obese donors who are unable to climb onto the couch are not acceptable.
- Donors with unexplained weight loss of a significant degree 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:

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

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

Pregnancy:

- Are excluded from donating for 1 year after the conclusion of their pregnancy.

Medication:

- Deferral of donors depends on the nature of the disease for which the drug was ordered.
- Consult a medical doctor about a donor's long term treatment.

Illness:

- Prospective donors with:
  - disease of the heart, liver, lungs, or
  - history of cancer, or
  - bleeding problems should be excluded, subject upon evaluation by a physician.
  - Donors who have had leukemia must never be accepted, 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 transfusion transmissible infections
- 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.
- Person who have a history of **malaria**, or Persons at high risk for acquiring or transmitting **AIDS** should not donate blood.
- Donors previously resident in an endemic area should be deferred for 3 years after becoming symptomatic or after leaving the endemic area.

Previous donation:

- An interval of at least
  - 4 months for men and
  - 6 months for women, is required before the next donation.
  - The recipient can also donate a blood after 12 months of recovery

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 not 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.

5.2. Collection of Blood

Before blood collection Basic information about the donor like

- date of donation
- full name,
- Address
- Sex
- age and
- the ABO and Rh blood group
- donor's medical history, must be obtained and signed by the phlebotomist who performs the procedure.

ETHIOPIAN RED CROSS SOCIETY
NATIONAL BLOOD TRANSFUSION SERVICE
ENROLLMENT FORM

Name .....
Age ..... Sex .....
Occupation .....
Reg. No. .....

City: .....
Woreda ..... Keb. ..... H. No. .....
Tel: Res. .....
Office .....
P. O. Box .....

Date	Pack No.	Wt.	B/P	Hct.	Vol.	Screened by	ABO	Rh.	Compon-ent Req.	Type of Donation	Remark

- Patient (recipient) identification also is an important step in blood collection.
- Blood collection should be carried out with the blood donor lying on a simple bed with a support for his head.
- The donor must not be left alone while donating blood.

Blood collecting bottles and bags

- A standard unit of blood contains 450 cc blood and 63cc of preservative solution.
- If lesser amounts of blood is to be collected the amount of the preservative should be reduced proportionally.
  - 350 ml blood bag with 49 ml of anti coagulant.

5.3 Anticoagulants in blood donation:

- Anticoagulant with preservative function is selected as anticoagulant in blood donation.
- Viable hemoglobin concentration & optimum oxygen content of RBCs is kept due to preservatives.
- The common anticoagulant preservative solutions are:-

- CPD (citrate, phosphate & dextrose) - For 21 days/at 2-8^{0}C
- CPDA- 1 (CPD with adenine) - For 35 days at 2-8^{0}C
- ACD (Acid Citrate Dextrose)------For 28 days /at 2-8^{0}C

- The survival of blood cells depends during storage on the delicate biochemical balance of nutrients specially glucose, PH, and ATP.

Note: Low temperature slows glycolysis, and reduce bacteria proliferation.

1. ACD (Acid Citrate Dextrose)

- Acts as an anticoagulant by binding Ca
- Composition: **Trisodium citrate:** - binds Ca & act as anticoagulant
  Citric acid: - maintains PH
  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 (Citrate Phosphate Dextrose):

- Acts by binding Ca. It is most widely used preservative.
- 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

3. CPD-A (Citrate Phosphate Dextrose - Adenine):

- It is a combination of **CPD** and **adenine**.
- Adenine provides a substrate from which red cells can synthesize ATP during storage.
- is more preferable than ACD.
- Survival of red cells is 80% after 28 days storage and 75- 80% after 35-days storage.

4. EDTA and **Heparin**:

- are not commonly used in Blood Banking b/c they have only anticoagulation function.
- Whole blood or red cell collected and stored in heparin solution must be used within 48 hours of phlebotomy.

Method for blood collection:

Capillary blood (micro blood):

☞ Used for blood grouping, Hgb & HCT determination

Venous blood (macro blood):

- ☞ Used for blood donation & serological tests (HIV, Hepatitis, Syphilis…)

Blood collection procedure:

1. Apply a pressure cuff to the upper arm a few inches above the elbow, and raise the pressure to between 80 and 100mmHg.
2. And select a large deeply situate vein for the vein puncture, usually near the bend of the elbow.
3. Clean very well the required part of the arm with cotton wool and 70% methylated spirits.
4. Make the vein puncture with the needle directed up wards in the line of the vein. If necessary secure the needle in place with a small strip of adhesive tape.
5. When the blood begins to flow, reduce the pressure of the cuff to 40-60 mmHg, and ask the donor to squeeze a small object.
6. Mix the anticoagulant with the blood as it flows into the bag continually until collection is complete.
7. When the blood has almost reached the 450 ml mark, reduce the pressure to zero and remove the object from the donor's hand.
8. Clamp off the tubing and remove the cuff.
9. Take the needle out of the vein, applying pressure with cotton wool.
10. Ask the donor to bend his arm up wards, with the cotton wool over the puncture wound.
11. Empty the blood in the tubing of the taking set in to – a clean dry test tube for grouping and screening the blood.
12. Mix the blood well with the anti coagulant by inverting the bottle at least four times, but not shake it as this could damage the cells.
13. Seal the tops of the collecting and pilot bottle with adhesive tape on which is written the group and the bottle number.
14. Label the sample with- the blood group, the bottle number and the date of collection.
15. Place a small pad of cotton wool and an adhesive plaster on the donor's arm as a dressing, making sure that the bleeding has stopped
    - See that the donor is given a rest and a drink, perhaps of tea or orange juice, but not alcohol to make up his fluid lose.
    - Thank the donor and give him a certificate of blood donation

Danger in taking blood:

- The greatest danger in taking blood is an air embolism
  - ⁻ can be caused by the air out let needle becoming blocked, either before or during the taking of blood.

- If the air out let is blocked, the following can happen.
  - The flow of blood can slow down and even stop
  - The blood may move in the opposite direction, returning to the arm
- Slowing down of the flow of blood can also be occurred due to:
  - A fall of the pressure cuff
  - A bend in the tubing of the taking set
  - The needle in the arm requiring adjusting

5.4 Donor reactions:

Include:

- syncope (fainting),
- weakness,
- excessive perspiration,
- dizziness,
- pallor, and nausea.
- Occasionally convulsions, loss of consciousness or involuntary bowl or urinary passage.

Note:

- At the first sign of the reaction, the phlebotomist should stop the phlebotomy
- Preliminary measures taken when donor reactions occur……

Syncope

- Place the donor on his or her back and raise the feet above the level of the bend.
- Loosen tight clothing, and ensure that the donor has an adequate air way.
- Apply cold compress to the donor's forehead or back of the neck.
- Refer if the condition is not improved

Nausea

- instruct the donor to breath slowly and deeply,
- Have emergency basin and damp towel available in case the donor vomits.

Convulsions

- the convulsions are rare, when they occur help should be summoned immediately.

Weakness, excessive perspiration, Dizziness, pallor

- Apply cold compress to the donor's forehead

Hematoma

- remove the tourniquet and the needle from the donor's arm ,

- place gauze and apply pressure for 7-10 minutes with the donor's arm held above the level of the heart.

Air-embolism

- this is very dangerous and always take care.
- If it happens, immediately call for help. Because, it cause rapid fall of systolic blood pressure, death can occur.

