

# **Division of Blood Transfusion Services**

**Ministry of Health and Family Welfare**



# **Basic Red cell serology – Immunohematology**

# Teaching Aims

- To understand the basis of Immunology and genetics which are closely involved in the understanding of blood group serology

# Antigen and Antibody

- **Antigen:**
  - A substance that on introduction give rise to a formation of antibody that reacts specifically with the same antigenic substance.
- **Antibody:**
  - A gamma-globulin product of an immune response, (also called immunoglobulin) that reacts with antigen against which it is stimulated.



# Characteristics of antigen

- Chemical structure of antigen
  - Antigens composed of oligosaccharides tend to stimulate IgM type of antibody production
  - Antigens which are primarily protein in nature, produce IgG antibody
- Degree of foreignness
- Number of antigens introduced
  - Higher the dose, greater the antibody production
- Route of administration
  - Intramuscular or subcutaneous route more potent

# Immunogenicity

- Ability of an antigen to stimulate the production of its corresponding antibody in a person who lacks the antigen
- Rh D most potent antigenic
- Fy<sup>a</sup> is poor antigenic

# Immunogenicity of antigens

Antigens	System	Immunogenicity (%)
D	Rh	70
C		0.2
c		04
E		3.3
e		1.1
K	Kell	10
k		1.5
Fya	Duffy	0.6
Jka	Kidd	0.1
Jkb		0.03
S	MNS	0.08
s		0.06

# Immunoglobulins

- Five types, viz. IgG, IgM, IgA, IgD & IgE.
- Blood group antibodies are mainly confined to IgG & IgM.
- IgG is a smaller molecule, with a pair each of heavy & light chains of amino acids.
- IgG antibody can just coat but not agglutinate the cells.
- IgM has 5 such pairs joined together by the J chain.
- IgM antibody agglutinate the cells bearing corresponding antigen.

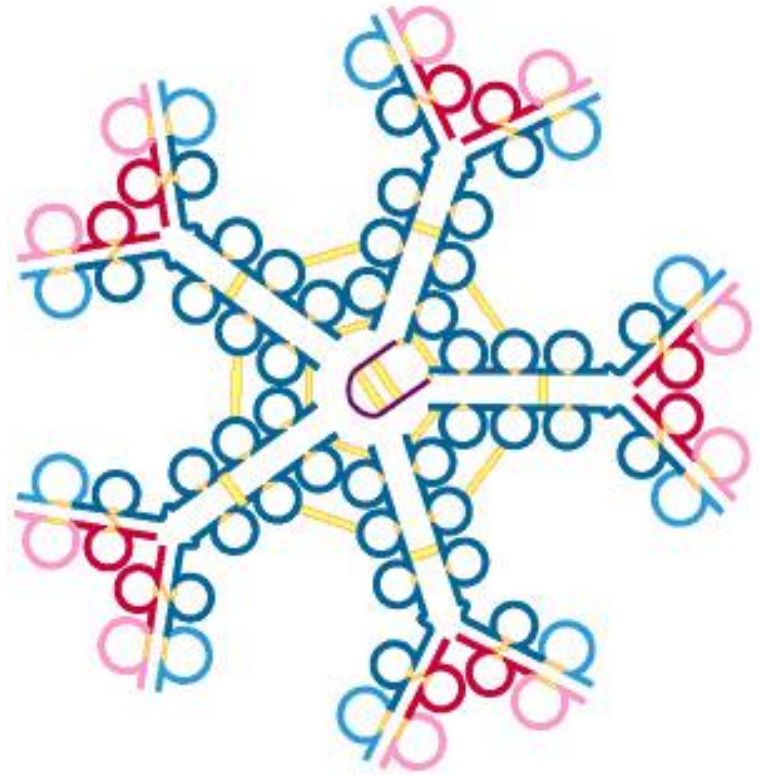


# Properties of human immunoglobulins

Properties	IgG	IgM	IgA
H chain, Class	Gamma	Mu	Alpha
Serum conc (mg/dl)	1000 – 1500	85 - 205	200 - 350
Molecular weight	150,000	900,000	180,000
Complement fixation	Occasionally	Yes	No
Structure	Monomer	Pentamer	Mono / dimer
Crosses placenta	Yes	No	No
Secretions	No	No	Yes

