2.2 Can we count the number of viruses?

Today we will see some of the procedures used to determine the number of viruses in a given volume and thus establish their concentration. This is called "quantification". Viral quantification is essential in research and development, to prepare vaccines, or to know the amount of viruses that we add to the tissue culture. But also in diagnosis, to evaluate the patient's response to antiviral therapy.

Various methods to quantify viruses have been described. They evaluate their infectivity, or their nucleic acid or their proteins, or they even count directly the number of particles. In this video we are going to talk about some of them.

Plaque assays

One of the most common methods for quantifying viruses is the plaque assay, especially with viruses that lyse the infected cells. It consists of infecting tissue cultures arranged in wells, or in plates, with dilutions of the sample virus. A first step is to remove the culture medium to facilitate optimal contact between the viruses and the cells. After a short incubation, the cultures are covered with semi-solid agar. This is to prevent that the viruses spread freely, so they will only infect cells adjacent to those already infected. After a few days we will see transparent circular areas developing, a sign that the viruses have lysed the cells. These are the so-called "plaques". The number of plaques depends on the number of virions in the inoculum, and it is assumed that each plaque was formed from a viral particle in the sample. They are counted manually, usually with the naked eye, being easier to see them after removing the semi-solid agar and staining the cells, for example, with crystal violet. The result is expressed as plaque forming units, or PFU, per ml. To calculate this value the best thing is to add the viral suspension in triplicate and make the average of the three values. Finally, we just need to divide this value by the dilution used and the volume added to the plate, as you can see in the example of the image.

Focus forming units

You'll be wondering how to make this assay if viruses do not cause lysis, right? The trick is to use specific antibodies against viral proteins, marked with a fluorescent dye. This type of testing with labelled reagents we'll see in the video 4.2. It is a faster method than the plaque assay, since the results can be seen between 24 and 72 hours after infection. But it is more expensive, as we will need more reagents.

Determination of TCID50

The TCID50 quantifies the amount of virus needed to destroy or cause any other type of cytopathic effect in 50% of the cells or infected cultures. It is considered more accurate than the previous methods because concentrations that produce 100% effect may vary widely, and the value of 50% is the most precise. A mathematical formula is applied, which we will discuss in video 4.1, which is used for many other measurements, such as to determine the infective dose 50, etc. The TCID50 value differs from the value of the plaque assays, and statistically, one TCID50 unit is equivalent to 0.69 PFU units.

Protein assays

The viral concentration can also be quantified determining the amount of protein, both total and specific, of a virus. This can be done through haemagglutination techniques, the Western Blot, or immunoassays or ELISA. We will talk about most of these techniques in other videos.

Some labs use other techniques, such as the following.

To quantify viruses by flow cytometry (which we'll talk about in video 4.4) two different fluorochromes are used: one to mark viral proteins and another to mark the viral nucleic acid. Quantitative PCR, which we will see in the video 3.3, measures the RNA, both associated to the virions and free. Finally, we can also quantify the amount of viruses using the transmission electron microscope that we just saw in the previous video.

Using any of these systems we can quantify approximately the number of virions per milliliter. Another interesting concept is that of the MOI that means multiplicity of infection. This is the number of virions to be added per cell during infection. Thus, if we add a million viruses to a million cells, the MOI is one.

As you can see, different strategies can be followed for quantifying viruses. Here we have talked about a just few, but there are many more.

Thank you very much for your attention.