5.4 How is diagnosis done in the lab?

Welcome to the last video of the course about Diagnostic Virology. During these weeks we have been learning many techniques. We are going to review briefly what we have seen:

We first saw the management of viruses in the laboratory. Then we saw a couple of videos on electron microscopy and viral quantification. Then we dedicated four videos to technologies that focus on nucleic acids, both viral and cellular. We destined the fourth block to see how to evaluate antibodies and cells of the immune response to determine if there is a viral infection. And finally, the fifth block was dedicated to biological assays.

But you might wonder: how are samples usually processed in the laboratory?

More than 60% of all cases of human infectious diseases diagnosed by doctors are viral infections. In clinical practice, both human as veterinary, it is essential that diagnosis is fast and accurate in order to control the disease, by antiviral therapy, or more frequently, implementing measures to prevent its transmission to other individuals.

More and more systems are being developed to allow the diagnosis by the bed of the patient, or POC (which is also called point-of-care). Ideally, the test should be fast, simple, sensitive, specific and low cost. To cut costs, the diagnostic tests are increasingly more miniaturized and automated. The analysis of the results is usually done by computer, to eliminate subjectivity and gain in precision.

The correct laboratory diagnosis depends on the sample being taken in the appropriate **moment** and **site**. As for the moment, samples of the affected organs must be taken as soon as possible after the appearance of the first clinical signs, as it is when the viral presence is greater. For the serological diagnosis it is preferable to take a blood sample after one week after infection and another, 3-4 weeks later, to check the increase in antibody titre.

The choice of the adequate sample is related to the clinical signs and understanding the pathogenesis of the disease that we suspect. As a general rule, samples should be taken from the epithelial surface of the entry site, whether it is the throat, the conjunctiva, or a wound. In the additional information you'll find a table that describes different conditions and types of samples to be taken.

If the diagnosis is not made by the bed or in the practice, we must send the samples to the laboratory. It is important to remember that the samples must be of quality, i.e., free of contaminants and representative of the site of the infection. The virus must be viable when it reaches the laboratory in case it needs to be cultivated, so shipping has to be quick, in refrigeration and in a suitable transport medium so that viruses do not desiccate.

Once in the laboratory the sample should be processed immediately or kept refrigerated. In general, we have to homogenize and centrifuge at low speed, then filter it through an adaptable 0.45 µm syringe filter to remove all gross debris. Three different approaches can be followed of which we have already spoken in previous videos: viral isolation, direct detection, and serology.

1. Viral isolation is performed in cell cultures, chicken embryos or in newborn mice, continuing with immunofluorescence techniques, PCR or other techniques of detection of nucleic acids.
2. Direct detection is performed by evaluating the viral components in cells, body fluids or infected tissues. We would do this by means of electron microscopy,

immunofluorescence techniques or even by PCR to detect the viral nucleic acids in the sample.

3. The aim of serology is to demonstrate the presence of IgM, which is an indication of a recent infection, or the increase of at least four times the titre of IgG. For this we can use techniques such as indirect immunofluorescence, serum neutralization, haemagglutination inhibition or ELISA.

This completes this course. We hope that it has been useful and that you have learned with it. Don't forget to take the corresponding test and to review those aspects that have not been clear. Thank you for your attention.