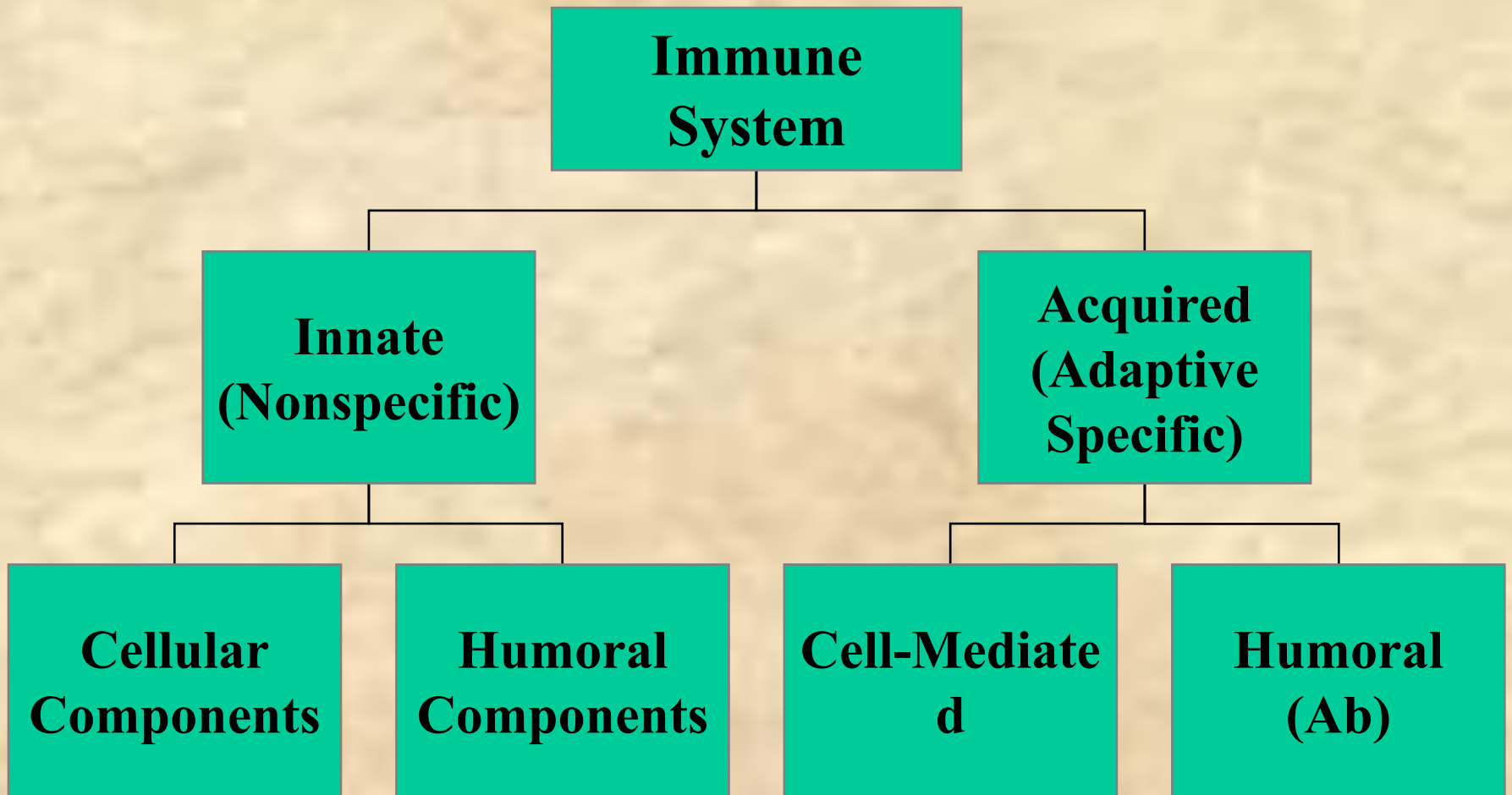


Overview of the Immune System

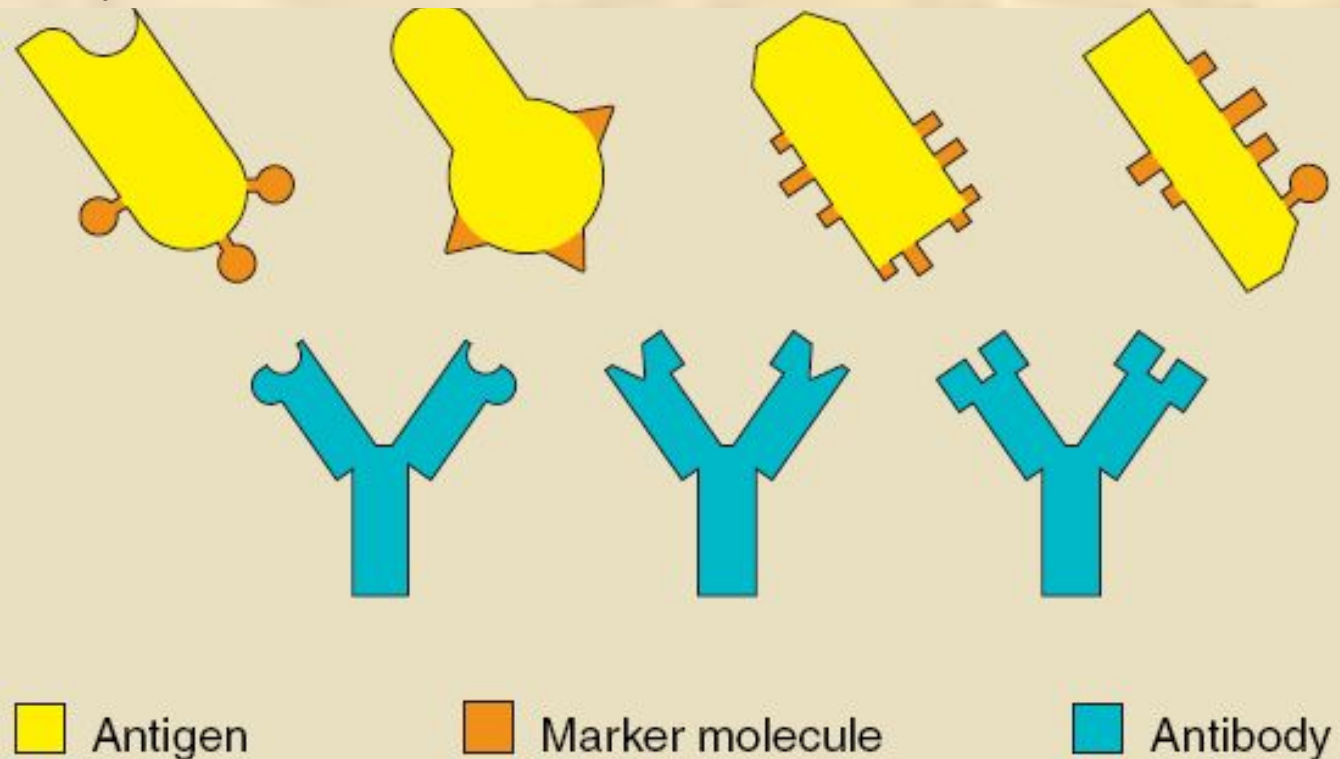


Acquired immunity

The immunity acquired during the lifetime of an individual is known as acquired immunity. Acquired immunity differs from innate immunity in the following respects:

- 1. It is not inherent in the body but is acquired during life.**
- 2. It is specific for a single type of microorganism.**

- **ANTIGEN** is a substance which, when introduced parenterally into the body, stimulates the production of an antibody with which it reacts specifically in an observable manner.
- The main property of the antigen is *immunogenicity*.



