Antibodies and Antigens in the Blood Bank

NAHLA BAKHAMIS

Outline

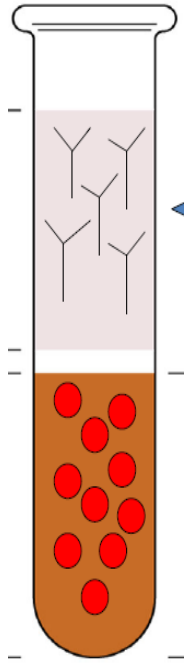
- Antibodies structure, classes and functions
- Most important Abs in the blood bank
- effective roles of Abs
- Zeta potential
- factors to influence agglutination in vitro

Why we study RBC Ag and Ab?

To avoid the immunological reaction between

Ag on donor RBC and

the naturally occurring Abs present in patient serum



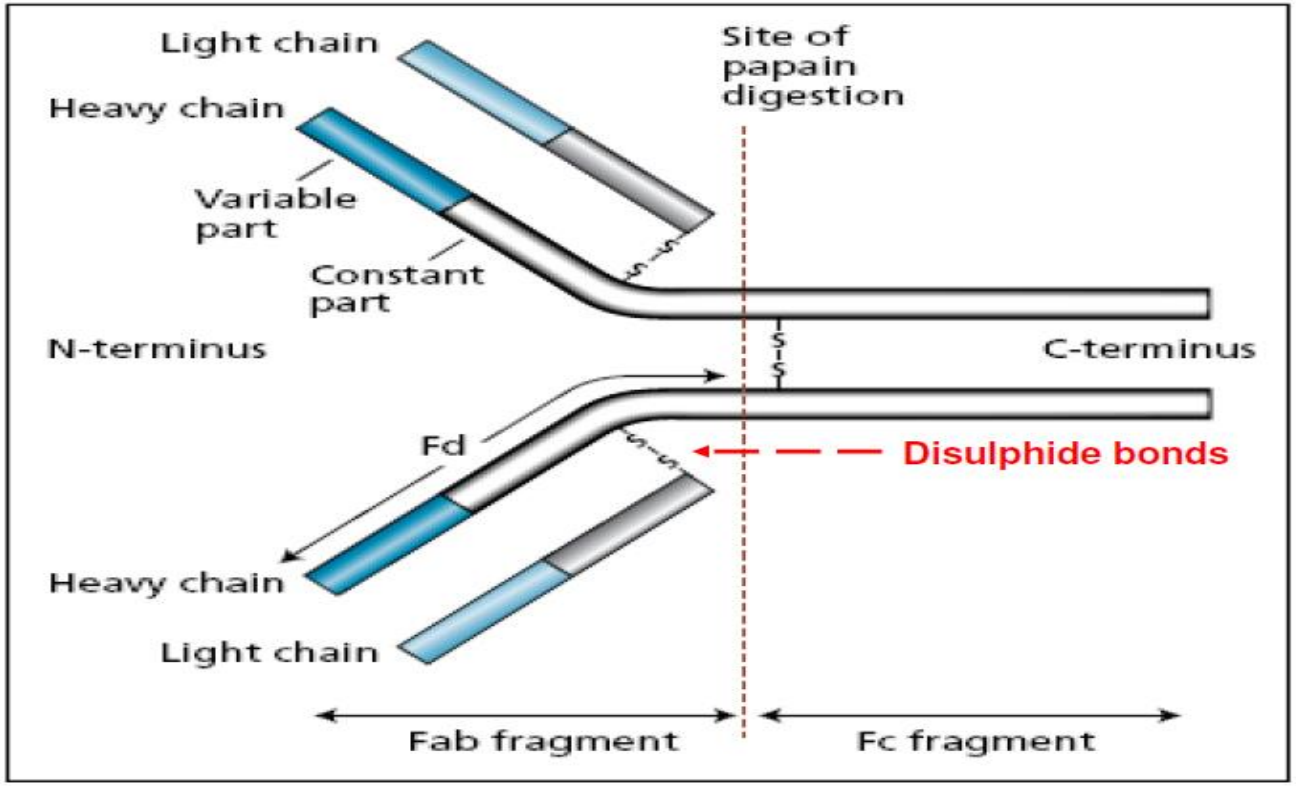
Antibodies

- Proteins that recognize and bind to a particular Ag
- Belong to a group of serum proteins; immunoglobulins (Ig)
- Found in all body fluids; plasma, saliva, tears .. etc

Antibodies

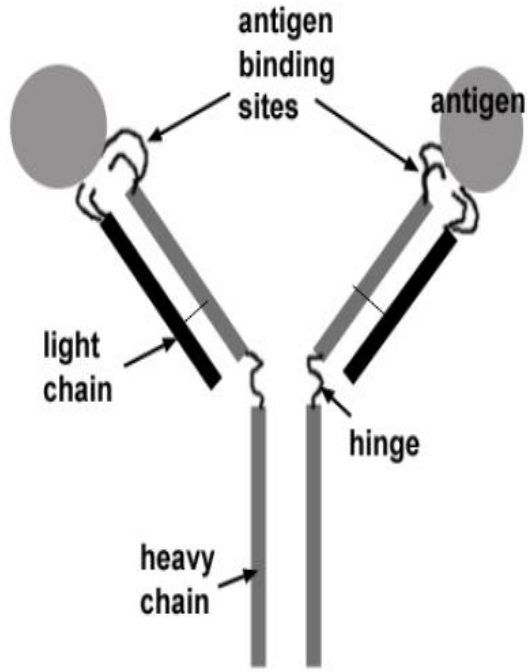
- five classes in each individual;
- each class of Ab has at least 2 Ag binding sites (Paratopes)

Antibody structure



Antibody structure

- Basic structure unit in all classes:
  - 2 identical light chain
  - 2 identical heavy chain
- holds together by Covalent disulphide bonding



