IMMUNOHEMATOLOGY

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IMMUNOHEMATOLOGY

- merges aspects of hematology, immunology & genetics

- serologic, genetic, biochemical and molecular study of antigens associated with membrane structures on the cellular constituents of the blood

- immunologic reactions involving all blood components and constituents

IMMUNOLOGIC PRINCIPLES

- **primary immunological components: *antigens* & *antibodies* → provides basis for blood bank testing and reactions**

CARDINAL RULE IN BLOOD BANK:

The antigens are found on the surface of red blood cells and the antibodies are found in serum or plasma

IMMUNOLOGIC PRINCIPLES

ANTIGENS

- substances that have the capability to stimulate the production of an antibody
- characteristics:
  1. Chemical nature – protein, CHO, lipopolysaccharide or nucleic acid
  2. Molecular weight > 10,000 daltons
  3. Complexity – more complex, > antibody stimulation
  4. Stability – if unstable → degrade → less Ab stimulation
  5. Foreign

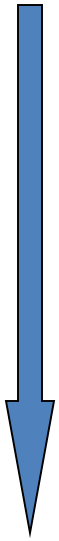
IMMUNOLOGIC PRINCIPLES

Chemical composition of antigens:

1. **Glycoproteins & lipoproteins – most potent**
2. **Glycolipids**
3. **Pure polysaccharides – not immunogenic except in humans and mice**
4. **Pure lipids & nucleic acids – not immunogenic but can be antigenic → serve as haptens**

IMMUNOLOGIC PRINCIPLES

Immunogenicity of Blood Group Antigens



A, B and D (Rho) – most immunogenic

Kell (K)

Duffy: Fy^a

Fy^b

Kidd: Jk^a

Jk^b

IMMUNOLOGIC PRINCIPLES

ANTIBODIES

- also called **immunoglobulins**
- characteristics:
  1. Protein
  2. Produced in response to stimulation by an antigen
  3. Specific for the stimulating antigen
- consists of 2 heavy chains & 2 light chains held together by disulfide bonds
- produce 3 fragments when cleaved by enzymes → 2 Ag-binding fragments (Fab) & 1 crystallizable fragment (Fc)

IMMUNOLOGIC PRINCIPLES

Classification of Blood Group Antibodies:

1. **Alloantibodies**
   - **Reacts with foreign Ag not present on patient's own RBC**
   - **Most produced as result of immune stimulation via transfusion or pregnancy (usually during delivery)**
2. **Autoantibodies**
   - **Reacts with an Ag on patient's own cells & with that same Ag on the cells of other individuals**

ABO BLOOD GROUP SYSTEM

- discovered by Karl Landsteiner; locus on chr 9
- single most important blood group for the selection and transfusion of blood
- widely expressed → tissues & body fluids including red cells, platelets & endothelial cells
- three antigens: A, B, H
- two major antibodies: anti-A and anti-B
- four phenotypes: A, B, AB, O → A & B Ag's autosomal co-dominant (expressed on grp A, B and AB red cells; O phenotype autosomal recessive (most frequent)

ABO BLOOD GROUP SYSTEM

ABO Antigens

- present on the surface of red cells as well as tissue and endothelial cells in the body
- found in soluble form in plasma & other body secretions in people known as ***secretors***
- inherited in simple Mendelian fashion from an individual's parents
- 3 possible genes that can be inherited: A, B, O
- A and B genes produce a detectable product
- O gene does not produce a detectable product

ABO BLOOD GROUP SYSTEM

ABO System

Phenotype	Antigen	Natural antibody	Genotype
A	A only	Anti-B	AA or AO
B	B only	Anti-A	BB or BO
AB	A and B	None	AB
O	None	Anti-A, Anti-B	OO

ABO BLOOD GROUP SYSTEM

- **A and B genes do not directly produce antigens → produce an enzyme called *transferase* → attaches a sugar molecule to the chemical structure of the antigen → sugar molecule responsible for specificity**

- **O antigen → no transferase → no antigen produced**

- **A and B antigens on surface of RBC → protrude from outermost layer of cell membrane**

