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CELL CULTURE CONTAMINATION — PART 3

EVALUATING CONTAMINATION PREVENTION SOLUTIONS FOR THE CO₂ INCUBATOR
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This is the third in our three-part series on CO₂ incubation.

Contamination of cell cultures by bacteria (including mycoplasmas), viruses, and fungi, or even cross-contamination by other cell lines, can result in a significant loss of resources for any research or pharmaceutical laboratory. The most effective way to reduce the risk of biological contaminants is the proper use of aseptic techniques when working with cells and reagents. Basic good laboratory practices are also important and include effective sterilization of equipment, media, and reagents; using dedicated media for each cell type; wearing gloves and lab coats; and keeping the laboratory free of dust and clutter. The CO₂ incubator, used to provide the ideal environment for cell culture propagation, also provides an

excellent environment for the growth of microbes, so it must be considered especially deserving of attention. Different methodologies exist for prevention and elimination of contamination in CO₂ incubators. The best options for your lab depend on the number and types of cells you grow, the number of personnel in your lab, and how the pros and cons of the method fit with your workflow.

Contamination prevention methods

It is virtually impossible to prevent microbes from entering the CO₂ incubator every time we open the door, unless the laboratory itself is a cleanroom facility. Microorganisms, primarily bacteria, are our constant compan-

“In 2008 the U.S. EPA certified that pure copper ‘kills 99.9 percent of bacteria within two hours.’”

ions. They circulate in the air and cover every part of our bodies. In fact, recent sampling using swabs of human skin recovered 10,000 microorganisms/cm².¹ We cannot help but shed bacteria from our skin, hair, and breath, and they fall on the culture vessels and into the incubator chamber.



◀ *Starting with a clean incubator and using proper aseptic technique is the best way to reduce the risk of biological contaminants.*

Therefore, in recent years, cell culture incubator manufacturers have introduced a number of different options to help prevent growth of unwanted microorganisms inside the incubator—even after they enter. An understanding of available methodologies will ensure that you select the technology best suited to your laboratory's requirements and work environment.

Pure copper touch surfaces

One approach for preventing microbial growth in the incubator requires no hands-on time or maintenance and is thousands of years old: pure solid copper. Ancient societies successfully used copper as a topical treatment for skin diseases and wounds. Today, copper has enjoyed a popular resurgence in the clinical sciences, and in 2008 the U.S. EPA certified that pure copper “kills 99.9 percent of bacteria within two hours.”² Copper has even been shown to be effective against bacterial spores.³

In recognition of copper's effective antimicrobial activity, incubators featuring 100 percent pure solid copper interior chambers have developed a strong following within the cell culture research community. Use of copper in CO₂ incubators makes even more sense with the understanding that higher temperature and higher relative humidity increase copper's antimicrobial effectiveness.⁴ Reflecting the increased interest in copper, more manufacturers are now offering variations of the copper solution, including copper plating and stainless steel alloyed with small amounts of copper. However, plating has the tendency to scratch and peel, leaving behind unprotected surfaces, and it is clear that alloys must contain a very high proportion of copper—greater than 60 percent—to be effective.^{5,6}

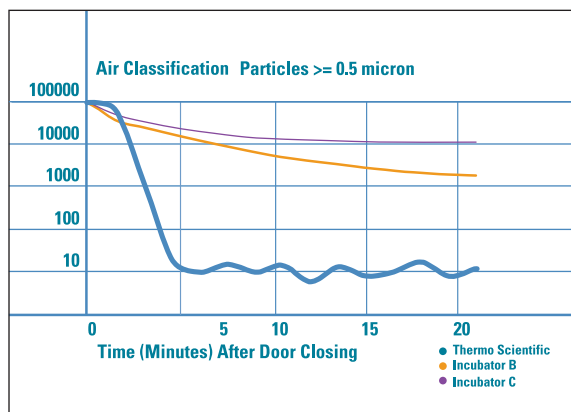
By what mechanism does copper kill? The details are not yet clear; however, we know that copper ions disrupt and damage the microbial cell membrane and either enter the cell or cause cytoplasmic contents to leak out. Reactive oxygen species cause further damage, and the DNA is degraded.⁴ Does this mean that copper surfaces present a risk to cultured cells growing in the CO₂ incubator? No! The copper ions do not become airborne, so they do not pose a risk to cultured cells. Copper kills only on contact.

Solid copper incubator chambers require no maintenance other than occasional cleaning, just like stainless steel. Over time the copper will tarnish due to oxidation, and this is good. The tarnish actually improves the antimicrobial efficacy. In fact, in tests on *E. coli*, tarnished 99 percent copper killed more than 100,000 bacteria in

60 minutes. The tarnish was then removed and the copper tested again. In this 60-minute test with untarnished 99 percent copper, fewer than 50 bacteria were killed.⁵ Alloys that do not tarnish have very low, if any, antimicrobial efficacy.

HEPA filter

High Efficiency Particulate Air (HEPA) filters are commonly used in many applications including health care and safety. They trap airborne pollutants including dust, allergens, and microorganisms, and some can trap volatile organic chemicals.



