

Antigen Antibody interactions

pg-1

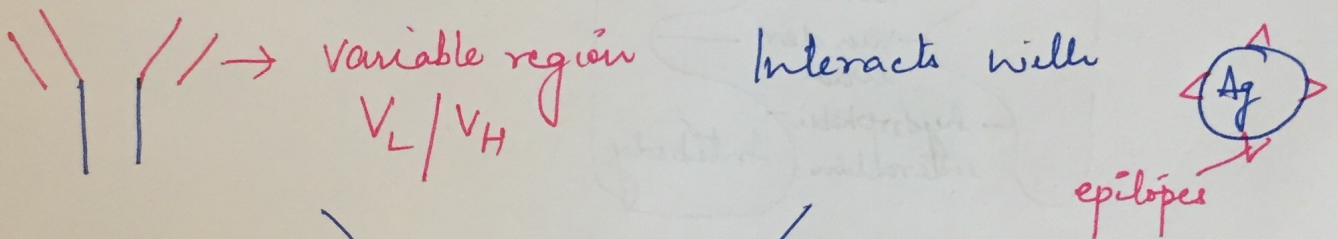
Course - B.Sc. (P) Life Sciences 6th Sem

Subject - Immunology

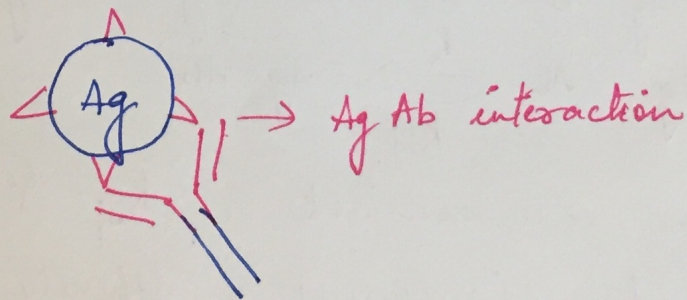
References - Textbook "Immunology - Kuby" 5th edition
Roitt's essential Immunology

Introduction

Ag-Ab are like enzymes and substrate interaction but not irreversible chemical interactions takes 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

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

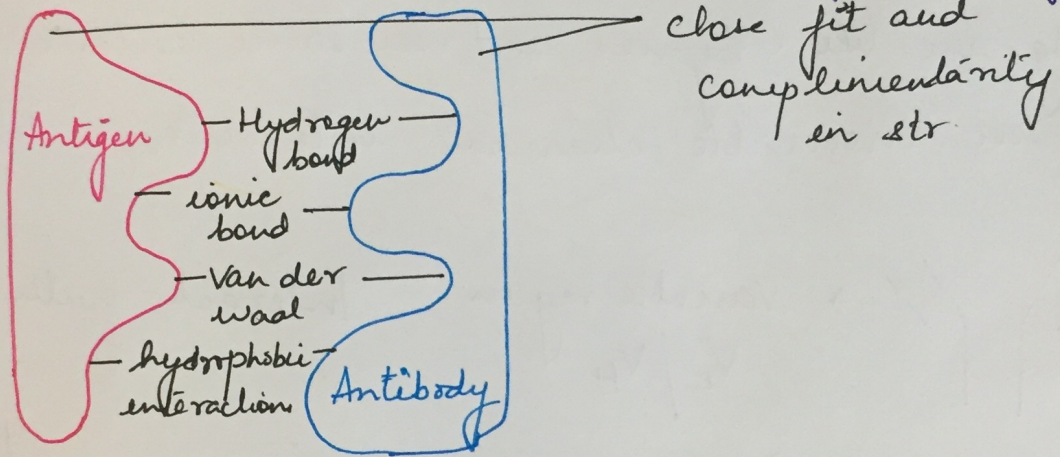
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- hydrogen bonds
- hydrophobic interactions
- ionic bonds
- Van der Waal interactions

Why is a strong interaction needed?

