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CELL CULTURE CONTAMINATION – PART 2

THE ROLE OF THE CO_2 INCUBATOR IN REDUCING CONTAMINATION

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This is the second in a three-part series on cell culture contamination.

As mentioned in the first installment of this three-part article series, biological contamination is a serious issue for every cell culture laboratory. With implications for the reliability of resulting data, it is vital that the occurrence of contamination is kept at an absolute minimum. Having discussed common sources of contamination, here we look at the use of the CO_2 incubator and its role in providing a safe environment, free from any potentially contaminating microorganisms.

"Good laboratory practice is the most effective way of preventing contamination."

Cell culture relies on the use of incubators to maintain the right conditions for keeping cells alive. The CO_2 incubator specifically aims to simulate mammalian physiological conditions. Therefore, the incubator combines the elements needed for cells to thrive: a stable temperature at 37 °C (98.6 °F), a controlled pH of 7.4 to 7.6 balanced with a controlled CO_2 level and a high relative humidity of 95 percent. Unfortunately, the ideal environment for mammalian cells also provides an ideal environment for a range of biological contaminants that are normal flora in and on our bodies. This is why it is so important to understand good laboratory practice and how choosing the right equipment can help reduce contamination. Certain CO_2 incubators, for example, have been designed to reduce contamination and can make a real difference in the laboratory setting.

Getting it right: Good laboratory practice

Good laboratory practice is the most effective way of preventing contamination. By wearing a laboratory coat with elastic cuffs to cover street clothes, washing hands thoroughly before beginning any work with cells and wearing disposable gloves, workers can greatly reduce the potential for contamination. As much as possible, culturists should also avoid touching items such as door handles, telephones, calculators, etc.; avoid wearing jewelry; and tie back long hair. Anyone suffering from a cold or other respiratory infection should wear a face mask to minimize the potential spread of infection.

Working areas and the tops of refrigerators, freezers, storage cabinets and benches should be kept clean, uncluttered and free of dust. Floors should be cleaned regularly, especially corners, to minimize dust and dirt that will circulate as a result of traffic in the room. In addition, laboratory equipment (e.g., mechanical pipettor, vortex, water bath, centrifuge) should be cleaned and regularly checked for signs of contamination.

There is no substitute for proper aseptic technique. Cultures and media should be opened only in the biosafety cabinet and should not be shared between personnel. A common route of contamination is entry by a liquid "bridge" that forms when a droplet of culture medium remains between the culture vessel and its lid or on the neck of a culture medium bottle. It is not uncommon for lab workers to relax their technique after a contamination-free period, as demonstrated graphically by Ian Freshney,[1] who also provides a complete discussion of aseptic best practices that should be standard in every lab.

The CO_2 incubator, as the home for your cultured cells, is a key point where good laboratory practice must be maintained. Remember that the perfect environment for mammalian cells is also an inviting environment for microbial companions. Since the route of entry for any incubator contaminant is through the open door, a working system needs to be established that keeps door openings to a minimum. Try to limit the number of people sharing an incubator to reduce sources of contamination. Set a standard and regular cleaning procedure (weekly or monthly) and immediately clean any spills using 70 percent alcohol.

Incubator design: Keeping contamination in check

The design of the CO_2 incubator can be an important factor in the reduction of contamination by biological agents. There are a number of helpful features that simplify cleaning and provide ongoing protection from contamination during routine use. These capabilities differentiate incubator choice, and it is important to recognize that similar options from different manufacturers will not necessarily provide the same results.

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Easy to clean

When the incubator is easy to clean and features minimal internal surfaces, the presence of microbes can be significantly controlled. An incubator with rounded corners eliminates cracks and edges where microbes can hide and prosper. Surfaces that are electro-polished remove tiny depressions that would otherwise serve as hiding holes for germs.

There are a few good rules to follow when cleaning an incubator:

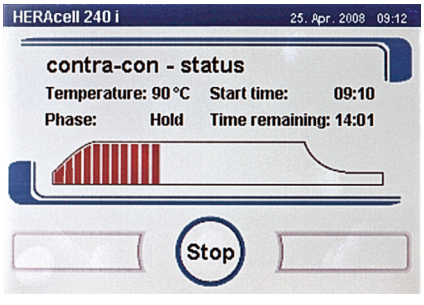
- Remove all cultures from the incubator and turn it off.
- Remove all separate internal components of the incubator and clean them using a soapy disinfectant.
- Sterilize all removable parts in an autoclave.
- Thoroughly clean all interior surfaces and then apply a disinfectant to all surfaces and allow it to dry. Any remaining residue can be removed using distilled water.
- Wipe all surfaces with 70 percent alcohol and allow them to air dry.
- Disinfect the outside doors and handles of the incubator using 70 percent alcohol.

External water reservoirs eliminate a potential breeding ground for contaminants inside the incubator.

If a water pan is present, adding a safe antimicrobial agent to the water is a good idea. Use distilled water in the pan and change it every week. Be sure to wear gloves and a lab coat throughout the cleaning procedure to minimize the introduction of new contaminating organisms.

