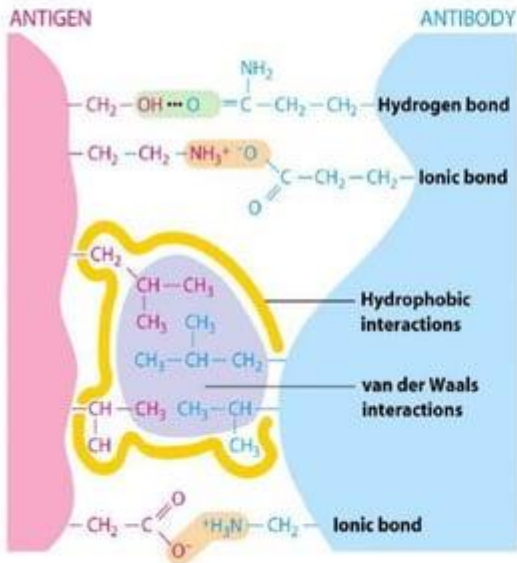


Chap. 6 – Antigen-Antibody interactions

Characterized as:

- Non-covalent interaction (similar to “lock and key” fit of enzyme-substrate)
- Does not lead to irreversible alteration of Ag or Ab
- This exact and specific interaction has led to many immunological assays used to:
 - » detect **Ag** or **Ab**
 - » diagnose **disease**
 - » measure magnitude of **humoral IR**
 - » identify molecules of **bio** and **med** interest

Ag-Ab interactions



Bonds:

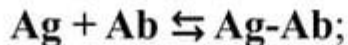
- Hydrogen
- Ionic
- Hydrophobic interactions
- Van der Waals forces

Each bond is weak; many are strong

To “hold” they must be close
→ requiring high amts of complementarity!

Measuring affinity of Ab to Ag

Assoc between CDR and monovalent Ag can be expressed as:



k_1 = forward (assoc) rate constant

k_{-1} = reverse (dissoc) rate constant

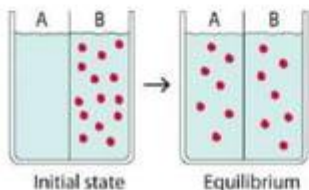
whereby $k_1/k_{-1} = K_a$
the assoc/equilibrium
constant

$K_a = \frac{[\text{Ag-Ab}]}{[\text{Ag}] [\text{Ab}]}$ value of K_a depends on k_1 ;
for small haptens, k_1 is high
for large protein Ag's, k_1 is lower

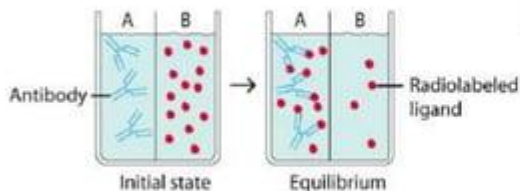
K_a determined by equilibrium dialysis

(a)

Control: No antibody present
(ligand equilibrates on both sides equally)

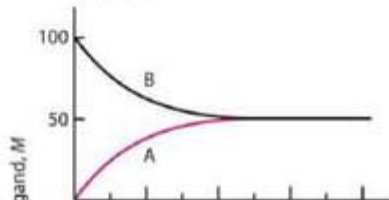


Experimental: Antibody in A
(at equilibrium more ligand in A due to Ab binding)

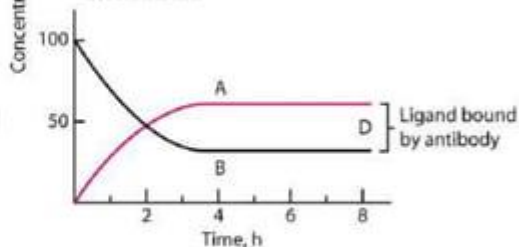


(b)

Control



Experimental



Cross-reactivity

Sometimes, Ab can “cross-react” with unrelated Ag....

(can occur if Ag's share an identical/similar epitope)

Often seen with polysaccharide Ag's

e.g. **ABO Blood groups – glycoproteins**

-persons lacking one or both of the blood (AB) Ag's will have serum Ab's vs.the missing Ag's

-these Ab's produced from cross-reactive MO Ag's!!

-provides basis for **blood typing tests**

-necessitates **compatible blood types** during transfusions, etc.