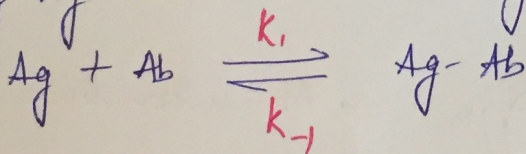
Firstly, a combination of all the above mentioned interactions or a large no. of same / one type interaction

Secondly, a close fit or complementarity in str. of Ag and Ab is also essential for strong binding



Affinity of the Ab for a single epitope on Ag

During Ag Ab interaction, following equation



k_1 - forward rate constant

k_{-1} - Reverse rate constant

$$K_a = \frac{k_1}{k_{-1}} \quad (\text{measure of affinity of Ab})$$

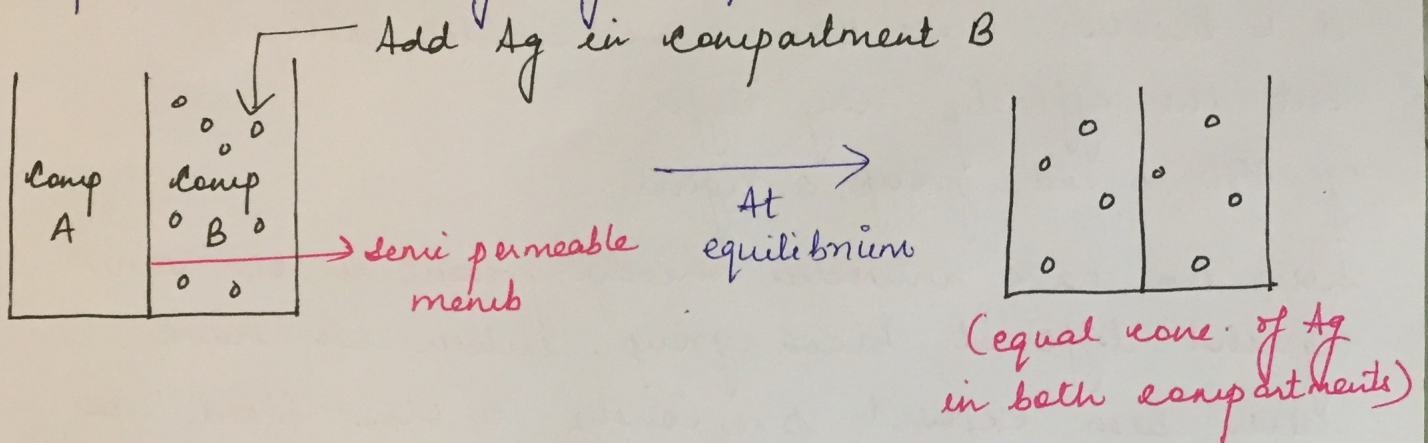
K_a varies for different Ag-Ab complexes

low affinity Ag-Ab K_a $10^4 - 10^5$ L/mol

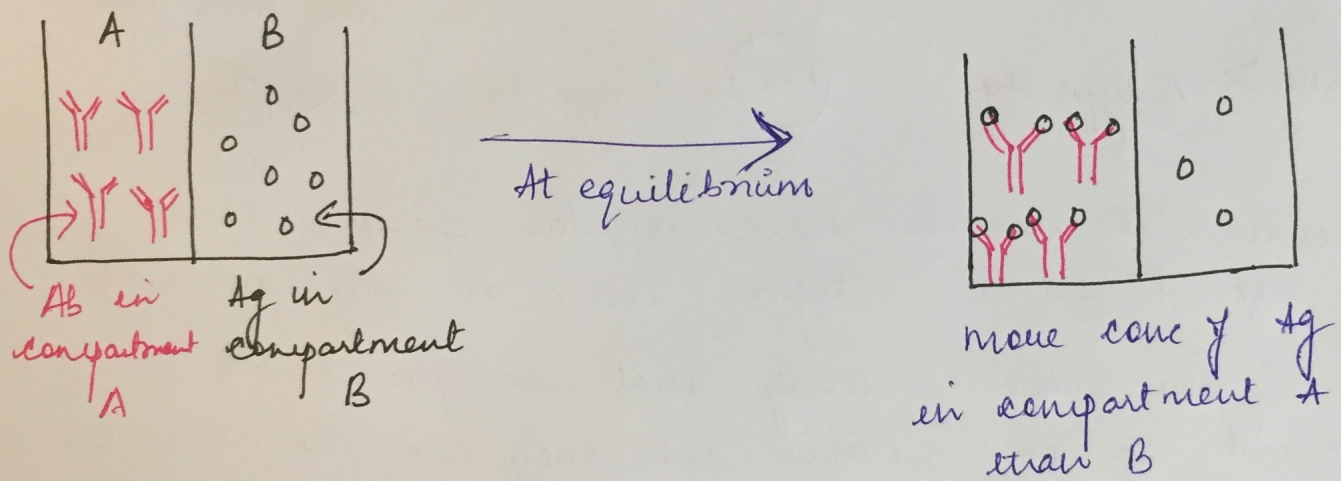
high affinity Ag-Ab K_a 10^{11} L/mol

Determination of Ag-Ab affinity by equilibrium dialysis (3)

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 ~~can~~ (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.

