GUIDE TO PROPER
CO_2 INCUBATOR USE AND PREVENTIVE MAINTENANCE

By: Adam Christensen

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When cells are grown for research in a lab setting, CO_2 incubators are used to create an ideal growth environment. Researchers depend on both the incubator and the lab manager responsible for operation and maintenance of the incubator.

Incubated cell cultures potentially represent months, even years, of valuable research that is lost if cells die. Therefore, incubator reliability and stability are of the highest importance. In addition to mechanical failure, contamination due to human error or from the surrounding environment also poses risks.

A lab manager can minimize risks in three ways:

1) Properly training all incubator users.
2) Implementing a preventive maintenance program.
3) Choosing the most advantageous location in the lab for the CO_2 incubator.

Not only is the CO_2 incubator important to the researchers, but it is also an investment for the institution or company. The lab manager's role in proper use and maintenance ensures the organization gets the most from its investment via research productivity and long life of the incubator.

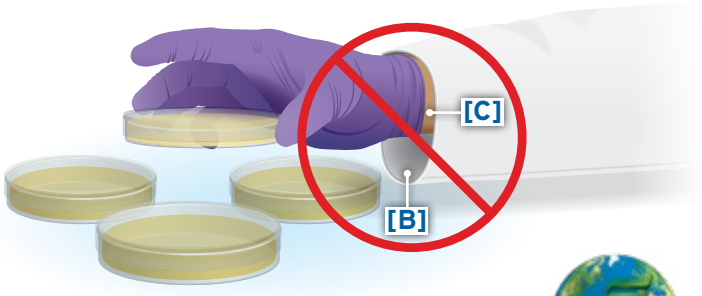
This guide will help lab managers understand risks and best practices as they develop training for CO_2 incubator users and establish a preventive maintenance program and setup criteria.

1) Training the General User

Multiple users interact with an incubator. Because each interaction adds risk of human error, all users should be trained on the proper use of the equipment. Training for the users should focus on minimizing the risks to cell health. All lab users should clearly understand the following best practices.

Proper Method for Introducing Materials Into the Growth Chamber

Proper personal protective equipment (PPE) must be worn. Also, good laboratory practice, aseptic technique, and standard operating procedures (SOPs) must be followed at all times based on your laboratory's biosafety level (BSL). Wearing gloves that fit over the cuff of the lab coat **[A]** is recommended. Loose cuffs increase the probability of cross contamination, due to excess cloth hanging from the arm **[B]**, which increases the likelihood of fabric brushing against culture materials. Also, the opening between the gloved hand and sleeved arm **[C]** allows access to the epidermis as well as potential contamination from a user shedding skin cells.



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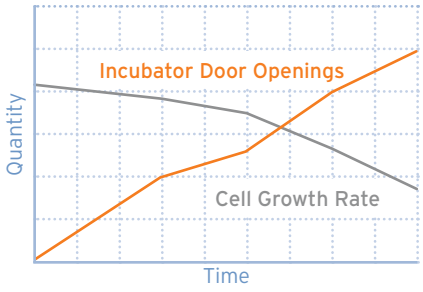
Gloves should be cleaned with an appropriate disinfectant, such as 70 percent ethanol, before handling materials. Culture flasks, plates, and petri dishes should be appropriately labeled before being placed in the growth chamber. When placing culture media into the chamber, it is recommended to set materials in the chamber starting from the back to avoid reaching over or distributing existing media. Make a note of which materials may have to be removed first, and place them toward the front of the incubator. Avoid stacking culture media, and position flasks with caps facing away from the door to prevent contamination. Prevent disruptions to the growth chamber by keeping door openings to a minimum.

Minimizing human error when using an incubator

Product technology aids in minimizing human error, but it can never replace good laboratory practice and a culture of accountability. A training program should be created for new users. Ongoing training sessions or refresh sessions can take place on a yearly basis to review Standard Operating Procedures (SOPs) and promote best practices as a means to reduce risk. **Documentation is the key to identifying trends, good or bad**. Documenting use and preventive maintenance helps staff remain accountable for their actions, and also acts as a reminder for tasks such as the weekly inspection of filters. Documentation may also help identify patterns, such as a negative effect on cell growth related to frequent openings of the incubator door. Proper training, documentation, and accountability, aid in creating a lab culture focused on results.



2) Establishing a Preventive Maintenance Program

A critical aspect of ensuring reliable incubator operation of is preventive maintenance. Preventing failure is better strategy than rushing to mitigate the negative effects of a failure or a contamination event. Not only can routine maintenance prevent failures, but it can also minimize contaminants and increase the life span of the incubator—both of which further increase research productivity.

If an incubator stops running, or conditions are not kept stable, the risk is cell death. While it's difficult to put a precise cost on the value of lost cell cultures, research time is wasted, work repeated, and learning is delayed. Such failure events can be minimized when the incubator is cared for properly.

