Antigen Antibody interactions

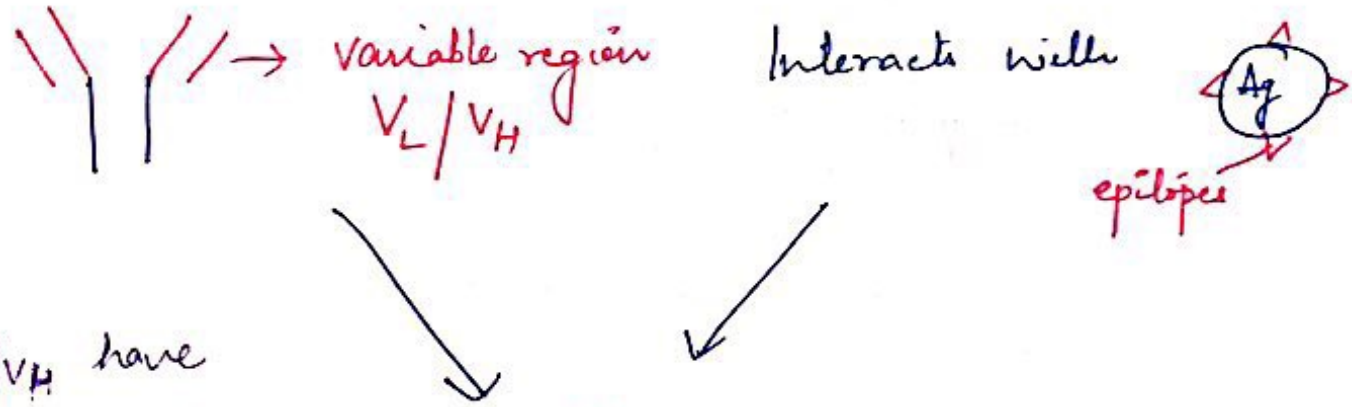
Course - B.Sc. (P) life Sciences 6th sem

Subject - Immunology

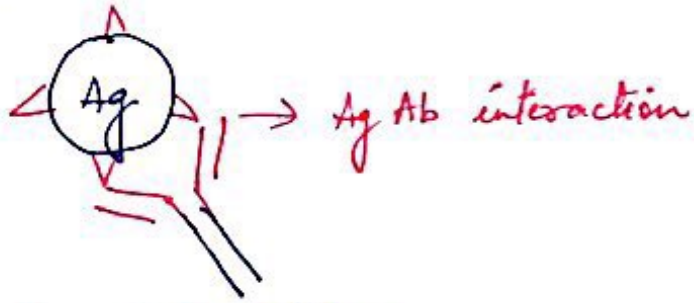
References - Textbook "Immunology - Kuby" 5th edition
Roitt's essential Immunology

Introduction

Ag - Ag are like enzymes and substrate interaction but not irreversible chemical interactions take place.



V_L/V_H have specific regions as CDR's which interact with epitopes on Ag.



Importance of such interactions

1. Immunological assays
2. Diagnosing diseases
3. level of humoral immune response (Ab are produced)
4. Identification of important molecules

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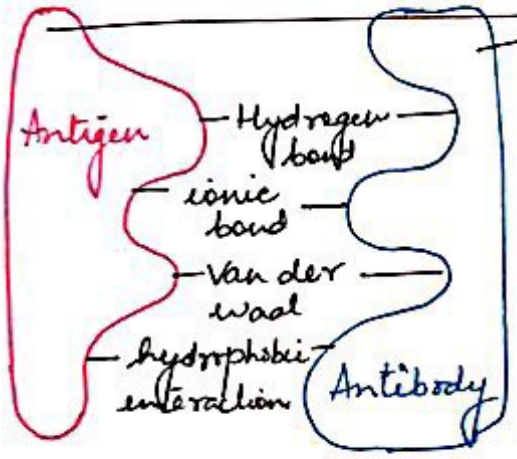
Types of non covalent enteractions b/w Ag and Ab.

- hydrogen bonds
- ionic bonds
- hydrophic enteractions
- Van der waal interactions

Why is a strong enteraction needed ?