ABO BLOOD GROUP SYSTEM

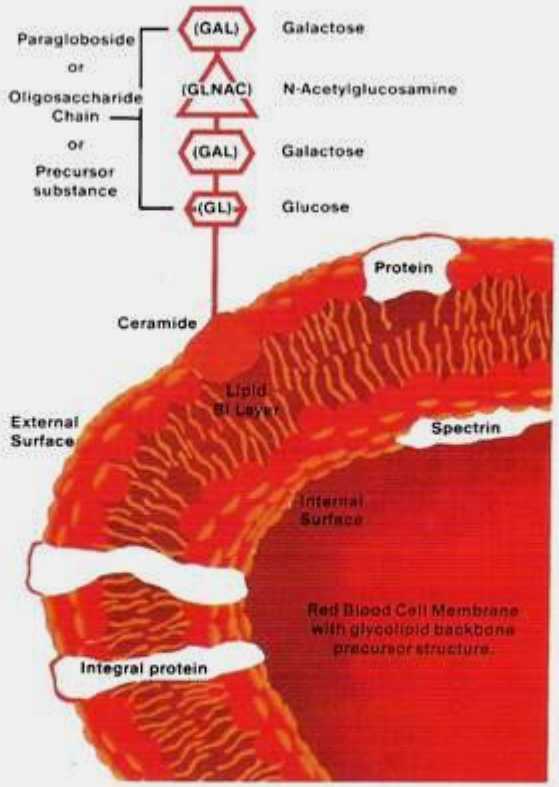


Plate 3. Red blood cell precursor structure (which represents a paragloboside).