# IgM Antibodies (Complete)

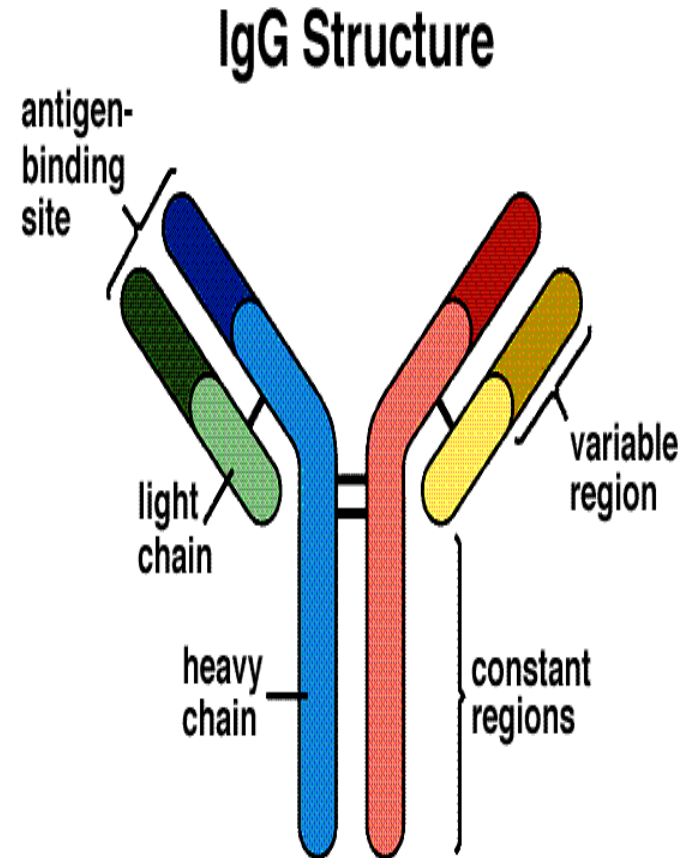
- Agglutinate in saline phase
- Pentavalent
- Usually naturally occurring
- Do not cross placenta
- React at temperature varying from 4 – 20°C
- Example: ABO antibodies




# IgG Antibodies (Incomplete)

Sylvia S. Mader, Inquiry into Life, 8th edition. Copyright © 1997 The McGraw-Hill Companies, Inc. All rights reserved.

- Agglutinate in IAT phase
- However, may cause agglutination in saline phase using albumin / enzymes
- Monovalent
- Usually immune in nature
- Can cross placenta
- React at 37°C
- Example: Rh antibodies

