4.1 Protection against viruses in a tube (Seroneutralization)

Welcome to a new block of videos of this course, in which we'll talk about the diagnosis of viral infection through the immune response that viruses cause in the host. Let's start seeing how to evaluate the protective role of antibodies. This is done using tests of serum neutralization or viral neutralization.

As you know, for a virus to infect a cell recognition must occurbetween the cell receptor and certain molecules or epitopes on the surface of the virus. Well, certain antibodies can surround these viral molecules and prevent that they are recognized by the receptor. These antibodies are called neutralizing antibodies and they are very important because they actually block the progress of the infection, so they are protective. Similar to all antibodies, they are very specific, and if the viral molecules vary, they won't recognize them and cease to be protective.

The neutralization test is considered the technique that best reflects the correlation in vivo and in vitro between viruses and antibodies. It is a quantitative technique, so we will make dilutions of the sample serum and we will add a constant amount of virus. After incubating to allow antigen and antibody to react, the mixtures are added to a susceptible system to see the residual infectivity of the virus. Susceptible systems can be experimental animals, eggs with embryo or cell cultures, where non-neutralized viruses produce a recognizable effect, such as death, injury, cytopathic effect, haemagglutination, syncytia formation, etc.

There are several ways to prepare serum dilutions, but they only differ in the proportions. Here we are going to make serial two-fold dilutions. We need to prepare a battery of 10 tubes, to which we add 250 µl of culture medium. Then we add 250 µl of the serum to the first tube, we mix well and pass 250 µl of the mixture the following tube, and so on. Whenever we go from one tube to the next one we dilute the amount of antibodies present in the serum by half. I.e. in the first tube the dilution is 1:2, in the next 1:4, in the next 1:8, and so all the way to the tube 10 where it will be 1:1024. We must follow the same procedure with the positive and negative control sera. Next, we add 250 µl of the appropriate concentration of virus to the 10 tubes (diluting again by half the amount of antibodies), we shake and incubate for 60 minutes at 37°C to enable the possible neutralizing antibodies in the serum to react with the epitopes of the virus that are recognized by the cell receptor.

As I have mentioned before, neutralization can be tested in experimental animals, eggs or in tissue culture. Here we are going to do it in tissue culture. We transfer 100 µl of each serum-virus mixture by quadrupled to wells of a 96-well plate in which we have cultivated cells susceptible to the virus and we incubate plates 2 or 3 days or the time necessary to observe the cytopathic effect with the microscope. If there are neutralizing antibodies in the serum there will be no effect. Of course we must compare the results of the sample serum with the positive and negative controls.

This quantitative method allows us to determine the so-called antibody titer, in this case of neutralizing antibodies titer, which is defined as the denominator of the highest dilution in which there is complete neutralization of the cytopathic effect in 50% of the wells. To determine this we apply a statistical formula. Thus, after entering the data in the formula the result indicates how much we need to dilute the serum to obtain protection in 50% of the wells, animals, or eggs, depending on the system that we have used.

This formula is very useful, and it is frequently used to find out the concentration of virus that produces death or infection in 50% of animals (lethal dose 50, or infective dose 50, respectively), in 50% of the wells (which is the TCID 50), etc.

As you can see, the serum neutralization or viral neutralization test is somewhat laborious and slower than the techniques that we will see in the following video, but it is very specific and sensitive and is considered a “gold standard” test for any serological titration.

Thank you very much for your attention.