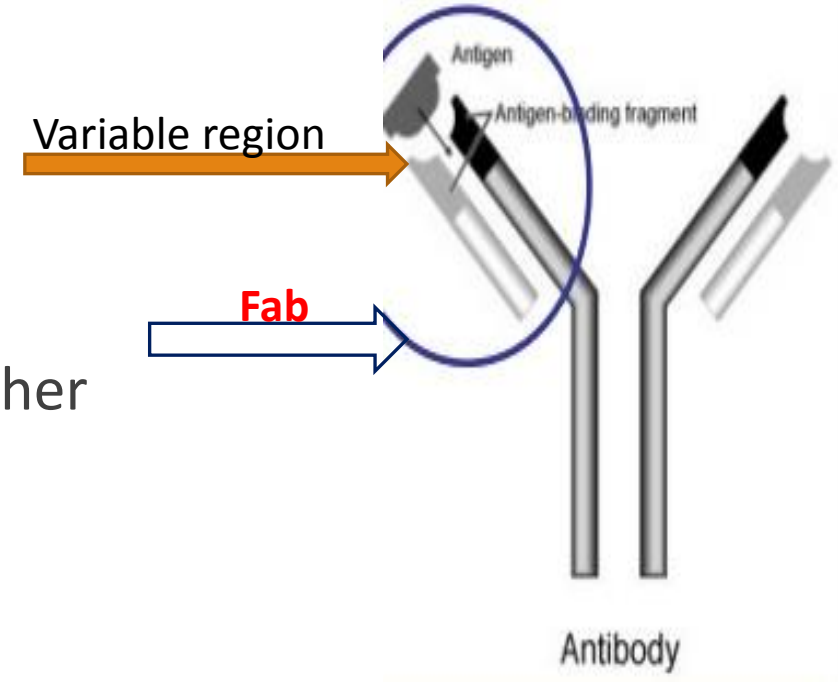
Antibody structure

Variable regions:

2 sections at the end of the ‘Y’ arm

Contain Ag binding sites (Fab)

Identical within the same Ab, dif form Ab to another

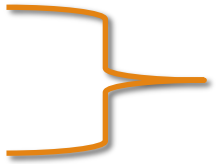


Antibody structure

- Constant region: lower part of Y arm

- Fc region: binds to Fc receptors on cells

  or activate complement pathways



Cell lysis

Antibody classes

- classification based on dif in heavy chains
- 5 types of heavy chains .. 5 classes;

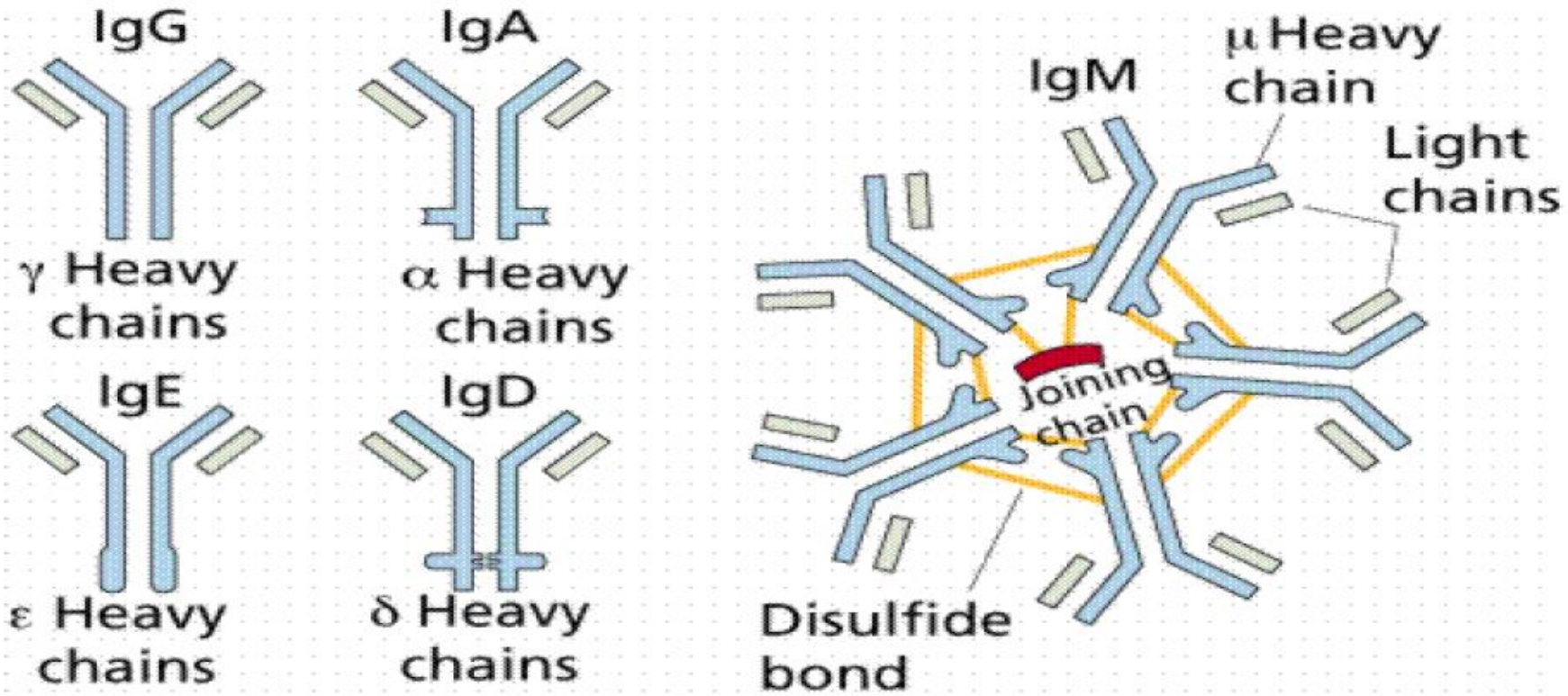
μ ; IgM	α ; IgA
γ ; IgG	ε ; IgE
δ ; IgD	

Antibody classes

- differ in their; distribution, physiochemical properties, concentration and function,

- within some classes there is also subclasses

Antibody classes



Antibody fragments

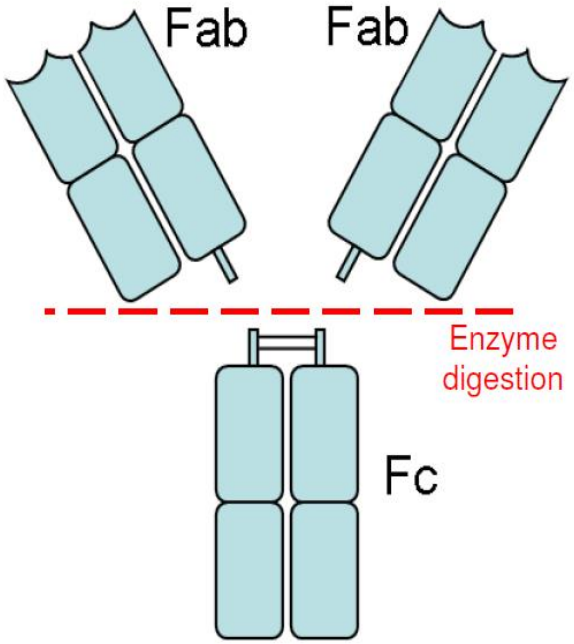
- Ab when treated with certain enzymes cleaved into;

