PHCbi

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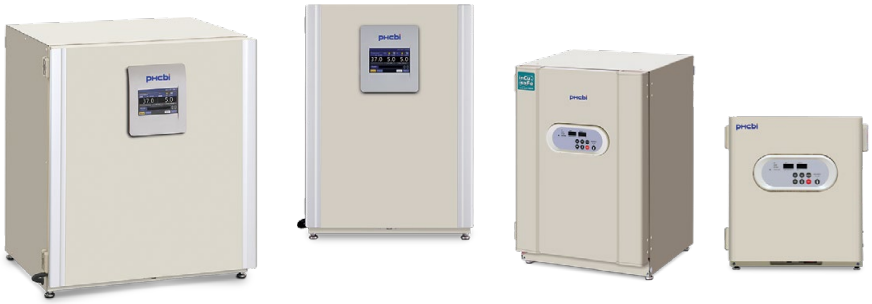
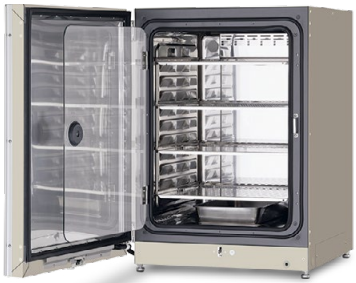
Contamination Control Methods in Multigas (CO_2 and O_2) Cell Culture Incubators

APPLICATION NOTE

AVAILABLE SYSTEMS AND COMPARATIVE BENEFITS



Left to right:
MCO-230AICUVL-PA | 10830-874, MCO-170ML-PA | 10854-386,
MCO-18ACL-PA | 10046-924, MCO-5ACL-PA | 10046-922



High Heat Sterilization
MCO-170AICUVDL-PA | 75856-512

Abstract

Any time the cell culture incubator door is opened, a migration of airborne contaminants occurs as the interior environment seeks equilibrium with the laboratory environment. During such door openings, airborne pathogens that enter the incubator typically come to rest on wall and shelf surfaces, on the surface of humidity reservoir water or in cell culture vessels. Despite the fact that cell culture vessels or roller bottles with filtered caps are used for cell culture processes inside the controlled environment of the incubator, contamination remains a threat to cell viability.

Once the inner door is closed, setpoint conditions must be restored. For this reason, contamination control in the cell culture incubator must be managed by both passive and active decontamination methods. CO_2 and multigas (CO_2 and O_2) incubators are designed to create a carefully blended environment configured to replicate *in vivo* conditions *in vitro*. Factors include precise temperature control and uniformity at all shelf locations, gas concentrations and elevated humidity to eliminate desiccation of cell culture media.

This application note describes contamination control among the PHCbi branded cell culture incubator line, and how user preference, protocol, and best practices are accommodated in product selection regardless of preferred method.

PHC Corporation of North America
Formerly Known as Panasonic Healthcare Corporation of North America

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The Cell Culture Solution

The cell culture solution is based on a series of mutually dependent systems working together to offer a safe, productive *in vitro* cell culture environment. We apply the following the following into a dynamic cell culture system designed to reward good laboratory techniques for the most critical and highly regulated protocols. Key features include the following:

- Choice of high heat sterilization or vaporized hydrogen peroxide (H_2O_2) decontamination
- Infrared (IR) CO_2 and zirconia O_2 sensor technology
- Self-compensating narrow bandwidth ultraviolet (UV) light
- Gentle airflow to minimize desiccation
- Intelligent microprocessor control
- Graphical monitoring and data collection

Manual, Passive and Active Contamination Control

Incubator design is intended to create an accurate *in vitro* model of the *in vivo* environment. Cells *in vivo* benefit from naturally occurring contamination defenses. The *in vitro* environment, however, is particularly susceptible to bacteria, yeasts, spores, fungi and other airborne pathogens introduced into the chamber during normal door openings.

Whenever contaminants enter the chamber, the threat to active cell cultures remains. We have established a range of contamination control methods to match a variety of user preferences.

The Productivity Advantage

Type	Description	Application
Manual	Manual, conventional, 70% ethanol solution	Requires intervention. Used on all PHCbi Cell-IQ™ incubators as desired.
Passive	InCu-saFe® copper enriched, stainless steel surfaces	Requires no intervention. Available on all PHCbi Cell-IQ incubators.
Passive	SafeCell™ UV Cycle in background, without ozone production	Requires no intervention if in automatic mode. Available on selected PHCbi Cell-IQ incubators.
Active	SafeCell UV Cycle without ozone production, 24 hour cycle.	Manually initiated. Available on selected PHCbi Cell-IQ incubators.
Active	High Heat Sterilization Cycle, 180°C (355°F)	Requires intervention. Available on selected PHCbi Cell-IQ incubators.
Active	Vaporized Hydrogen Peroxide (H_2O_2) Cycle	Requires intervention. Available on selected PHCbi Cell-IQ incubators.

