Smart Notes

What features are essential when considering a CO_2 incubator for housing an in-chamber live cell imaging system?

Cell biologists use a live cell imaging system inside a cell culture incubator to obtain information on cell behavior during normal cell growth. The goal is to observe the cells under *in vivo*-like conditions rather than on a cold microscope stage, so all of the considerations that make a quality environment for cultured cells still apply. Thus, the incubator must provide optimal conditions for growth, just like in a standard CO_2 incubator.

Following a door opening, it is imperative that all incubator parameters recover quickly to set conditions and these conditions must be homogenous throughout the chamber. This includes not only temperature, CO_2 gas and humidity, but in addition, the extra heat generated by the imaging system must be dissipated. Effective, continuous contamination control methods are especially important to help protect the sensitive imager from invading microorganisms. Proven, continuous contamination control features include in-chamber HEPA filtration and 100% pure copper.

In-chamber sensors and probes are critical to ensure that the incubator is reacting to the same conditions experienced by cultured cells. Active air circulation is the only way to ensure homogeneous conditions and to aid dispersion of the heat generated by the imaging system.

Keep in mind that in-chamber imaging systems are very heavy. An empty imager may weigh 52 lb (23.6 kg).[1] A reinforced shelf is recommended to help keep the imager level and secure.

Ideal environment

Factors that affect cell growth include temperature, atmosphere and relative humidity.[2] For proper cell growth, these parameters should be optimal, stable and uniform throughout the incubator chamber. However, these conditions can be affected by 3rd party electronics that generate extraneous heat. Therefore, it is critical to choose an incubator with all sensors and probes located in the incubation chamber, to accurately measure and appropriately react to the same conditions experienced by the cultured cells. In contrast, some CO_2 incubator manufacturers position the sensors in a "by-pass loop"[3] which removes samples of air from the chamber and passes them through tubing to sensors in an external compartment. This by-pass prevents the sensor from reacting in 'real-time' to the conditions experienced by the cells. And this by-pass loop cannot be decontaminated and so presents a continual source of microbial contaminants.

Active air circulation — with in-chamber sensors — provides the fastest recovery of all parameters following a door opening.[2] Live cell imaging systems typically include a moving camera which generates heat. To deal with this, some live cell imaging manufacturers recommend an incubator temperature setting 1-2˚C lower than normal. A circulating fan will help dispel this heat, and dual temperature probes provide security to prevent overheating.

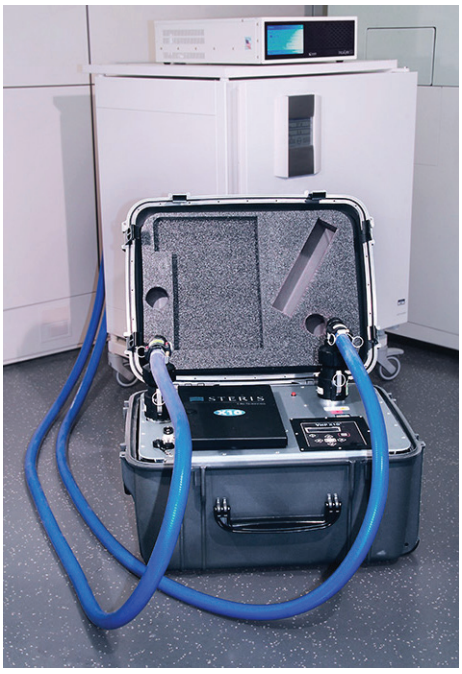

Figure 1:
A Thermo Scientific™ Heracell™ 240i CO_2 incubator housing an Essen™ IncuCyte™ system is decontaminated using a biodecontamination system (Steris Life Sciences). Image courtesy of Essen BioScience Inc., a Sartorius company. (Used with permission.)

Controlling contamination

An in-chamber imaging system requires no manual manipulation by the user; the camera does all the work, and the culture vessels do not move. Therefore, the risk of contamination due to handling or aerosols is minimal, and is no higher than in a standard CO_2 incubator.[4]

As in any incubator, circulating airborne contaminants remain a risk, so a CO_2 incubator featuring proven, effective continuous contamination control will also help protect the imaging system and contents. In-chamber HEPA filtration, coupled with gentle air circulation, will capture all particles regardless of size.[5] HEPA filtration should establish ISO Class 5 conditions in 5 minutes following a 30-second door opening and the filter needs replacing only once per year. A 100% pure copper chamber is proven effective and naturally easy to maintain. Ultraviolet (UV) light, sometimes used as a disinfectant, is not effective in a CO_2 incubator due to the high humidity.[6] In any case, the UV light would only disinfect areas where it is actively shining for at least ten minutes, so areas where the light does not reach – such as beneath the imager or in the by-pass loop – are never disinfected.

A CO_2 incubator manufacturer may claim their models are better suited for in-chamber imaging due to on-board, automated chemical disinfection systems such as hydrogen peroxide vapor. However, there is no proof of the efficacy of such automated systems for decontaminating live cell imaging systems and the hydrogen peroxide droplets must contact every microscopic spot to completely eliminate contamination. Also, the manual handling required to set up such an on-board system is significant[7] and such chemicals can leave white residue which may affect the imaging functions. For these reasons, most labs would not run any automated chemical disinfection more than once per year. Since most in-chamber imaging manufacturers recommend annual maintenance for the imager, this is an ideal time to remove the imager and run a high temperature sterilization cycle designed to provide 12-log sterility assurance according to the U.S. and EU Pharmacopeias.[8,9] As an alternative, a 3rd party Vaporized Hydrogen Peroxide (VHP®, Steris Life Sciences) procedure can be performed by trained technicians who will guarantee neutralization of the dangerous chemical and elimination of all microbial contaminants, as shown in Figure 1. This biodecontamination is approved by the U.S. Environmental Protection Agency (EPA).[10]

For live cell imaging systems residing in a CO_2 incubator, choose an incubator with in-chamber sensors, proven continuous contamination control and minimal manual handling.

References

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