Cross reactivity - Sometimes, Ab against one type of Ag (4)
can also bind or cross react with some other Ag.

- it is because these can have similar epitopes

but the affinity may differ

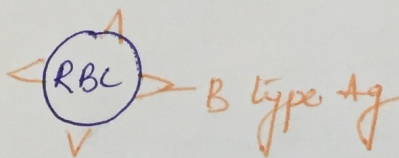
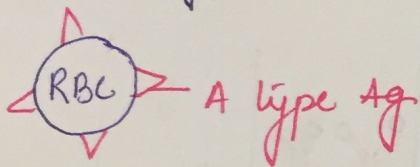
eg ABO blood group antigens

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 enteritinal 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 its enteritinal 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.

Vaccines produced for vaccinia virus that causes scumpox ⁽⁵⁾
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 haemagglutination

Diagnosis of bacterial infection - eg Salmonella typhi

produces Ab in infected person against the bacterial Ag.

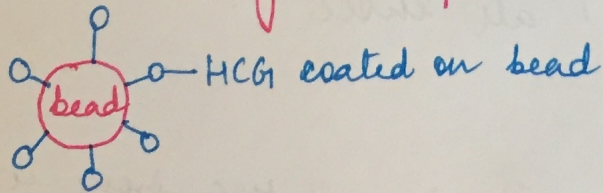
The serum of the patient shows agglutination
with bacterial Ag in the lab.

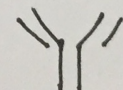
Passive agglutination - Soluble Ag cannot cause agglutination
but can be made to do so. It is done by coating
these Ag on the surface of RBC or latex beads.

Agglutination Inhibition assay - eg original home
pregnancy kit

kit components (2 components)

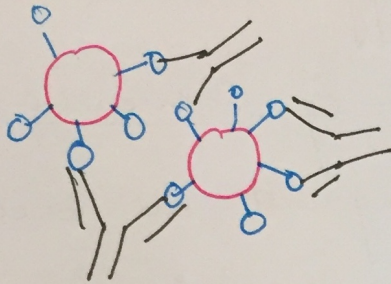
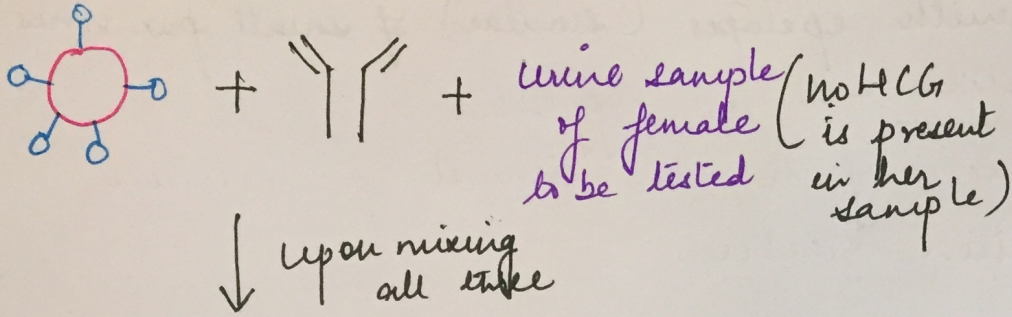
① human chorionic gonadotropin (HCG) coated on latex bead



② Anti HCG (antibody against HCG) 

These 2 components are provided separately in the kit

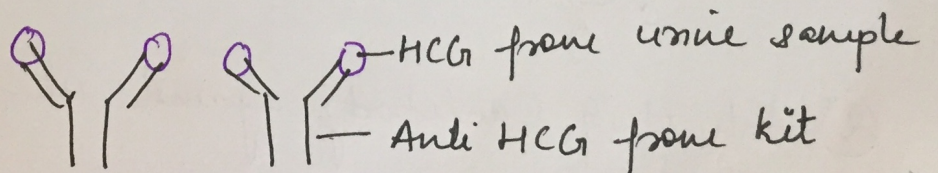
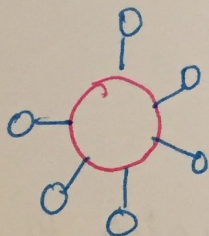
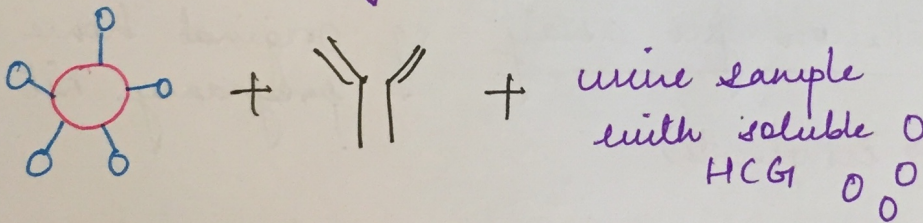
Case I - When female is not pregnant