ABO BLOOD GROUP SYSTEM

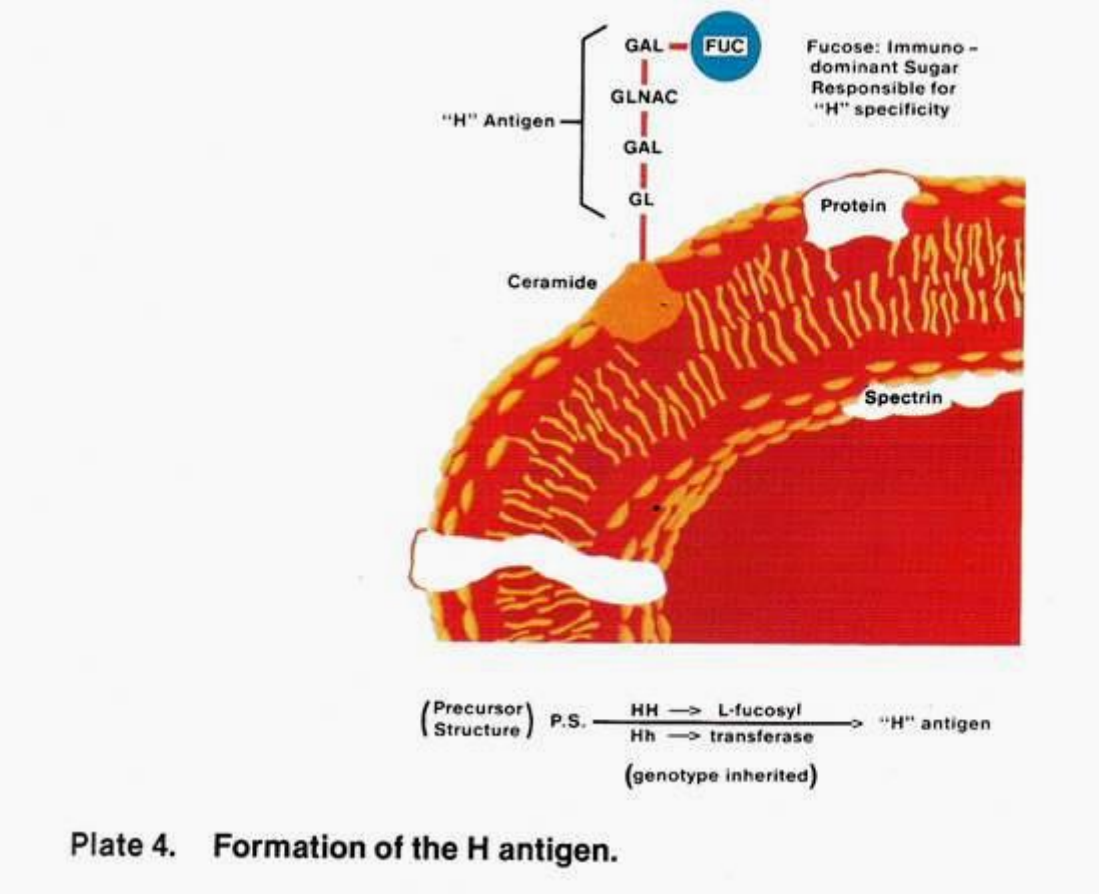


Plate 4. Formation of the H antigen.

ABO BLOOD GROUP SYSTEM

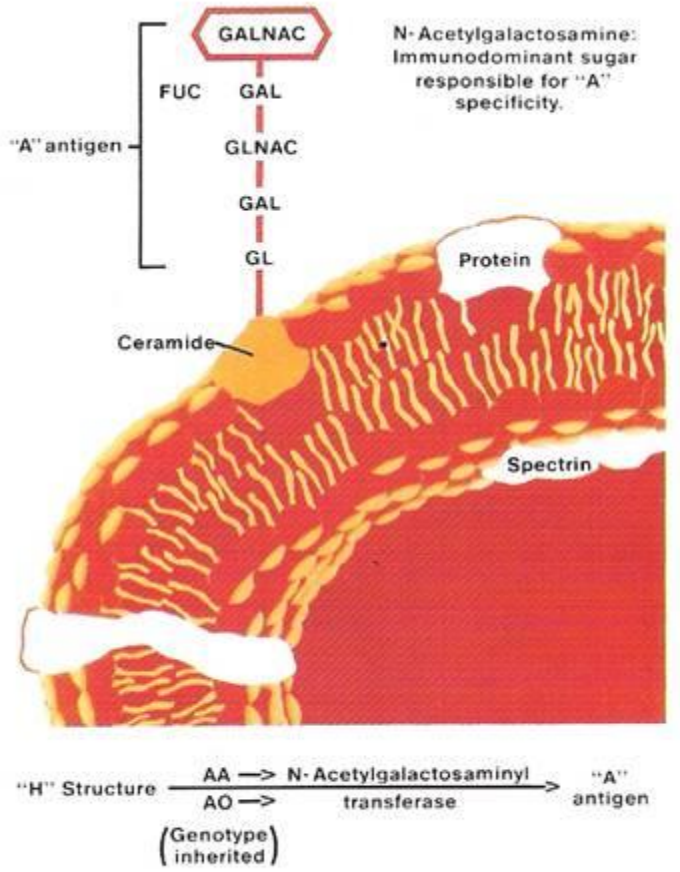


Plate 5. Formation of the A antigen.