▲ *The Thermo Scientific Forma Steri-Cult and Steri-Cycle incubators incorporate in chamber HEPA filtration systems that continuously filter the entire chamber volume every 60 seconds and provide Class 100 (ISO Class 5) cleanroom conditions within 5 minutes following a door opening.*

A HEPA filter is made of randomly arranged borosilicate fibers. The filter traps pollutants via three different mechanisms: interception and impaction (which trap particles larger than 0.4 μm) and diffusion (which traps tiny particles, especially those that are smaller than 0.1 μm). The tiny particles collide with air molecules in Brownian motion. The collisions slow the speed of the particles, and they become stuck in the filter.⁷ Thus, particles between 0.1 and 0.4 μm are hardest to capture. This is why HEPA filters are rated according to the most penetrating particle size of 0.3 μm, which they remove with at least 99.97 percent efficiency.⁸ Particles larger and smaller than 0.3 μm are actually caught even more efficiently.

Well known for use in biological safety cabinets, HEPA filtration is ideal for use inside a CO₂ incubator to protect cultured cells from airborne contaminants that enter through the incubator door. HEPA filters are generally

inexpensive and easy to replace and last six months to one year. Some incubators offer a convenient alarm to remind you to replace the filter. The soiled filter can simply be autoclaved with other laboratory waste prior to disposal. When evaluating incubators with a HEPA option, be sure the HEPA filter meets requirements to trap 99.99 percent of particles. The more quickly the filter cycles through the entire air volume in the chamber and conditions are recovered after a door opening, the greater its protective value.

Methods for elimination of contamination

Periodically, a cell culture incubator should be cleaned and decontaminated to completely eliminate all microbial life. This requires removing all cultures and is generally done once every week to every two months. Some CO₂ incubators offer automated methods to perform this task. The great advantage of these automated systems is that they simplify cleaning the unit by eliminating the need to separately autoclave removable parts or use germicidal cleaners.

High-temperature decontamination

Many direct-heat incubators now offer a high-heat decontamination cycle that runs overnight. These processes aim to eliminate the need for removal, separate autoclaving, and reassembly of shelves and other incubator components.

However, it is important to ask exactly what is required to prepare for the decontamination cycle offered. Not all incubators use CO₂, humidity, and oxygen sensors that are compatible with these high

temperatures, so these sensors must be removed prior to the procedure and replaced afterwards, which takes time and also poses a risk of reintroducing contamination.

It can be confusing to compare the different manufacturers' approaches, since temperatures range from moist heat at 90°C to dry heat at 180°C, and there are no ISO guidelines for sterilization of an empty chamber. Thus, the best way to evaluate the different incubators is to look for data generated by independent testing laboratories showing elimination of test organisms.

UV light

Ultraviolet (UV) light is a well-known procedure for disinfection of biosafety cabinets used in cell culture laboratories. UV light is occasionally offered as a decontamination mechanism in some CO₂ incubators. However, the U.S. CDC, NIH, and NSF no longer recommend UV as the sole method of disinfection.⁸ There are several reasons for this. It is commonly recognized that the cleanliness, temperature, and size of the bulb will affect the UV light output, so a particular bulb may not be providing the amount of germicidal activity that is advertised. This is especially relevant to CO₂ incubators operating at high humidity levels, because the germicidal effects of the UV light drop off precipitously with relative humidity above 70 percent.⁹ Also, the amount of germicidal light emitted from any UV lamp decreases with the bulb's age, so it is difficult to determine if it has been truly effective.⁸

One paper shows a 24-hour UV decontamination in an empty incubator that is as effective as high-heat decontamination in eliminating bacteria and fungi;

however, according to the manufacturer's instructions, the UV cycle must be immediately followed by a complete cleaning of the chamber with 70 percent isopropyl alcohol,¹⁰ which itself would eliminate these organisms. Thus, it is difficult to determine whether the elimination of microbial life was due to the UV or due to the alcohol disinfection. Another problem with using UV is that anything that blocks the light (including dust particles, shelves, and air ducts) prevents effective disinfection. This means that in order for UV light to decontaminate a CO₂ incubator, all internal components must be removed and autoclaved separately for decontamination.¹⁰

Sample	Run 1		Run 2	
	Total Cells	Log Reduction	Total Cells	Log Reduction
Positive control	2.46x10 ⁶	0	1.52x10 ⁶	0
Door	<2	>6.08	<2	>5.88
Floor	<2	>6.08	<2	>5.88
Left Side	<2	>6.08	<2	>5.88
Right Side	<2	>6.08	<2	>5.88
Back	<2	>6.08	<2	>5.88
Ceiling	<2	>6.08	<2	>5.88
Positive control	2.31x10 ⁶	0	2.36x10 ⁶	0

▲ *Elimination of Bacillus subtilis spores verifying the ContraCon 90°C moist heat decontamination system. A concurrent test showed zero growth in broth, proving complete eradication. Test procedures were performed by CAMR (Porton Down, UK).*

Toxic chemicals

Antimicrobial compounds of various types can be used to clean the interior of CO₂ incubators, and different chemicals have been employed as disinfectants. Some examples include chlorine vapor, hydrogen peroxide vapor, formaldehyde, and ozone. The problem with use of chemicals in the cell culture incubator is that it is difficult to be sure that all traces of the chemicals have been eliminated, and additional safety precautions may be required that are difficult to implement in the lab. Experimental evidence shows that even very low amounts of volatile organic compounds are highly soluble in culture media and result in cytotoxicity and expression of stress proteins.¹¹ Use of these approaches should be considered only when administered by trained professional service providers, so they are not recommended for routine use.

Conclusion

When evaluating options for controlling and eliminating contamination in your CO₂ incubator, consider ease of use and proven effectiveness (with independent data) as among your primary concerns. The best methods are those that require little hands-on time to be effective. A combination of a continuous contamination prevention strategy with a periodic decontamination method and good aseptic technique will ensure that your valuable cells continue to grow securely, contributing to your research or production goals.

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