IMMUNOLOGY

REVERSE PASSIVE HAEMAGGLUTINATION,

The agglutination of antibody coated red blood cells. So in this the red blood cells have an antibody coupled. This is then added to a 'sample'. If the antigen to which the antibody (attached to RBC) is complementary is present, then there will be 'reverse passive agglutination'

THE COMPLEMENT FIXATION TEST,

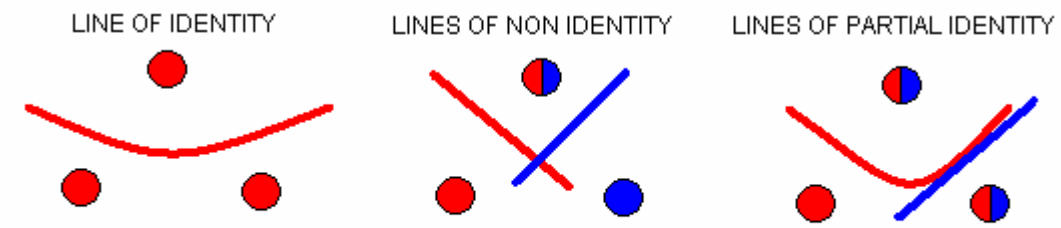
You have RBC with antibody bound. However first antigen to which the antibody is specific is added to a sample of serum. If the serum has antibody to the antigen then, complexes will form and thus will activate complement and then deplete it from the serum. Consequently the RBC with antibody bound is added. If there is any residual antigen, then the consequent activation of complement will cause lysis due to formation of the MAC. So if the serum has plenty of antibody, it will require a higher dilution before full lysis is observed

THE AGGLUTINATION INHIBITION TEST,

Latex beads with an antigen attached will agglutinate when the antibody complementary to the antigen is present. However, if the antibody is introduced into a 'sample' that contains soluble antigen, then upon introduction of the antigen bound latex beads, there will be no agglutination since the antibody has already bound to antigen. Thus the soluble antigen 'inhibits agglutination'. e.g pregnancy test.

IMMUNODIFFUSION (WITH PRECIPITATE FORMATION),

Antigens and antibodies are able to diffuse through agar. A concentration gradient will be established from the wells. Close to the Ab well, the small number of Ag will bind multiple Ab forming small complexes that remain soluble. Close to the Ag well, small amounts of Ab each bind 2 Ag forming small soluble complexes, At some point between the wells, the Ab and Ag will be 'equimolar', extensive cross-linking can occur forming large complexes that will precipitate (a white line)



The top wells contain antigen. Different colour represents different antibody
The lower wells contain antibody, again the colours determine specificity

- **Line of identity –** 2 antibodies recognising the same Ag – continuous arc forms between wells

- **Line of non identity –** 2 Ab's recognise different Ag so two lines form crossing each other
- **Line of partial identity –** line of identity forms for the shared antigen. A line of non identity also forms next to well with extra Ab. So a line of identity results with a 'spur' = line of non identity

BLOOD GROUP DETERMINATION BY AGGLUTINATION,

The ABO blood groups can be determined by agglutination of RBC with specific anti-A and anti-B sera. If agglutination occurs upon addition of anti-A confirms A antigen present on RBC etc…

DIRECT ANTIGLOBULIN TEST (THE DIRECT COOMBS TEST)

This is a test for 'non agglutinating' Rh anti-D IgG antibody on the RBC in Haemolytic disease of the new born. This is INDIRECT AGGLUTINATION. The antigen to which the antibodies added to the RBC sample bind, is in fact the Fc component of IgG. Thus the anti-human IgG binds to the Fc portion of anti-D IgG antibodies bound to RBC (which themselves are unable to agglutinate) to cause agglutination. [It is important that the sample is 'washed' as otherwise other soluble antibodies will interfere with the result]

VIROLOGY

VIRAL INFECTIVITY ASSAY

The infectivity of a virus preparation can be measured by the ability of the virus to form a plaque in a continuous lawn or sheet of host cells. 'FOCI OF CYTOPATHIC EFFECT' are observed – not necessarily lysis, but rounding, proliferation or disintegration of cells. The number of fce indicates the number of infectious virus particles in the initial sample. This is the virus titre and is expressed as the number of 'PLAQUE FORMING UNITS'

INFECTIOUS CENTRE ASSAY

In the early stages of the infectivity assay, no free virus can be found (eclipse phase). Thus if an aliquot of the cell solution is taken and the cells are assayed with further indicator cells, then the consequent plaques will represent the virus that has been released from the infected cells (the so called 'INFECTIOUS CENTRES') and is related to the initial virus titre

INFLUENZA HAEMAGGLUTINATION ASSAY

Influenza is able to efficiently agglutinate fowl RBC. Serial dilutions of the virus are made and equal volumes of fowl RBC are added. The endpoint occurs when insufficient virus is present to cause agglutination. (ie agglutination occurs when the [virus] is sufficient) NB – agglutination is caused DIRECTLY by the virus haemagglutinin and is independent of complement etc…

VIRUS NEUTRALISATION ASSAY

To confirm a diagnosis of virus, the assaying of the virus is not the only means and is often more difficult. This method acts to find the highest dilution of serum, which when mixed with a standard virus suspension will prevent the usual effect of that virus on cells – ie prevention of its 'cytopathic effect'.

INFLUENZA HAEMAGLUTTINATION INHIBITION ASSAY

Special example of virus neutralisation. Serial dilutions of serum performed. Standard virus is added, then after a period, a standard amount of fowl RBC is added. When high amounts of antibody are present in the serum (diagnostic of recent infection), a higher dilution is required before virus is able to cause haemagglutination