5.5 Whole blood and blood components:

I. The whole blood (preparation, storage, and clinical indication):

- Only human blood and its components are used for transfusion into humans
- Transfusions are the introduction of either
  - Whole blood
  - blood components (RBCs, Plts, plasma or WBCs) or
  - blood derivatives (albumin, gamma globulin, Factors VII,VIII,Von willebrand,or Immune globulins and prothrombin) directly into the blood stream.
- Whole blood- complex tissue ,composed of cells and plasma
- Blood components-prepared from blood by mechanical method especially by centrifugation
- Blood derivatives-separated by more complex process

The use of component therapy:

- ✓ Gives a better form of treatment (it reduce circulatory overload)
- ✓ Often permits considerable economy in the use of blood
- ✓ Minimizes the risk of immune reactions

General whole blood (w/b):

- Contains all cellular elements and coagulation factors
- Freshly drawn w/b maintains all its properties for a limited time.
- Upon storage a number of changes occur,

Example:

- increase in O_2 affinity and
- loss of viability of RBC.

Indications for whole blood

- ❖ To provide both O_2 carrying capacity and blood volume expansion.
  Example: In treatment of massive hemorrhage (patient becomes hypovolumic).
- ❖ Used as a source material for blood component preparation.
- ❖ For exchange transfusion in new born.

- Whole blood less than 4-5 days old is often the component of choice
- A unit of whole blood is – 450ml
  - 63ml anticoagulant
  - HCT of 35-40%
  - Red cells of 180-225ml volume
- Whole blood can be stored at 1^{o}C – 6^{o}C for 21-35 days if the preservatives are ACD, CPD or CPD-A.
  - ☞ At this temperature, platelet, leukocytes and coagulation factors become ineffective.

Modified whole blood:

- Platelets are removed
- Have the same storage T^{o} as the general whole blood

Blood components

- Component make blood use more economical by using one unit one can treat:-
  - ☞ Anemia with the packed cells
  - ☞ Platelet deficiency with plate late preparations
  - ☞ Clotting factor and other plasma deficiencies

II. RBC concentrates (packed red cell):

- Is the product remaining after the removal of most of the plasma from freshly drawn whole blood by centrifugation.
- Giving RBC component is recommended in the case of anemia & other factors that reduce O_2 carrying capacity. (for those patients needing additional red cell mass for oxygen-carrying capacity due to blood loss or a disease process that reduces RBC count).
- Giving RBC component is not recommended for individuals that :
  - Can compensate anemia
  - Have nutranemia e.g Iron deficiency anemia, pernicious anemia (lack of vit B_{12} & follic acid)
- RBC components can be stored at 1-6^{o}C for 21-35 hours using ACD, CPD CPD-A anticoagulants.

Indications for whole blood:

- The use of red blood cells offer several advantages over the use of whole blood:
  - ✓ The possibility of circulatory overload is minimized
  - ✓ The incidence of transfusion reactions from donor antibodies is reduced
  - ✓ The volume of anticoagulants and electrolytes is reduced
  - ✓ The incidence of transfusion reactions to plasma components is minimized

- ✓ Each blood donation can be used as components and fractions, thereby serving the needs of more than one recipient.

- The commonly available RBC preparations are
  - Concentrated red cell suspension
  - Frozen/ Deglycerolized red cells
  - Leukocyte poor red cell suspension

- The red cell prepared in this form contain the same:
  - ☞ red cell mass and
  - ☞ oxygen carrying capacity as whole blood with approximately one half of the volume.
- The final Hematocrit of the product should be between 70-80% in 250-300 ml of volume
- Transfusion of 1 unit red cell increases the HCT of nonbleeding patient by 3% and the hemoglobin by 1 g/dl

	Whole blood	**Packed red cells**
Vol (ml)	500 ± 25	300 ± 25
HCT	40 ± 5	70 ± 3
Plasma vol (ml)	300 ± 25	100 ± 25
Red cell vol (ml)	200 ± 25	200 ± 25

A. Leukocyte poor red cells:

- Is preparation of blood in which 70% of the WBCs has been removed by retaining 80% red cells.
- It decreases WBC associated antibodies (e.g HLA)
  - Inverted centrifugation
  - Saline dilution
  - Filtration method
  - Freezing
- But, none of the 4 methods can remove leukocytes totally. If the original number of leukocytes decrease by 25-30%, leukocyte antibody associated hemolytic reactions can be prevented.
- Storage = 1-6^{o}C with the same expiry date of whole blood if the preparation is closed system.

  Clinical indications:

  Leukocyte poor red cells are recommended when:
  - ➢ WBC Ab are suggested

- Patient undergo dialysis (to reduce the risk of alloimmunization to leukocyte antigens)

B. Washed Red Cells:

- A clinician may order washed RBCs:
  - for a patient with febrile & allergic reactions to transfused units
  - for a patient with IgA - anti-IgA reactions
- Washed cells are usually prepared with an automated cell washing device, though they can be prepared by a manual batch centrifugation process.
- Storage of washed red cells is not recommended b/c of risk of contamination.
- But if storage is obligatory, store at 1-6^{o}C like whole blood.

C. Frozen-thawed red cells:

- Includes freezing (-160^{o}C) & then warming (37^{o}C)
- Is efficient in removing leukocytes, platelets & other contaminants.
- Methods:
  - Using high glycerol concentrations(40%w/v glycerol)
    - Mostly used and it is based on cryoprotective ability of glycerol (prevent the damage of RBC membrane from rupture due to the binding ice crystals formed while freezing at -65 ^{o}C).
    - The only drawback of this system is that it requires a large volume of wash solution to deglycerolization.
  - Using low glycerol concentrations(14%w/v glycerol)
    - The cryop`rotective ability of glycerolis minimal and a rapid freezing procedure is required
    - Liquid nitrogen is routinely used for this method with the frozen units being stored at -160^{o}C
  - Agglomeration method
    - Use dextrose and it is not commonly used
- Storage of frozen-thawed red cells => -65 to -85^{o}C for 3-10 years.

Clinical indications:

Because of the marked reduction of plasma proteins, platelets and leukocytes; the frozen-thawed red cells are indicated for the following circumstances:

- for special transfusion circumstances, such as autologous use,
- to minimize severe allergic transfusion reactions
- to store very rare RBC units based on their specific phenotype b/c frozen RBCs have an expiration date of 10 years.

Apheresis: is the transfusion of blood, from which some components have been removed, back into a patient.

If we need to remove platelets => Plateletapheresis

>> leukocytes => leukapheresis

>> red cells => red cell apheresis
>> plasma => Plasmapheresis
>> to separate all blood components (cells) => cytoapheresis

Storage: 22^{o}C for 24hr in an open system
22^{o}C for 5 days in closed system

III. Platelet concentrate:

- Platelets can be prepared from a whole blood unit or collected from an apheresis donor.
- Clinical indications:
  - ☞ Patient having thrombocytopenia
  - ☞ patients whose platelets are adequate in number but dysfunctional.
- Platelet Concetrate of the same ABO group should be used
- Rh (D) incompatibility has no effect on platelet survival
  - ☞ but the extent of RBC contaminating in PC may be sufficient to immunize Rh (D) negative women in reproductive age group.
    - In such cases Rh (D) negative PC should be given
- Each unit of PC:
  - contains 5.5x10^{10} platelet in 50-65ml of plasma
  - represents 60-80% of the platelet present in a unit of whole blood.
  - increases the platelet count by approximately 5,000-10,000 / ml in an average adult.
- The normal adult dose is 6-10 units, and for child 1 unit/ 10kg
- Platelet should be stored at:
  - 1-6^{0}C with agitation for 72 hours if prepared in a close system
  - 20-24^{0}C for
    - 24 hours if prepared in an open system
    - 5 days if prepared in closed system
- Refrigerated PC don't maintain function or viability as well as the PC stored at room temperature.

IV. Leukocyte (granulocyte) concentrate:

- Leukocytes can be prepared from whole blood collections, but the most common collection technique leukapheresis.
- Granulocytes are most commonly used as a treatment for bacterial sepsis in a neutropenic patient when no other therapeutic intervention has been successful.