Firstly, a combination of all the above mentioned enteractions on a large no of same/one type enteraction

secondly, a close fit on complimentarily in str of Ag and Ab is also essential for strong binding



Affenity of the Ab for a single epitope on Ag

During Ag Ab enteraction, following equation

$$Ag + Ab \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Ag\text{-}Ab$$

k_1 - forward rate constant k_{-1} - Reverse rate constant

$$ka = \frac{k_1}{k_{-1}}$$ (measure of affenity of Ab)

ka varies for different Ag - Ab complexes

low affenity Ag - Ab ka $10^4 - 10^5$ L/mol

high affenity Ag - Ab ka 10^{11} L/mol

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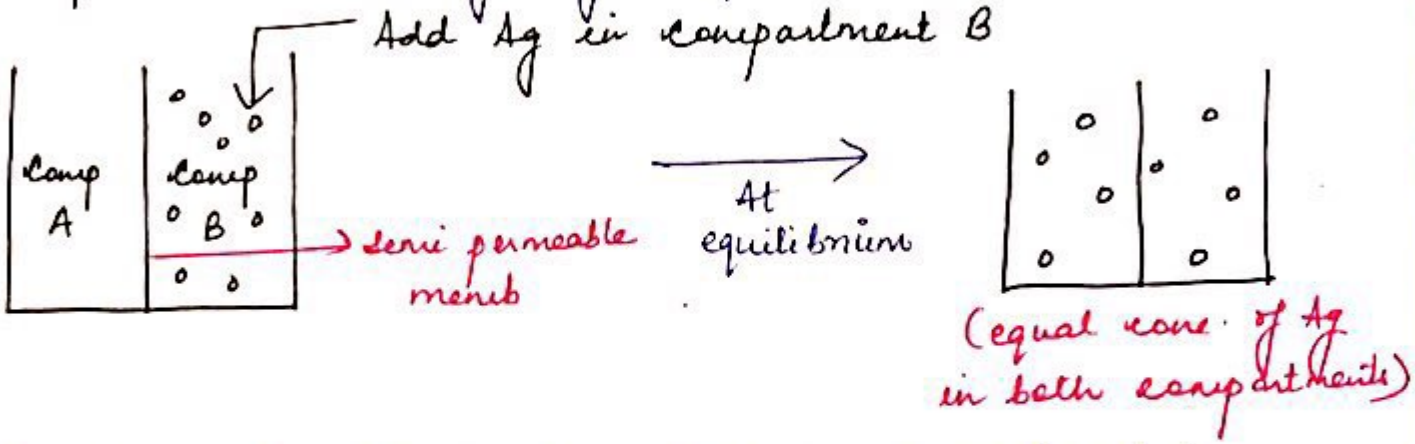
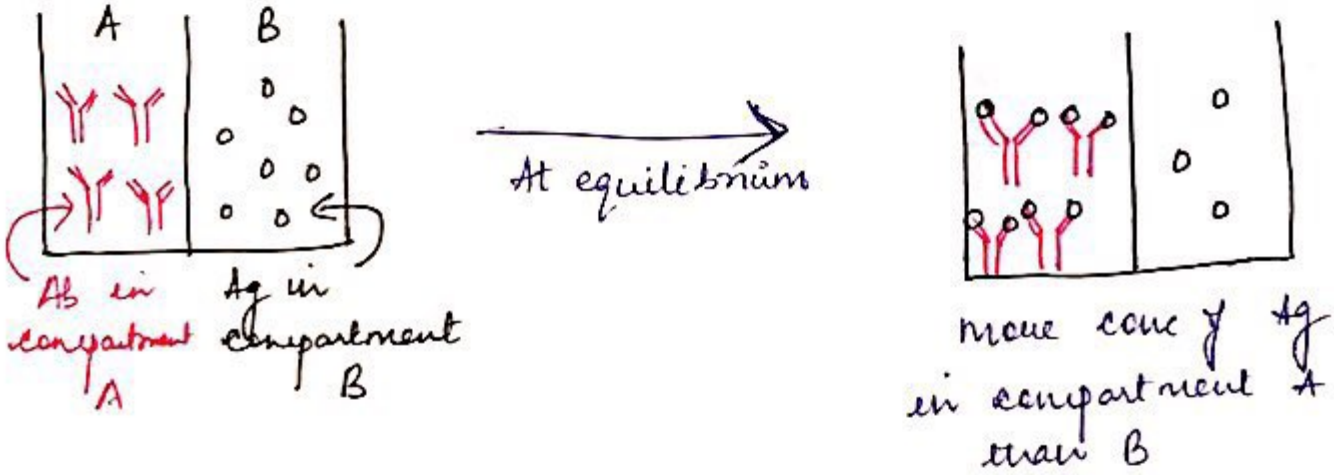
Determination of Ag-Ab affinity by equilibrium dialysis

Setup - (1) when only Ag is present



Setup 2 - when Ab is also added in compartment A



Scatchard equation - based on repeated equilibrium dialysis with a constant conc of Ab and varying concentration of Ag.