ABO BLOOD GROUP SYSTEM

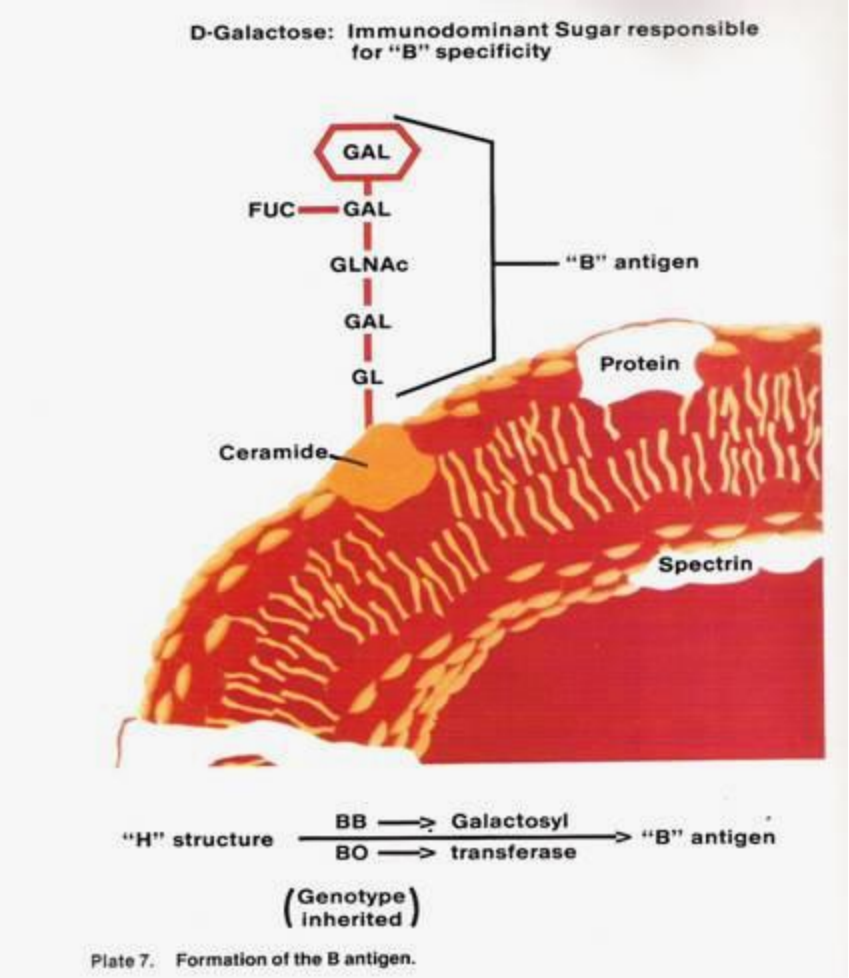


Plate 7. Formation of the B antigen.

ABO BLOOD GROUP SYSTEM

H Antigen

- required to produce either A or B antigens
- possible genetic combinations: HH, Hh, or hh
- HH or Hh (+) → produce H Ag → 99.99% of Caucasians
- hh → does not produce H Ag → Bombay phenotype (Oh)
- anti-H antibodies rare – found only in individuals with Bombay phenotype

ABO BLOOD GROUP SYSTEM

Example of determining offspring blood types from known or suspected genotypes:

		Genotype parent #1 (AO)	
		A	O
Genotype parent #2 (AB)	A	AA	AO
	B	AB	BO

Phenotypes of possible offsprings: A, AB, B

ABO BLOOD GROUP SYSTEM

Frequencies of ABO Blood Groups:

Blood Group	Frequency
O	45%
A	41%
B	10%
AB	4%

ABO BLOOD GROUP SYSTEM

ABO Subtypes:

1. **A variants (A_1, A_2)**
   - **A_1 most common (80%) & most antigenic**
   - **A_1 and A_2 differentiated using antisera specific for A_1 Ag (anti-A_1 lectin) prepared from seed known as *Dolichos biflorus* → (+) reaction with A_1 but not A_2**
   - **Anti-A → reacts with both A_1 & A_2 but more strongly with A_2**

ABO BLOOD GROUP SYSTEM

ABO Subtypes:

2. **Weak A and weak B phenotypes**
3. **Null phenotypes:**

   (a) Bombay (Oh)

   - **No A, B or H Ag on red cells & secretions**
   - **With anti-A, anti-B & anti-H in their sera**

   (b) para-Bombay

   - **Absent or only trace A,B & H Ag's detected on rbc w/ normal expression in secretions & body fluids**

ABO BLOOD GROUP SYSTEM

ABO Antibodies

- **Natural antibodies → antigenic stimulus is environmental → exposure occurs from birth**
- **Newborns → without ABO antibodies of their own; begin to produce Ab with detectable titer at 6 months of age**
- **Other characteristics of ABO antibodies:**
  1. **IgM**
  2. **Reacts at room temp. after an immediate spin**

ABO ROUTINE TESTING (slide or test tube method)

DIRECT OR FORWARD TYPING

- test for antigens
- patient's cells containing unknown antigens tested with known antisera
- antisera manufactured from human sera
- antisera used:

Antisera	*Color*	*Source*
Anti-A	Blue	Group B donor
Anti-B	Yellow	Group A donor
Anti-A,B	Clear	Group O donor

