4.2 The most widely used method for viral diagnosis

Welcome. In several serological techniques the amount of antibodies may be quantified faster than in the viral neutralization test we saw in the previous video, although we cannot evaluate the protective effect they have. These techniques have in common that one of the two reagents, the antigen or, more frequently, the antibody, are marked with a molecule, which may be fluorescent, a radioactive isotope, a heavy metal, or an enzyme. Depending on the marker they are called in one way or another, although the principle is always the same. In this video we will see the tests that use enzymes as markers, in particular one called ELISA, and don't think it's because someone with that name invented it.

In all the tests that use markers, first we have to adsorb or bind antibodies or antigens to a surface that holds them, called solid phase, and the process of adsorption or fixation is called "coating". In the case of the ELISA, the solid phase can be a polystyrene plate, which typically has 96 flat bottom wells. After having coated and washed the wells, the sample to be tested is added in duplicate, i.e. in two wells. The sample may be viral antigen, if the plate is coated with antibodies, or serum if it is coated with viral antigen. We incubate at the appropriate temperature to favour the union antigen-antibody, usually at 37°C. We wash carefully but thoroughly, to remove the reagents that are not retained to the solid phase. We continue adding reagents, incubating and washing as many times as required by the protocol of each particular type of ELISA. The penultimate reagent is usually the "conjugate", which are antibodies bound to an enzyme; that means, they are "labelled". After another incubation and washing, we add the substrate of the enzyme.

The most commonly used enzymes are peroxidase, alkaline phosphatase and luciferase. In all cases it will produce a coloured or uncoloured reaction, whose intensity depends on the amount of enzyme present in the well. We quantify this intensity with the corresponding instrument.

As always, we must not forget to include positive and negative controls. Let's see some types of ELISA.

Indirect ELISA

In the indirect ELISA we coat the well with antigen, we add the test serum, i.e., sample, and the conjugate which in this case are antibodies anti-immunoglobulins of the same species as the sample, marked with the enzyme. This antibody is called secondary antibody. You know that you must incubate and wash between each step. If there are specific antibodies, colour will develop by adding the substrate of the enzyme.

Competitive ELISA

Competitive ELISA is an ELISA technique that can be adapted to detect antibodies or antigens. First let's see how it is done to quantify antibodies. The first step is to add the problem serum to a constant amount of virus, which is the antigen. After incubating, the mixture is added to the wells coated with antibodies. If there were many antibodies in the sample there will be little free virus for binding the antibodies in the well. The last step is to add an anti-virus conjugate, and obviously, the substrate of the enzyme. In the case of a positive sample with high titre, there will be little colour signal in the sample well. That is why it is called competitive.

We could also do this test in a different way, coating with the antigen, instead of with antibodies. In this case, we would simultaneously add the sample serum and an enzyme-labelled anti-virus conjugate. Both types of antibodies compete for binding the virus, so, if the titre of the test serum is high, there is no space for the conjugate and after washing and adding the substrate there will not colour development, or this will be very subdued as we can see in this image.

I’m sure you now know how to make the appropriate modifications to detect viral antigen instead of serum antibodies.

Sandwich ELISA

The last type of ELISA which we will be talking about is sandwich ELISA. The wells are covered with antibodies and the sample is the problem virus. If it corresponds with the antibodies, it stays attached to the well. It is detected by an anti-virus conjugate: the more colour in the wells, the more virus there is.

Before we finish this video, it is convenient for you to know that there are systems that allow differentiating vaccinated animals from infected animals, which is very important in veterinary medicine. To do this, the vaccine has to be designed in a way that it does not contain all the viral antigens, though, of course, it must contain those that stimulate a neutralizing response that we saw in the previous video. These systems are called DIVA, and we employ two different types of ELISA. Stop the video to see their rationale.

Thank you for your attention!