  FC and Fab

- they reflect the 2 main roles of Ab;

  Fab: bind specifically to Ag

  Fc: stimulates its elimination



Antibodies in the Blood Bank

- the most imp classes; IgG and IgM

- they can destroy transfused Ag-positive RBC, cause anaemia and transfusion reaction

IgM antibodies

- form most of anti-A and anti-B antibodies

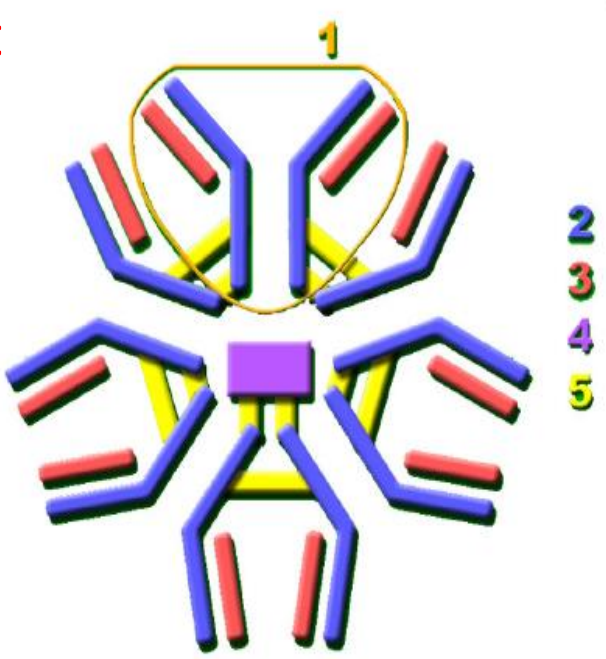
- IgM Ab against A/B blood gp are Naturally occurring

  few months after birth

- probably as a result of exposure to ABH Ag-like substance in the diet or environment

IgM antibodies

- very effective in destroying Ag bec can fix complement
- pentamer molecule (5 Ig unites)
- so capable of binding up to 10 epitopes ??
- Pentavalent Ab .. Why ?

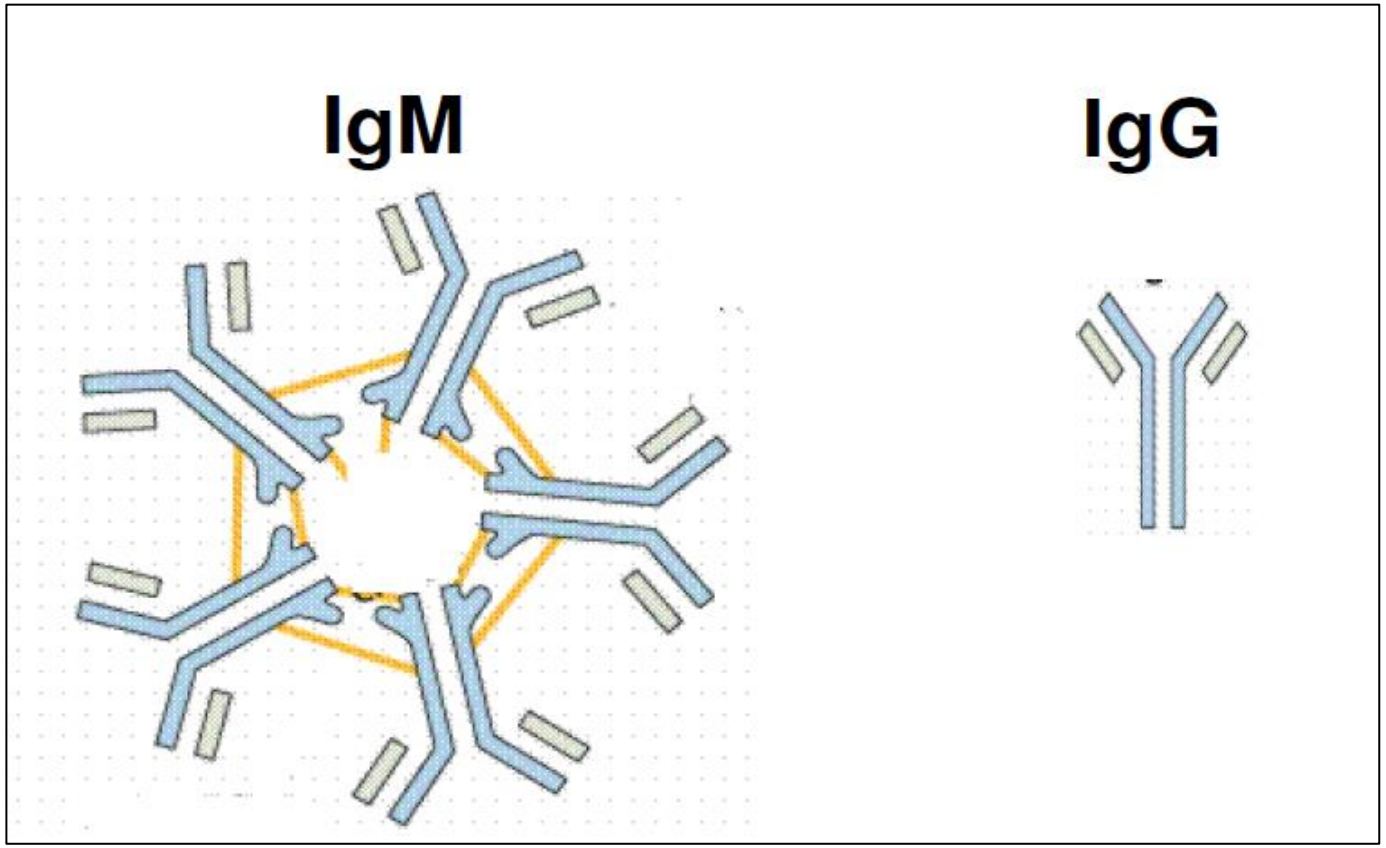


IgG antibody

- known as immune Ab
- formed in response to transfusion with non-self (incompatible) RBC
- monomer
- can cross the placenta
- 4 sub-classes (IgG1-4)

IgM vs. IgG

	IgM	**IgG**
Type of heavy chain	Mu	Gamma
MW (kd)	900	150
Placental transfer	No	yes
Complement fixation	++++	+
Production	Natural	Immune
Temperature required	Cold Ab (22C)	Warm Ab (37C)



