Neutralization test for virus

NEUTRALIZATION TEST FOR VIRUS Neutralization of a virus is defined as the loss of infectivity through reaction of the virus with specific antibody. Virus and serum are mixed under appropriate condition and then inoculated into cellculture, eggs or animals. The presence of unneutralized virus may be detected by reactions such as CPE, haemadsorption/haemagglutination, plaque formation, disease in animals. The loss of infectivity is bought about by interference by the bound Ab with any one of the steps leading to the release of the viral genome into the host cells.

There are two types of neutralization;- Reversible neutralization - The neutralization process can be reversed by diluting the Ab-Ag mixture within a short time of the formation of the Ag-Ab complexes (30 mins). It is thought that reversible neutralization is due to the interference with attachment of virions to the cellular receptors eg. the attachment of the HA protein of influenza viruses to sialic acid. The process requires the saturation of the surface of the virus with Abs.

Stable neutralization - with time, Ag-Ab complexes usually become more stable (several hours) and the process cannot be reversed by dilution. Neither the virions nor the Abs are permanently changed in stable neutralization, for the unchanged components can be recovered. The neutralized virus can be reactivated by proteolytic cleavage. Stable neutralization has a different mechanism to that of reversible neutralization. It had been shown that neutralized virus can attach and that already attached virions can be neutralized.

The number of Ab molecules required for stable neutralization is considerably smaller than that of reversible neutralization, Kinetic evidence

shows that even a single Ab molecule can neutralize a virion. Such neutralization is generally produced by Ab molecules that establish contact with 2 antigenic sites on different monomers of a virion, greatly increasing the stability of the complexes. An example of stable neutralization is the neutralization of polioviruses, whereby, the attachment of the antibody to the viral capsid stabilizes the capsid and inhibits the uncoating and release of viral nucleic acid.

Viral evolution must tend to select for mutations that change the antigenic determinants involved in neutralization. In contrast, other antigenic sites would tend to remain unchanged because mutations affecting them would not be selected for and could even be detrimental. A virus would thus evolve from an original type to a variety of types, different in neutralization (and sometimes in HI) tests, but retaining some of the original mosaic of antigenic determinants recognizable by CFTs.

Because of its high immunological specificity, the neutralization test is often the standard against which the specificity of the other serological techniques is evaluated. Before the neutralization test is carried out, the known components that are to be used must be standardized. To identify a virus isolate, a known pretitred antiserum is used. Conversely, to measure the antibody response of an individual to a virus, a known pretitred virus is used. To titrate a known virus, serial tenfold dilutions of the isolate is prepared and inoculated into a susceptible host system such as cell culture or animal.

The virus endpoint titre is the reciprocal of the highest dilution of virus that infects 50% of the host system eg. 50% of cell cultures develop CPE, or 50% of animals develop disease. This endpoint dilution contains one 50% tissue

culture infecting dose (TCID50) or one 50% lethal dose (LD50) of virus per unit volume. The concentration of virus generally used in the neutralization test is 100 TCID50 or 100 LD50 per unit volume. The antiserum is titrated in the neutralization test against its homologous virus.

Serial twofold dilutions of serum is prepared and mixed with an equal volume containing 100TCID50 of virus. The virus and serum mixtures are incubated for 1 hour at 37oC. The time and temperature for incubation varies with different viruses. The mixtures are then inoculated into a susceptible host system. The endpoint titration contains one antibody unit and is the reciprocal of the highest dilution of the antiserum protecting against the virus. Generally 20 antibody units of antiserum is used in the neutralization tests.