Factors Influencing Immunogenicity

- ***Foreignness*** - only antigens which are foreign to the individual induce an immune response
 - ***Size*** - usually antigens have a molecular weight of 10,000 or more (20-30 kD).
 - ***Susceptibility to tissue enzymes:*** Only substances which are metabolized and are susceptible to the action of tissue enzymes behave like antigen.
 - ***Chemical Composition***
 - Primary Structure
 - Secondary Structure
 - Tertiary Structure
 - Quarternary Structure
- Sequence determinants
- Conformational determinants
-
- ```
graph LR; A[Primary Structure] --- B[Sequence determinants]; B --- C[Secondary Structure]; C --- D[Conformational determinants]; D --- E[Tertiary Structure]; E --- F[Quarternary Structure];
```

# Chemical Nature of Immunogens

- Proteins
- Polysaccharides
- Nucleic Acids
- Lipids
  - Some glycolipids and phospholipids can be immunogenic for T cells

# Types of Antigen

- On the basis of capability of antibody formation antigens are classified as under:
  - *T-cell independent antigens*
  - *T-cell dependent antigens*

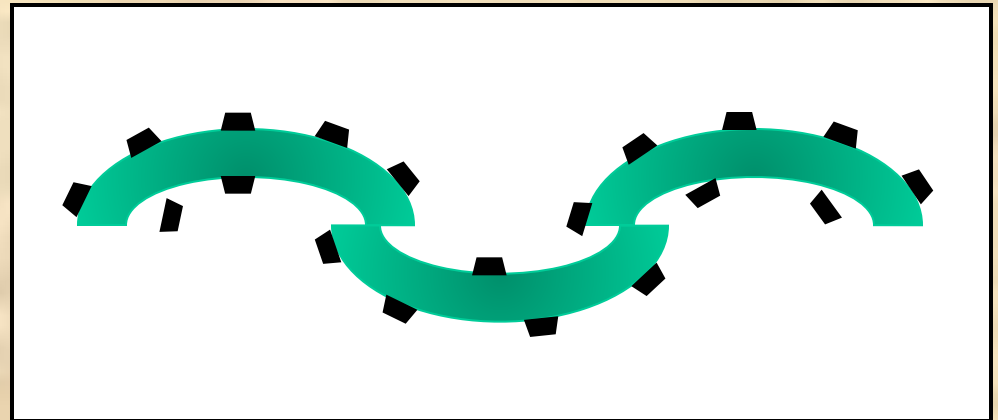
# Types of Antigens

## T-independent

- **Polysaccharides**

- **Properties**

- Polymeric structure
- Polyclonal B cell activation
- Resistance to degradation



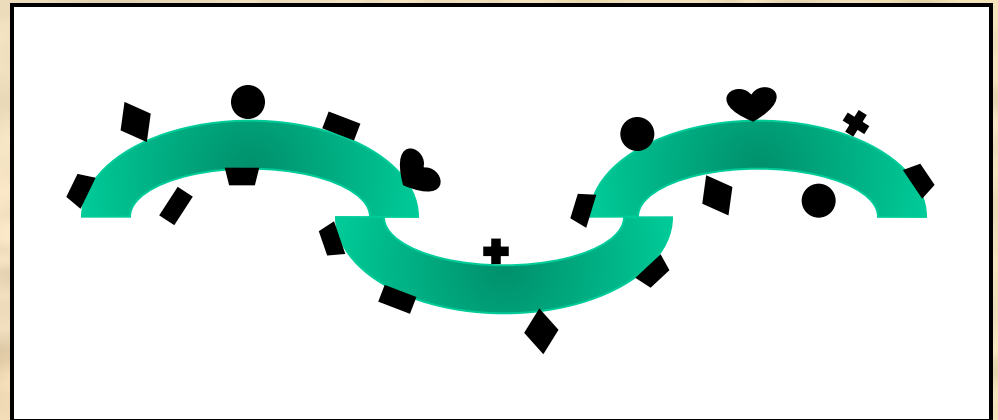
- **Examples**

- Pneumococcal polysaccharide, Lipopolysaccharide
- Flagella

# Types of Antigens

## T-dependent

- **Proteins**
- **Structure**
- **Examples**
  - **Microbial proteins**
  - **Non-self or Altered-self proteins**





# Types of Antigens

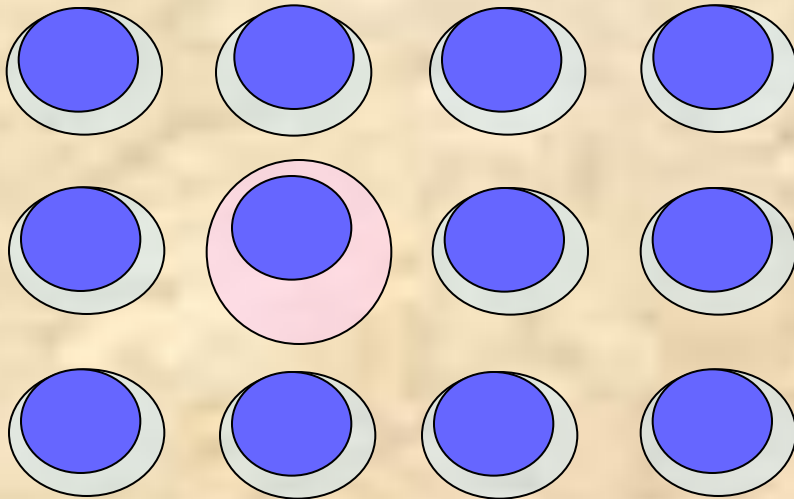
- Depending on the size and capacity to induce antibody production antigens can be divided into:
- **Complete antigen**. It is able to induce antibody formation and produce a specific and observable reaction with the antibody so produced, e.g. proteins.
- **Partial antigen** (also called *hapten*): *Haptens* are substances which are unable to induce antibody production by themselves, but are able to react specifically with antibodies, e.g. lipids, nucleic acid, sulfonamide, penicillin, etc.

# Superantigens

- **Definition** – they can induce very powerful immune response (examples - Staphylococcal enterotoxins, Streptococcal pyrogenic exotoxins)

## Conventional Antigen

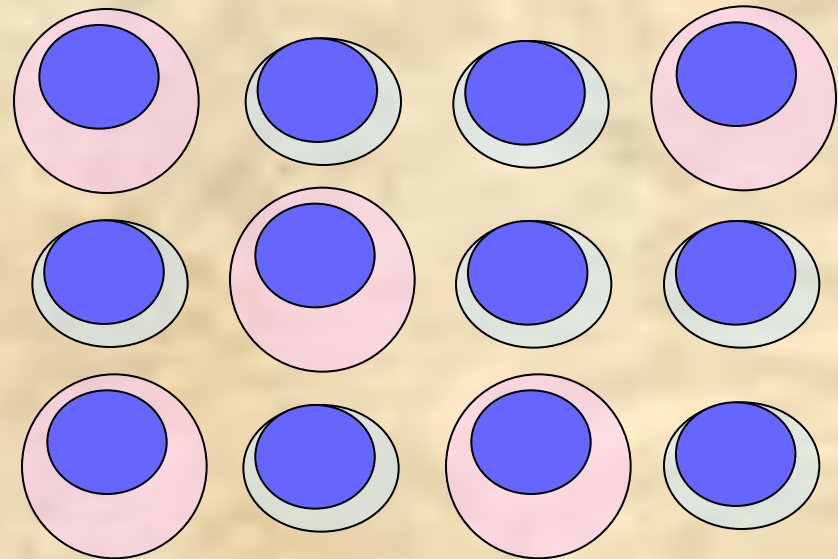
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**Monoclonal/Oligoclonal  
T cell response**