Preventive Maintenance Program Overview

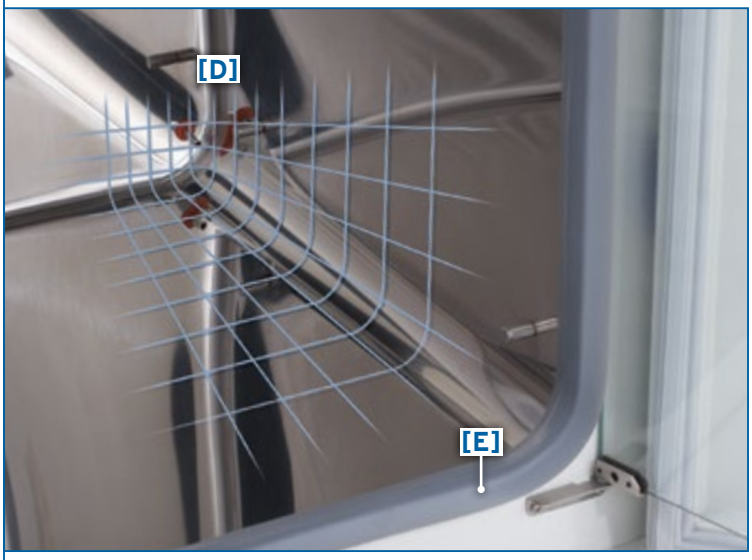
Consulting **a manufacturer's preventive maintenance checklist** is a good first step. This is a list of specific tasks and special instructions, and the frequency at which they are performed to keep the incubator running at optimal conditions. It is important to know that the frequency of tasks can vary from lab to lab based on the cleanliness of the lab environment, the number of people using the lab, and even the type of cell cultures. The lab manager must take these factors into consideration when setting the frequency of tasks for their lab. In addition to the preventive maintenance checklist, SOPs should document what to do when a problem is discovered during preventive maintenance.

Frequent Exterior and Interior Cleaning

The frequency of cleaning the exterior and interior of the CO_2 Incubator depends on the type of cell cultures being stored and potential for contamination within the lab.

The exterior of the incubator must be cleaned periodically to remove dust and other contaminants which have settled on surfaces. These contaminants have the potential to enter the growth chamber due to air turbulence created by opening the incubator door. The exterior may require cleaning as frequently as once per week in high traffic labs, or as little as once per month in low traffic labs with fewer door openings. Use a cleaning agent such as a solution of mild household detergent or bleach and single distilled water.

Cleaning the stainless steel interior one to two times per month is recommended. Incubators designed with one continuous interior surface **[D]** can reduce the risk of contamination. Use an appropriate disinfectant, such as 70 percent isopropyl alcohol, 70 percent ethanol, or similar non-corrosive disinfectant. Pay special attention to seams and crevices in which contaminants may accumulate. For example, an irregular surface such as the incubator door gasket **[E]** is a high risk area for contamination. Clean the door gasket carefully, or remove it for autoclaving.



Cleaning the Water Pan

Cleaning the pan and changing the incubator water in the pan of the is one of the most critical preventive maintenance tasks. This must be done on a **weekly basis** to minimize contaminants and maintain the humidity in the incubator. When refilling the pan with water, use only single distilled water.

Prevent Desiccation

If the cell culture environment does not provide adequate humidity levels, moisture can evaporate from the cell culture medium and cause cells to dry out, an avoidable risk to cell health. Refilling the water pan weekly is important for preventing desiccation.

Minimize the Risk of Contamination

Contaminants, such as bacteria, molds, viruses, or mycoplasmas, are the single greatest risk to the cells in your incubator. Modern incubators offer a range of designs and features that can reduce your biological contamination risk.

HEPA Filtration

The most important feature to minimize contamination risk is the closed loop HEPA filter. HEPA filtration is the primary means by which a virtually contamination-free environment is maintained. To work effectively, HEPA filters should be changed according to the preventive maintenance schedule. Lab SOPs should indicate how frequently a filter should be checked i.e., weekly or bi-weekly. The average life of an air inlet filter **[F]** is three to six months.

As the lab manager, you will need to determine the frequency of filter changes based on the number of users and cleanliness of the laboratory. NuAire recommends changing the gas supply line filter(s) **[G]** every fifth tank or when the filter is discolored. The air pump capsule **[H]** must be replaced every two years.

Understanding HEPA Filtration

A CO_2 incubator equipped with HEPA filtration is essentially a miniature cleanroom. The atmosphere of the growth chamber is continually cycled through a HEPA filter to produce Class 100 air in the growth chamber.

Each time the incubator door is opened, contaminants such as mold spores can potentially enter the growth chamber. If spores settle onto growth media, mold can damage cultures and disrupt research.

In a CO_2 incubator manufactured by NuAire, contaminants introduced during a door opening are quickly removed from the growth chamber. NuAire incubators HEPA filter the entire growth chamber atmosphere once every 20 minutes. This continual filtration produces ISO Class 5 air quality.