- Leukocyte preparations should not be stored, but should be infused as soon as possible after preparation. If storage is necessary it should be at 20-24^{0}C for no longer than 24hr (without agitation).

5.6 Plasma components:

- **Plasma** is a fluid portion of one unit of blood collected and separated in a closed system and intended for intra venous use.
- The therapeutically useful plasma products are:
  - Fresh Frozen plasma (FFP)
  - Ordinary plasma (OP)/ single donor plasma
  - Cryo depleted plasma
- All plasma products have to be stored at -18^{0}C or colder

I. Fresh Frozen plasma (FFP)

- Is the plasma obtained from a unit of whole blood after centrifugation
- It is then separated and frozen solid at a temperature that will adequately maintain the labile coagulation factors in a functional state.
- The indications for FFP are for:
  - replacement of labile and stable coagulation factors (factor V,IX, II, XI & VIII)
  - deficiencies of antithrombin III
  - deficiencies of protein C and protein S
  - treatment of thrombotic thrombocytopenic purpura (TTP)
- FFP Should be stored frozen at –30^{0}C or colder for 12 months
  - beyond this period the factors VII may have decreased
  - To maintain adequate levels of coagulation factors

❖ If not used in 12 months time may be re designated and relabeled ”Plasma” and has 4 more years of shelf life at –18^{0}C or colder.

II. Single donor plasma (SDP)/Ordinary plasma (OP)

- is deficient in labile coagulation factors.
- It is separated from single whole blood unit at any time during its storage and up to 5 days after the expiration of the original unit.
- OP is different from FFP in that it contains high levels of K and ammonia since it is prepared after long contact with red cells. FFP that is not used within the 12 month dating period can be converted to OP (sometimes called **Stored plasma**).
- is stable up to 5 years after preparation if kept frozen at -18^{0}C or colder.

Indications:

- ☞ In the treatment of stable coagulation factors deficiencies
- ☞ For volume and protein replacement under special circumstances

III. Cryoprecipitated Antihemophilic Factor (Factor VIII Concentrate):

- Is a cold- precipitated concentration of factor VII, anti hemophilic factor (AHF), obtained after the plasma has been thawed (obtained from FFP).
- Contains:
  - Factor VIII: C (pro coagulation factor)
  - Factor VIII: VWF (Von-Willebrand's factors)
  - Fibrinogen factor XIII
  - Fibronectin factor
- Each unit has at least 80μl of factor VIII, 250 mg of fibrinogen in a volume of 15 to 20 ml.
- Cryo:
  - has a shelf life of 12 months at a temperature of -18^{0}C or colder (preferably -30^{0}C) and
  - it must be transfused within 6 hours after thawing at 37^{0}C.

Indications:

- ☞ In the management of classic hemophilia (factor VIII deficiency)
- ☞ In the management of factor XIII deficiency
- ☞ In the management of von willebrand's disease and
- ☞ source of fibrinogen for the treatment of hypo fibrinogenemia.

IV. Cryo depleted plasma (CDP)

- CDP is the supernatant plasma following removal of cryo precipitate.
  - immunoglobulin and coagulation factors are the same as that of FFP,
  - fibrinogen concentration and levels of the labile coagulation factors V and VIII are markedly reduced.

Indications:

- o CDP is indicated for patients requiring volume expansion or protein replacement when labile clotting factors are not required

V. Factor IX Concentrate (Prothrombin Complex):

- Is prepared by fractionation of pooled plasma and contains factors II, VII, IX and X with a minimal amount of total protein.
- Should be stored 1 - 6^{o}C for the recommended expiration date by manufacturer.
- The risk of transmission of hepatitis & HIV is high when using factor IX concentrate.

Indications:

- For the prevention and control of hemorrhagic episodes in congenital factor IX deficiency (hemophilia B), congenital factors VII and X.

VI. Human Serum Albumin:

- Is prepared from normal human plasma by cold ethanol plasma fractionation, then treated with heat inactivation which removes the risk of hepatitis and HIV transmission.
- Can be stored for up to 3 years at 1 – 6^{o}C.

Indications:

- In the treatment of hypovolemic shock due to hemorrhage or surgery
- In the treatment of burns as a protein and fluid replacement
- In neonatal hyperbilirubinemia, to assist in binding unconjugated bilirubin

VII. Immune Serum Globulin (Gamma globulin):

- Is prepared by cold ethanol fractionation from pooled plasma.
- It is the concentration of plasma gamma globulin in aqueous solution and contains primarily IgG, although small amount of IgM and IgA may be present.
- Can be stored for up to 2 years at 1 – 6^{o}C.

Indications:

- To provide passive antibody protection after exposure to certain disease
- For prophylaxis in congenital antibody and immune deficiency disorders

TABLE 3-5 Requirements for Storage, Transportation, and Expiration for Select Components

Component	Storage	Transport	Expiration	Additional Criteria
Whole blood	1–6°C	Cooling toward 1–10°C If intended for room temperature components, cooling toward 20–24°C	CPD: 21 days CPDA-1: 35 days	
Red blood cells Red blood cells, leukocytes reduced	1–6°C	1–10°C	CPD: 21 days CPDA-1: 35 days Additive solution: 42 days Open system: 24 h	
Deglycerolized RBCs	1–6°C	1–10°C	Open system: 24 h Closed system: 14 days or as FDA approved	
Frozen RBCs	≤65°C	Maintain frozen state	10 yr	
RBCs irradiated	1–6°C	1–10°C	Original expiration or 28 days from date of irradiation, whichever is sooner	
Rejuvenated RBCs	1–6°C	1–10°C		Follow manufacturer's written instructions
Washed RBCs	1–6°C	1–10°C	24 h	
Platelets Platelets pheresis Platelets irradiated	20–24°C with continuous gentle agitation	20–24°C	5 days in a closed system	Maximum time without agitation 24 h
Pooled platelets	20–24°C with continuous gentle agitation	20–24°C	Open system: 4 h Closed system: original outdate	Maximum time without agitation 24 h
Granulocytes	20–24°C	20–24°C	24 h	Transfuse as soon as possible
Cryoprecipitated AHF Pooled cryoprecipitate (closed system)	≤18°C	Maintain frozen state	12 mo from original collection	Thaw the FFP at 1–6°C Place cryoprecipitate in the freezer within 1 h
Cryoprecipitated AHF or pooled cryoprecipitated AHF, after thawing	20–24°C	20–24°C	Open system: 4 h Single unit: 6 h	Thaw at 30–37°C
Fresh frozen plasma Plasma frozen within 24 h after phlebotomy Plasma, cryoprecipitate reduced	≤18°C	Maintain frozen state	12 mo from collection	Place plasma in freezer within 8 or 24 h and label accordingly
Thawed plasma	1–6°C	1–10°C	5 days from when original product was thawed	Closed system: 5 days

CHAPTER SIX

THE ANTIGLOBULIN TEST (COOMB'S TEST)

- It was introduced by Coomb's in 1945.
- Is a sensitive technique to detect **incomplete Abs** which are:
  - Abs that are sensitized but which fail to agglutinate RBCs suspended in saline at room temperature, mainly IgG.
  - are agglutinated by the anti-IgG in antiglobulin serum through the linking of the IgG molecules on neighboring red cells.
- Red cells can also be agglutinated by a reaction of complement components on their surface with anti-complement serum.

