

FACT SHEET

Best Practices for CO₂ Incubator Maintenance

To best support the integrity and health of cell cultures and ensure accurate responses predictive of the *in vivo* environment, new CO₂ incubators offer improved environments for cell culture. A well-designed, properly functioning incubator will ensure that cells grow well, contamination problems are rare and cleaning and maintenance are easy.

The following best practices will keep a CO₂ incubator working optimally and help laboratories avoid common mistakes that may result in project delays. While these recommendations are generally applicable for any CO₂ incubator, user manuals should be consulted.

Reducing contamination

Cleanliness is critical for preventing contamination in cell cultures. Dust and dirt can be carried by air currents created by movement in the lab. Normal indoor room air contains 100–1000 microorganisms per cubic meter, all circulating at any given moment, and most of which come from the trillions of normal flora that live in and on the skin. This means that contaminants can enter each time the incubator door is opened. The lab should be cleaned at least once a month, including cleaning and disinfecting the biological safety cabinet, water bath, centrifuge, microscope and all corners of the lab and around equipment. Cardboard storage in or around refrigerators and freezers should be eliminated as cardboard can get wet and breed fungi. Items should not be stored on top of the incubator because dust and dirt could be swept inside the chamber via air currents created during door opening.

Incubator disinfectants

While many disinfectants are available, not all are safe for cells. Some strong disinfectants emit fumes that enter the incubator and affect cell growth. These fumes contain volatile organic chemicals (VOCs) that can induce expression of heat shock and other stress proteins. Common laboratory chemicals such as phenol, isoamyl alcohol and beta-mercaptoethanol are VOCs, but laboratory cleaning products and disinfectants, and even floor cleaners and waxes, produce harmful vapors.

Thermo Fisher Scientific's technology team tested a number of disinfectants that fulfilled the following criteria: broadly effective against a range of microorganisms and harmless (noncorrosive) to incubator components. A quaternary ammonium disinfectant best fulfilled the requirements. The basic version is widely available from several manufacturers. Some examples include Lysol No Rinse (Reckitt Benckiser), Conflikt (Decon Labs) and Fermacidal-D (Labotect). A 2% solution of the same quaternary ammonium disinfectant that is used to disinfect the incubator interior is added to the water pan. Cleaners containing chlorine should be avoided, because chlorine bleach and derivatives with oxidizing activity can corrode stainless steel and copper and are toxic to cultured cells.

Cleaning the incubator

Regular cleaning (see Figure 1) is necessary to protect cells from contamination and to keep the incubator functioning properly. Guidelines include:

Change the incubator water (not just refill it, but empty and add fresh, sterile, distilled water) at least every other week.

Clean the incubator one to two times per month (depending on the number of users). It is not necessary to autoclave everything; spray or wipe down the incubator with 70% ethanol, especially the water pan (do not spray ethanol on sensors). Allow to air dry.

Check the incubator once per week and discard unused cultures.

If any room air vents are blowing onto the incubator, redirect the air if possible, as air conditioning ducts can contain mold.

Remove anything stored on top of the incubator and clean the top of the unit every two weeks to remove dust. Wipe down the doors and handles with 70% ethanol.

Clean all spills immediately.



Figure 1: A CO₂ incubator should be easy to clean with minimal handling.

Importance of correct water

Tap water with even small amounts of chlorine can corrode stainless steel or pure copper and may contain bacteria and minerals. Deionized or ultrapure type 1 water is very aggressive; because it contains few ions, the water actively pulls ions from the stainless steel, pure copper, glass door and other incubator components, causing pitting and corrosion. Reverse osmosis water can vary significantly in terms of quality because purification is a percent-removal process. Thus, if the starting water has 500 ppm, the finished water might be 50 ppm; if the starting water has 150 ppm, the finished water would only have 15 ppm. Best results are obtained with sterile, distilled water with a pH between 7 and 9. For best operation and long life, conductivity of 1–20 microSiemens/cm (resistivity of 50 K-1 M Ohm-cm) is recommended.

Commercial antimicrobial agents may be added to the the pan water to prevent microbial growth. Examples include Aquaguard-1 (Promokine), Aqua EZ Clean (T-Pro Biotechnology) and SigmaClean (Sigma Aldrich).

General incubator maintenance

Other than regular cleaning and disinfection, incubators generally require minimal maintenance. Replace HEPA filters (see Figure 2) every six months to a year, depending on the number of users, cleanliness of the unit and laboratory and incubator design. Handle HEPA filters only by the external housing, without touching the filter medium. Before replacing a HEPA filter in a CO₂ incubator, inspect the medium to ensure there are no breaks or tears. The HEPA filter should be located in the incubation chamber for best function and be easy to replace without using tools. Replace gas inlet filters (where the CO₂ gas enters the incubator) every six months to one year.

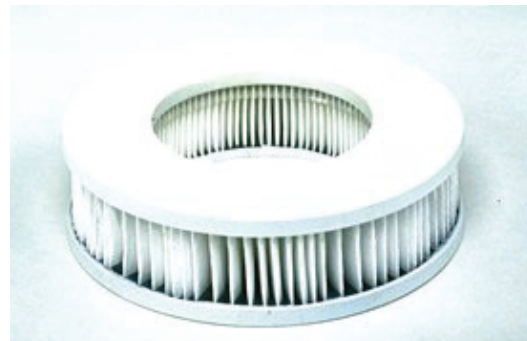


Figure 2: Typical HEPA filter installed in an incubator chamber.

CO₂ calibration should be monitored regularly using a CO₂ analyzer or handheld sensor, from once per month to quarterly, depending on traffic in and out of the incubator.

The frequency with which the heat sterilization cycle is run depends on the cleanliness of the lab, how many people use the incubator, how often the door is opened and how convenient it is to shut the incubator down overnight. Most users use this function from one time per month to once every six months.

Perhaps most important for incubator maintenance is keeping a high level of water in the water pan. A drop in humidity leads to evaporation of water from the culture medium. When water evaporates, the carefully balanced salts, minerals, amino acids, etc., in the growth medium become too concentrated, which can result in toxicity and cell death. Low humidity can damage the CO₂ sensor as well.

Conclusion

Choosing an incubator that provides ideal conditions for sensitive research and development is critical. The incubator should be easy to maintain and successfully control contamination.

Sources:

American Laboratory article by Mary Kay Bates, M.S. (<https://www.americanlaboratory.com/914-Application-Notes/189342-Best-Practices-for-CO2-Incubator-Maintenance/>).

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