Antibody Avidity - When complex Ag having multiple types of epitopes are mixed with Ab (polyvalent sites)
The strength of such multiple interactions b/w multivalent Ab and Ag is called avidity
- Affinity is for individual binding sites
- pentameric IgM has low affinity but high of avidity and binds efficiently to Ag than IgG that has high affinity for a particular Ag.

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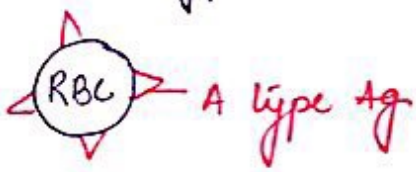
Cross Reactivity – Sometimes, Ab against one type of Ag can also bind or cross react with some other Ag.

- it is because these can have similar epitopes but the affinity may differ

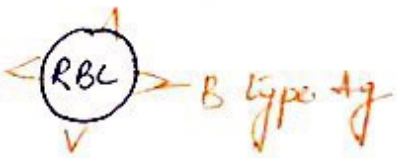
eg ABO blood group antigens

how we have antibodies already present in our serum against different blood group, when we have never been exposed previously to other blood types.

A blood gp



B blood gp



AB blood gp (has both type)

- The intestinal microbes have similar epitopes that are like A and B antigen (these are glycoproteins)
- It is the cross reactivity that we produce Ab against Ag on microbes but they can also recognise similar epitopes on RBC (A/B or AB).
- A blood gp person will have Ab against Ag B. because of cross reactive Ag in all intestinal microbes that are similar to antigen B epitopes

other examples

Streptococcus pyogenes has Ag that produce Ab which also recognise myocardial and skeletal muscle protein of infected person. The Ab produced to fight bacterial Ag also attacks the heart and kidney cells of the infected person.

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Vaccines produced for vaccinia virus that causes cowpox it cross reacts with epitopes (similar) of small pox virus

Agglutination Reaction - Ab are agglutinins

prozone effect - excess of Ab can inhibit precipitation and agglutination reaction

Blood typing - because of haemoagglutination

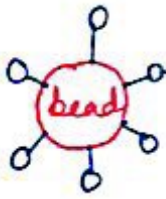
Diagnosis of bacterial infection - eg Salmonella typhii produces Ab in infected person against the bacterial Ag. The serum of the patient shows agglutination with bacterial Ag in the lab.

Passive agglutination - Soluble Ag cannot cause agglutination but can be made to do so. It is done by coating these Ag on the surface of RBC or latex beads.

Agglutination Inhibition for assay - eg original home pregnancy kit

kit components (2 components)

(1) human chorionic gonadotropin (HCG) coated on latex bead



(2) Anti HCG (antibody against HCG) 

These 2 components are provided separately in the kit

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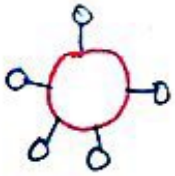
Case I - When female is not pregnant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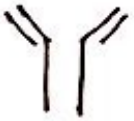
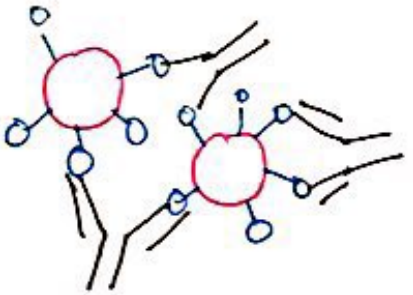

+ Urine sample of female to be tested (no HCG is present in her sample)

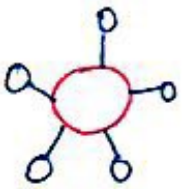
↓ upon mixing all three



(clumping or agglutination can be observed in female who is not pregnant)

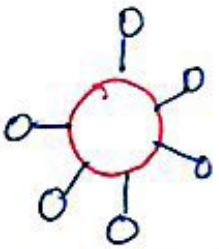
This is because no HCG in soluble form is present in the urine sample therefore, anti HCG and HCG on bead is free to agglutinate

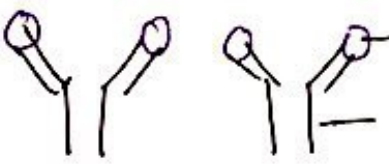
Case II - When female is pregnant (her urine sample has HCG)


