2.1 Can we see viruses?

Welcome back. As you know, the viruses are very small agents. If we want to see them we need to use the electron microscope. This is an instrument that uses a beam of electrons as the source of illumination. As the wavelength of an electron may be 100,000 times smaller than that of the photons of visible light, the electron microscope has a higher power of optical resolution, and you can see the structure of smaller objects.

The electron microscope has allowed the first identification of the agent of important viral processes, such as of the Ebola disease, severe respiratory syndrome (or SARS) coronavirus, and others caused by hitherto unknown viruses. It is also used to identify fastidious viruses, that do not grow well in culture, such as, for example, intestinal viruses, or for those for which there are no specific reagents, or to distinguish the different viruses in the dual infections.

The original electron microscope, which is the one used in diagnosis, is called "transmission electron microscope" or "TEM. Samples most frequently used are the urine or faeces from the patient, but other types of samples may also be used. A drawback of the electron microscope is its low sensitivity, since there has to be at least 10 million viruses per milliliter. For increasing the sensitivity we need to concentrate the virus particles present in the sample, usually by centrifugation: first at low speed to settle the heavier elements of the sample, including cells and bacteria, and then by centrifuging the supernatant at high speed, to settle the viruses. Another way to concentrate the viruses is using antibodies, they aggregate viruses and allow spinning at a slower rate. Specific antibodies can be used when we suspect a specific virus, or a pool of non-specific immunoglobulins, that group viruses indiscriminately.

So we already have the viruses concentrated in a small volume. To see them, we deposit the sample on a special grid coated with carbon. Negative staining is used to see the viruses. It is named that way because reagents cannot penetrate in the interior of structures and they stay on the outside, marking the silhouette, for example, of the viruses.

If the sample that we use are cells or tissues these are too thick for the electron beam to be able to cross them, and we have to prepare thin sections. This is a problem in itself, since if the infection is focal, the area where the viruses are located may be missed.

All this seems complicated. But with practice, in the specialized laboratories it takes 2.5 to 3 hours to complete it. If we have followed carefully the steps of the technique we will have the reward of seeing viruses of different morphologies, as we see in these images.

If we want to confirm which virus it is, or locate it in the different cell structures, we can use gold-labeled monoclonal antibodies. These react with specific proteins of the virus or cell if we want to see its location. Different monoclonal antibodies can even be marked with particles of gold of different sizes to be able to identify more than one protein simultaneously.

Look how well we can see here the gold particles marking these rhabdoviruses. You can recognize these viruses easily by the bullet shape that they possess.

As with all techniques, new types of electron microscopes have been developed.

With the scanning electron microscope (or SEM) the surface of the sample is analyzed with a concentrated beam of electrons to produce the image. The electrons interact with the atoms of the sample, producing different signals that contain information on the topography and composition of the surface. It has a resolution of nanometers! It can be combined with the

electron cryomicroscopy, which freezes the sample rapidly in liquid nitrogen so that the structures will not alter. This technique allows many photos at different angles, and a computer program reconstructs the 3D structure. As you can see in these images, the resulting images of these two techniques can be colored artificially, and they are spectacular.

In this video we've seen applications of the electron microscope, how to prepare samples, how can proteins be located using antibodies and different variants of this instrument. On the internet you can find many images of viruses seen under a microscope. We encourage you to look for some.

Thank you very much for your attention.