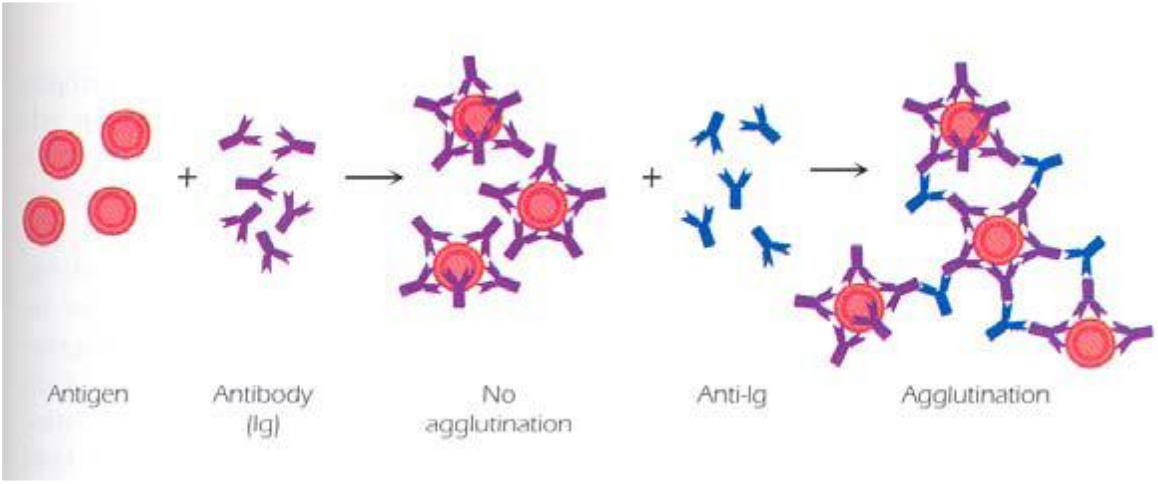


Fig 6.1 Representation of the Antiglobulin (Coomb's test) test.

- The anti globulin reagent is prepared by immunizing animals, often rabbits with human gamma globulin (antibody), or beta globulin (components of complement).
- The ***antiglobulin*** test depends on the following basic premises (principles):
  - Antibodies are globulins.
  - The antihuman globulins (AHG) bind to the Fc portion of sensitizing antibodies and form bridges between antibody-coated red cells, resulting in visible agglutination.
  - After addition of IgG the RBCs should be washed to remove the excess.

Types of AHG:

1. Broad spectrum (polyspecific):

- prepared by combining anti-IgG and anti-complement.
- The reagent may also contain antibodies of other specificities such anti-IgM, anti-IgA, anti C_3, or anti C_4.

- The anti-C_3 in the antiglobulin reagent (AHG)
  - binds to the C_3 on the sensitized red cells;
  - bridges the gap between the cell – bound human C_3 on adjacent red cells.
  - helps in detecting IgM antibodies,
  - enhance the reactions seen with complement binding antibodies.

2. Monospecific:

- contains only a single antibody:
  - anti-IgG or
  - only anti-complement.
- It is advantageous to detect RBC bound IgA, IgM and/or complement components by this test.
- The antiglobulin test can be used to detect invivo or invitro red blood cell sensitization

Types of AHG tests:

- the direct antiglobulin test (DAT)
- the indirect antiglobulin test (IAT).

5.1 Direct antiglobulin test (DAT):

- It is the detection of in **vivo** sensitized RBC. e.g HDN

Clinical significance:

- ☞ Diagnosis of HDN
- ☞ Diagnosis of autoimmune hemolytic anemia
- ☞ Investigation of drug sensitized RBCs
- ☞ Investigation of transfusion reactions.

Principle:

- ❖ Cause agglutination of human RBCs that have been sensitized in vivo by globulin antibodies or complement components. The coomb's serum which contains AHG and anti complement component will combine these antibodies already present on the RBCs suspension causing visible agglutination or hemolysis. That means,

- ❖ Washed red blood cells from the patient are directly reacted with AHG serum.

Specimen:

2-5% RBC suspension

Equipments and reagents:

- Test tubes
- Centrifuge
- Microscope
- Microscopic slides
- AHG
- Physiological saline

Procedure:

- Place one drop of a 2% to 5% saline suspension of cells to be tested (which are supposed to be sensitized in vivo/ called coated red cells) in a labeled tube.
- Wash 3 or 4 times with saline to remove excess IgG or C_3. After last wash decant completely.
- Add one or two drops of antiglobulin reagent and mix.
- Centrifuge at 3,400 rpm, for 15 sec
- Gently re suspend the red cells and examine macroscopically and microscopically for agglutination or hemolysis.

  (The tube should be held at an angle and shaken gently until all cells are dislodged. Then it should be tilted gently back and forth until an even suspension of cells or agglutinates is observed.

Control:

- To control for inadvertent contamination of the AHG,
  - Add one drop of IgG- sensitized red blood cells to any tubes that have been recorded as negative and re-centrifuge.
  - If the patient's cells were washed adequately in the first stage of the test, the control cells should be agglutinated and the negative result on the patient is valid.

6.2 Indirect antiglobulin test (IAT)

- It is the detection of Ab that may cause RBC sensitization in **vitro**. e.g D^u testing
- The sensitizing antibody or complement acts as the antigen for the antiglobulin reagent.
- IAT used in cross- matching, to detect antibodies that might reduce the survival of transfused red cells and Du technique, in the detection of Du antigen.

Clinical Significance:

- Detection and identification of unexpected Antibodies.
- Cross matching
- Detecting RBC Antigens not demonstrable by other techniques etc….

Principle:

- The Antibody-containing serum is incubated with specific RBCs which, following washing (to dilute unbound/ free globulin), are reacted with antiglobulin serum to see whether RBC sensitization has occurred.

Equipments and Reagents:

- Test tubes
- Centrifuge
- Microscope
- Microscopic slides
- AHG
- Physiological saline
- Anti-D

Procedure:

- Place 2 to 4 drops of the serum under test (patient's serum) in a test tube.
- Add one drop of washed 5% RBC suspension of the test cells (donor's RBC, screening RBC, etc).
    - Optional: 2 drops of 20-22% bovine albumin may be added to the mixture.
        - Albumin is important to increase sensitization of RBCs & decrease the incubation period.
- Mix well and incubate at 37^0C for 15 to 30 minutes.
- Centrifuge immediately on removal from the incubator for 15 seconds at 3400 rpm: examine for hemolysis and agglutination using an optical aid, and record results
- Wash 3 or 4 times in large amounts of saline. Decant each wash as completely as possible.
- Add 1 or 2 drops of antiglobulin reagent and mix well.
- Centrifuge at 3400 rpm for 15 sec.
- Examine for agglutination using an optical aid and record results

Control:

- Add one drop of known sensitized red cells to all negative test. Centrifuge at 3400 rpm for 15 sec: examine for agglutination. If no agglutination is seen, the test result is invalid and must be repeated.

Factors Affecting the Indirect Antiglobulin Test:

- The IAT can be affected by anything that alters the tenacity with which antibody attaches to red cells or that affects the amount of antibody that attaches to red cells. Factors that can affect the IAT include:
    - Incubation time and temperature
    - pH
    - Ionic concentration
    - Affinity constant of the antibody
    - Proportion of antigen and antibody

Incubation Time and Temperature

- In general, antibodies that are not detected in the 37°C incubation phase or IAT are not thought to be clinically relevant.

pH

- The optimal pH for red cell antigen–antibody interactions is usually considered to be in the physiologic range of pH 6.8 to 7.2.

Ionic Concentration

- Reducing the ionic concentration of the environment in which antigens and antibodies react allows the rate of binding to increase. This occurs as the natural shield effect from positive and negative ions is weakened.
- The use of a low–ionic-strength saline (*LISS*) solution reduces the time needed for suitable levels of antibody to be bound in vitro for detection and identification.

Affinity Constant of the Antibody

- Every red cell antibody has characteristics that are peculiar to that antibody. One of those characteristics is the *affinity constant*, also called the *equilibrium constant.*
- The affinity constant is partly responsible for the amount of antibody that binds to red cells at the point of antigen–antibody equilibrium. As a general rule, the higher the affinity constant, the higher the level of antibody association during the sensitization phase of antigen–antibody reactions.