Other MO cross-reactions: 1) *Streptococcus pyogenes*

2) *Vaccinia* virus

Immunologic tests:

1. Precipitation Rxns:

-Ab's and Ag's in aqueous soln's form a lattice => **Precipitin**

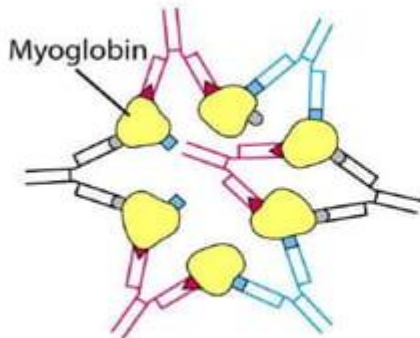
Lattice formation requires:

1) polyvalent Ab's

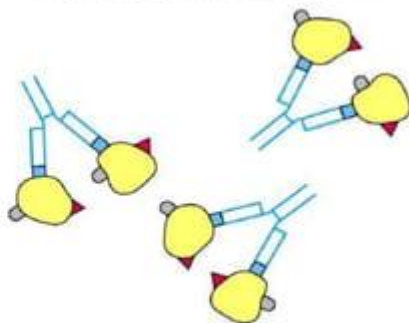
2) Ag must be bivalent, polyvalent



POLYCLONAL ANTISERUM



MONOCLONAL ANTIBODY

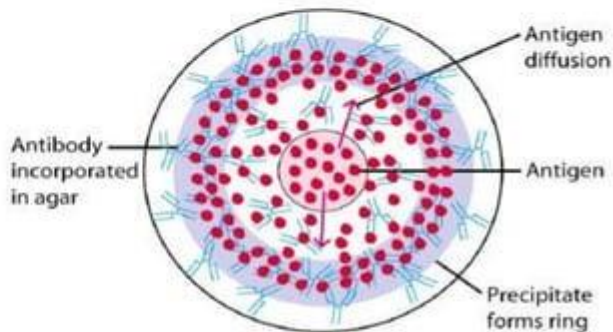


Precipitation rxns, once popular, have been replaced by faster, more sensitive tests

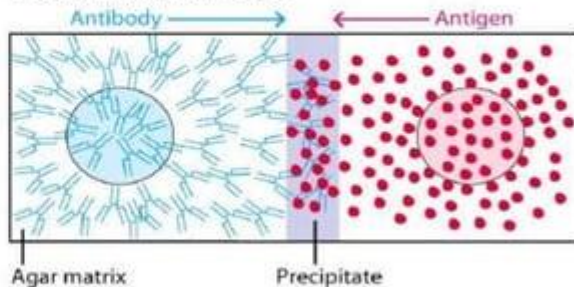
Immunologic tests:

Precipitation rxns in gels

RADIAL IMMUNODIFFUSION



DOUBLE IMMUNODIFFUSION

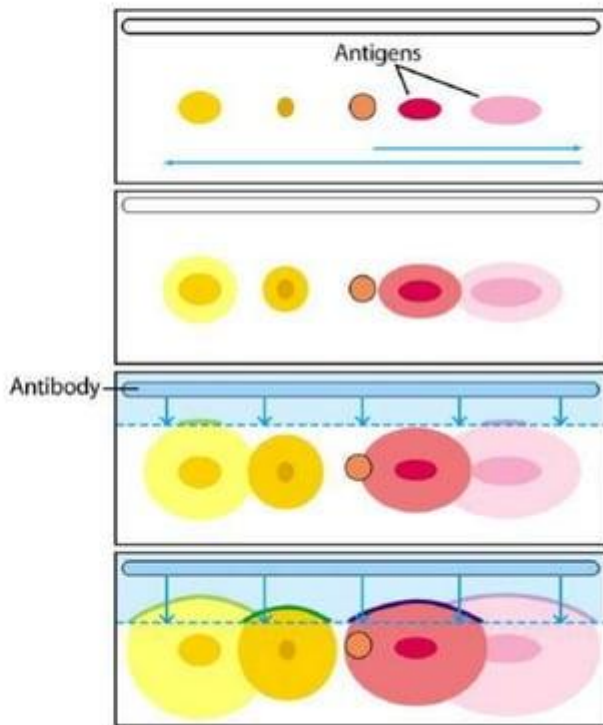


Immunologic tests:

