Clinical Biochem 2 BMS302 Learning Objectives Answered

MODULE ONE : TOPIC ONE

write the equations illustrating the competition between labelled and unlabelled antigen to a specific antibody

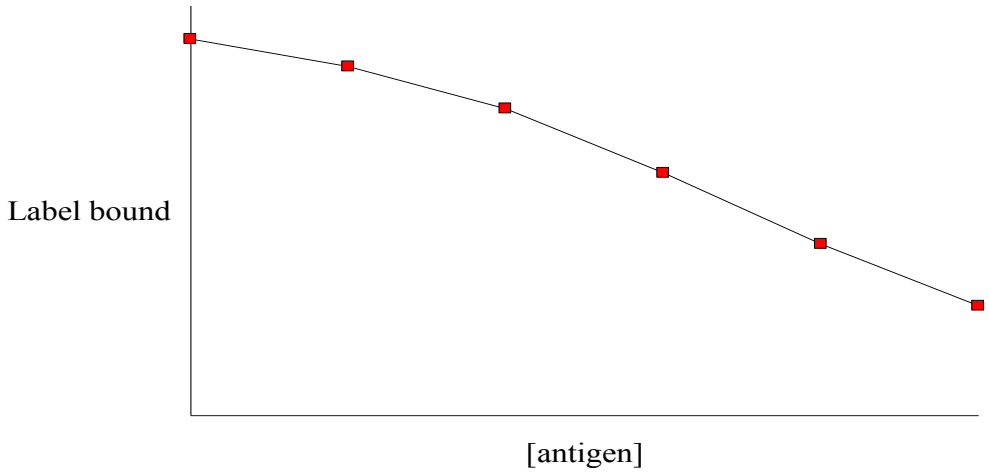
Ag + Ab AgAb
Ag* + Ab Ag*Ab

identify experimental conditions for competitive binding immunoassay

1. There must be fixed concentrations of antibody (Ab) and labelled antigen (Ag*) – the only variable is the amount of unlabelled antigen (Ag)
2. A limiting amount of antibody (Ab) – that is there must be less antibody than there is labelled antigen. This means that the labelled Ag* and the unlabelled Ag will need to compete for the binding to the limiting amount of Ab.

describe the relationship between the amount of labelled antigen complexed to antibody (the label bound) and the concentration of unlabelled antigen.

- At low levels of unlabelled antigen (Ag) – which is the variable – higher levels of labelled antigen (Ag*) will bind to the antibody (Ab).
- As unlabelled antigen (Ag) levels increase – less labelled antigen (Ag*) will bind to the antibody (Ab).
- This means there is an inverse relationship between the levels of Ag* bound and the levels of Ag.
- Standards must be made with unbound antigen (Ag) and run in parallel and a standard curve plotted.



The more unlabelled antigen (Ag) added the less labelled Ag* binds to Ab.

distinguish between antigens, immunogens and haptens.

- Antigens: molecules that bind to specific antibodies.
- Immunogens: substances that induce antibody formation.
- Haptens: non-immunogenic moelcules which become immunogenic after attachment to a carrier.

NB. The bigger the molecule the more immunogenic it is likely to be. Smaller molecules only become immunogenic after attachment to a carrier – usually a protein.

describe conjugation and identify the range of carrier proteins for conjugation to haptens

- Conjugation is the attachment of a small non-immunogenic compound (hapten) to a carrier protein in order to make it immunogenic.
- The bond must be stable and this is achieved through covalent linkages via a conjugation reaction.
- Protein carriers include
    - globulin fractions
    - albumin
    - haemocyanin
    - ovalbumin
    - fibrinogen
- The hapten attaches to the amino acid side chains on the surface of the carrier protein. For eg.
    - ε-amino groups of lysine
    - phenolic hydroxyl groups of tyrosine residues
    - sulphydryl groups of cysteine
    - imidazole groups of histidine residues

- Conjugation should take place in an area of the hapten that is least important for immunological recognition.
- Conjugation should not overly change the tertiary structure of the protein.

explain the procedures used to raise polyvalent antisera

NB. Polyvalent antisera is not used much anymore – not sensitive or specific enough

High affinity anti-hapten antibodies are raised from rabbits, guinea pigs, goats, mice and sheep.
Antigen is administered to young fully developed animals in multiple small doses.

define a monoclonal antibody and describe hybridoma technology in production of monoclonal antibodies