Proportion of Antigen and Antibody

- The speed with which antigen–antibody reactions occur depends on the amount of antibodies present and the number of red cell antigens available. Increasing the serum-to-red cell ratio may increase the test sensitivity because more antibodies are available to combine with the red cell antigen sites.

CHAPTER SEVEN

THE CROSS MATCH (COMPATIBILITY TESTING):

- It is a procedure performed before transfusion to select donor's blood that will not cause any adverse reaction, (hemolysis /agglutination) in the recipient.

7.1 Purpose of Cross Match:

- It helps the patient to receive maximum benefit from the transfusion of red cells, which will survive maximum in his circulation.
- to detect - ABO & Rh compatibility
  - 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.
- to detect major errors in ABO grouping, labeling, and donor & recipient identification.
- Cross match will not:
  - prevent immunization of the patient
  - guarantee normal survival of transfused erythrocytes
  - detect all unexpected antibodies in a patient's serum.

7.2 Types of Cross Match:

1. Major cross-match:

- Involves mixing **recipient's serum** with the **donor's red cells**.
- Is much more critical for assuring safe transfusion than the minor compatibility test.
- called major b/c the Abs in the recipient's serum are most likely to destroy the donor's RBC

2. Minor cross match:

- Involves mixing the donor's serum with patient's red cells
- Called minor because
  - any Ab in the donor's serum will be diluted by the large volume of the recipient's blood

- the destructed RBCs of the patient may be compensated by the transfused RBC of the donors

Steps for compatibility testing:

- Accurate Patient Identification
- Proper sample collection and handling
- Review of the recipient's past blood bank records
- Careful **ABO**/ **Rh** determination
- **Antibody screening** of the recipient (cross matching of the donor unit).
- In cases when the recipient possess a clinically significant Antibody, donor units must be:
  - Screened for the corresponding Ag and should be negative
  - Cross- matched
- Finally, during the actual transfusion :
  - careful observation of the recipient's vital signs and
  - post transfusion hematocrit and Heamoglobin levels must be considered.

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 group as that of the recipient.
- However, Rh positive recipients may receive either Rh positive or Rh negative blood.
- Avoid transfusing group O blood to those who are not group O, particularly when needing to use whole blood because group O blood can contain dangerous immune anti-A and anti-B haemolysins (particularly in tropical countries). The risk is reduced when using concentrated red cells.
- Whenever possible blood of the patients own blood group should be given. Otherwise the following rules should be applied.
  - **Group A patient**- Should receive group A blood, if not available group O
  - **Group B patient**- Should receive group B blood, if not available group O
  - **Group O patient**- Can only receive group O blood
  - **Group AB patient**- Should receive from group AB, if not possible can receive blood from group A, B, and O.

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.				

7.3 Procedure for cross matching:

- The safe full cross match for non- emergency transfusions of blood requires that the donor's cells be mixed with the patient's serum in the following phases of separate tubes, using :
    1. Saline
    2. Albumin
    3. Anti-human globulin
    4. Enzyme

1. Saline tube technique:

- The red cells from the donor are suspended in saline and mixed with the patient's serum.
- show the presence of any complete antibodies
- important to detect **IgM** antibodies of ABO system and other potent cold agglutinins at 25^{o}C or low
- Agglutination in the saline tube is usually caused by:
    - anti-A or anti-B antibodies and
    - Occasionally by Lewis, MNSs, Lutheran and kell antibodies.

2. Albumin technique:

- The red cells from the donor's suspended in saline, are mixed with the patient's serum, and albumin is added.
- The tube is incubated at 37^{0}C
- shows the presence of any incomplete antibodies (**IgG**)
- the antibodies react in albumin or any other protein medium
- Agglutination in the albumin tube is often caused by:

- mostly Rh antibodies,
- Lewis, MNSs, Lutheran and P antibodies, and
- occasionally by anti-kell.

- Reaction caused by anti- A or anti- B antibodies usually occur in albumin as well as in saline.
- Disadvantage of Albumin technique:
    - Albumin seen microscopically seems to be false agglutination for inexperienced lab workers.
    - Albumin Agglutinating Factor (AAF) - an autoagglutinin will be formed against Sodium Caprylate (which is used as stabilizer in preparations of bovine albumin) and gives false agglutination.

3. Enzyme technique:

- is a very sensitive one for the detection of some low affinity Rh antibodies, which are not detected by other methods including the antiglobulin technique.
- Can detect clinically significant IgG and saline inactive IgM. As a result, it has cumulative effect.
    - Two stage technique:
        - RBCs pretreated with enzyme and they are mixed with patient serum
            - **Enzyme pretreated RBCs + Patient serum**
    - One stage technique:
        - Involves enzyme, patient's serum and donor's red cell incubated together.
            - **Enzyme + RBCs + Patient serum**
- Fail to detect certain Abs in MNS and Duffy BGS.
- There will be non-specific agglutination when:
    - ☞ RBCs over treated or undertreated by the enzyme
    - ☞ Enzyme not properly used
- The most widely used enzymes are Bromlein, Papain, Trypin, Ficin…
- If blood is compatible, we will proceed to the next step.

4. Anti-human globulin technique:

- A more concentrated suspension of red cells is mixed with the patient's serum and incubated at 37^0C and then AHG is added.
- is highly efficient for the detection of most kinds of incomplete antibodies.
- A positive test detects the presence of antibodies of:
    - rhesus, kell, kidd, S and Lewis
- Anti globulin is essential for detection anti-Duffy.

- ❖ No agglutination or hemolysis in any of the 4 techniques **=> compatible blood**
- ❖ Hemolysis or agglutination in at least one of the 4 stages **=> incompatible blood**
- ❖ In most cases enzyme technique is ignored because the enzymes are expensive.

Procedure:

A. Standard major cross matching:

- Is cross-match that is performed in three techniques (Saline, albumin and AHG) within 45 to 60 minutes.

Clinical significance:

- detects unexpected (irregular) antibodies in the recipient/ donor serum

Principle:

- Serum of the recipient is tested against the red cells of the donor under different conditions in order to establish their compatibility.

Type of specimen:

- Recipient serum (plasma) not older than 48 hrs
- Donor's washed red cells (2 - 5%)

Equipments and reagents:

- Test tubes
- Centrifuge
- Microscope
- Microscopic slide
- Normal saline
- 20% albumin
- AHG (Coomb's reagents)

Procedure:

1. Label two test tubes as **S** for saline cross matching and **P** for protein cross match.
2. Place 2 drops of fresh recipient serum (less than 48hr old) into each tube.
3. Select donor's unit of the same ABO and Rh type as that of the recipient and prepare 2-5% saline red cell suspension.
4. Add 1 drop of donor's red cell to each tube and mix.
    - Saline cross match incubation

5. Allow tube S to stand at room temperature for 15 – 30 minutes.
6. Centrifuge at 3400rpm for 15 sec and examine macroscopically for hemolysis and for agglutination using an optical aid. (Examine negative reactions microscopically). If there is no agglutination proceed to protein cross match.
    - Saline cross match immediate spin
7. Immediately centrifuge tube S at 3400rpm for 15 sec without incubation and observe for agglutination.
    - Protein cross match
8. Add 2 drops of 22% albumin into tube P.
9. Mix and incubate at 37^{o}C for 15 – 30 minutes.
10. Centrifuge at 3400rpm for 15 sec or at 1000rpm for 1 minute and examine macroscopically for hemolysis and for agglutination using an optical aid.
    - Antiglobulin cross match
11. If no agglutination is observed in tube P (protein cross match) or if it is still compatible, wash the cells 3 or 4 times with saline decanting completely after the final wash.
12. Add 1 or 2 drops of antiglobulin reagent
13. Mix and centrifuge immediately at 3400rpm for 15 sec
14. Examine for reaction using an optical aid and record result.
15. Add 1 drop of known sensitized red cells (Coomb's control cells) to all negative tests. Centrifuge at 3400rpm for 15 sec and examine for agglutination. If no agglutination is seen, the test result is invalid and must be repeated.