IgM vs. IgG

Effector roles of Ab

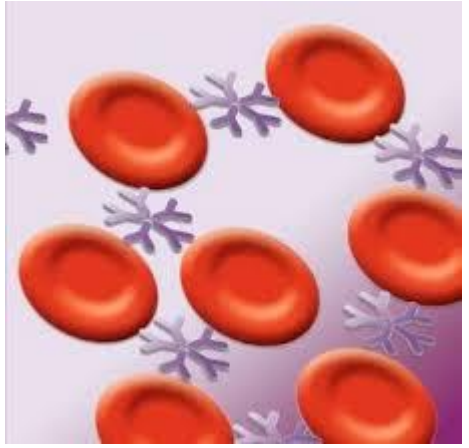
- Induction of physical changes
- Cell lysis
- Phagocytosis
- Inflammation

Effector roles of Ab

- Induction of physical changes :

Ag-Ab binding to the surface of the RBC result in their clumping together

or Agglutination



Cell lysis

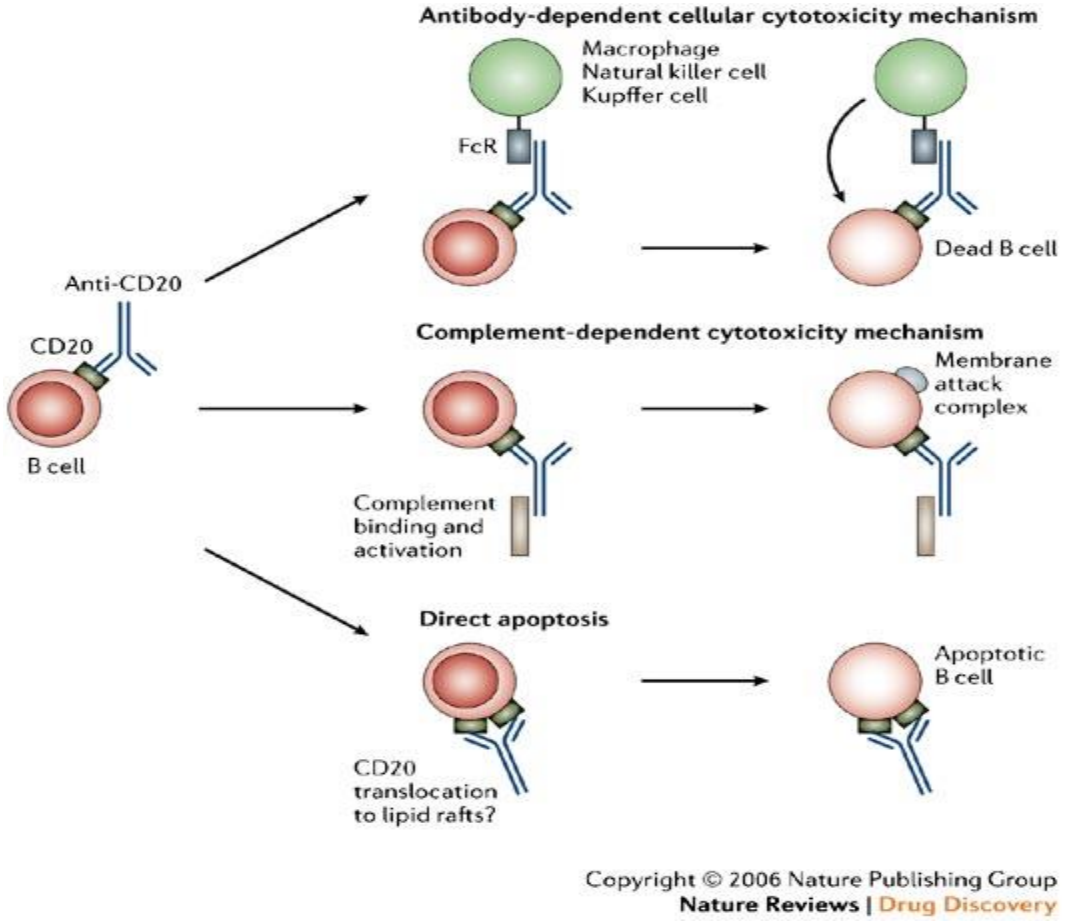
1. IgM and IgG activate complements → serious of reactions resulted in cell lysis (C3b)

2. ADCC; antibody-dependant-cellular-cytotoxicity **(only IgG)**

ADCC

- binding of FC region of IgG to certain receptor of lymphocytes
- activation of this lymphocytes results in releasing of its toxins
- toxins results in RBC lysis
- only mediated by IgG