(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)



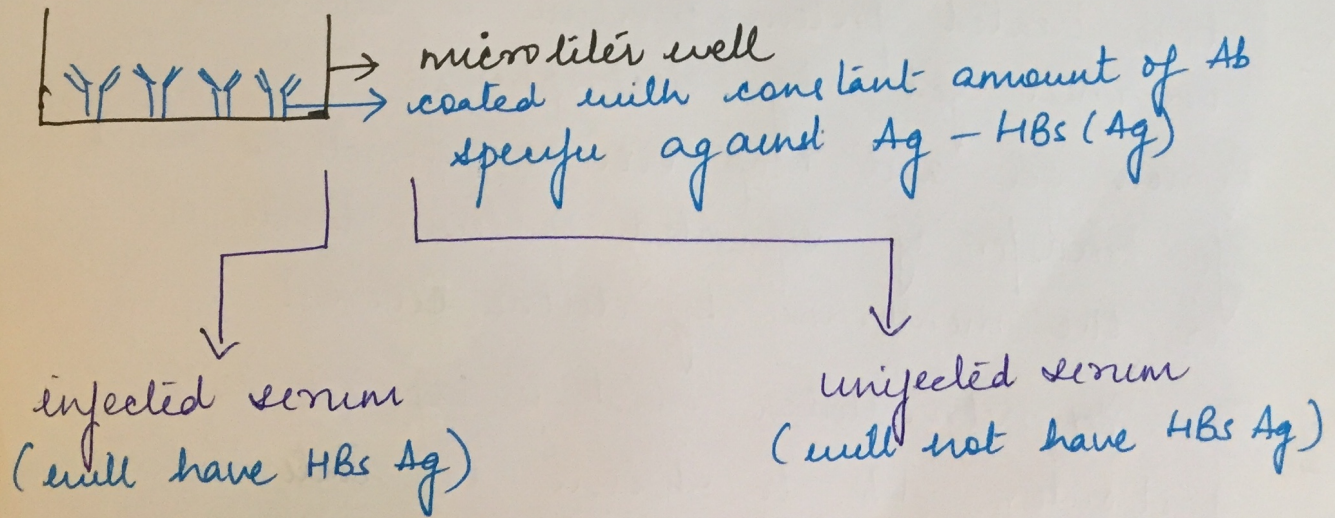
No agglutination as no Anti HCG binds to this

Radioimmunoassay (RIA)

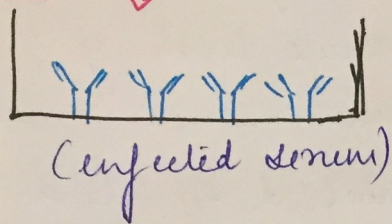
- Technique developed by Berson and Rosalyn
- used to determine very less conc./minute levels of molecules like insulin, drugs, vitamins, serum proteins
- it uses radiolabelled Ag and unlabelled Ag that compete with each other for binding to the limited amount of Antibody.
- Radiolabelled Ag binds to 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.
- Radio labelled 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

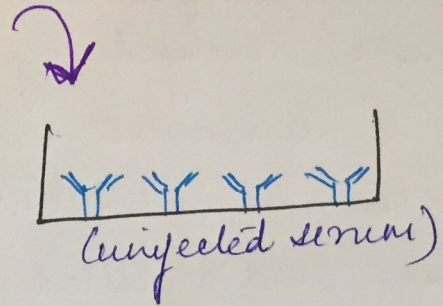
HBsAg - surface Ag on hepatitis B.



Unlabelled
HBsAg
is also
present



radio-labelled
HBsAg
added
in both



↓
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 mem.
for transfer of separated proteins bands on
to the membrane by electric current

step 4 - The ~~at~~ nitrocellulose mem. is exposed to
particular Ab to observe its interaction
with different bands of Ag on the membrane

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