+

+ urine sample with soluble HCG

↓ upon mixing all three





No agglutination as no Anti HCG binds to this

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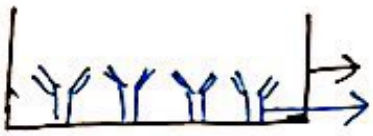
Radioimmunoassay (RIA)

- Technique developed by Berson and Rosalyn
- used to determine very less conc./minute levels of molecules like insulin, drugs, vitamins, serum proteins
- it uses radiolabelled Ag and unlabelled Ag that compete with each other for binding to the limited amount of Antibody.
- Radiolabelled Ag binds to Ab and only few sites is available for unlabelled Ag
- Radiolabelled Ag is added from outside whereas unlabelled Ag is called from serum to check its level in the body.
- Radiolabelled ratio will decrease if there is more conc. of ~~tab~~ unlabelled Ag
- Ag is are generally labelled with I^{125}, H^3.

Solid phase RIA eg hepatitis B virus

HBsAg - surface Ag on hepatitis B.



microtiter well coated with constant amount of Ab specific against Ag - HBs (Ag)

infected serum (will have HBs Ag)

uninfected serum (will not have HBs Ag)

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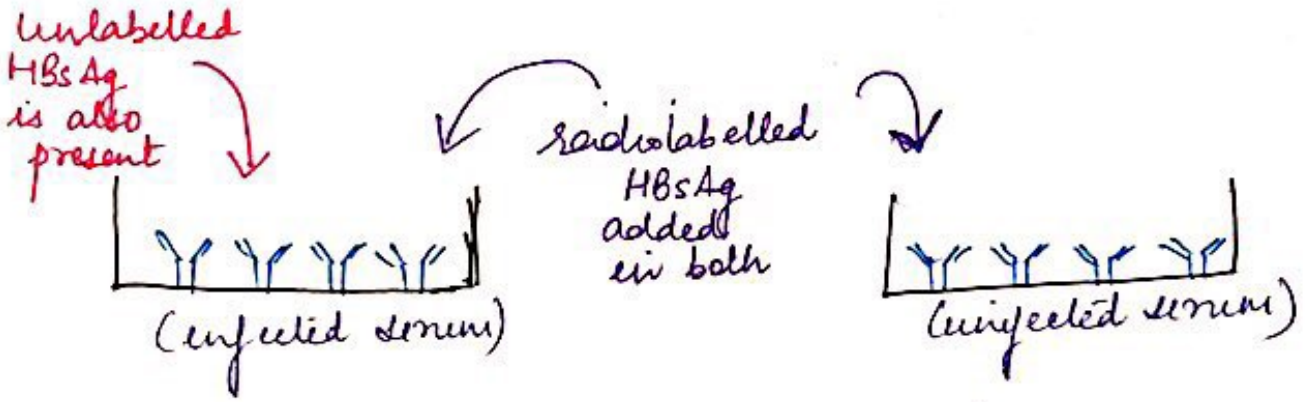

labelled and unlabelled will compete with the constant amount of Ab coated on the well and ratio is calculated

Only labelled HBsAg is present in this case

Western blotting - similar to southern blotting but is used for protein mixture

- protein mixture (Ag) is electrophoretically separated on PAGE gel electrophoresis, it has 5 steps

Step 1 - The protein Ag (mixture of proteins) is treated with SDS (sodium dodecyl sulphate) a detergent

Step 2 - this treated protein mixture is separated on PAGE gel by electrophoresis

Step 3 - Remove gel and keep over a nitrocellulose membrane for transfer of separated proteins bands on to the membrane by electric current

Step 4 - The nitrocellulose membrane is exposed to particular Ab to observe its interaction with different bands of Ag on the membrane