Results:

- No hemolysis or agglutination is seen in any phase of the cross match.

  — the blood is compatible and can be issued with the completed cross-match label.
- If there is agglutination or hemolysis in any of the tubes

  — the blood is incompatible, and must not be issued for the patient.

Type of Incompatibility	**Saline tube**	**Albumin tube**	**Anti- globulin tube**
ABO incompatibility (anti A and Anti-B.)	Shows strong agglutination	Shows agglutination	show no agglutination
Rhesus incompatibility (anti-D ,c, e)	does not usually show agglutination	Shows agglutination	Shows agglutination

Anti- Duffy and anti kidd incompatibilities	Does not usually show agglutination	does not usually show agglutination	Shows agglutination
Anti- Lewis incompatibility	Shows agglutination	Shows agglutination	Shows agglutination

Table 7.2 Incompatibility investigation during compatibility testing.

B. Emergency cross match:

- Performed when there is no enough time to perform the standard cross match
- Takes about 25 to 30 minutes and
- Does not include antiglobulin test.

C. Rapid direct slide cross match:

(Request for un cross matched blood)

- Takes only 3 or 4 minutes
- Plasma is used instead or serum.
- Not safe and must only used in extreme emergencies
- Standard cross match should be carried out while the transfusion is in progress.

Procedure:

- Take 2 volume of patient's plasma on a slide
- Add 1 volume of donor's whole blood of the same group as the patient and mix.
- Leave for 2 minutes and examine microscopically for agglutination

Results:

- If the cells show agglutination the blood must not be given and will usually indicate that the wrong ABO group blood is being cross marched.

Sources of errors in cross-matching:

- Rouleaux
- Auto agglutinins
- Infected donor cells
- Anti- A1
- Over centrifugation
- Dirty glass wares etc..

CHAPTER EIGHT

THE TRANSFUSION REACTION

- Also called adverse reactions to transfusions
- Are any unfavorable responses by a patient following transfusion of blood, blood components or derivatives.

  Example:
  - Facial flushing
  - Chest/back pain,
  - Chills,
  - Fever ,
  - Cyanosis, Dyspnea

8.1 Types of Transfusion Reaction

A .Hemolytic reactions-

-abnormal destruction of RBCs of either the donor or recipient

B. Non hemolytic reactions-

-not usually associated with RBC hemolysis

a. Febrile reactions (pyogenic reactions)

b. Allergic reactions

c. Bacteriogenic reactions

d .Circulatory overload

Characteristics non hemolytic reactions:

- shortened post transfusion survival of RBCs
- febrile reactions,
- allergic response and
- disease transmission.

Cause of haemolytic transfusion reactions (HTR)

- incompatible blood,
- blood under great pressure
- transfusion of hemolyzed ,overheated or frozen blood,

- HTR can be:
  1. immediate or
  2. delayed.

1. Immediate Type (Acute):

- hemolysis evidence is obtained during or immediately after blood is infused due to destruction of donor cells by the recipient's Abs.
- are the most serious and potentially lethal,
- Most commonly are caused by Ag-Ab reaction between the patient's serum and the donor's red cells and vice versa, of transfusing ABO incompatible blood.

Two types:

a. intravascular
b. extravascular

a. Intravascular-

It is due to:

- ABO incompatibilities due to clerical errors (Usually)
- Antibodies to other blood group antigen systems may also be involved in immediate HTR (Rarely)

b. Extra vascular-

It is due to:

- ☞ Coating of red cell antigen by incompatible Abs which results in the removal of the red cells in liver & spleen.
- ☞ Any IgG, non agglutinating, non-complement binding form of antibody

Causes of immediate hemolytic transfusion reactions:

1.Failure to recognize antibodies present recipient serum/plasma prior to transfusion
2.Failure to recognize donor antibodies before transfusion
3.Administration of blood considered compatible on the basis of an incompatible cross match
4.Erroneous identification of recipient, donor or both etc...

2. Delayed HTR

- Are not so dramatic and are usually noticed a week or two after a transfusion.
- Abs to Ags other than ABO,(Rh, Kell, Duffy, Kidd) Ag systems are commonly responsible.
- occur because of an anamnestic response (previously encountered Ags)
  - transfusions or
  - pregnancies.
- The Abs screen on the patient's serum and the major cross-match may well have been normal during the initial testing.
- They are subtle in their manifestations and present

- only as a mild jaundice or
- as a failure of the Hb to rise as expected.

- Are not preventable ,b/c
  Abs -are not present or
  -below the detectable level

2. Non-Hemolytic transfusion reactions:

- not usually associated with RBC hemolysis

Characteristics non hemolytic reactions:

- shortened post transfusion survival of RBCs
- febrile reactions, allergic response and disease transmission.

a. Febrile reactions (pyogenic reactions)

- The patient develops fever and chills during or after transfusion
- The rise in body temperature may be due to leukocyte Abs ,platelet Abs or Pyrogens
- Prevented by giving leukocyte and platelet poor RBCs

b. Allergic reactions

- Most common type is urticaria (an itchy rash)
- Characterized by flushing of the skin & dyspenia.
- Commonly caused by the interaction b/n transfused IgA and class specific anti-IgA in the recipients plasma.
- controlled by giving anti-histamines (corticosteroids).

c. Bacteriogenic reactions

- Caused by bacteria that may contaminate solutions or equipments before sterilization
  - Transfusion of bacterially contaminated blood components
  - Common problem for platelet concentrates stored at room temperature

Signs of bacteriogenic reactions

- ✓ Rapid onset of chills & fever
- ✓ Vomiting
- ✓ Diarrhea

- ✓ Profound hypotension
- ✓ Shock

To minimize the risk of infection of blood

- Blood should be maintained at refrigerator temperature at all times during storage
- the container should not be opened or punctured to obtain a sample
- the recommended storage time must not be exceeded
- Blood should be examined routinely for
  - unusual color (turbidity) or
  - the presence of hemolysis, both of which may suggest bacterial contamination.

d. Circulatory overload

- Results from the transfusion of a patient with a normal blood volume but a reduced RBCs volume (when plasma-red cell ratio is high)
- The total circulatory volume becomes too great for the pumping action of the heart

- Cause congestive heart failure manifested by:
  - Coughing
  - Cyanosis, and
  - Difficulty in breathing

8.2 Laboratory tests to be done when transfusion reaction occurs:

- Check the identification of the patient and transfused unit
- Obtain a post transfusion specimen from the patient and visually examine it for hemolysis
- Perform direct Antiglobulin test from post transfusion sample, taken as soon as possible after the reaction has taken place.
- Re-type the RBCs of both donor and recipient for ABO and Rh grouping.
- Re-cross-match blood from each unit transfused using serum from both pre-and post-transfusion specimens from the patient.

TRANSFUSION TRANSMITTED DISEASES AND SCREENING ASSAYS:

- There are three main types of infectious agents known to be transmitted by transfusion.