- Monoclonal antibodies are a single homogenous (all the same) population of antibodies that are highly specific for a SINGLE epitope on a multivalent antigen.
- Immunisation with a single pure antigen triggers a population of lymphocytes that each produce a single antibody.
- If you take one of these antibody forming cells you can produce a clone in vitro which will produce a large amount of the monoclonal antibody you require.
- Because the lymphocytes cannot grow in tissue culture due to the Hayflick limit, a hybridoma is created – a hybrid of the antibody producing lymphocyte and a multiple myeloma cell.
- Hybridomas have the following characteristics
    - Ability to constantly divide in a tissue culture – due to the myeloma cells potential immortal qualities.
    - Ability to produce a single antibody – because of the lymphocyte.

define antibody titre and know its importance in an immunoassay

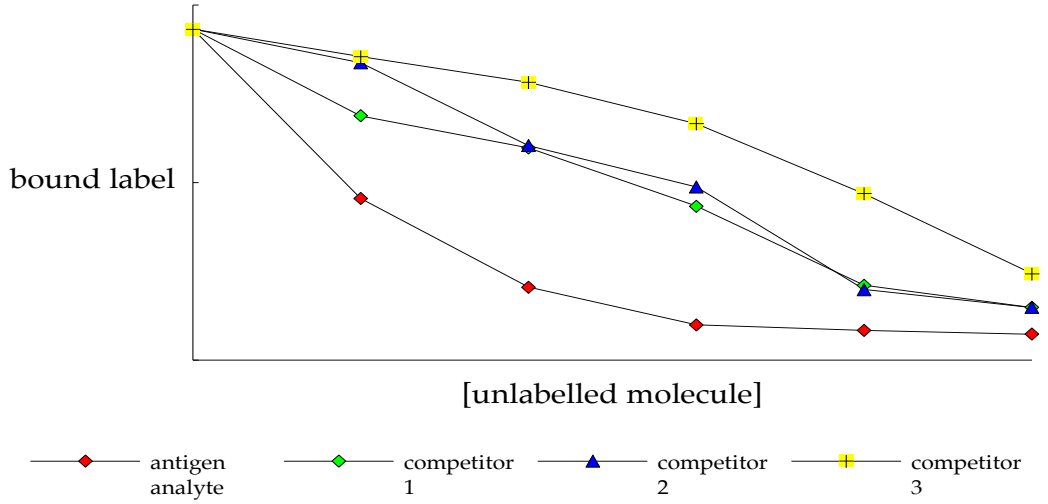
- Antibody Titre: is the final dilution of antibody used in the assay when the antigen is present. In other words it is the appropriate dilution that allows the antibody to be limited.

distinguish between affinity and avidity of antigen-antibody binding

- Affinity: the strength of a SINGLE non-covalent Ab-Ag bond
- Avidity: is the strength of the multiple binding between Ag and Ab.
- Sensitivity: the minimum detection limit of an assay. The least concentration of Ag which can be distinguished from a sample that contains no Ag.

define specificity and cross-reactivity in relation to antigen-antibody interaction and know the importance in relation to immunoassays. describe the experimental methods to assess the extent of cross-reactivity.

- Specificity: the capacity of an antiserum to recognise its own antigen in the presence of others.
- Cross reactivity: is when other antigens bind to an antibody that it is not directed towards. (Antigen A will be displaced by Antigen B)
    - FSH, TSH, LH, hCG all have different beta chains but similar alpha chains and this can result in cross reactivity.
    - To overcome these issues a competition assay between a labelled version of the antigen, an unlabelled version, and then some known cross reacting molecules.



- If there is no cross reactivity – increasing concentration of cross reacting molecules will have no effect on the amount of lable bound to antibody.
- If there is 100% cross reactivity the cross reacting molecule will follow the displacement line for the actual Ag.
- Most cross reacting molecules will be somewhere in the middle.
- To work out the midrange (the concentration of cross reacting molecule needed to reduce labelled antigen binding to 50% of its original level)
    - Draw a horizontal line across from the small mark midway down the vertical scale and see where it cuts the experimental lines.
    - Draw vertical lines down from these intersection points to the horizontal axis.
    - Compare this figure with the concentration of antigen required to cause a similar 50% reduction.

distinguish between heterogeneous and homogeneous immunoassays

NB. For most immunoassays, the parameter that is always measured is the amount of labelled antigen (Ag*) which is bound to the antibody.

- Heterogenous assays
    - Binding of the Ab-Ag* does not change the signal
    - Separation of the Ab-Ag* and the free Ag and Ag* is required.
    - Difficult to automate