## Superantigen

---

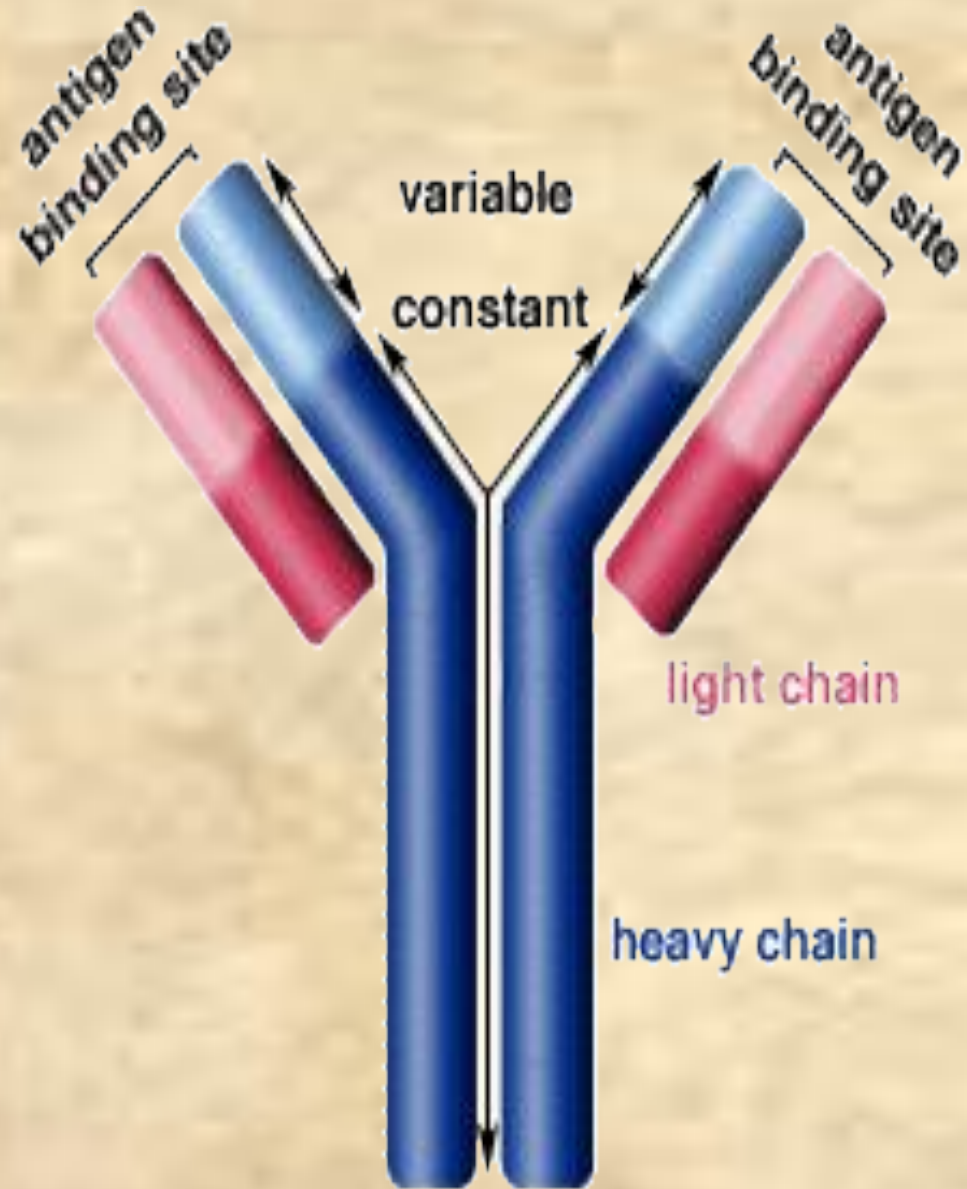


**Polyclonal T cell response**

**Antibody** is humoral substance ( $\gamma$ -globulin) produced in response to an antigenic stimulus.

**Antibodies are:**

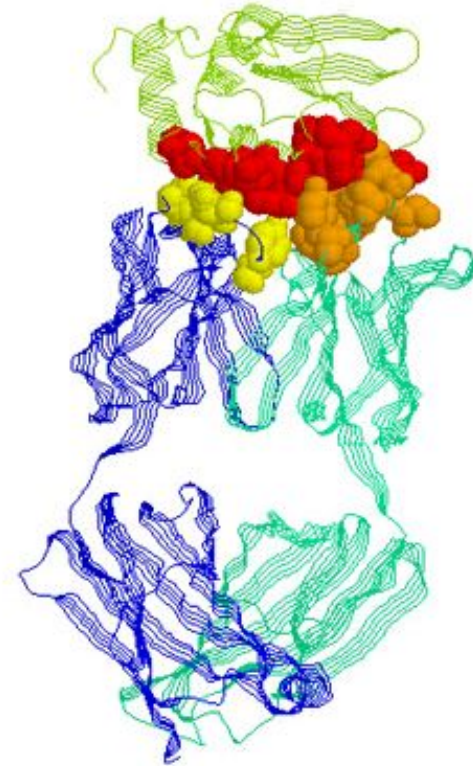
- **Protein in nature.**
- **Formed in response to antigenic stimulation.**
- **React with corresponding antigen in a specific and observable manner.**



# Antibodies

- Immunoglobulins are synthesized by plasma cells.
- Immunoglobulins make 20-25% of the total serum proteins.
- Five groups of immunoglobulins have been distinguished: *IgG*, *IgA*, *IgM*, *IgD* and *IgE*.

<http://www.med.sc.edu:85/chime2/lyso-abfr.htm>

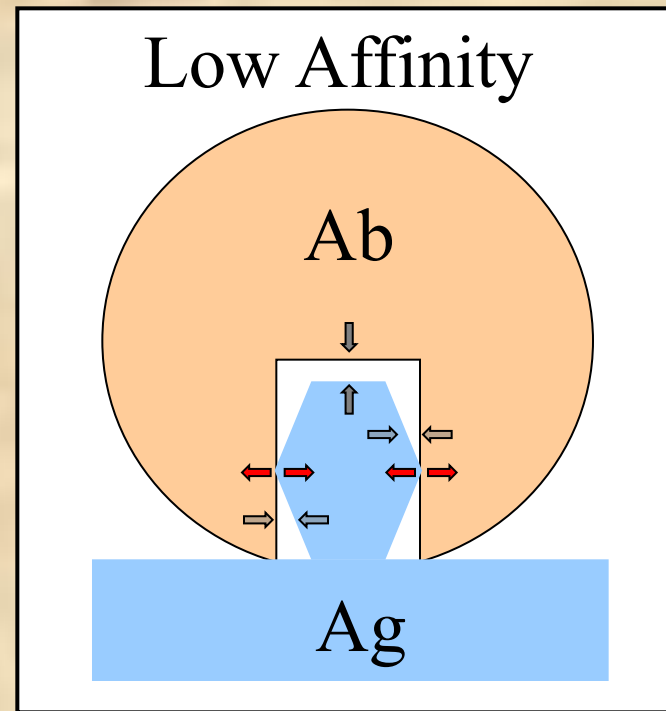
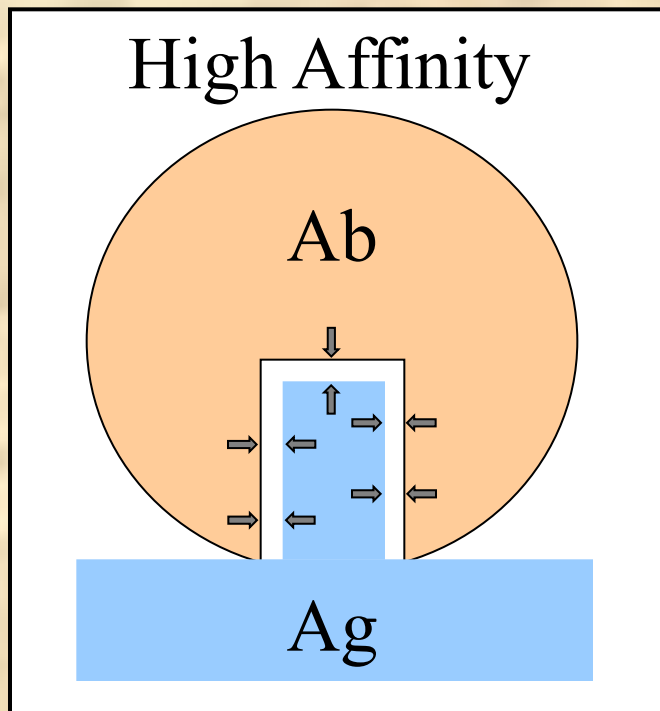


MDL

Source: Li, Y., Li, H., Smith-Gill, S. J.,  
Mariuzza, R. A., Biochemistry 39, 6296, 2000

# Affinity

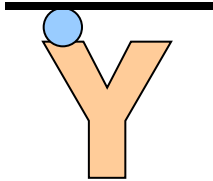
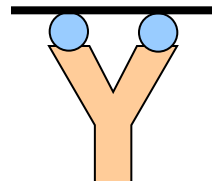
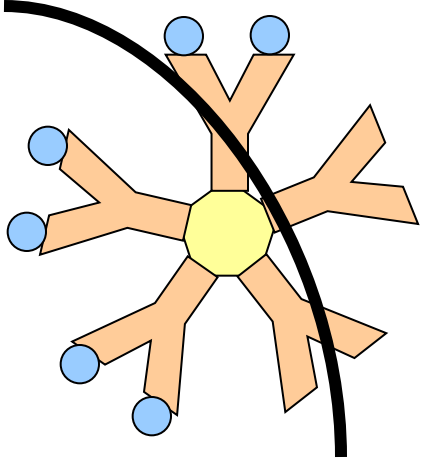
- Strength of the reaction between a single antigenic determinant and a single Ab combining site