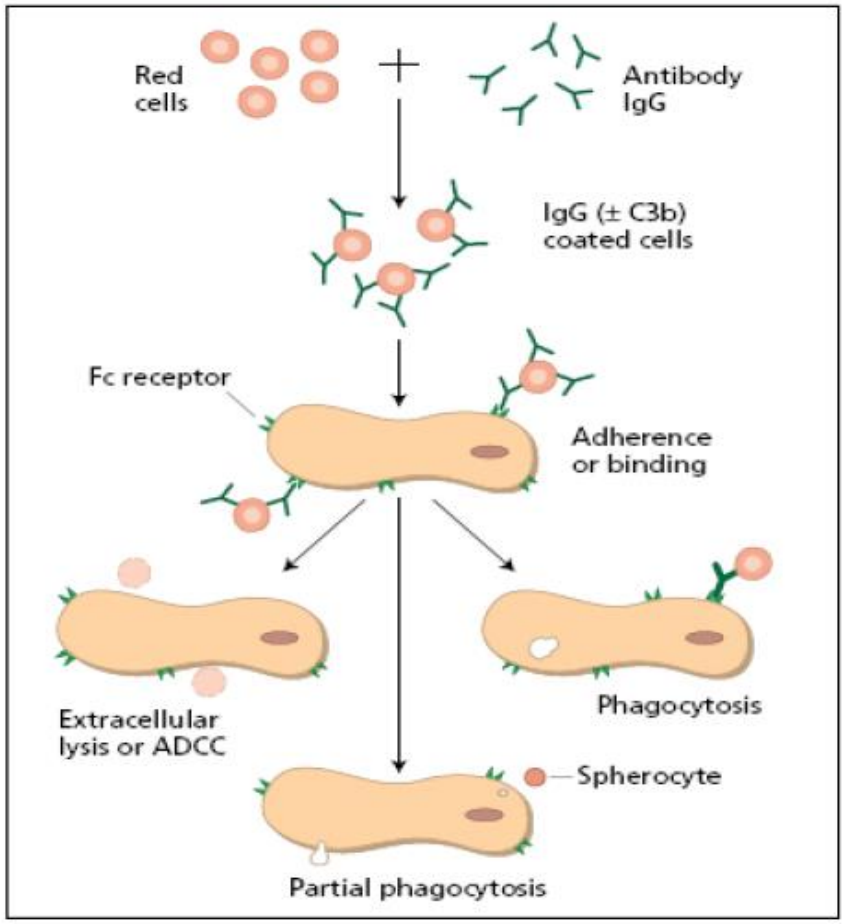
Consequences of RBC Ag-Ab reaction



Consequences of RBC Ag-Ab reaction

Agglutination

Lysis by phagocytosis



Ag-Ab reaction in the blood bank

- to determine blood gp
- Ab screening
- compatibility testing

Detected by haemolysis or agglutination

Haemagglutination

- the major technique used in blood bank

- heam: blood agglutination: clumping

  = red cell agglutination

haemagglutination

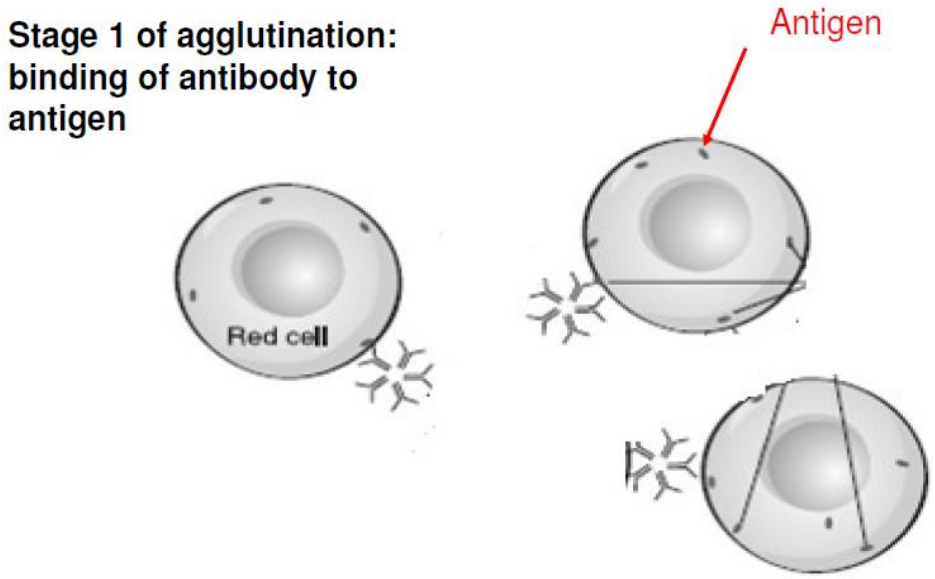
- Agglutination of RBC by Ab occur in 2 steps:

1 - sensitization

2 - agglutination

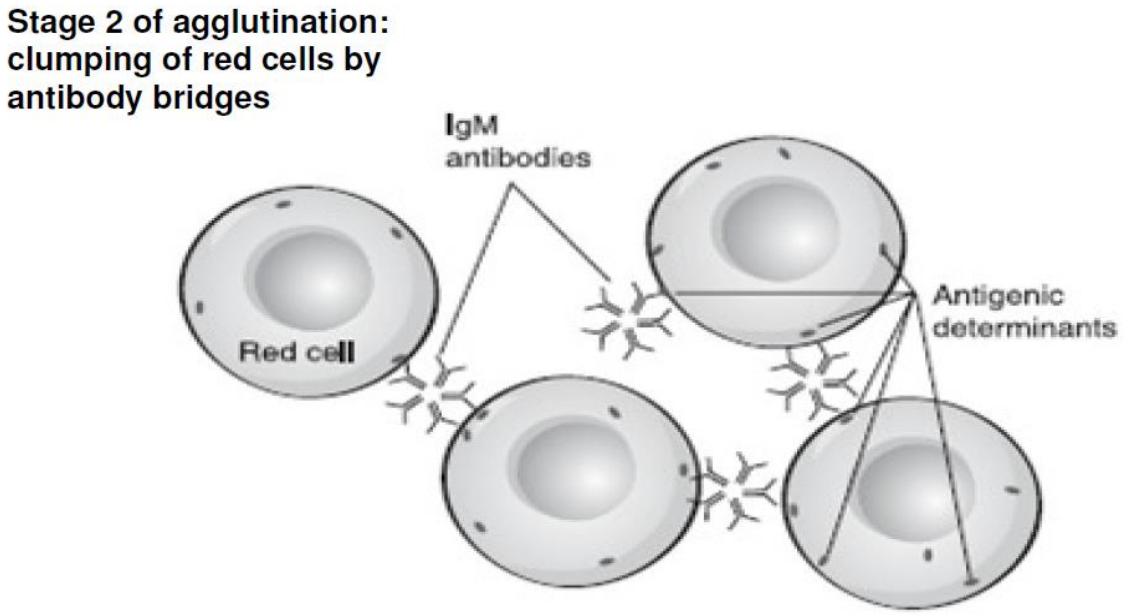
Sensitization

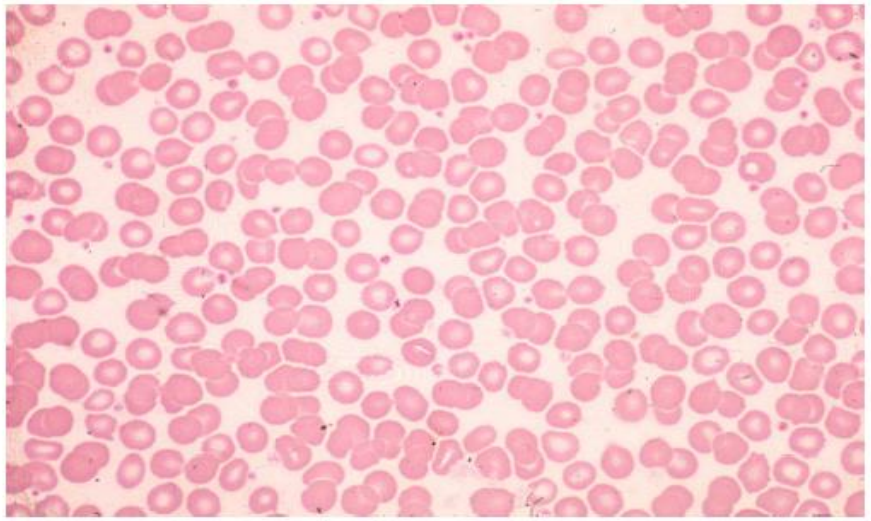
- first stage of agglutination; Ab bind to Ag