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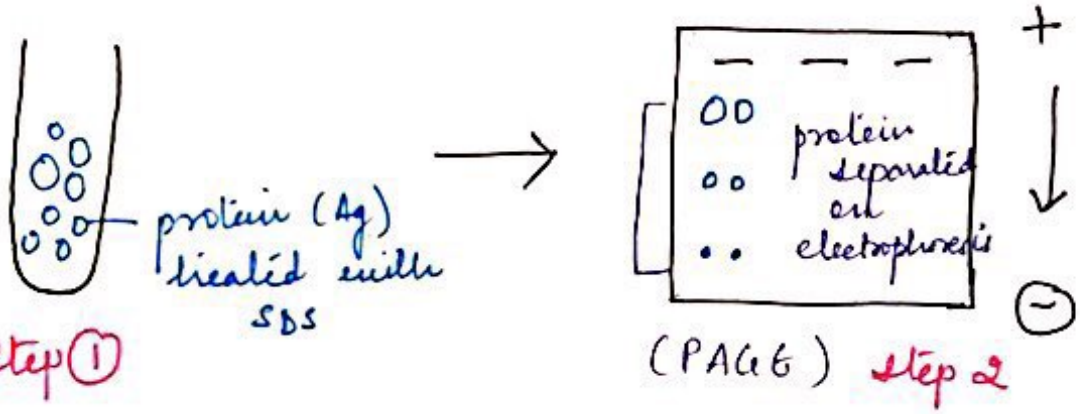
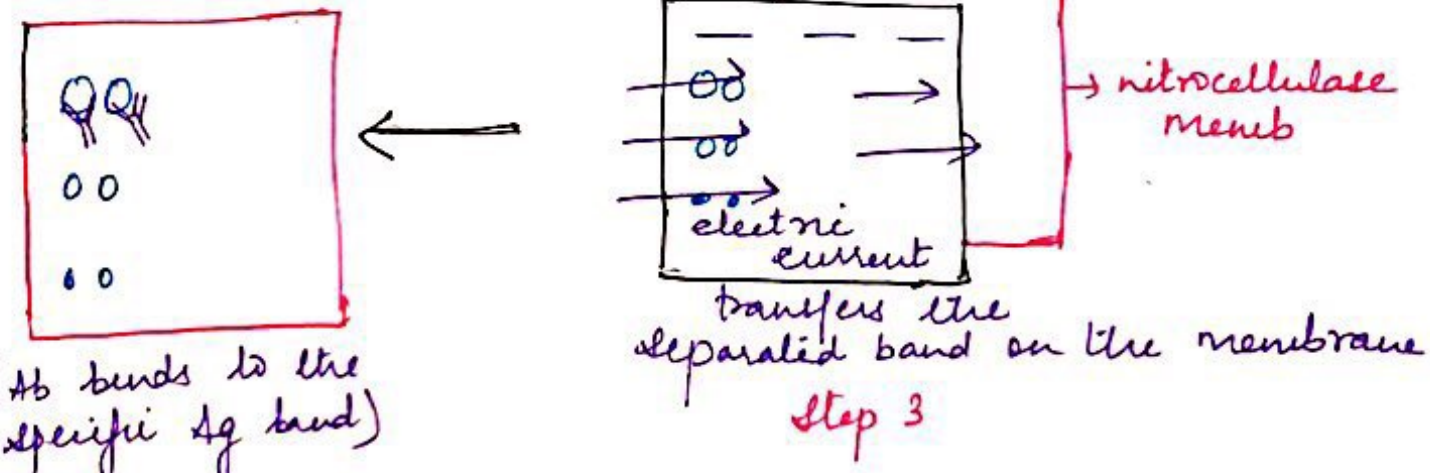
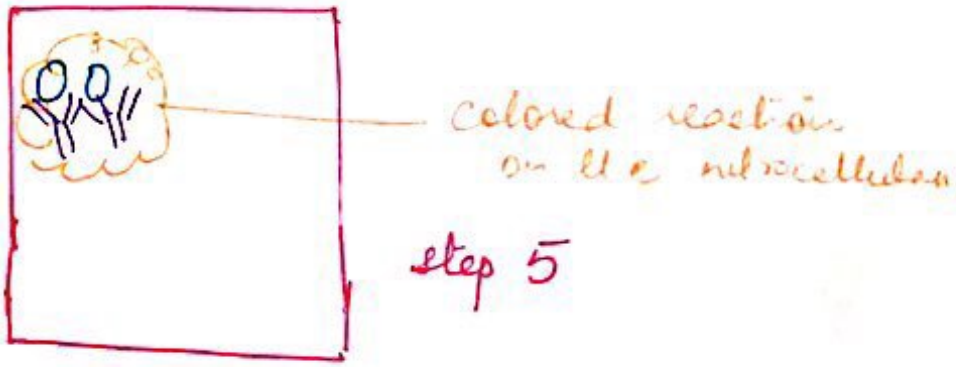
Step 5- To observe the Ag-Ab complex, either the Ab is radiolabelled and its result is seen on Xray film or the Ab is bound to an enzyme substrate to give a colored product at site of Ag Ab binding







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