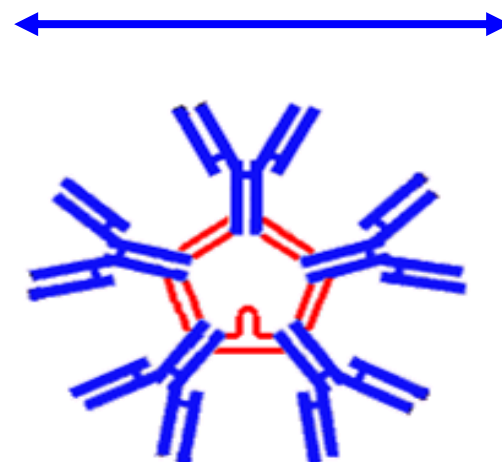


14 nm



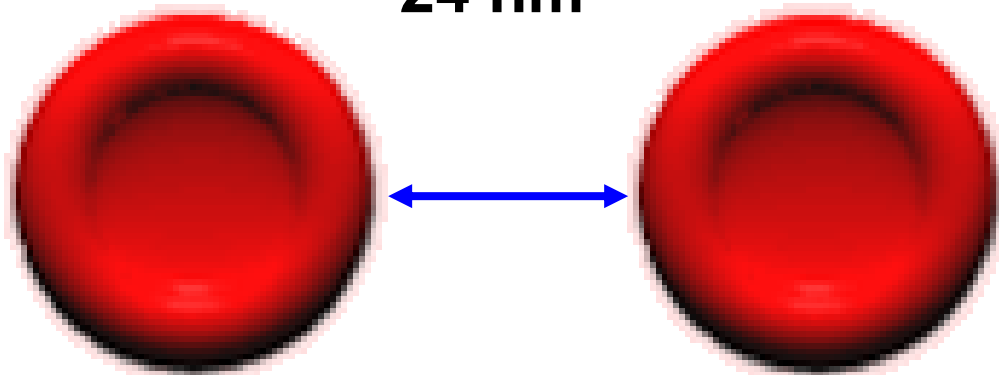
**IgG**

35 nm



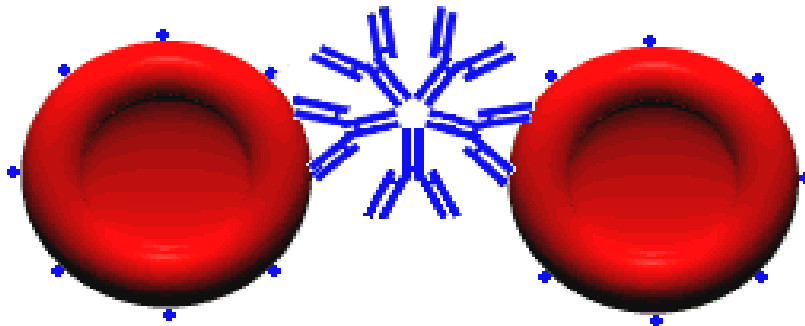
**IgM**

24 nm



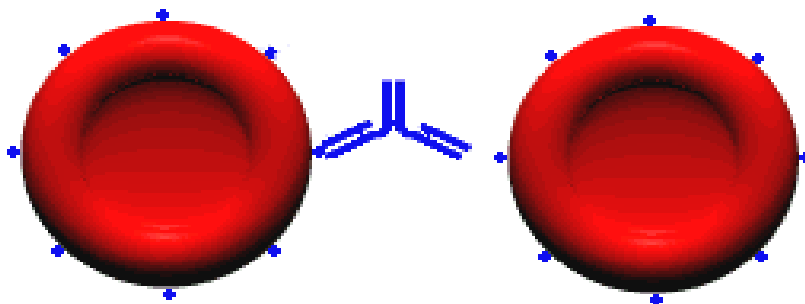
**Intracellular distance**

# Complete and Incomplete antibody



Complete Antibody (IgM)