ABO ROUTINE TESTING

Anti-A,B

- **not a mixture of anti-A and anti-B**
- **separate Ab that reacts with both A and B antigens**
- **used in forward grouping for two purposes:**
  1. **confirms the results of the anti-A and anti-B**
  2. **will show a (+) reaction with weak subgroups of A and B that do not react with the anti-A and anti-B**

ABO ROUTINE TESTING

Reaction Patterns for ABO Groups

Blood group	Agglutination with Anti-A	Agglutination with Anti-B
A	+	-
B	-	+
AB	+	+
O	-	-

ABO ROUTINE TESTING

INDIRECT/REVERSE TYPING

- known antigen (cell) vs. unknown antibody (patient's serum)
- serum is combined with cells having known Ag content in a 2:1 ratio
- uses commercially prepared reagents containing saline-suspended A_1 and B cells

ABO ROUTINE TESTING

Reaction Patterns for ABO Groups

Blood Group	Agglutination with A cells	Agglutination with B cells
A	-	+
B	+	-
AB	-	-
O	+	+

ABO ROUTINE TESTING

Stages of Hemagglutination

First Stage:

- red cell sensitization
- Ag and Ab held by non-covalent interactions

Second Stage:

- formation of stable latticework → basis of visible reaction

ABO ROUTINE TESTING

Grading of Agglutination:

Negative (0)	**No clumps or aggregates**
Weak (+/-)	**Tiny clumps or aggregates barely visible macroscopically or to the naked eye**
1+	**Few small aggregates visible macroscopically**
2+	**Medium-sized aggregates**
3+	**Several large aggregates**
4+	**One solid aggregate**

ABO ROUTINE TESTING

Causes of Discrepancies in ABO Testing:

A. Technical

1. **Incorrect ID/recording**
2. **Patient/donor serum not added**
3. **Reagent contamination**
4. **Under-/over-centrifugation**
5. **Hemolysis**
6. **Warming of test mixture**

ABO ROUTINE TESTING

Causes of Discrepancies in ABO Testing:

B. Red Blood Cells

1. **Missing or weak A/B antigen**
2. **Acquired B Ag – colon or gastric CA, intestinal obstruction**
3. **Polyagglutinable RBC**
4. **Ab-coated RBC – post-transfusion incompatibility; autoimmune hemolytic anemia**
5. **Maternal-fetal agglutination – mismatched transfusion**

ABO ROUTINE TESTING

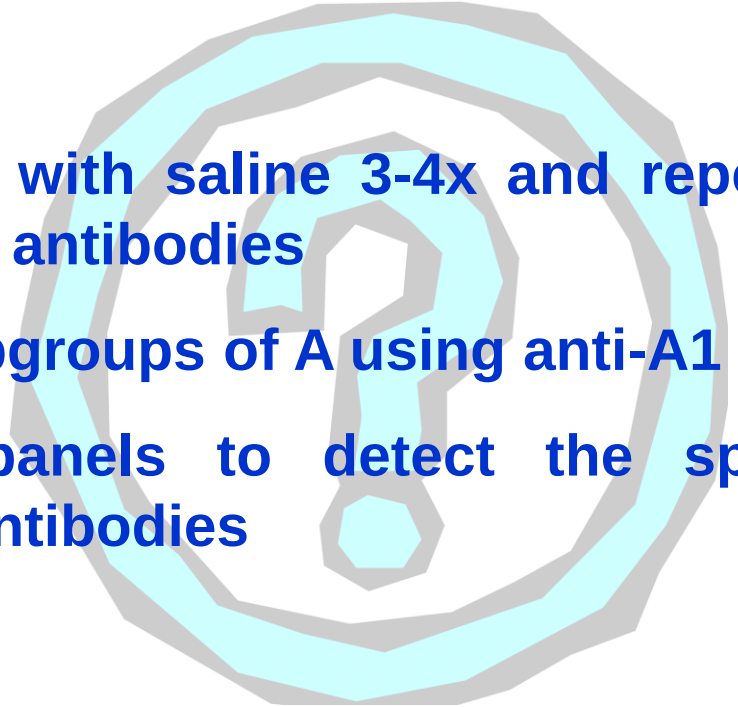
Causes of Discrepancies in ABO Testing:

C. Serum

1. **Roleaux formation – presence of plasma expanders, monoclonal gamma globulins**
2. **Anti-A1**
3. **Unexpected alloantibodies**
4. **Expected antibody absent – hypogammaglobulinemia, extreme ages, immunosuppression**

ABO ROUTINE TESTING

WHAT TO DO?