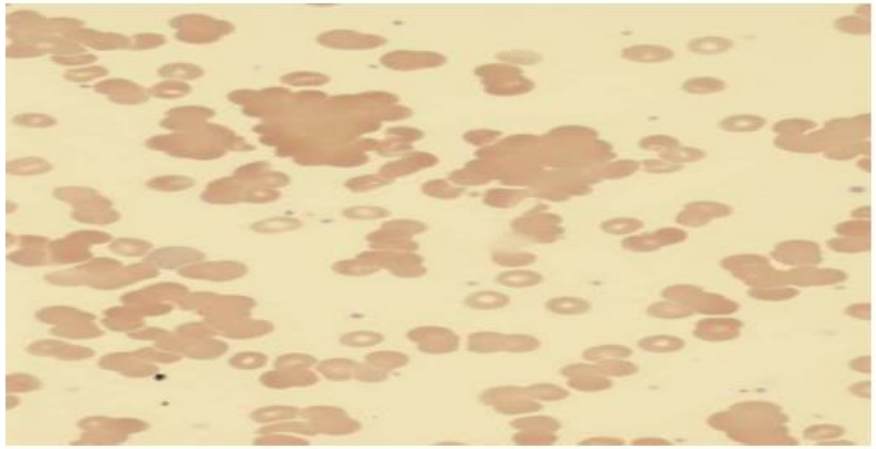
Agglutination

- when sensitized cells clump together resulting in the formation of visible agglutination





No agglutination



agglutination

Complete/incomplete Ab

- only IgM can bring about the 2 stages of Agglutination so the reaction is visible (Complete Ab)

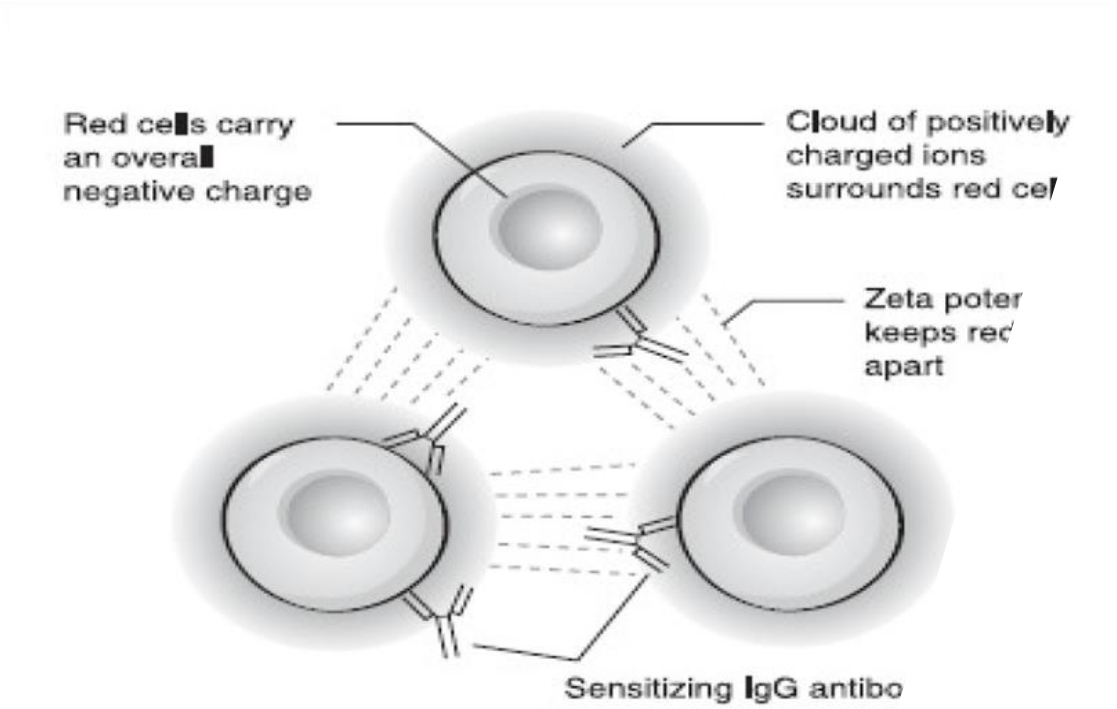
- IgG can bring sensitisation to RBC but not Agglutination (incomplete Ab)

Zeta potential

- RBC carry –ve charge on their surface resulted in repulsive force (Zeta potential)
- cause RBC to stay apart (imp in vivo)
- in vitro we need to ↓ zeta potential to facilitate Agglutination