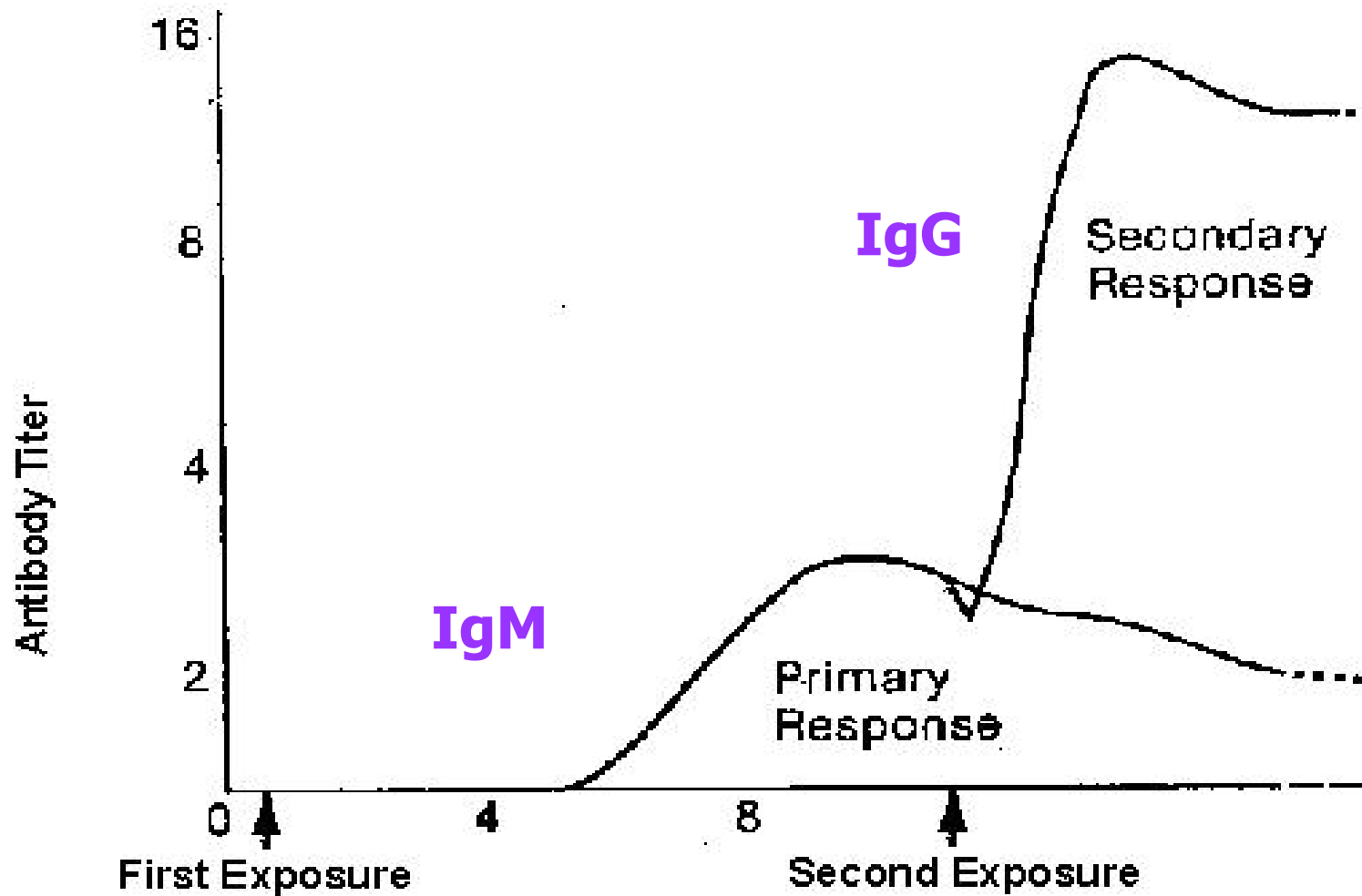
**Complete antibody -IgM**



Incomplete Antibody (IgG)

**Incomplete antibody -IgG**

# Antibody (Immune) Response



# Naturally occurring Vs Immune Antibodies

Feature	Naturally occurring	Immune
Antigen stimulus	Obscure, possibly from microbial origin	Human red cell antigens
Type of Immunoglobulin	IgM	IgG
Optimum temperature	< 22° C	at 37° C
Clinical significance	Acute HTR	HDN, DHTR
Examples	ABO antibodies	Rh, Kell, Kidd, Duffy antibodies