2. **Immuno-electrophoresis**: Incorp electrophoresis
w/ double diffusion
- An Ag mixture is 1st separated by charge
 - Then, “troughs” are cut ||to direction of elec field and antisera is added to trough
 - Ag's and Ab's diffuse towards each other to produce precipitin bands
 - Used to detect: a) presence/absence of specific proteins or Ig classes
- b) immunodeficiency or immunoproliferative disorder

Immunologic tests:

Immuno-electrophoresis:



Immunologic tests:

3) **Agglutination reactions** – simple,
inexpensive, but sensitive!

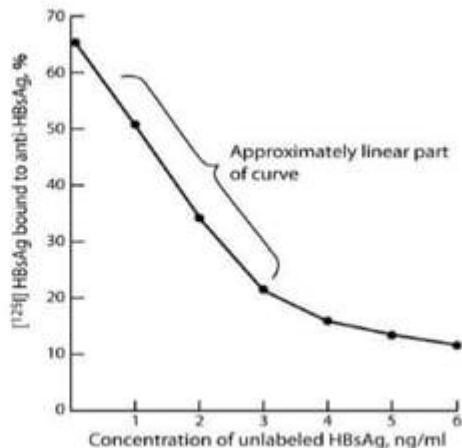
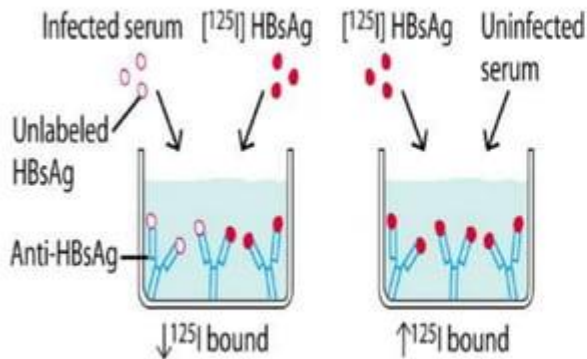
Several types exist:

- a) Hemagglutination of RBC's
- b) Bacterial Agglutination
- c) Passive Agglutination
- d) Agglutination Inhibition

Immunologic tests:

4) **Radioimmunoassay (RIA)**– very sensitive test; used for measuring hormones, serum proteins, drugs, etc. at low [C]’s ($\leq 0.001\text{ug/ml}$)

measures “**competitive binding**” of radiolabelled Ag + unlabelled (test) Ag to high affinity Ab



Immunologic tests:

5. **ELISA tests:** dep on enzyme conugated to 2 Ab reacting with a specific substrate to produce a color rxn. Most sensitive of tests for Ag/Ab!!

Variations of ELISA's:

Allows for qualitative or quantitative testing.

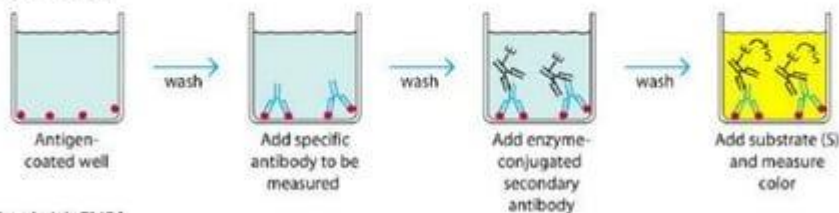
Each one can be used for qualitative detection of Ag or Ab

Also, a **std curve** based on known [C]'s of Ag/Ab can be prepped and an unknown [C} can be ascertained

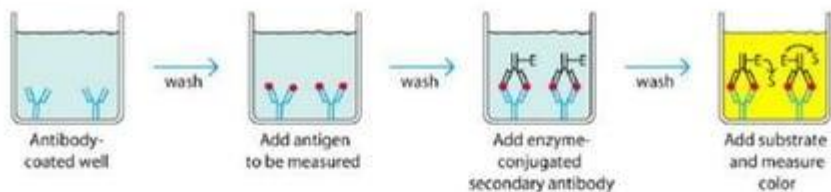
- a. Indirect ELISA
- b. Sandwich ELISA
- c. Competitive ELISA

Immunologic tests: Types of ELISA's...