*Affinity* =  $\square$  attractive and repulsive forces

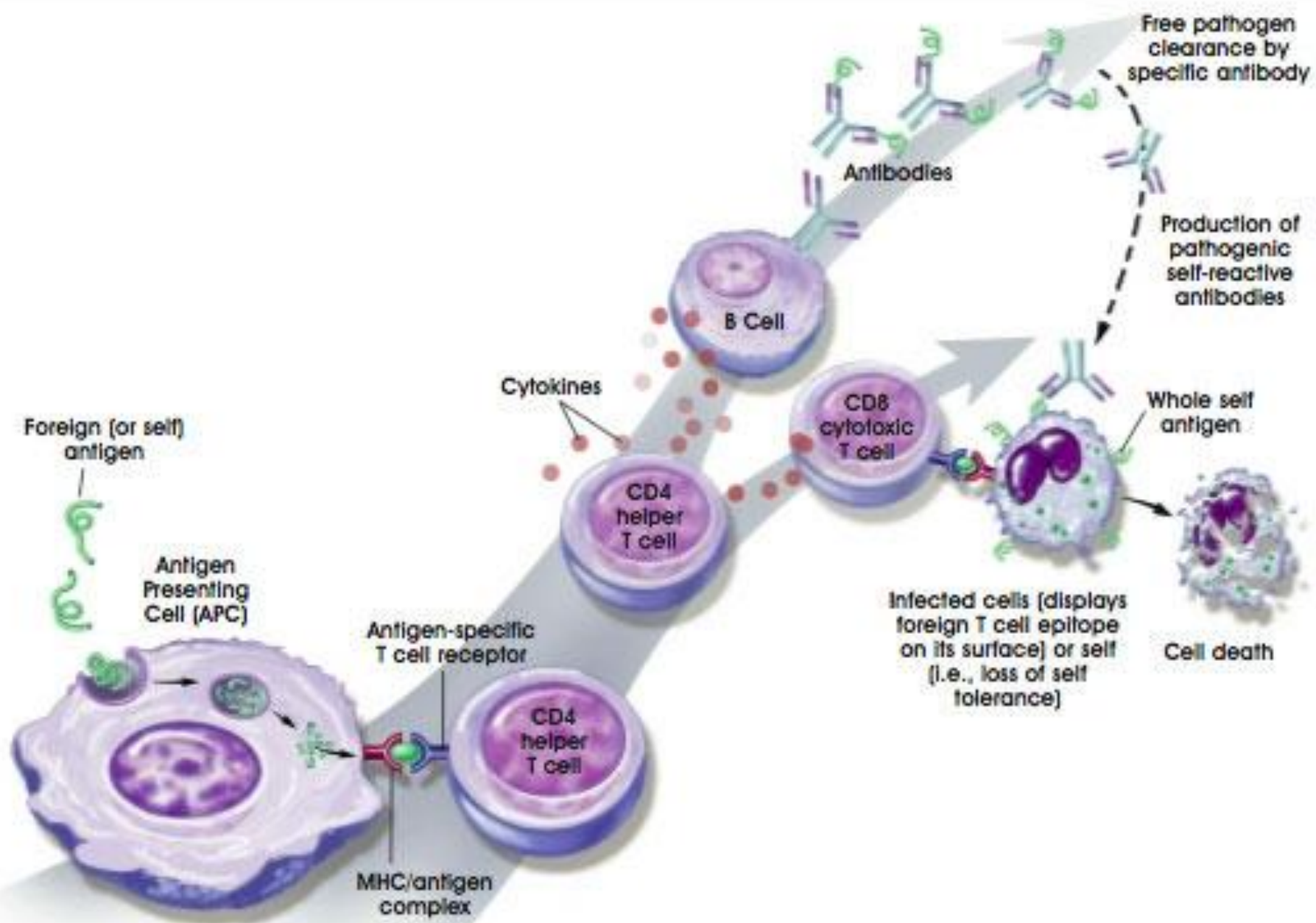
# Avidity

- The overall strength of binding between an Ag with many determinants and multivalent Abs

|                                                                                                                                  |                                                                                                                         |                                                                                                                              |
|----------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|
|  <p><math>K_{eq} = 10^4</math><br/>Affinity</p> |  <p><math>10^6</math><br/>Avidity</p> |  <p><math>10^{10}</math><br/>Avidity</p> |
|----------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|

# Specificity

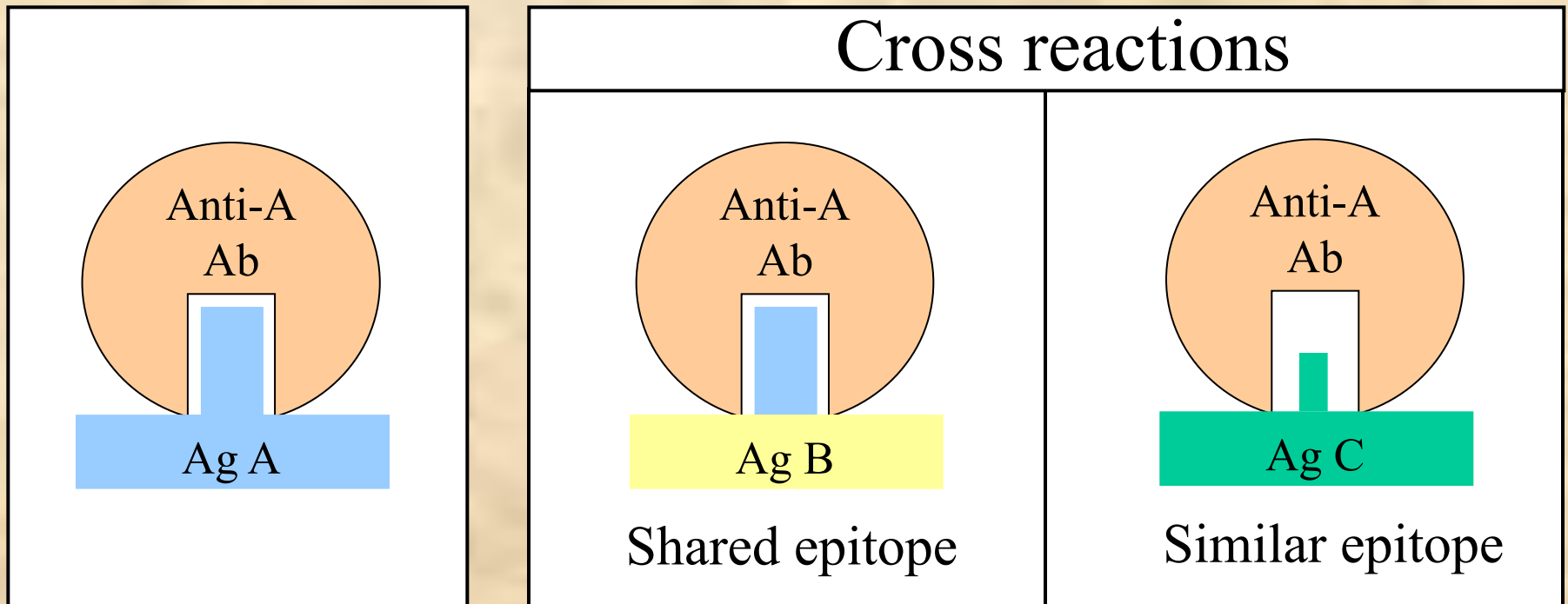
- The ability of an individual antibody combining site to react with only one antigenic determinant.
- The ability of a population of antibody molecules to react with only one antigen.