# Clinical Significance of Antibody

## Clinically significant

- ABO
- Rh
- Kell
- Duffy
- Kidd
- Ss

## Clinically insignificant

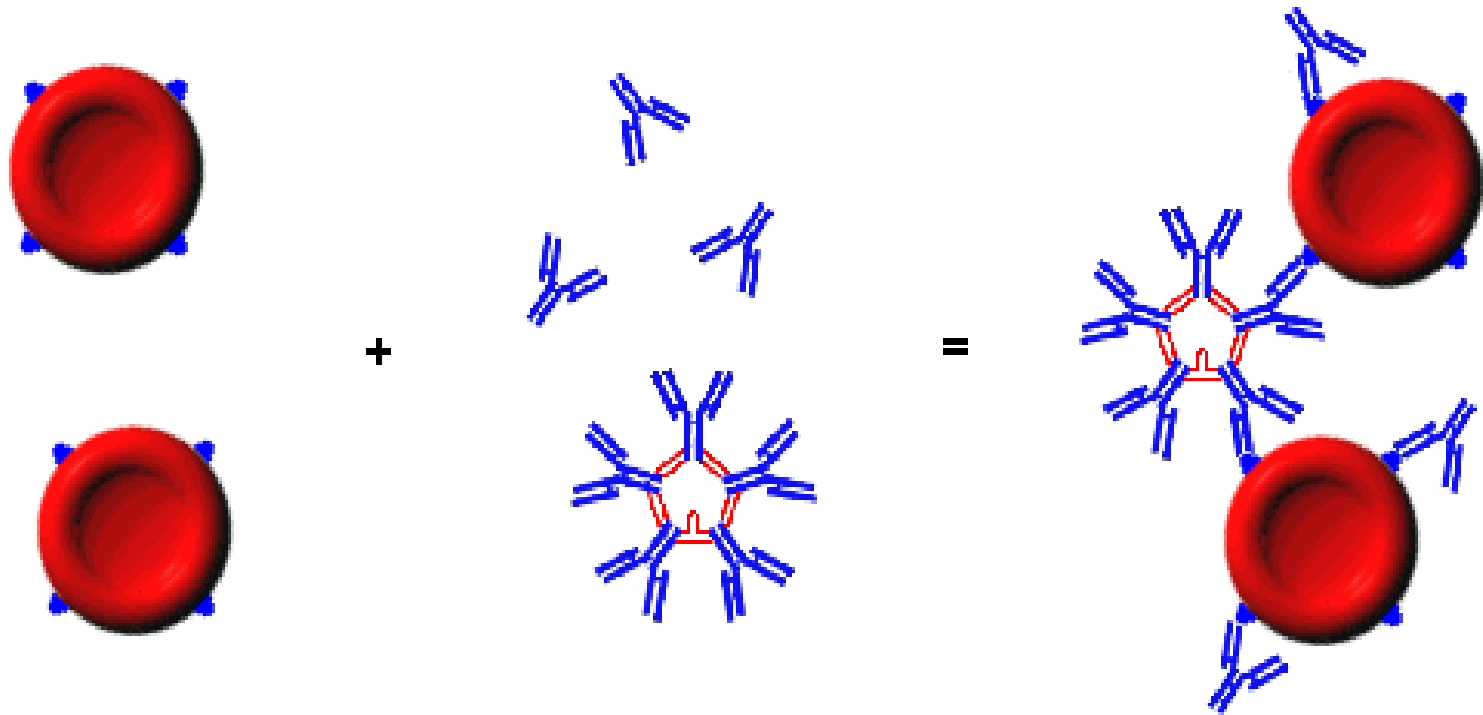
- Lewis
- M,N
- P<sub>1</sub>
- Lutheran
- A<sub>1</sub>



# Types of Antigen-Antibody Reactions

- Agglutination
- Sensitization
- Haemolysis
- Neutralization (inhibition)
- Precipitation
- Immunofluorescence
- Complement fixation
- Radio Immunoassay

# Antigen-Antibody Reaction



Erythrocytes with  
antigen "blue"

Antibody against  
antigen "blue"

- Binding to erythrocytes
- Agglutination of erythrocytes
- Eventually complement activation

# Stages of Ag-Ab reaction

## Stage of sensitization

- Only coating of red cells with IgM antibody without causing agglutination
- Bond holding Ag-Ab complex may dissociate & re-associate till the state of equilibrium is reached

## Stage of agglutination

- Characterized by formation of bridges between sensitized red cells resulting in visible aggregate of red cells