Incubators without HEPA filtration are more susceptible to problems related to contamination.

Sterilization Cycle

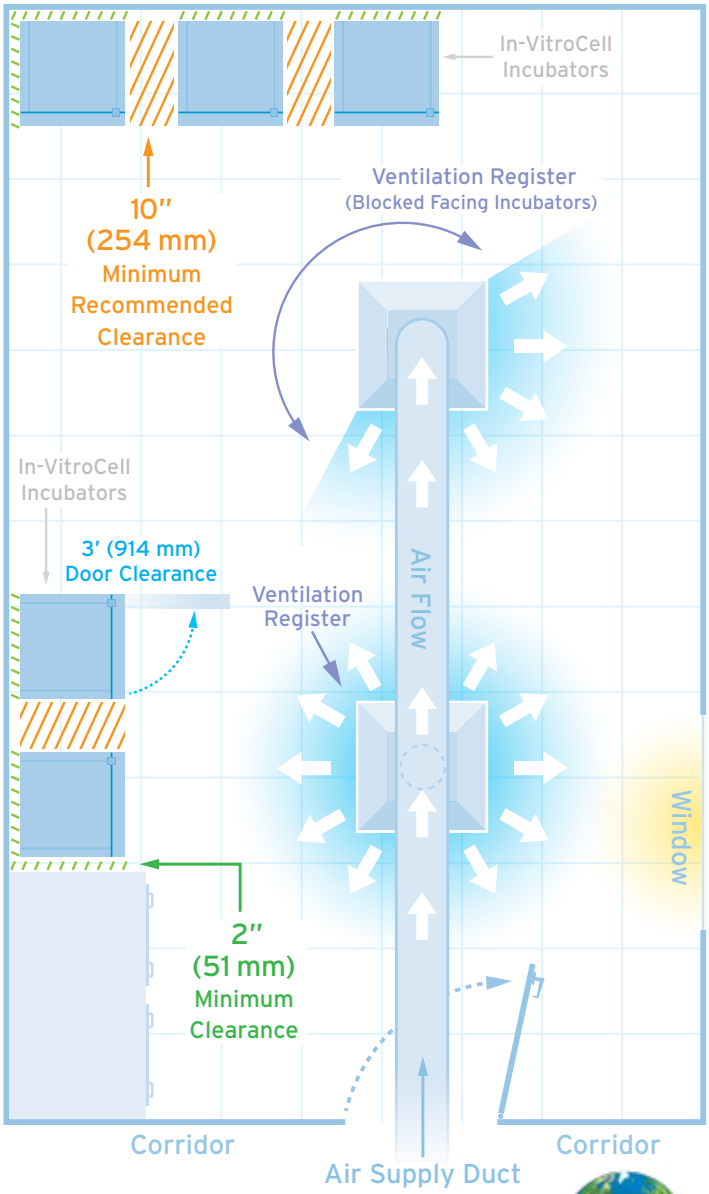
For laboratories that grow potentially hazardous microorganisms such as the HIV virus, an incubator with a 145° C sterilization cycle can help minimize risk. The sterilization cycle lasts from 8 to 14 hours and is often run overnight or during a similar period of low usage.

Choose an incubator designed for optimum ease-of-use with a 145° C sterilization cycle. For example, NuAire CO_2 incubators feature shelves and internal hardware able to withstand 145° C. This saves time since there is no need to remove shelves prior to the sterilization cycle, and also sterilizes these components along with the growth chamber.

Use of a 145° C sterilization cycle is based on laboratory SOPs, after evaluation of contamination risk. The sterilization cycle should not be used as a replacement for routine cleaning.

3) Considerations for Placement of the Incubator

Careful consideration should be given to the location of the incubator in the lab. Choose a low-traffic area with a stable temperature, away from sinks or water sources. Locate the incubator away from strong air currenct such as an air conditioning vents that may be a source of contaminants, windows which may be a source of localized high temperature due to sunlight and away from other equipment that might produce heat such as an autoclave. Also select a location near a reliable power source.



Conclusion

The lab manager plays an important role in minimizing risks to the incubated cell cultures entrusted to their care. Risks to cell cultures can be greatly minimized by training users on the best practices when interacting with the incubator, strictly following a preventive maintenance program, and wisely choosing the location of the incubator.

About the Author

Adam Christensen is a family man first and attempts to be a service technician second. Born and raised in the heart of MN with a fishing pole in hand. Trained by experts at South Central College in Mankato, MN for HVAC, he ended up putting time and effort into the Biological industry at NuAire. Learning about Biosafety cabinets, incubators, centrifuges and eventually taking hold of the Ultralow Freezer responsibilities in the service department. Adam has now become a customer service specialist in the Biological and Ultralow industry.

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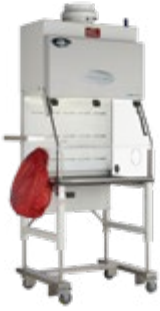

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