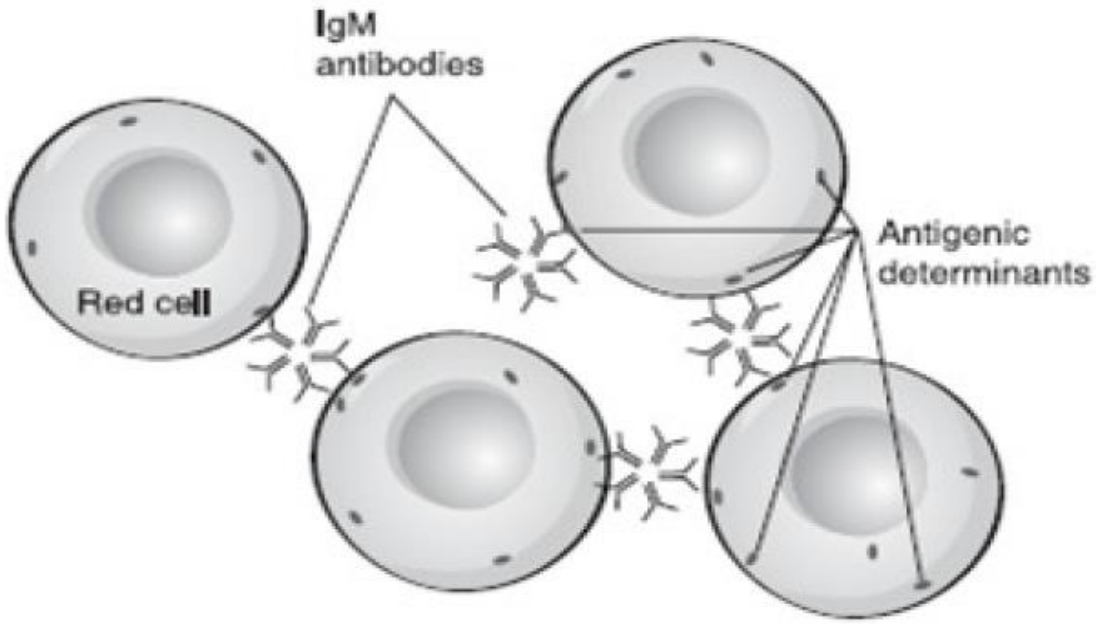
Zeta potential

IgG can not form bridge bec it is small in size

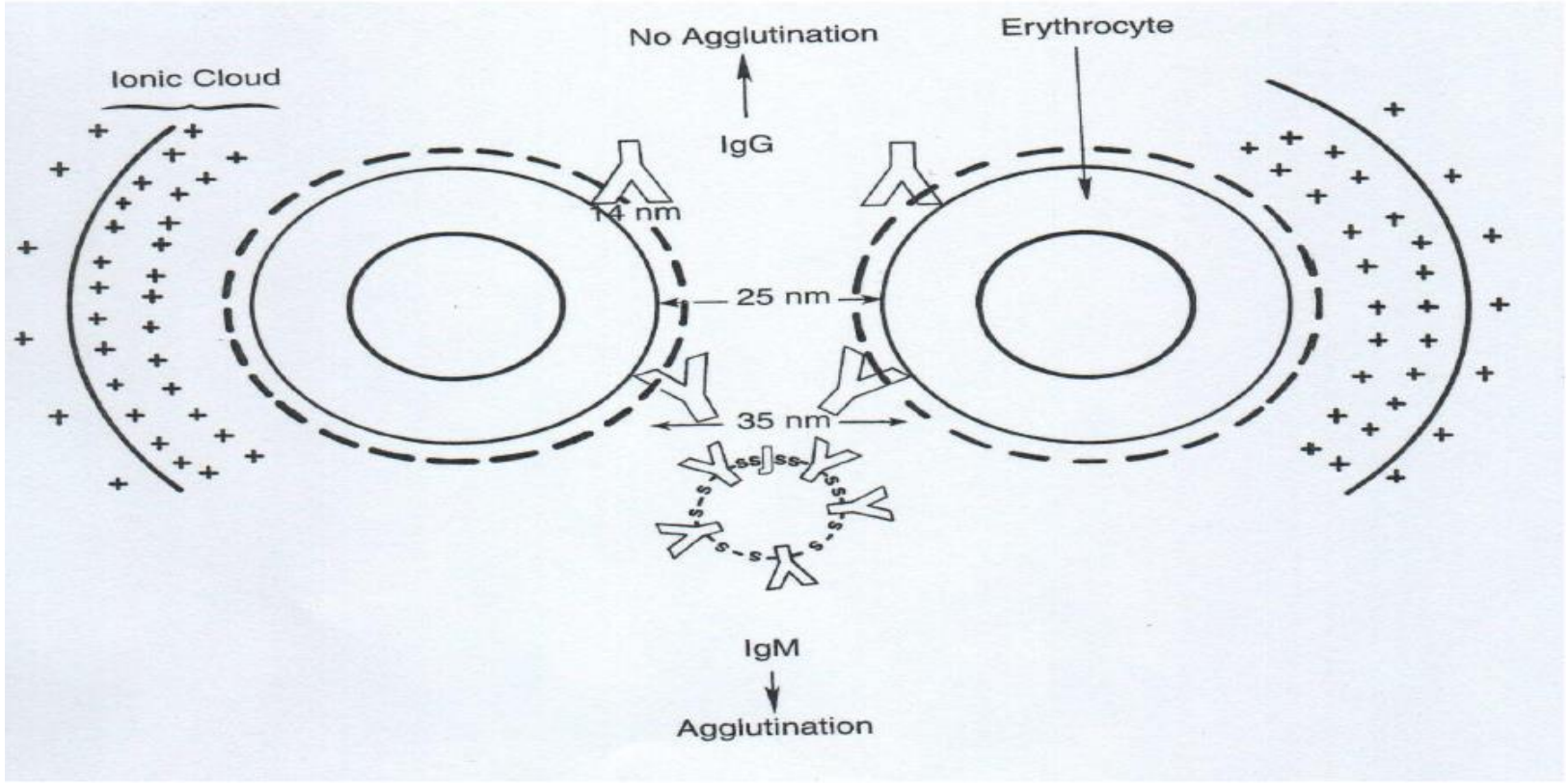


Zeta potential

IgM can form bridges despite zeta potential bec its larger size



Zeta potential

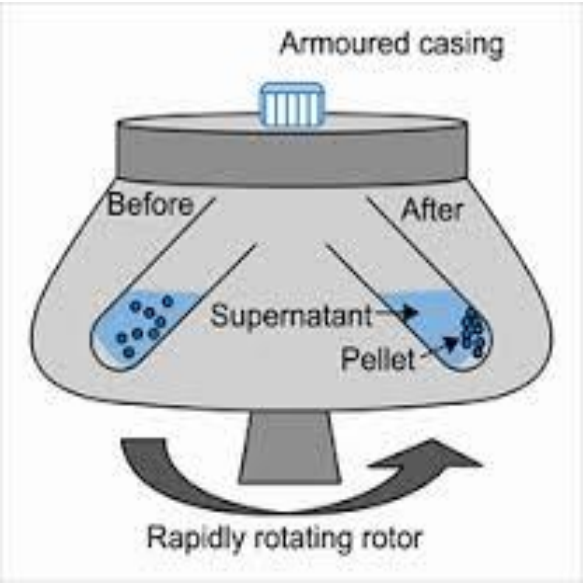


Factors influence agglutination reaction

- To help IgG in accomplishing the second stage of agglutination
- To enhance IgG and IgM agglutination

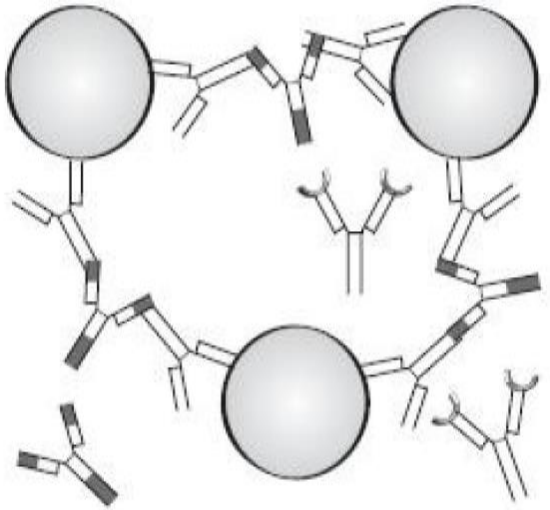
Centrifugation

- ↓ reaction timing by bringing reactants together
- allow RBC to overcome repulsive force
- places Ag and Ab in close proximity