1. Wash cells with saline 3-4x and repeat all tests and test for antibodies
2. Test for subgroups of A using anti-A1 and anti-A
3. Use cell panels to detect the specificity of abnormal antibodies

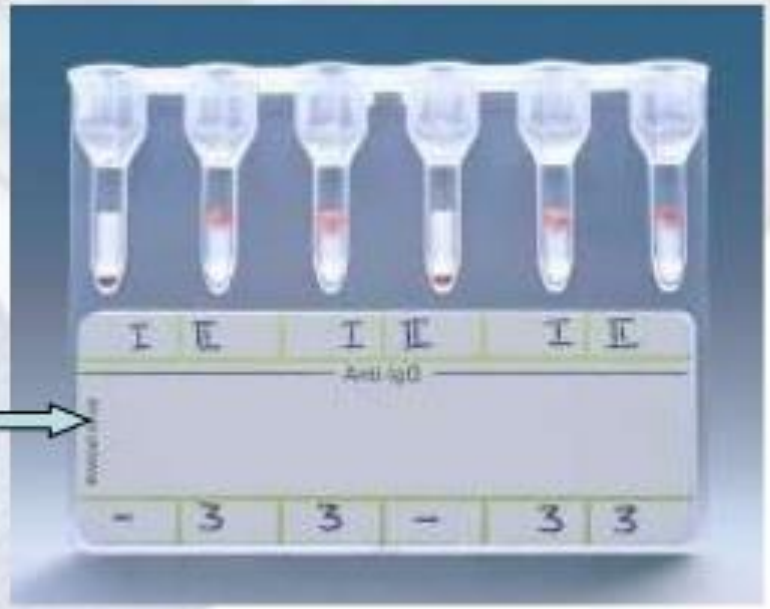
DiaMed

Add Reactants

Serum/plasma/
red cells

Principle
Reaction
Chamber

Gel and
Reagent

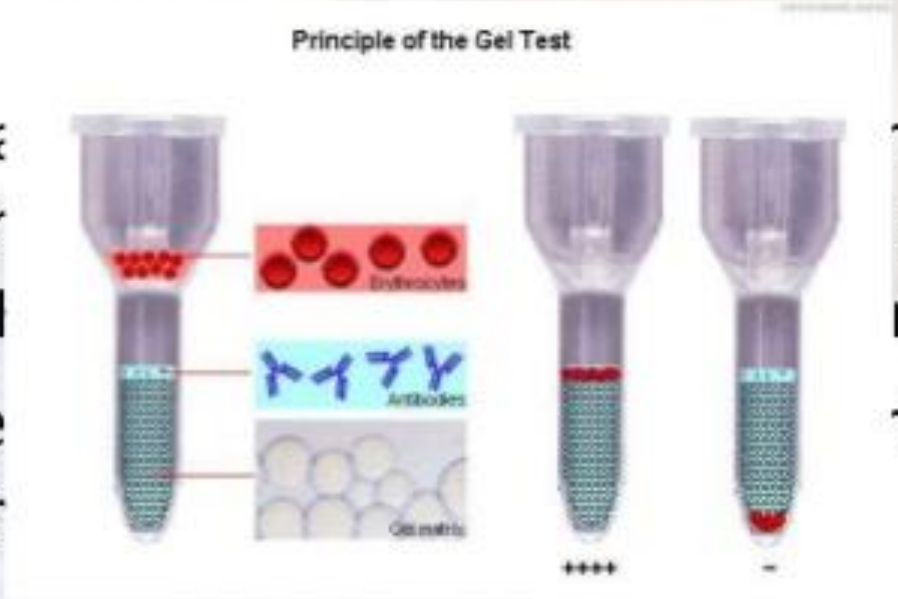


6 Microtubes in Plastic Card

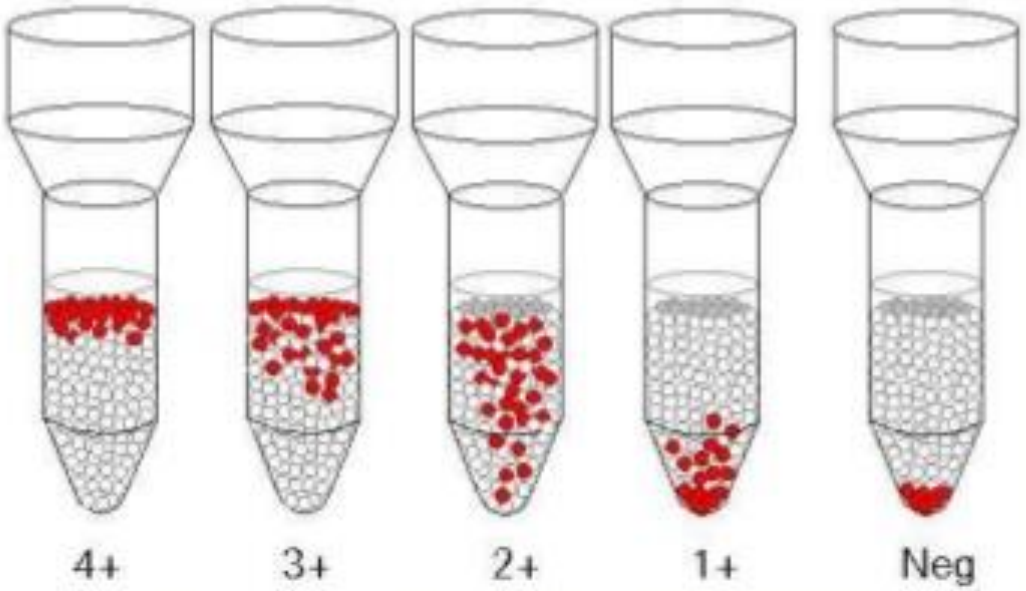
DiaMed