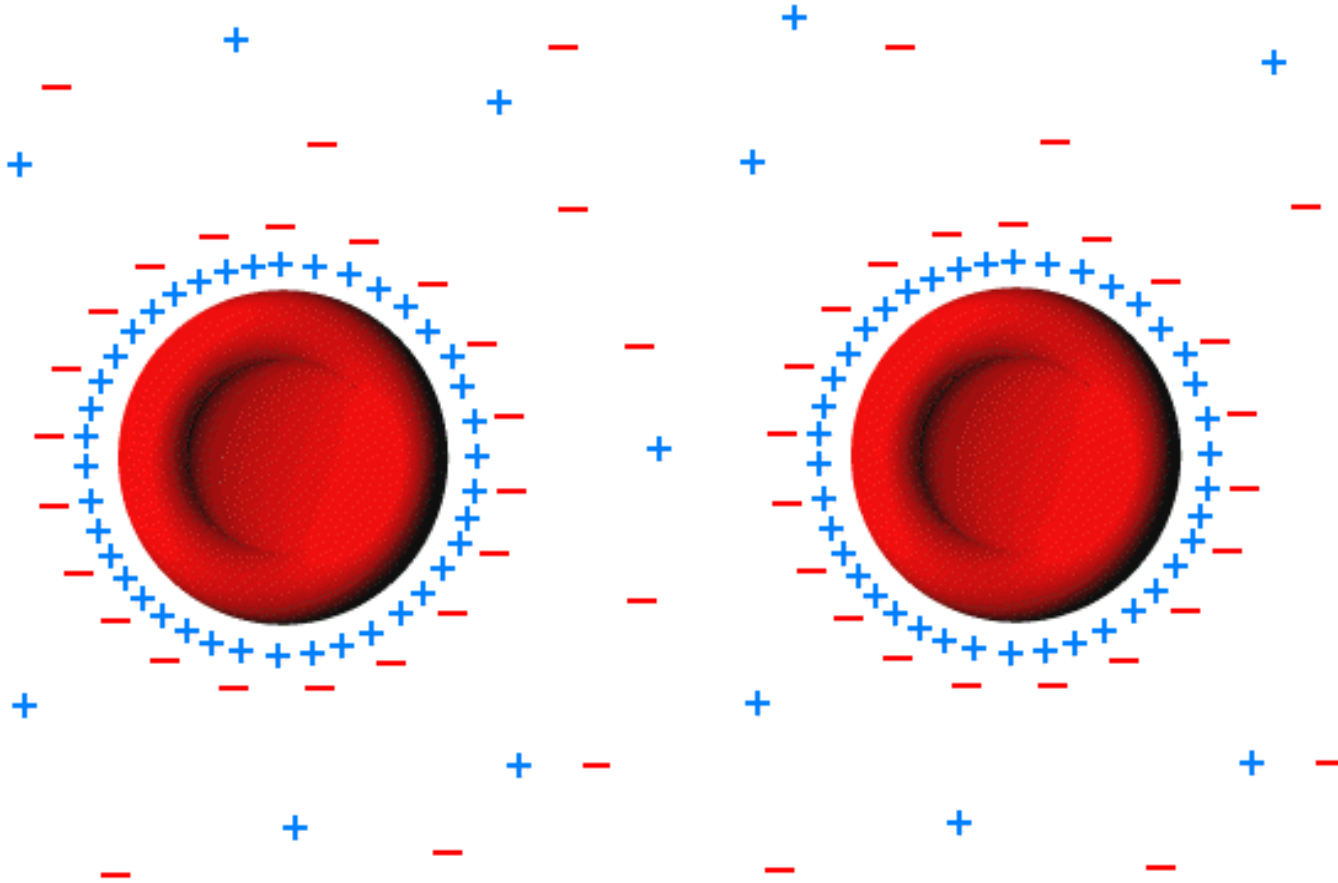
# Factors affecting stage of sensitization

- Antigen – Antibody ratio
  - Two volume of serum and one volume of 5% red cells
  - Sensitivity of test depends upon number of antibody molecules bound per red cells
- pH
  - Most antibodies detected at neutral pH
- Temperature
  - IgM antibodies react optimally at cold temp while IgG at 37°C
- Incubation time
  - Time needed to reach Ag-Ab reaction at equilibrium
  - Too short incubation – weaker reaction
  - Prolonged incubation results in dissociation of antibody

# Factors affecting stage of agglutination

- Size & Class of antibody
  - IgM antibody, being a pentamer can bind antigen sites up to 35 nm apart
  - IgG antibody, being a monomer can bind antigen sites up to 14 nm apart
- Antigenic sites
  - Antigens located on surface of red cell membrane (ABO) result in strong agglutination
  - Antigens embedded in membrane (Rh antigens) result in weaker agglutination
- Zeta potential
  - Electrostatic repulsive force between red cells