  They are: ***Viruses, Bacteria, Protozoa infection***

Viral Infections

- Hepatitis A,B,C
- HTLV
- HIV
- West nile virus
- Herpes and parvovirus

Parasitic infections

- Malaria
- Chagas
- Toxoplasmosis
- Babesiosis
- Leishmaniasis

Bacterial infections

- Syphilis
- Lyme disease (Borreliosis)

SCREENING ASSAYS

Types of screening assays

- The choice of markers to screen for depends on the infectious agents that are screened.
- Screening for ***specific antibody*** is usually most appropriate for HIV,HCV and Syphilis.
- Screening for ***specific antigens*** (HBsAg) is necessary for HBV.
- The three main kinds of primary screening assay are available to detect infectious disease markers in donated blood:

  1. ELISA/EIA

2. Particle agglutination assay
3. Simple rapid assay

- Criteria for selecting the most appropriate assays for screening of blood:
  1. Principle of the assay
  2. Complexity of the assay
  3. Incubation time
  4. Sensitivity
  5. Specificity
  6. Suitability for different situation
  7. Availability and
  8. Cost

8.3. HEMOLYTIC DISEASES

8.3.1 Hemolytic Transfusion reaction (HTR)

- Is a condition in which there is intravascular destruction of transfused RBCs
- Is mainly caused by antibodies of the ABO and Rh system.
- Clinical consequences include
  - Dissiminated intravascular hemolysis(DIC)
  - Hypotension
  - Irreversible shock and renal failure

8.3.2 Autoimmune Hemolytic Anemia (AIHA)

- Brought about through the interaction of red cells and auto antibodies.
- Classified into four groups
  - warm-reactive auto antibodies
  - cold- reactive auto antibodies
  - paroxysmal cold hemoglobinuria and
  - drug induced hemolysis.
- The diagnosis depends on the demonstration of auto antibodies on the patient's red cells using the DAT.
- The autoimmune antibodies are either
  - 'warm antibody' type or
  - 'cold antibody' type.
- The warm antibodies are active at 37^0C
- The cold types are optimally active at 2^0C but with a temperature range which may go as high as 32^0C.
- A positive DAT will be found in both types;
  - In the cold type , complements rather than the bound antibody sensitizes the cells and give rise to the positive DAT
  - In the warm type, the attached antibody sensitizes the cells, although occasionally it may be complement**.**

8.3.3 Hemolytic Disease of the Fetus and New born (HDFN)

- Originally known as erythroblastosis fetalis results from blood group incompatibility in which maternal antibodies destruct fetal red cells.
- Initially observed in babies from D- negative women with D-positive mates.
- Initial pregnancies were not usually affected.
- Infants from subsequent pregnancies were often still born or severely anemic and jaundiced.
- 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.

Rh immune globulin (RhIG)

- Protects D- negative mothers against the production of anti- D following delivery.
- Anti-C, anti-c, anti-E, anti-e are not protected by RhIG and can cause HDN.

Overview of HDFN:

- HDFN is a condition in which the red blood cells of a fetus or neonate are destroyed by Immunoglobulin G (IgG) antibodies produced by the mother.

Factors that must be present for HDFN to occur:

- ☞ The mother must lack the Ag, and, following exposure to the Ag should produce Abs of the IgG class.
- ☞ The Fetus must possess the Antigens
- ☞ The Antigen must be well developed at birth

Etiology of HDFN:

- The placental barrier limits the number of fetal RBCs entering the maternal circulation during pregnancy and thus reduces the chances of Ab production during pregnancy.
- At the time of delivery, a fetal RBCs escape into the maternal circulation (known as feto maternal hemorrhage (FMH).
- Immunization can result from fetal RBC exposure following:
  - — Abortion
  - — Ectopic pregnancy, or
  - — Abdominal trauma
- Ags on the fetal RBC can stimulate the maternal immune system which results in the production of IgG antibodies.

- In a subsequent pregnancy the IgG antibodies cross the placental barrier by active transport mechanism.
- The antibodies bind to the fetal antigens which results in RBC destruction by macrophages in fetal liver and spleen.
- Hemoglobin liberated from the damaged RBCs metabolized to indirect bilirubin.
- If the RBC destruction continues, the fetus becomes increasingly anemic
- Fetal liver and spleen enlarge as erythropoiesis increases in an effort to compensate for the RBC destruction.
- Erythroblasts are released into the fetal circulation

  — Erythroblastosis Fetalis
- If this condition is left untreated, cardiac failure can occur accompanied by hydrops fetalis, or edema and fluid accumulation in fetal peritoneal and pleural cavities.
- Thus the greatest threat to the fetus is cardiac failure resulting from uncompensated anemia.
- Following delivery, red blood cell destruction continues with the release of indirect bilirubin
- The new born liver is deficient in glucuronyl transferase
- The indirect bilirubin binds to tissues results in jaundice.
- Bind with tissues of the CNS and cause permanent damage (kernicterus)

  — resulting in deafness, mental retardation, or death.
- HDN is often classified into three categories based on Antibody specificity:

  — Rh

  — ABO and

  — Other (non-Rh) Antibodies.

