## 1.6 A typical lab

Hello again! We have already seen the three major systems to grow viruses: cell cultures, experimental animals, and embryonated eggs. We have already commented that the first one mentioned, the cell cultures, is the most common, and almost all virology laboratories have infrastructures to grow them. Let's see what they involve.

First of all let's talk about the minimum equipment that we need in our laboratory.

One fundamental is the laminar flow hood or biosecurity hood. In the laminar flow hood we do not use Bunsen burner or any other type of burner. Sterility is provided by a constant, unidirectional air flow filtered through special filters that trap particles. Obviously, in addition we need to follow sterile techniques so that cultures are not contaminated. There are three types of hoods. The type I would be comparable to a gas extraction hood. Type II is the most common for cell culture. Type III is what you've seen in some movies. You will recognize it because it is hermetically sealed, it includes gloves and is used for microorganisms that require Biosafety level 4, that we saw in a previous video. Before starting work and at the end of it we must clean the hood thoroughly with 70% alcohol.

We also need a  $CO_2$  incubator, which provides the right temperature to cells and an atmosphere with lower  $O_2$  content than what we breathe, because the majority of the cells that are used come from inside the body, where the concentration of  $O_2$  is lower than the atmospheric. In addition, it usually has a high humidity content, usually 95%, so that the flasks with cells do not desiccate.

In our laboratory we need an inverted microscope, which has the objectives underneath to be able to focus correctly the flasks with the cell culture.

Other less specific material is a water-bath, to bring the culture media to the right temperature so when we add it to cells, we do not shock them; and a centrifuge, or rather, centrifuges for tubes of different volumes: 15 or 50 ml and 1.5 ml microtubes. In addition, we need a refrigerator at 4°C, a -20°C freezer and a system to preserve ultrafrozen cells, such as a -80°C freezer or liquid nitrogen.

In addition to the apparatus, we need containers in which to grow viruses. Today they are made of plastic and single-use. Many times rectangular flasks are used. We set them down on their larger side, so that the cells have more surface to grow. They are known as T25 if the surface of this large side is  $25 \text{ cm}^2$ ,  $775 \text{ if it is } 75 \text{ cm}^2$ , etc. Other times round boxes similar to the Petri dishes for bacteria are used or even 6-, 24-, 48-, or 96-well plates. None of this should close tightly, because it is necessary that there is exchange of gases, eliminating the  $CO_2$  that is produced through cellular respiration, and using the  $O_2$ . T flasks may have a cap with a filter to facilitate this exchange of gases but which avoids the penetration of bacteria or fungal spores.

We must also use disposable pipettes to add accurate amounts of liquid, tips of micropipette, tubes of different volumes, including 1.5 ml microtubes, and cryotubes that can withstand freezing.

A very important aspect is the culture medium. The cells are very sensitive to changes of pH, osmolarity, salinity, etc., so the culture medium has to be rigorously controlled. The culture medium contains essential amino acids, carbohydrates, vitamins and minerals. Usually it is supplemented with fetal bovine serum (FBS) and antibiotics and antifungal agents.

In this video we aimed to describe what we need in a basic Virology laboratory, including the equipment and consumables (i.e. that are used up with use). In the following video we will see what happens to the virus-infected cells. Thank you for your attention.