Automatically coordinated processes within PHCbi cell culture incubators work together to maintain optimum *in vitro* conditions including temperature and CO_2 and CO_2/O_2 control while mitigating contamination. When repeatable and precise experiments are required, PHCbi incubators offer a complete selection of solutions to meet your needs.

Selection Summary

Model		MCO-230AICUVL-PA 10830-874	MCO-170AICUVL-PA 10119-820	MCO-170AICUVDL-PA 75856-512	MCO-170ML-PA 10854-386	MCO-5M-PA 10046-930	MCO-18ACL-PA 10046-924	MCO-5ACL-PA 10046-922	MCO-80ICL-PA 10046-928
Volume	cu.ft. | liters	8.1 | 230	5.8 | 165	5.8 | 165	5.7 | 161	1.7 | 49	6.0 | 170	1.7 | 49	30.1 | 851
Gas Control									
Sensor	CO_2	IR, Dual Wave**	IR, Dual Wave**	IR, Dual Wave**	IR, Dual Wave**	TC*	TC*	TC*	Solid-State IR**
Sensor	O_2	—	—	—	Zirconia	Zirconia	—	—	—
Manual Wipe Down Contamination Control Method									
Ethanol	70%	Anytime	Anytime	Anytime	Anytime	Anytime	Anytime	Anytime	Anytime
Passive Contamination Control Method									
InCu-saFe		Standard	Standard	Standard	Standard	Standard	Standard	Standard	Standard
SafeCell UV**		Standard	Standard	Standard	Optional	Optional	Optional	Optional	Optional
Active Contamination Control Method									
SafeCell UV**		Standard	Standard	Standard	Optional	Optional	Optional	Optional	Optional
High Heat		—	—	Standard	—	—	—	—	—
Vapor	H_2O_2	Optional	Optional	—	Optional	—	—	—	—

*TC – Thermal Conductivity
**IR, Dual Wave includes a dual array sensor; IR Solid State includes a single sensor.
***SafeCell UV can function in automatic mode (in the background - passive) or with user intervention (active)

Manual Cleaning

Surface disinfection with a 70% solution of ethanol in water offers a quick, cost-effective and time-proven method of killing contaminants on incubator interior surfaces. Known as a "manual wipe down" this type of decontamination method is widely used in clinical and research laboratories. The manual wipe down requires that all cell cultures, shelves, trays, pans, plenum components and other peripherals be removed, leaving the incubator surfaces exposed to the 70% ethanol spray. Concentrations of ethanol below or above 70%, including pure ethanol, are less effective due to rates of evaporation, cellular membrane penetration rates and other characteristics that inhibit the process.[1] Once the incubator is ready for cleaning, interior components can be autoclaved or manually wiped down in advance of returning the incubator to service.

InCu-saFe Contamination Control

We use inCu-saFe copper enriched stainless steel alloy for all interior surfaces of the cell culture incubator. This passive decontamination method is the first line of defense against airborne contaminants that migrate to interior surfaces during door openings. InCu-saFe has the germicidal properties of 100% copper, but does not permit discoloration over time or diffusion of airborne oxidized copper.

InCu-saFe Construction for Germicidal Protection

PHC Corporation of North America offers exclusive use of inCu-saFe interior surfaces within a technical design created to eliminate contamination sources and to mitigate the effect of airborne contaminates introduced through normal use.

- Selected to provide natural germicidal protection without rust or corrosion, inCu-saFe inhibits the growth of fungi, mycoplasma and bacteria when exposed to humidity and CO_2.
- Interior components have been reduced in most recent Cell-IQ series models including integrating molded shelf channels into the walls for easy cleaning.
- When components are removed, all interior surfaces are exposed for conventional wipe down.
- Large curve corners and electro-polished surfaces are easy to clean.
- Copper is infused into the stainless steel to assure that germicidal properties will remain throughout the life of the incubator.

How InCu-saFe Inhibits Mycoplasma: Survival Results

Mycoplasma Strain	Negative Control	Conventional Type 304 Stainless Steel	InCu-saFe	Conventional Copper C1100
Mycoplasma fermentans PG18	no survival	survival	no survival	no survival
Mycoplasma orale CH19299	no survival	survival	no survival	no survival
Mycoplasma arginini G230	no survival	survival	no survival	no survival
Mycoplasma hominis PG21	no survival	survival	no survival	no survival

The chart above summarizes test results with four strains of mycoplasma. Results demonstrate how inCu-saFe offers germicidal properties of conventional C1100 copper while maintaining and discoloration-resistant properties of conventional Type 304 stainless steel. Detailed test results are available from PHC Corporation of North America.