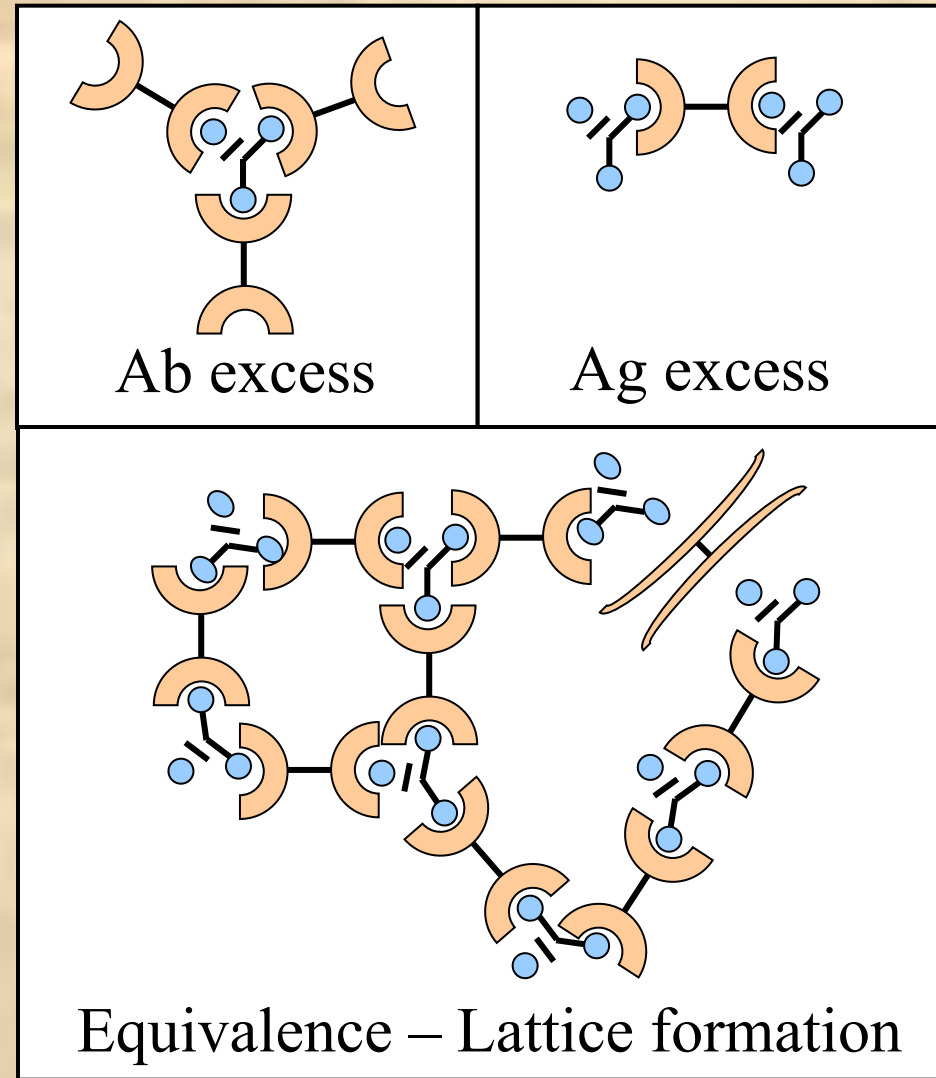
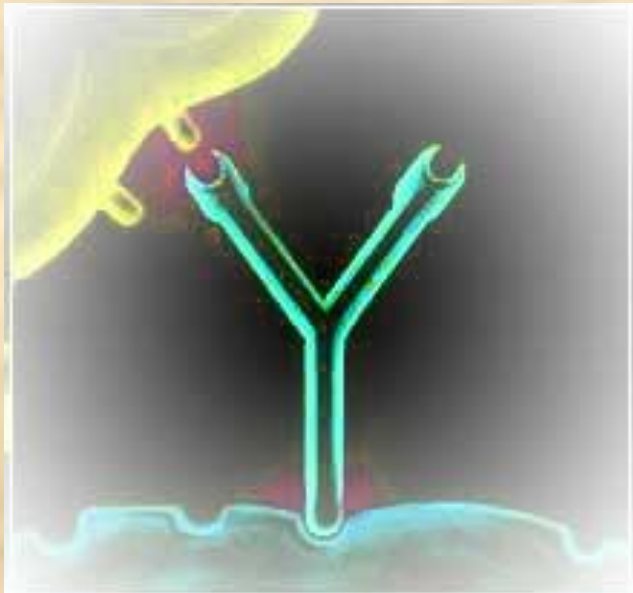
# Cross Reactivity

- The ability of an individual Ab combining site to react with more than one antigenic determinant.
- The ability of a population of Ab molecules to react with more than one Ag



# Factors Affecting Measurement of Ag/Ab Reactions

- Affinity
- Avidity
- Ag:Ab ratio
- Physical form of Ag

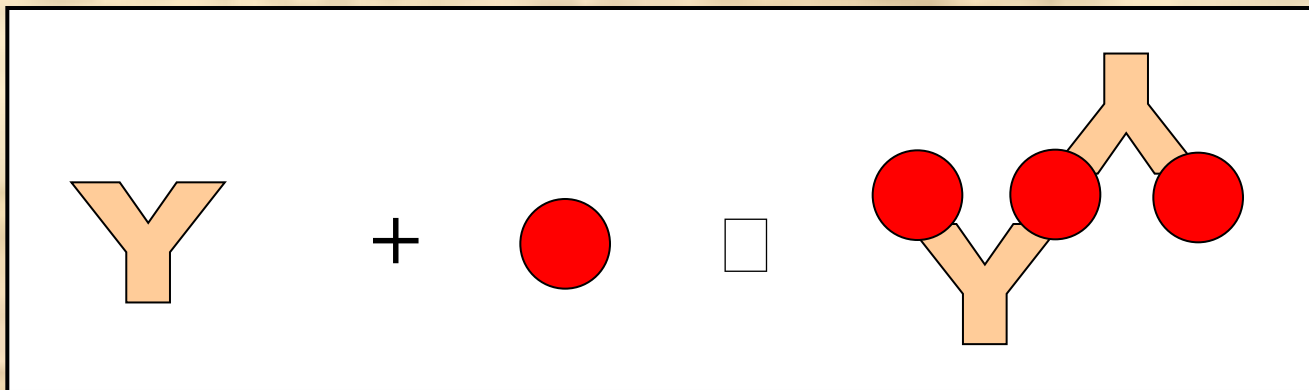


# Tests Based on Ag/Ab Reactions

- All tests based on Ag/Ab reactions will have to depend on lattice formation or they will have to utilize ways to detect small immune complexes
- All tests based on Ag/Ab reactions can be used to detect either Ag or Ab

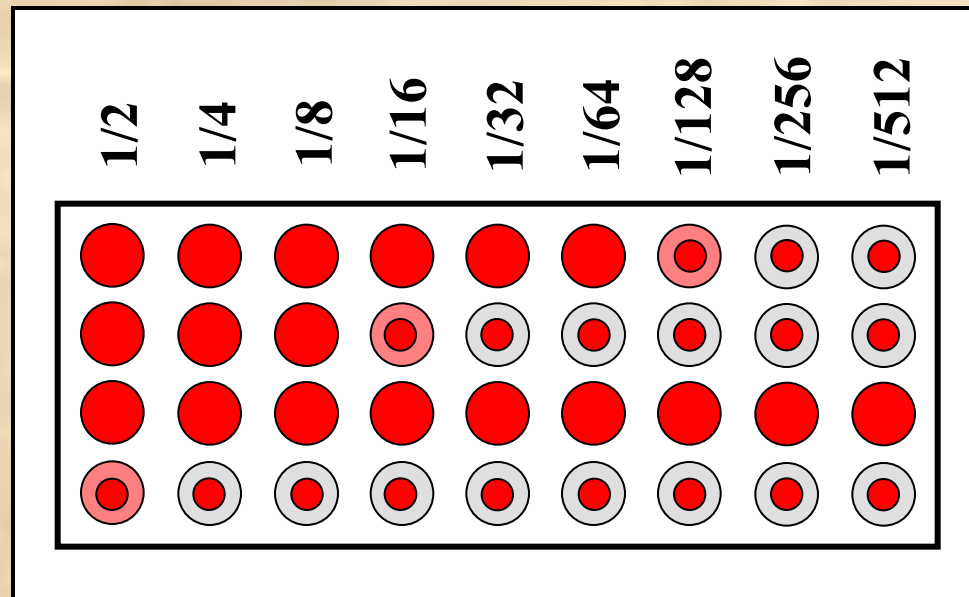
# Agglutination/Hemagglutination

- **Definition** - tests that have as their endpoint the agglutination of a particulate antigen
  - Agglutinin/hemagglutinin
- **Qualitative agglutination test**
  - Ag or Ab