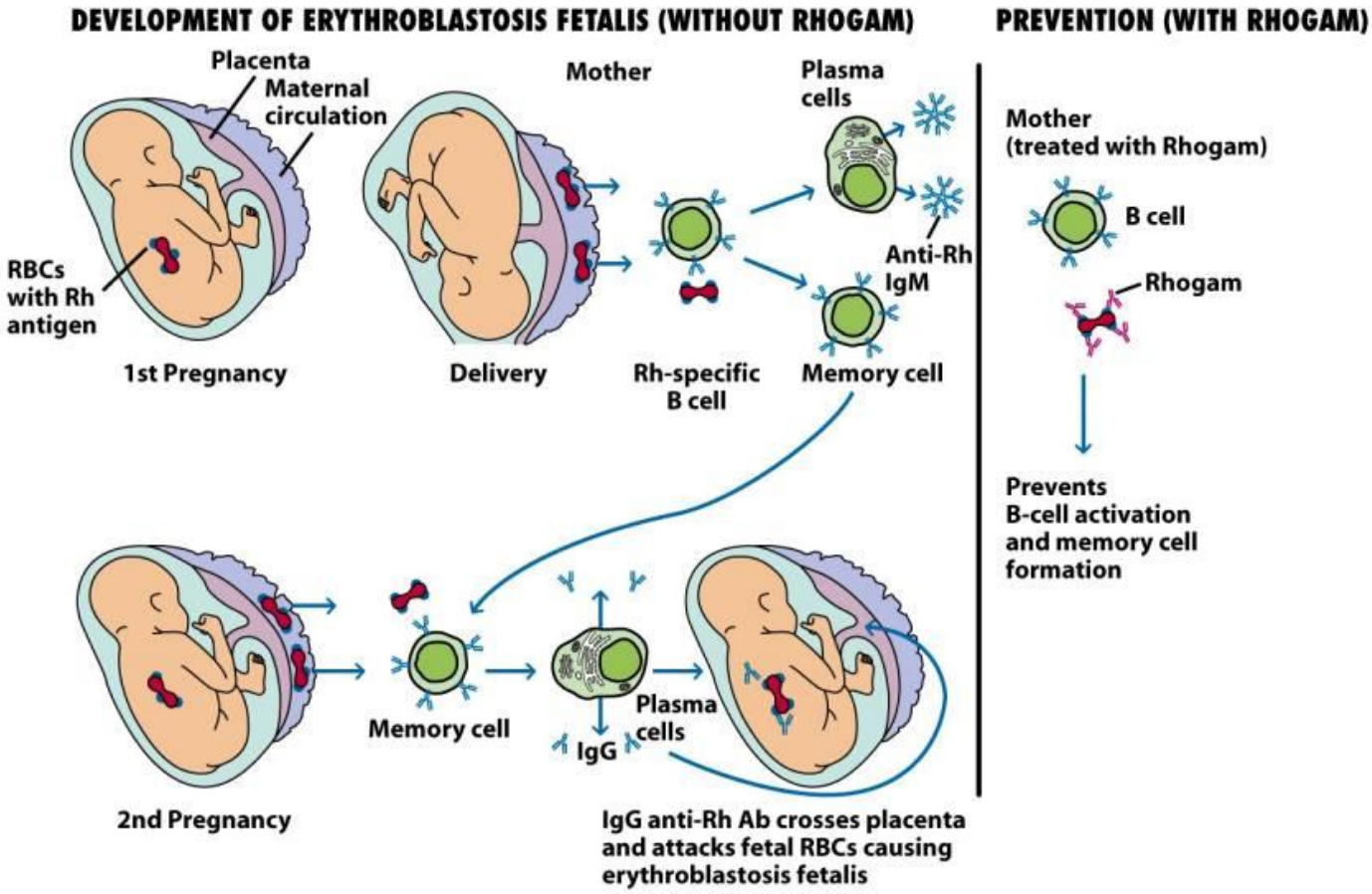


Fig.6-1 Development and prevention of HDN

1. HDFN due to RH

- Anti- D is responsible for the most severe cases of HDFN.
- Most severe than ABO HDFN.
- In most cases, D-Negative women become alloimmunized (produce anti- D) after the first D- Positive pregnancy.
- In rare cases, alloimmunization occurs during the first pregnancy but not result in clinical signs of HDFN.
- Unless the mother is sensitized to Rh antigen in previous transfusion, Rh HDFN can’t occur in the first pregnancy.
- In some cases, the maternal anti-D binds to fetal D-positive red blood cells and causes a positive direct antiglobulin test (DAT).
- 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,
  - the mother's ability to respond to these foreign antigens.
    - About one third of mothers are nonresponders, they fail to form anti- D despite intentional repeated injections of Rh (D) positive erythrocytes.
- Moderately affected infants develop signs of:
  - jaundice, and
  - corresponding elevations in bilirubin levels, during the first few days of life.
- Severely affected D- positive infants,
  - experience anemia in utero and develop jaundice within hours of delivery.

2. HDFN due to ABO

- Occurs most frequently in group A or B babies born to group O mothers.
- Is due to the increased incidence of IgG ABO Abs in group O individuals compared to other ABO groups.
- ABO incompatibility often affects the first pregnancy because of the presence of non-RBC stimulated ABO Abs.
- Red blood cell destruction by ABO Abs is more common than by anti-D.
- Fortunately, most cases are sub clinical and do not necessitate treatment.
- Some infants may experience mildly elevated bilirubin levels and some degree of jaundice with in the first few days of life.
- These cases can usually be treated with phototherapy.
- Mild red blood cell destruction, despite high levels of maternal antibody occurs because:
  - A or B substances are present in the fetal tissues and secretions and bind or neutralize ABO antibodies, which reduces the amount of ABO antibody available to destroy fetal red blood cells.
  - Poor development of ABO Antigens on fetal or infant red blood cells.
  - Presence of reduced number of A and B antigen sites on fetal or infant red blood cells.

- IgM and IgG are produced by the mother but IgG (which is weaker than IgM in complement fixation and in causing lysis of RBCs) can only pass the placenta and cause HDN

- This also explains why the DAT is only weakly positive in most cases of ABO HDN.

3. Alloantibodies causing HDN other than anti-D

- Other Rh-system antibodies are known to cause HDN alone or in combination with anti D.
- Anti-c is the second most common cause of HDN, followed by anti-K.

Assessment of HDFN:

A. Prenatal Assessment:

- 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 & Du,**
  - **alloantibody screening test**
  - **Antibody titer determination**
    - 4x rise in Ab is said to be significant
  - **Amniocentesis (examining amniotic fluid of the mother)**

❖ Indications of Amniocentesis:

- Antibody titer (if Ab titer is significant)
- Excess bilirubin production due to RBC destruction in the fetus cross the placenta and enter the mother's aminotic fluid. So the mother's aminothic fluid is tested for bilirubin (yellow color) in spectroscopy and this presence of bilirubin in aminotic fluid indicated HDN.

  - **Ultrasound**
    - Done after Aminocentesis
    - Detect edema, effusion or ascites and indicates the damage (enlargement) of the fetus spleen, liver and bonemarrow.

B. Postnatal Investigation:

- 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 DU positive and the mother D or DU negative

2. DAT on cord or infants blood:

- In ABO HDN, DAT is usually negative or weakly positive; due to:
  - ☞ weak Ag-Ab interaction which cause the antigen to be removed during the washing phase of the DAT.
  - ☞ too low antibody titer to be detected.
- In Rh HDN, DAT gives positive result.

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.
- It is based on the resistance of fetal hemoglobin for acid elution
  - Mother's blood is taken which contains fetal and maternal Hgb and smear is prepared from it.
  - While maternal Hgb is washed during acid elution, fetal red cells are resistant for acid red cell washing. Therefore, after the acid elution, the **bright red** staining red cells (due to the resistant Hgb) are fetal red cells. But maternal red cells which have soluble hemoglobin appear **'ghost' cells**.

❖ If mother and fetus are both ABO and Rh incompatible, it's protective for the fetus from HDFN. **How???**

8.4 Prevention of HDN:

- Fetal red cells in the maternal circulation might be destroyed by administration of suitable quantity of IgG anti- D to prevent Rh immunization of the mother, given to Rh-negative women within 72 hours of delivery.
- This dramatically decreases the incidence of anti-D HDN.
- Combined prenatal-postnatal treatment is more effective than postnatal treatment alone in suppressing Rh immunization.
- Candidates for this prophylaxis are:
  - mothers who are Rh-negative and D^u - negative, and
  - have an Rh-positive or D^u positive new born.
  - The screening test for alloantibodies is negative for anti D antibody.
  - DAT on cord or infant's cells is negative. If DAT is positive perform elution test to establish anti – D is not the coating antibody.
- All Rh-negative women who have abortions are candidates unless the father or fetus is known to be Rh-negative.
- The following are **not** RhIG candidates:
  - Rho-negative women
  - Who deliver Rh-negative babies.
  - Whose serum contains anti-Rho (D).
  - Who are Rho- positive or Du- positive
- RhIG:
  - Is a concentrate of IgG anti-D prepared from pools of human plasma.
  - Is given intramuscularly:
    - to non-sensitized D-negative women at 28 weeks of gestation (ante partum) and
    - again within 72 hours of delivery (post partum) of a D positive infant.

Mechanism of action

- Suppresses the immune response following exposure to D positive fetal red blood cells and prevents the mother from producing anti-D.

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 acid elution stain) x 50

$$\% \text{ of fetal cells} = \frac{\#\text{ fetal cells}}{2000 \text{ adult cells}} \times 100$$

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

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

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

Example:

- KB reported as 1.2% fetal cells
- 1.2 x 50= 60ml fetomaternal hemorrhage
- $\frac{60 \times 2}{30}$ = 4 vials

Treatment of Infants Suffering from HDFN:

- For infants who develop hyperbilirubinemia and/or anemia due to HDFN, **exchange transfusion** is usually carried out.

Exchange transfusion:

- is a continuous removal of small amounts of blood from the neonate with simultaneous continuous infusion of donor blood until a one or two-volume exchange is accomplished.
- Helps to reduce the concentration of bilirubin and incomplete antibodies
- Provides the infant is with compatible donor red cells.

- To give exchange transfusion to an infant clinical and laboratory findings must be considered.
  - Cord Haemoglobin(<10g/dl) and
  - raised serum bilirubin are strong indicators for treatment.
- For compatible exchange transfusion, donor's blood should be:

- cross-matched with the maternal serum and
- lack the RBC Ag corresponding to the maternal Abs
- ABO group and Rh type compatible with the infant's blood group.

- If the mother's antibody is not available, group O Rh negative red blood cells must be selected.

Phototherapy

- The use of light to degrade bilirubin in mildly jaundiced infants
    - Phototherapy (light treatment) is the process of using ***ultraviolet*** light to eliminate bilirubin in the blood. These light waves are absorbed by the baby's skin and blood and change the indirect bilirubin into direct bilirubin, which can be easily excreted by the newborn.
- Usually for ABO incompatibility