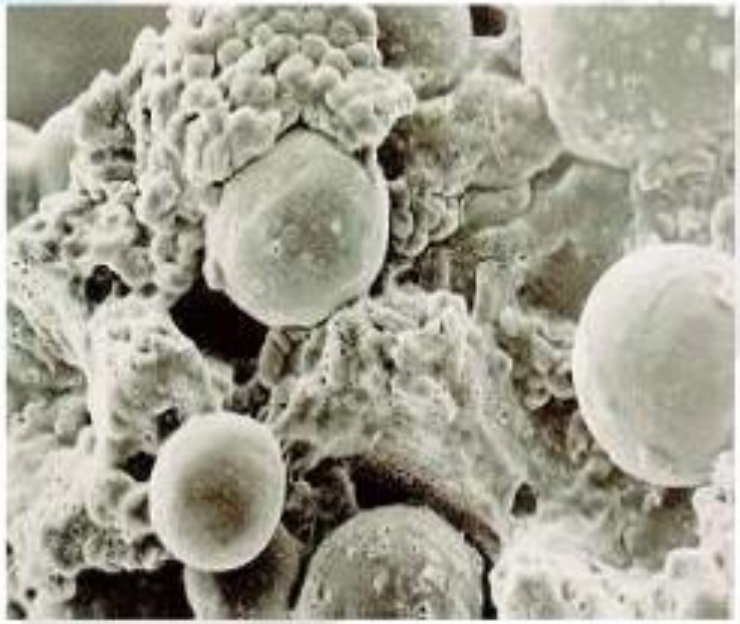
Principle of Gel Technology

- Sephadex gel matrix acts as a sieve.
  - Large aggutinates remain on or near the top of gel interface.
  - Smaller agglutinates pass partway through gel , depending on size.
  - Unagglutinated cells pass to base of microtube to form a button.
- Cells are alwa…nat serum does not con…eliminates the 'WASH PI…nique.
- Grading of re…ribution of RBCs through…



Grading of Reaction






ThankYou!