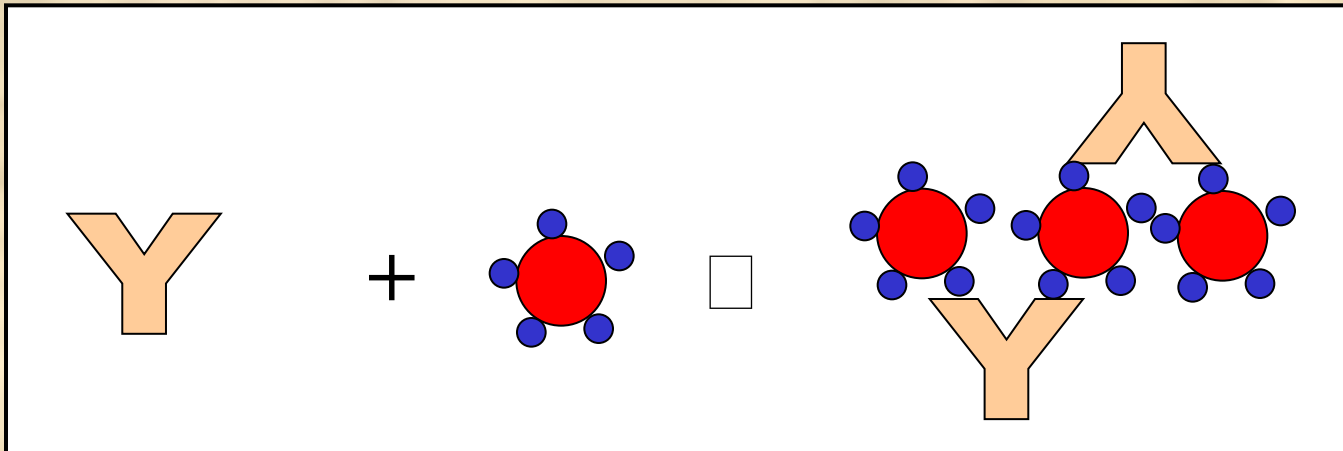
# Agglutination/Hemagglutination

- Definition
- Qualitative test
- Quantitative test
- Applications
  - Blood typing
  - Bacterial infections
    - Fourfold rise in titer
- Practical considerations
  - Easy
  - Semi-quantitative



# Passive Agglutination/Hemagglutination

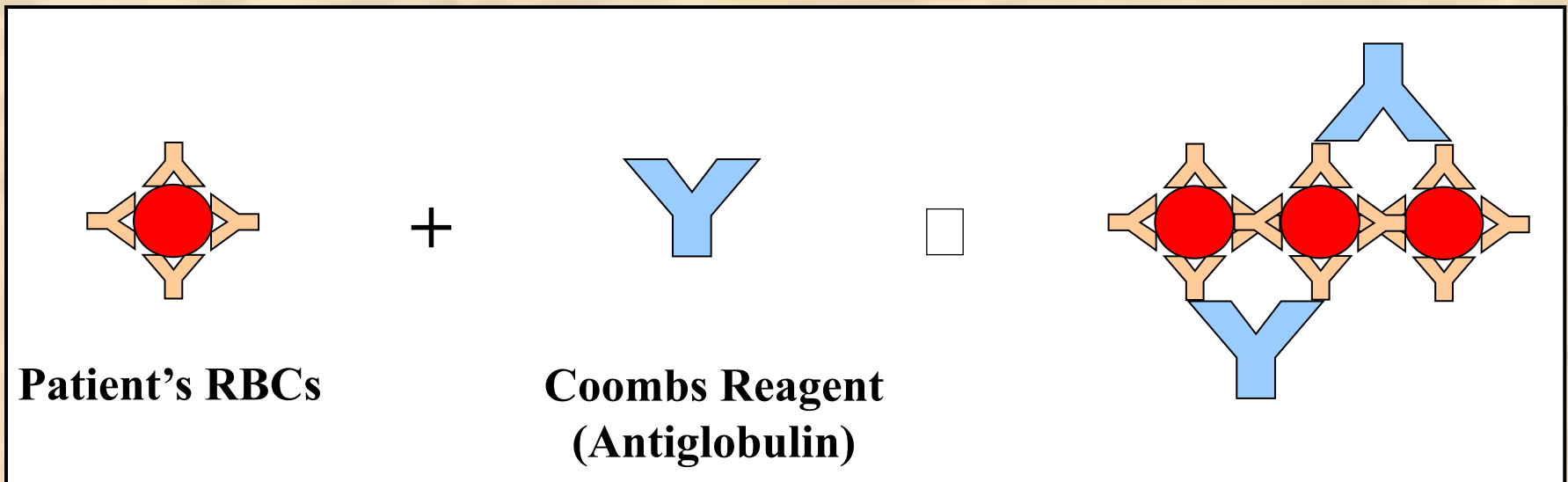
- **Definition** - agglutination test done with a soluble antigen coated onto a particle



- Applications
  - Measurement of antibodies to soluble antigens

# Coombs (Antiglobulin) Tests

- Incomplete Ab
- Direct Coombs Test
  - Detects antibodies on erythrocytes



**Applications:** *1. Detection of anti-Rh Ab*  
*2. Autoimmune hemolytic anemia*

# Coombs (Antiglobulin) Tests

- Indirect Coombs Test
  - Detects anti-erythrocyte antibodies in serum

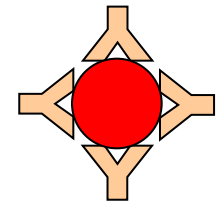
Step 1

  
Patient's  
Serum

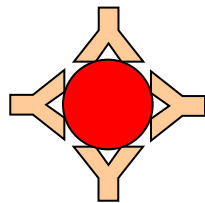
+

  
Target  
RBCs

□



Step 2

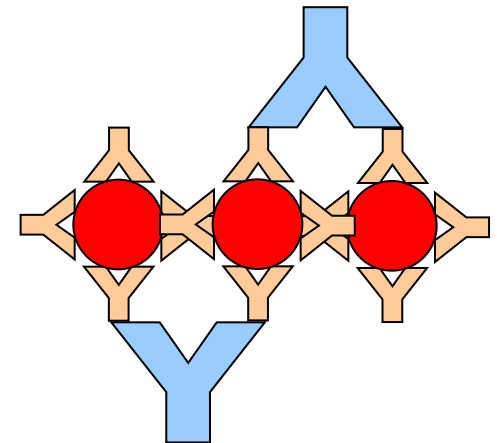


+



□

Coombs Reagent  
(Antiglobulin)