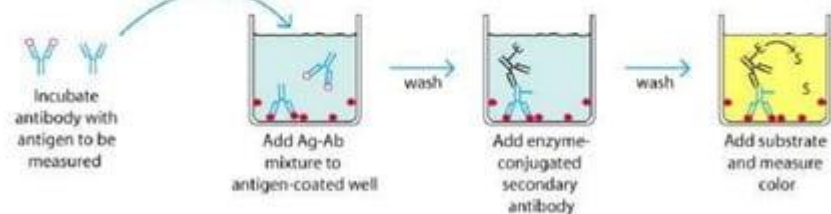
(a) Indirect ELISA



(b) Sandwich ELISA



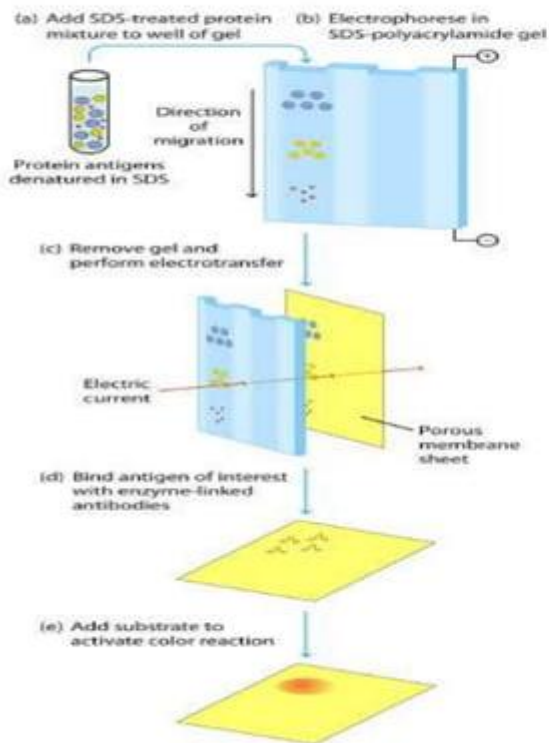
(c) Competitive ELISA



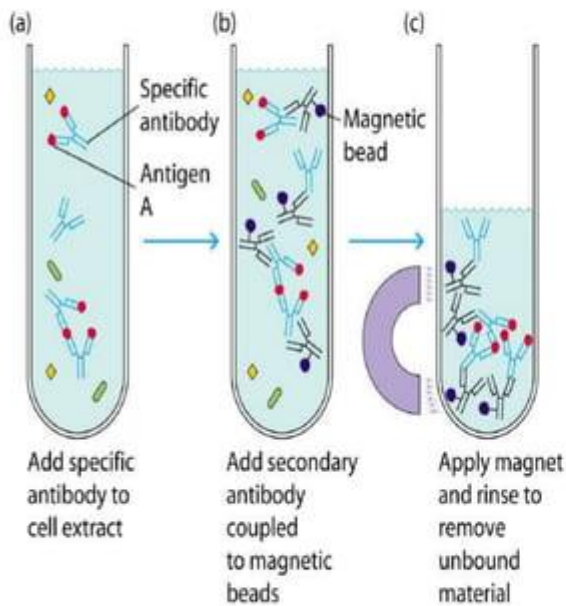
Immunologic tests:

6) Western Blot

- Used to id specific proteins in mixtures
- Proteins are separated on SDS-PAGE
- Proteins then transferred to membrane
- Membrane flooded w/ radio-labelled or enz-linked poly/monoclonal Ab's specific for protein



Immunologic tests: 7) Immunoprecipitation



- Provides a quick and sensitive test for finding proteins/Ag's
 - Especially in low [C]'s
- Binds Ab to synthetic bead support → centrifuged
- Or 2° Ab w/ bead or magnetic bead → collect by magnetism

Immunologic tests: 8) Immunofluorescence

- Provides a quick method for the id of pathogens and lymphocytes
 - Ab's are conjugated with a fluorescent dye (fluorescein, rhodamine, phycoerythrin)
 - If Ab's bind to specific Ag's, they can be illum w/ UV light and emit bright colors
 - There are currently 2 methods employed:
 - Direct staining
 - Indirect staining

Direct and indirect Immunofluorescence