SafeCell UV Efficacy

The SafeCell UV decontamination system arrests and destroys contaminants within the incubator chamber. The SafeCell UV system is based upon an isolated, narrow bandwidth (253.7nm) ozone-free ultraviolet lamp interlocked with the incubator door. This multifaceted approach to contamination control is designed to destroy airborne particulates introduced during door openings, as well as contaminants that may grow in the water reservoir. With active and passive systems working together in our performance model, contaminants that inevitably enter the chamber through routine door openings or other means are intercepted and destroyed while cell cultures continue uninterrupted.

Added Benefit: UV Safe and Effective During *In Situ* Operation

During normal operation when cells are being incubated within the chamber, the UV lamp is visibly isolated from the cell culture chamber by a plenum cover on the back wall and over the humidity pan. This permits UV decontamination of circulated, humidified air and humidity pan surface water to continue without damaging the cells. The UV cycle is factory set to glow for 10 minutes following each door opening, and automatically adjusts for ON time as the lamp ages. The lamp ON time is programmable from 0 to 30 minutes, depending on user preference. The position of the UV lamp, as well as the relationship between the lamp, plenum, humidity reservoir and airflow system, is integral to the performance of the Cell-IQ incubator.

A decontamination cycle with the SafeCell UV light can be programmed to operate up to 24 hours.

Vaporized Hydrogen Peroxide (H_2O_2)

H_2O_2 vapor decontamination yields a 6 log reduction over a range of organisms tested.[2] This process uses H_2O_2 vapor in conjunction with narrow-bandwidth ultraviolet light and copper enriched stainless steel to broaden CO_2 and CO_2/ O_2 incubator applications to highly regulated, environmentally sensitive cell cultures.

Throughout the entire cycle, the incubator airflow system continues to gently circulate interior air assuring 100% vapor contact with all interior surfaces. This ultimately creates a breakdown of H_2O_2 into harmless water and oxygen as it passes over the UV lamp, converting all H_2O_2 to less than 1 part per million following the completion of the decontamination cycle. No ozone is produced from this decontamination process and the door interlock prevents access. Shape and location of interior components permit the components to remain in the chamber during the decontamination process, bypassing the need for a separate autoclave cycle or manual wipe down. Once the cycle is complete, the door locking system is released and the incubator is returned to use.

High Heat 180°C (355°F)

The high heat sterilization system manages time and temperature for a proven method of sterilization at 180°C (355°F).[3]

During this process, thermal cabinet insulation maintains lower exterior cabinet surface temperatures and minimizes heat transfer to the lab and other surrounding equipment. The insulation and dual heater* decontamination system is energy efficient. Removal of interior components, CO_2 sensor and UV light are not required. The high heat sterilization cycle is controlled through the incubator microprocessor control system. Total process time required is 11 hours. Outer door is locked automatically upon initiation of the decontamination cycle and unlocked upon completion.

*Dual Heater - the high heat sterilization process utilizes the incubator's primary heater (heater jacket system) plus the high heat sterilization cycle heater to achieve 180°C.

Decontamination Turn-Around Time

H_2O_2 vapor and high heat 180°C (355°F) sterilization are offered for customer preference. Both require periods of downtime during which cultures must be removed and placed in other incubators.

- The high heat sterilization cabinet design includes high tech insulation and door gaskets to withstand 180°C (355°F) while maintaining relatively cool surface temperatures.
- The H_2O_2 vapor design requires no special consideration for materials such as metal surfaces, gaskets, outlets, sensors or other interior components.
- Regardless of process used, all cell cultures must be removed prior to the process.
- Interior components are easily rearranged for decontamination.
- Initiation of the overnight heat decontamination sequence requires a measure of advance administrative planning to accommodate the culture relocation and downtime.
- The H_2O_2 cycle can be completed in less than 3 hours, start to finish. The incubator can be returned to service immediately upon completion.
- During normal operation, the combination of inCu-saFe and SafeCell UV decontamination controls continuously scrubs the incubator for airborne and waterborne pathogens that can cause contamination or cross contamination among cultures.

1) http://www.americanpharmaceuticalreview.com/Featured-Articles/184449-Pharmaceutical-Facility-Sanitization-Best-Practices-Considered/

2) Contact PHC Corporation of North America for test data

3) http://www.cdc.gov/hicpac/disinfection_sterilization/13_10otherSterilizationMethods.html

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