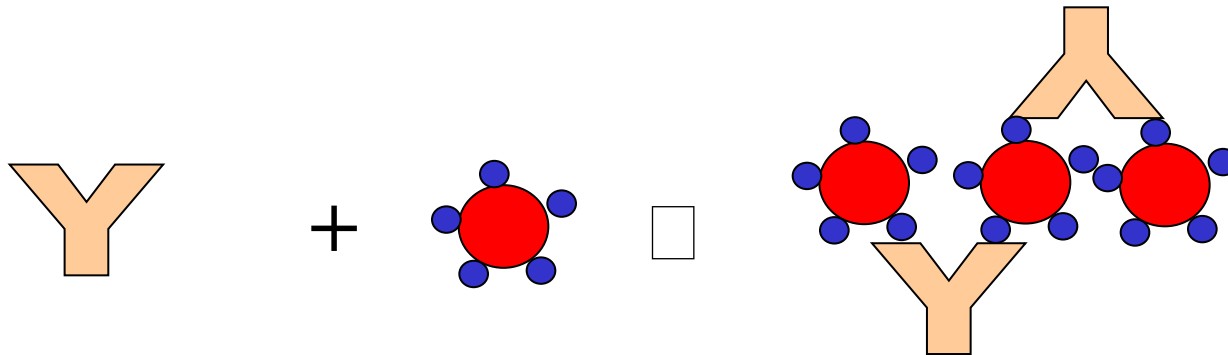




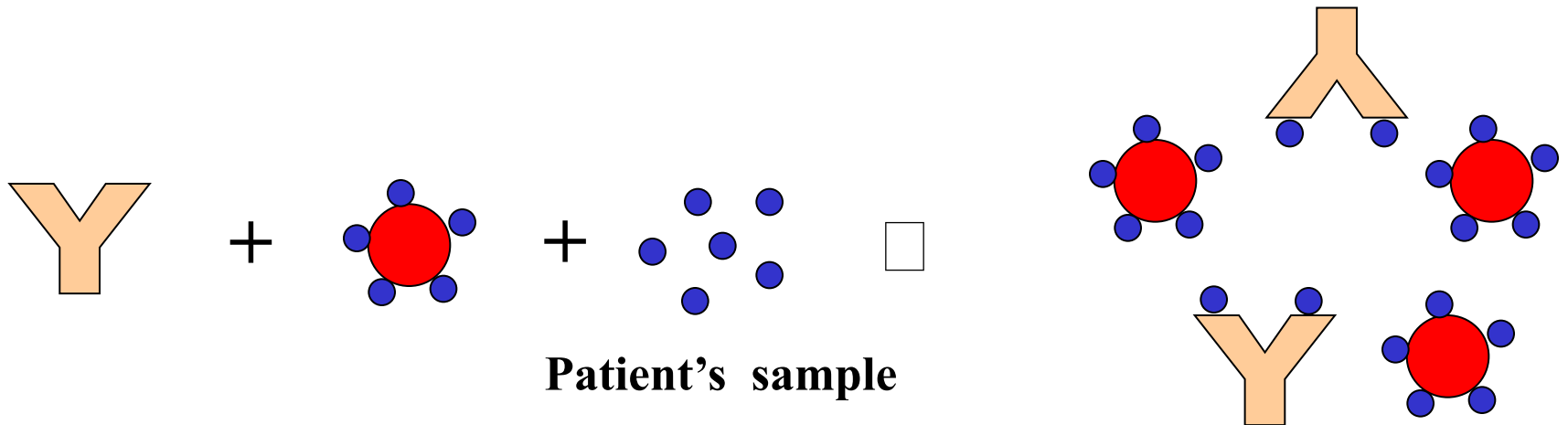
# Agglutination/Hemagglutination Inhibition

- **Definition** - test based on the inhibition of agglutination due to competition with a soluble Ag
- ***Applications:*** Measurement of soluble Ag

Prior to Test



Test



# Precipitation reactions

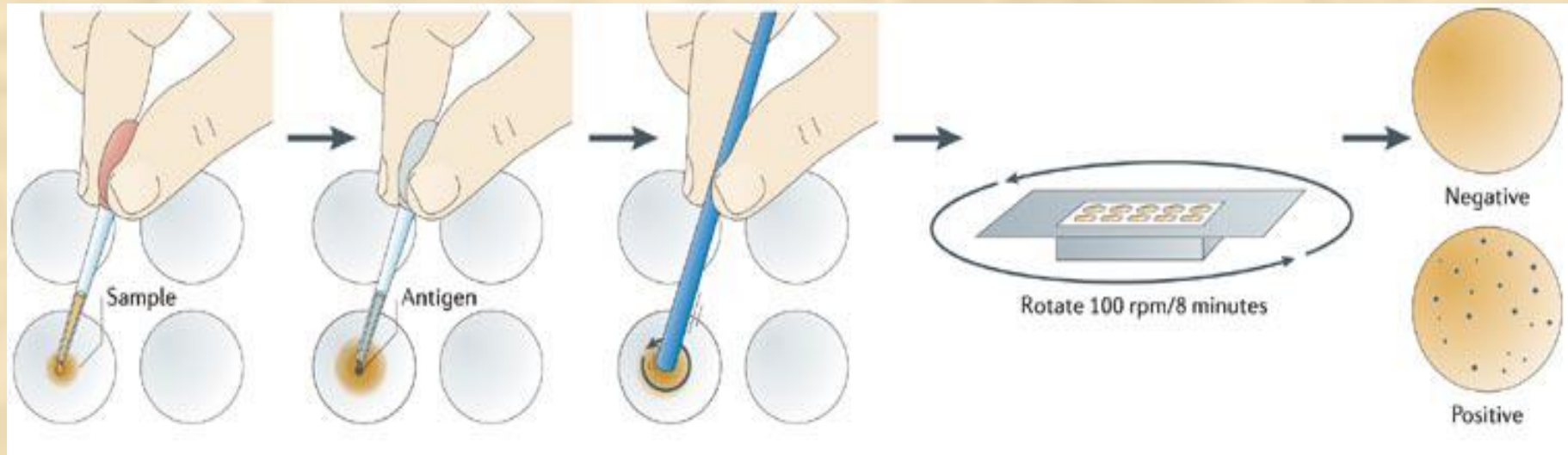
When a soluble antigen combines with its antibody in presence of electrolytes (NaCl) at a suitable temperature and pH the antigen antibody complex forms insoluble precipitate.

## *Uses of precipitation reaction*

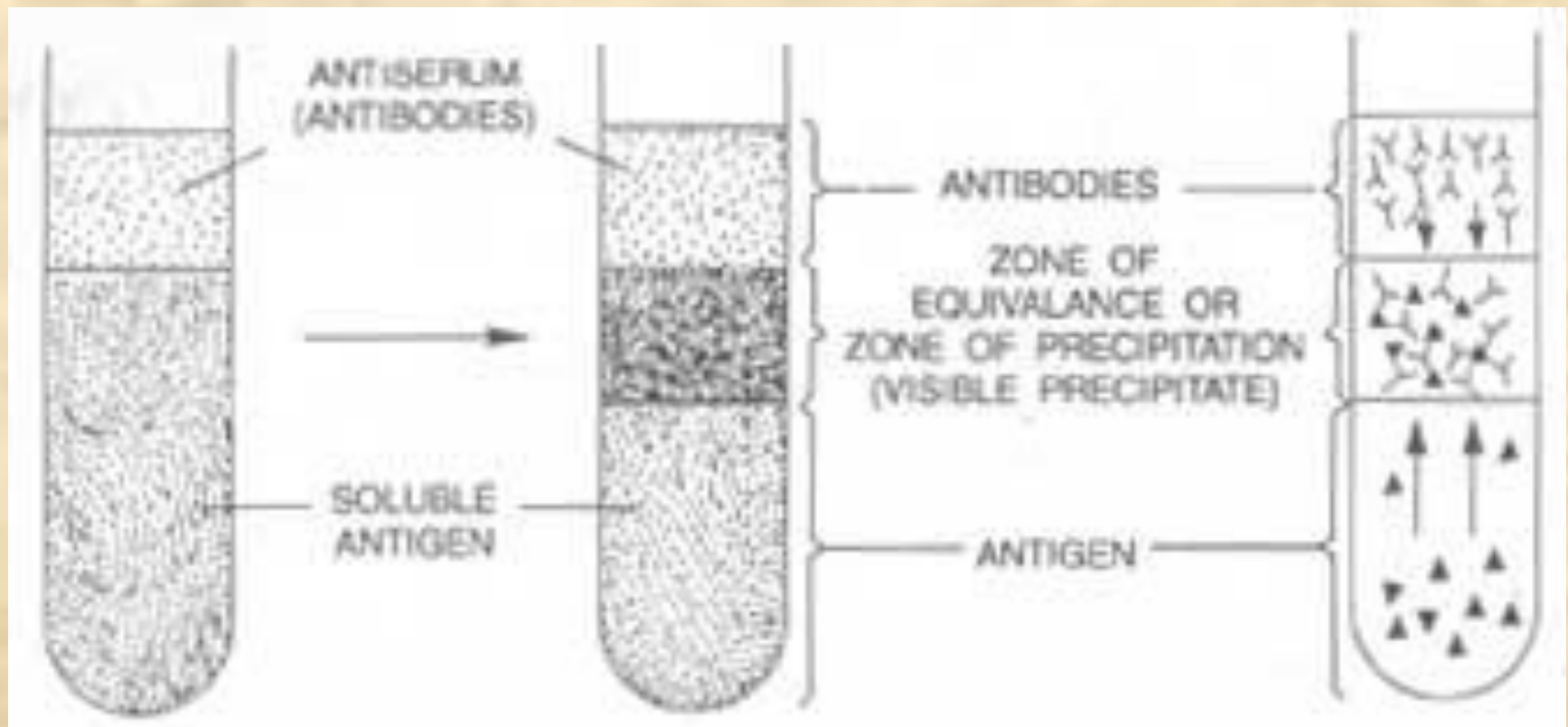
- Identification of bacteria
- Identification of antigenic component of bacteria in infected animal tissue (Ascoli test)
- Standardization of toxin and antitoxins
- Demonstration of antibody in serum
- Medicolegal serology for detection of blood, serum, etc.