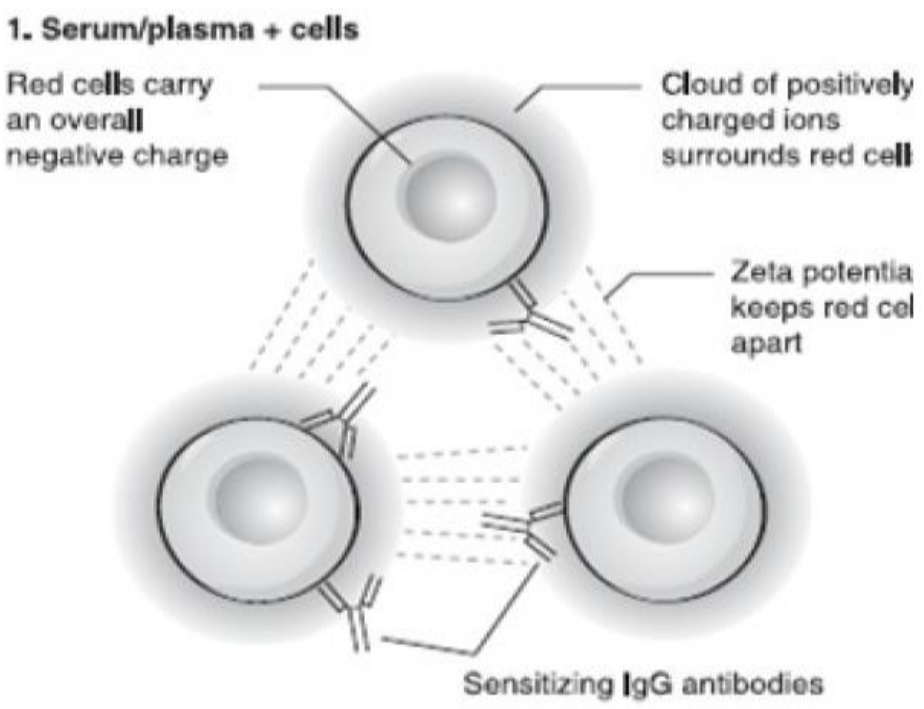


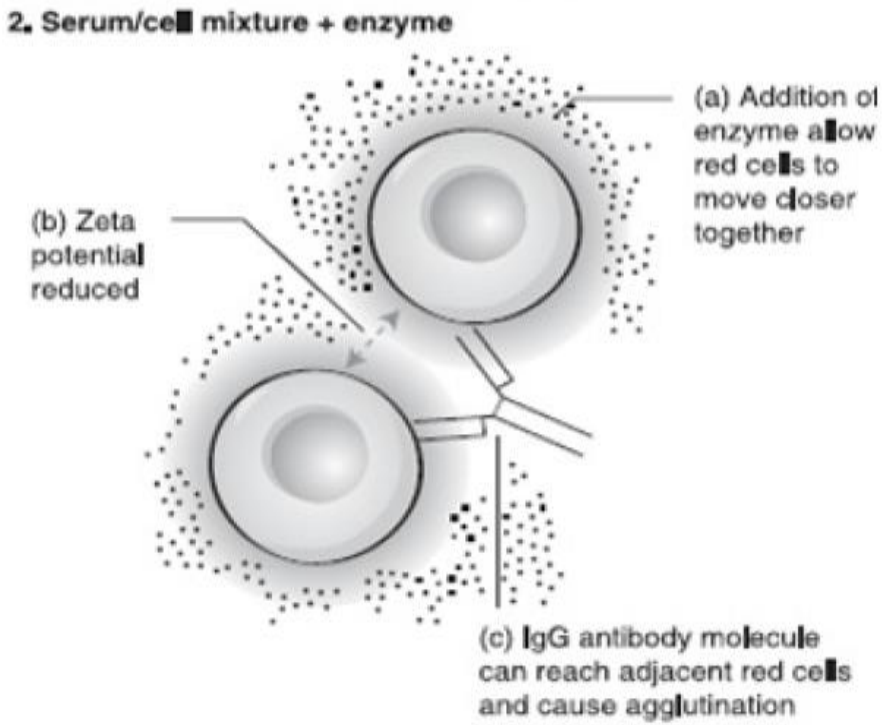
Enhancement media

- required for IgG but not necessary for IgM
- increase reactivity of IgG by reducing zeta potential
- eg; albumin, low ionic strength solution (liss)
  polyethylene glycol, proteolytic enzymes and
  anti-human globulin reagent (AHG)



Addition of enzymes





Other factors

- concentration of the reaction
- pH
- Temperature
- ionic strength
- Ab isotype
- RBC Ag dosage

Effect of pH

The ideal pH range is 6.5-7.5 similar of the pH of normal plasma or serum

Temperature

- dif Ab classes exhibit optimal reactivity at dif Tem.
- IgM react best at > 22C (cold Ab)
- IgG usually require 37C (warm Ab)

Detection of Ag-Ab reaction

- obtaining the right sample (most imp)

- dif tests may require dif sample types
- eg; measurement of complement fixation require serum sample
- complement activity requires presence of divalent ions Ca+2, Mg+2 which are chelated by some anticoagulants

Take home messages

- ✓ 5 classes of Abs (at least 2 Paratobes in each) differ in their heavy chain
- ✓ Light and heavy chains linked together by Covalent disulphide
- ✓ functions of variable, constant;
  Fab: bind specifically to Ag
  Fc: stimulates its elimination
- ✓ Main differences bet. IgM and IgG
  IgM; naturally, bind to up to 5 Ag, cold can fix complement and reach the 2nd step of agg
  IgG; immune, only 2 Ag, warm Ab, cant reach the 2nd stage of Agg by it self
- ✓ 2 steps of agglutination; sensitization and agglutination
- ✓ Complete/ incomplete Abs ?
- ✓ Factors influence agglutinations;
  centrifugation, Liss, AHG, enzymes …etc

Thanks for your attention ..

And happy to answer your questions ???