High-temperature disinfection

A convenient option available on some incubators is an automated high dry-heat or moist-heat disinfection cycle. Most of these are designed to be run overnight with an empty incubator. High-heat disinfection offers an effective way to ensure that the interior of the incubator is free of



On demand decontamination cycles eliminate the need for autoclaving or use of toxic chemicals inside the incubator.

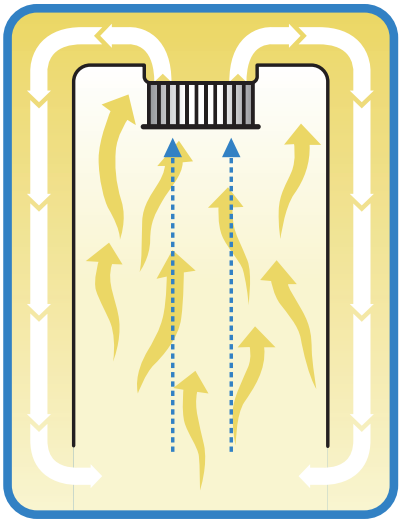
germs when your cells first enter the incubator after a cleaning cycle. There are different options available from different manufacturers, but not all of these are similarly effective,[2,3] and users should decide which option will best suit their needs.

While the disinfection cycle does not eliminate the need to routinely clean the incubator, it can remove the need to separately autoclave internal components and eliminates the risk of "resident" contamination.

Air filtration

A popular feature available on some CO_2 incubators is a HEPA filter, which will filter the internal air, removing microbes, particulates, aerosols and even, in some cases, volatile organic chemicals. A Class 100 HEPA filter should quickly provide clean-room air quality so that any contamination entering through the door is removed.

Consider the time that it takes to achieve maximum air quality after a door opening, especially if frequent door openings are inevitable. This recovery time can vary depending on the manufacturer.[4] HEPA filters require little maintenance but should be replaced on a regular basis (i.e., every six months) for best results.



▲ *In-chamber HEPA system surrounds cultures with Class 100 (ISO Class 5) cleanroom-like air quality.*

"... the perfect environment for mammalian cells is also an inviting environment for microbial companions."

Antimicrobial surfaces

Historically, copper has been used as a way to control microbial contamination, including that of bacteria, viruses and fungi. Ancient civilizations including the Aztecs, Greeks and Romans used copper as a topical treatment for skin diseases and wounds,[5] and copper vessels have long been safely used to store water and other foods. Copper can inactivate enzymes and damage proteins in the cell, since Cu^{2+} ions penetrate the pores of cell membranes and react with the -SH groups of enzymes, thus altering protein structure. Stainless steel and aluminum do not inhibit microbial life, and alloys with minimal copper content show much less benefit. The antimicrobial effect is directly related to the amount and quality of the copper used. Pure copper has been proven to be the most effective antimicrobial surface material, with an ability to inactivate methicillin-resistant *Staphylococcus aureus* in only 1.5 hours. Alloys containing less copper, such as brass, show a much slower response and considerably less antimicrobial effect overall.[5]

Some CO_2 incubators offer an option of copper interiors to inhibit growth of any germs that may enter the incubator when the door is opened. As noted above, only 100 percent pure copper will eliminate microbial contaminants effectively within minutes. The Cu^{2+} ions in solid copper will not become airborne, so cultures in dishes and flasks are not at risk. This is a great way to have continuous antimicrobial protection in the cell culture incubator that will last the life of the incubator while requiring minimum maintenance. Imam El-Danasouri of California Reproductive Laboratories, a long-time user of copper-lined incubators, explains, "...copper incubators reduce the possibility for infection in the humidification water or on the incubator walls."[6]

Protection from external environment

Most incubators come with a solid-glass inner door to protect samples from inadvertent exposure to the outside environment. A divided gas-tight inner door will further help to minimize exposure and speed recovery to set conditions after a door opening. This divided inner door may offer three or six separate smaller doors that allow access to specific sections of the incubator without disturbing other areas. The divided inner door reduces any opportunity for microorganisms to enter the incubator, and minimizes the loss of heat, atmosphere and humidity from the incubator.

External water source

Water is required for life, including, of course, microbes. While they enjoy the warm, humid atmosphere inside the CO_2 incubator, the water pan is especially inviting. Antimicrobial compounds, copper wire and even pennies are often added to the water pan. Laboratories with advanced applications or particularly valu-

"... the disinfection cycle does not eliminate the need to routinely clean the incubator..."

able samples may also consider an incubator design that moves the humidifier water source outside the incubator, thus entirely removing this source of contamination from the incubator interior.

Conclusion: Fighting a winning battle

The biological contamination of cell cultures is an occasional problem for every cell culture user. Contamination costs millions of dollars in lost time and materials every year, which could otherwise be spent on research and development. While cell culture contamination cannot be totally eliminated, because microbes are our constant companions, carefully controlled processes can be implemented that reduce the impact of an episode. Good aseptic technique, a clean laboratory and an understanding of the routes of contamination, including entry to the CO_2 incubator itself, are crucial. Manufacturers of CO_2 incubators are partners in the process and now offer many options that help to minimize contamination of the incubator.

In the third and final article of this series, we will expand on the preventive measures and technologies mentioned here, enabling you to confidently maintain a contamination-free culture environment.

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