

The ideal CO₂ incubator for cell cultures

In your eyes, what defines the ideal incubator for cell cultures? AtmoSAFE has talked to the head of the in vitro Institute for Molecular Biology, Prof. Dr. Gerhard Unteregger, about the requirements made of a CO₂ incubator.

AtmoSAFE: Professor Unteregger, let's get straight to the point. What are the essential quality requirements made of an **incubator** for **cell culture**?

Gerhard Unteregger: Well, above all a constant and high humidity – ideally at least 95 % - as well as fast recovery times after the door has been opened. The most important thing, however, is a constant CO₂ supply to the incubator, since nearly all cultures are kept by means of a CO₂/HCO₃ buffer system. If the CO₂ supply should fail just for a short time, the pH value in the culture medium drops very quickly to an alkaline level.

AtmoSAFE: What does “alkaline“ mean in the context of **cell culture**?

Gerhard Unteregger: The pH value in the cell culture medium is maintained at a constant level through the CO₂ supply, generally within a neutral range. Even the slightest hyperacidity through the metabolism products of the cell can lead to heavy impairment of cell vitality and irreversible loss of function. The alkalinisation of the medium in turn usually causes the cells to die immediately. The user should therefore open the door of the **incubator** as little as possible and make sure before the weekend that sufficient CO₂ is in the bottle cylinder. An automatic CO₂ gas bottle cylinder changeover has been shown to be extremely helpful in practice.

AtmoSAFE: Before, you mentioned humidity in the **incubator**. What should users take into consideration here?



Memmert CO₂ incubator



Professor Dr. rer. nat. Gerhard Unteregger, diploma in biology, in vitro Institute for Molecular Biology

Gerhard Unteregger: Since the **cell cultures** are normally cultivated in an open system, the culture medium is in equilibrium with the ambient air. If humidity there is below 95 % for a longer period, water evaporates increasingly from the **cell culture** medium. Then, the osmotic balance in the cell is lost. Incidentally, an uneven position of the containers can also result in part of the culture left high and dry, so to speak. Normally, the humidification of cultures takes place through a water tray on the floor of the **incubator**. With an active, that is, controllable humidification, the humidity can be controlled with high precision, especially as the danger of sample contamination through the water in the bottom tray is avoided, and the humidity level recovers more quickly after the door has been opened.

AtmoSAFE: Contamination is an important keyword for users.

Gerhard Unteregger: Correct. In this area, there are many worries – both justified and unjustified – because if a sample is contaminated, a lot of time and money may potentially be lost. I have a very clear attitude about this: contamination fundamentally has no place in the laboratory and therefore neither in the **incubator**. And if this should still be the case, then something has gone wrong beforehand. If the “good cell culture practice“ is observed uncompromisingly, then these problems simply do not occur!

AtmoSAFE: Can you give us an example of how contamination could occur?

Gerhard Unteregger: On every square centimetre of your scalp, more than 1 million germs are tumbling around. If you unconsciously scratch your head while working on the clean bench, your hands – even if you are wearing gloves – are contaminated, and there is a great risk that these germs will then be transferred to the cell cultures or to sterile instruments: in this way, contamination is pre-programmed! There is a wide range of contamination causes, which we examine specifically in our seminars, and you would be surprised by what routine errors can creep in, even with experienced laboratory technicians.

AtmoSAFE: What is your opinion on the subject of copper as an antibacterial material in the **incubator**?

Gerhard Unteregger: I believe that if you work cleanly then you don't need it. In the field, you can often hear the opinion that a copper incubator does not need to be cleaned as often as an **incubator** made of stainless steel. This is not correct, of course, germs can also colonise copper surfaces. The important thing is that the surface is as smooth as possible and that all parts in the interior can be removed.

AtmoSAFE: Professor Unteregger, one final question to finish with. You are one of the international experts calling for a "good cell culture practice". Can you describe to us briefly what this means in practice?

Gerhard Unteregger: Cells in vitro are incredibly sensitive and react to different conditions with instability and changed vitality. In order to keep test results comparable, the highest possible level of standardisation is necessary, and this inevitably results in working in accordance with SOPs (Standard Operating Procedures). Manufacturers in turn face the challenge of developing serum-free media, providing defined environments without changes in pH values or humidity, and developing optical, non-invasive control and cell-count instruments.

AtmoSAFE: Professor Unteregger, thank you for talking to us.

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