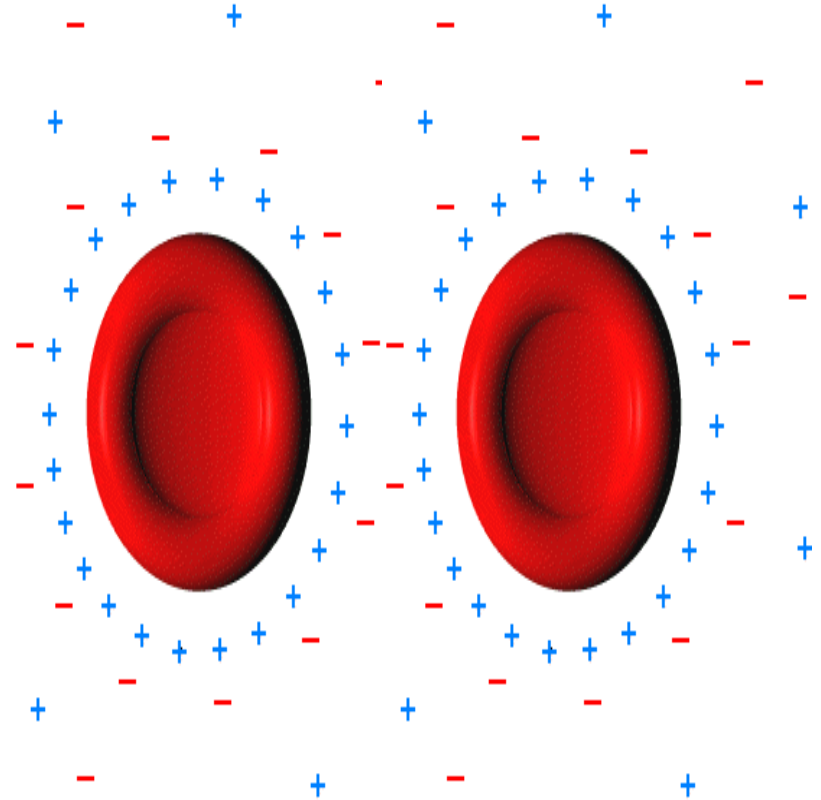
# Electron Cloud Around Each Cell



**zeta potential**

# Effect of adding LISS

- Reduction in zeta potential using LISS
- RBCs come closer together
- Strong agglutination
- Reduction in incubation time



# Red Cell Serological Techniques

- ABO and Rh (D) typing
- Weak 'D' or D<sup>u</sup> testing
- Compatibility testing
- Indirect antiglobulin test
- Direct antiglobulin test
- Antibody screening & identification



# Genetics: Basics

- Gene
  - A biological unit of inheritance
  - Dominant gene – always expressed as antigen regardless of whether it is in homozygous or heterozygous state
  - Recessive gene – produce antigen only when in homozygous state
- Chromosome
  - Linear arrangement of genes
- Locus
  - Hypothetical seat of gene on a chromosome
- Allele
  - An alternative form of gene at particular locus

# Genetics: Basics

- Genotype
  - Sum total of genes present on chromosome regardless of whether or not they produce detectable products.
  - Determined through testing of genes & family study
- Phenotype
  - Detectable products (antigens) demonstrated through direct testing only

# Genotype Vs Phenotype

Phenotype	Genotype
A1	<i>A1A1</i> <i>A1A2</i> <i>A1O</i>
A2	<i>A2A2</i> <i>A2O</i>
B	<i>BB</i> <i>BO</i>
O	<i>OO</i>

# Genetics: Basics

- Homozygous:
  - presence of identical pair of allelic genes on both chromosomes
  - double dose of antigens
  - stronger reaction with corresponding antibody
  - example: KK
- Heterozygous:
  - different alleles of genes on pair of chromosome
  - weaker reaction with corresponding antibody
  - example: Kk

# Learning Outcomes

- You will now understand the basic immunology and genetics related to blood banking