# ***TECHNIQUES OF PRECIPITATION REACTION***

***Slide test.*** When a drop of antigen and antiserum is placed on a slide and mixed by shaking, floccules appear.

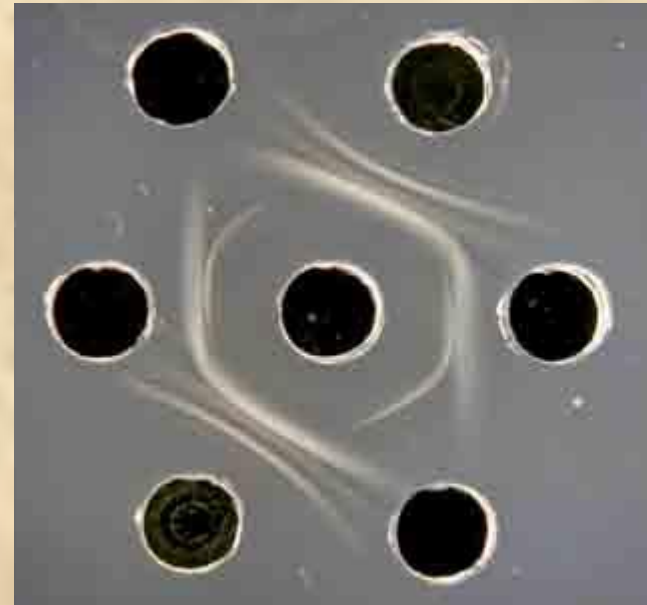


- **Ring test.** The antigen is layered over serum in a narrow tube. The reaction is visible as a white zone at the junction of two clear fluids.



# *TECHNIQUES OF PRECIPITATION REACTION*

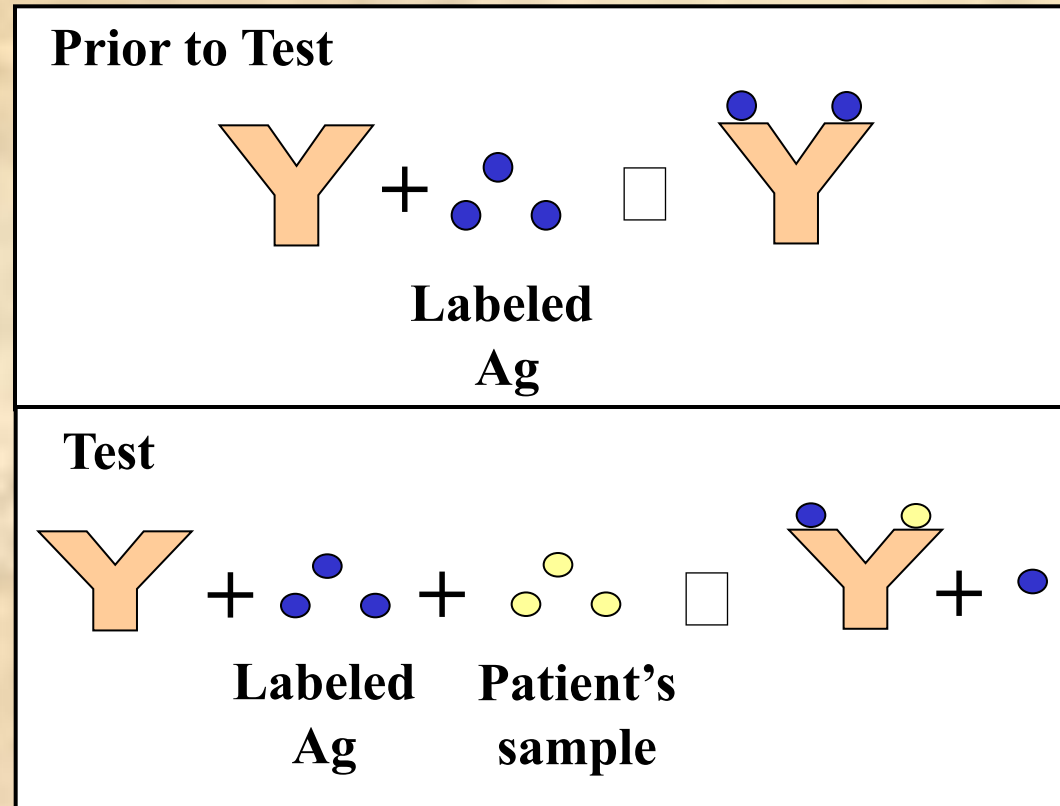
- ***Tube test.*** The Kahn test for syphilis is an example of tube flocculation test.
- ***Gel diffusion.*** The precipitate is relatively fixed by agar medium and is easily visible.



# Competitive RIA/ELISA for Ag

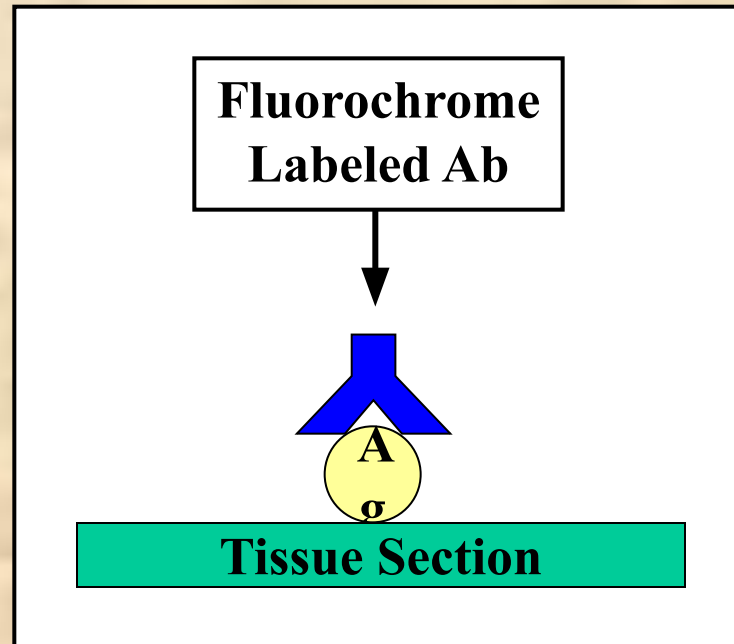
- Method

- Determine amount of Ab needed to bind to a known amount of labeled Ag
- Use predetermined amounts of labeled Ag and Ab and add a sample containing unlabeled Ag as a competitor



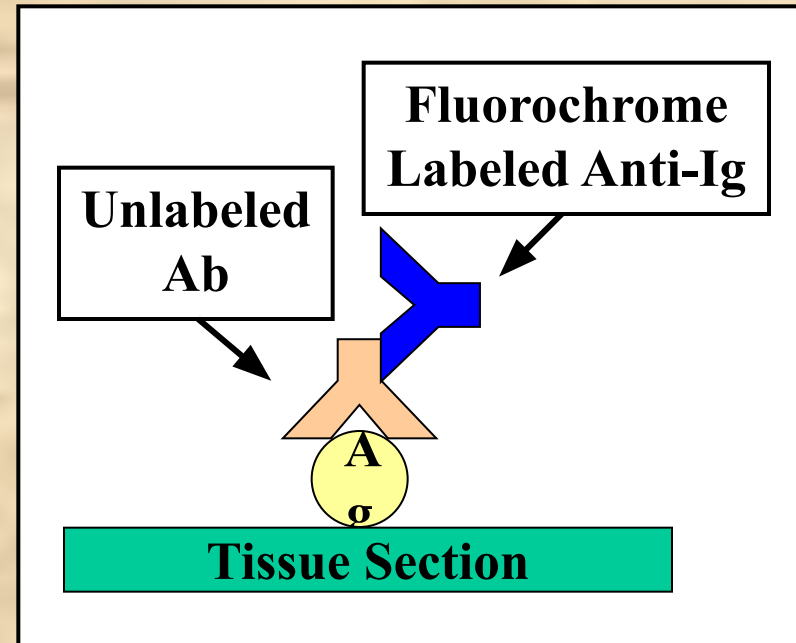
# Immunofluorescence

- Direct
  - Ab to tissue Ag is labeled with fluorochrome



# Immunofluorescence

- Indirect
  - Ab to tissue Ag is unlabeled
  - Fluorochrome-labeled anti-Ig is used to detect binding of the first Ab.
- Qualitative to Semi-Quantitative





# Complement Fixation

- Methodology
  - Ag mixed with test serum to be assayed for Ab
  - Standard amount of complement is added
  - Erythrocytes coated with Abs is added
  - Amount of erythrocyte lysis is determined

