

Application Note

A cell culture CO₂ incubator with continuous contamination control: Part 2

DESIGN FEATURES AND EVALUATIONS

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The dynamic nature of the cell culture CO₂ incubator presents a significant design challenge for the achievement of superior control of the purity of the incubator environment. Part 1 in *American Biotechnology Laboratory* (vol 20, no.1, pg. 16) reviewed the major concerns pertinent to the achievement of superior contamination control. Part 2 describes the development and validation of the model MCO-20 AIC (manufactured by **SANYO Electric Biomedical Co., Ltd.**, Osaka, Japan; distributed by **SANYO Scientific**, Itasca, IL) approach to optimizing the cell culture environment through a process of Active Contamination Control™.

The cell culture CO₂ incubator incorporates a number of known and newly developed findings to create an envelope of activities to achieve continuous contamination control throughout the active processes involved. It employs an isolated, narrow-bandwidth ultraviolet (UV) light (Figure 1) to destroy air-borne contaminants in the incubator chamber, as well as water-borne organisms in the humidity water reservoir. Integrated with copper-enriched interior surfaces and components that inhibit the growth of organisms without suffering surface discoloration, the incubator offers an optimum cell culture environment that eliminates the need for high heat decontamination, frequent chamber cleaning, and associated downtime.

Active Background Contamination Control is an integrated process combining several proven techniques in arresting and destroying particulates in the chamber including:

- An isolated, narrow bandwidth (253.7 nm) ozone-free UV lamp with door interlock (U.S. patent 6255103)
- A copper-enriched stainless steel interior chamber with copper-enriched stainless steel shelves, brackets, and plenum components (patent pending)
- A patented direct-heat (air) heating system with three independent heating zones (Figure 2) (U.S. patent 5519188)
- A directional air-flow and containment plenum surrounding a UV-exposed (U.S. patent 5519188) humidity reservoir in a removable, stainless steel pan (Figure 3).

The multifaceted approach to contamination control destroys air-borne particulates introduced during door openings, as well as contaminants that typically grow in the water reservoir. With active and passive systems working together, contaminants that inevitably enter the chamber through routine door openings or other means are intercepted and destroyed while cell culture continues uninterrupted.

UV protection

Because cell cultures are incubated in clear media vessels, the use of conventional UV light for contamination control in the cell culture incubator has not been possible previously. UV light will destroy cell cultures, and ozone gas emissions from the UV lamp are toxic to cells. Ozone buildup, combined with unprotected UV exposure, can create user and environmental hazards in the laboratory. UV light cannot reach hidden interior surfaces.

The MCO-20AIC is the first cell culture incubator

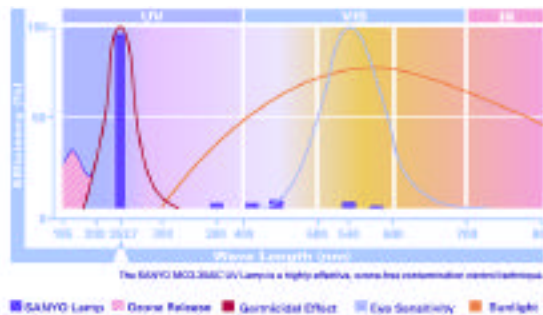


Figure 1 Unlike typical germicidal lamps, the patented, long-life SafeCell™ UV lamp is designed to deliver straight line performance at approx. 253.7 nm for maximum germicidal efficiency and long life.

to incorporate an active cycle UV function within the chamber for contamination control purposes. Marketed as SafeCell™ (**SANYO Electric Biomedical Co., Ltd.**), the UV lamp generates a narrow 253.7-nm bandwidth emission that is toxic when directly applied to microorganisms, but outside the 185-nm bandwidth that generates ozone toxicity.

UV light affects DNA by causing pyrimidine dimers to form when adjacent pyrimidine bases on the DNA strand become covalently linked (i.e., chemically bonded to one another). The dimer disrupts the normal replication of the DNA or transcription to make protein. Cells can usually repair themselves by a rather complex mechanism involving several enzyme groups because the second strand of DNA is unaffected by the mutation, so replacement DNA can usually be synthesized.¹

Because the UV lamp is visibly isolated from the cell culture chamber by a plenum cover, UV sterilization of air and water remains in process while cell culture continues uninterrupted. The lamp “on” time is programmable from 0 to 30 min 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 incubator.

Lamp life

The SafeCell UV lamp is an instrument-grade bulb specifically fitted with UV-resistant boots to protect seals from breakdown. Lamp “on” time is monitored by the microprocessor controller. The bulb is easily replaced when required; no tools are needed. The lamp cycle is factory set to glow for 5 min following each door opening. The cycle is programmable, and may be set to 100% on for overnight exposure of an empty chamber if desired.

Copper-enriched stainless steel interior surfaces

Conventional incubators with copper-bonded interiors or seamless copper interior inserts offer passive resistance to contamination through the natural process of copper oxidation, which destroys microorganisms attached to the surface. The copper surface is subject to discoloration through oxidation and reactions to cleaning agents. Although the copper surface is effective, other interior components in conventional

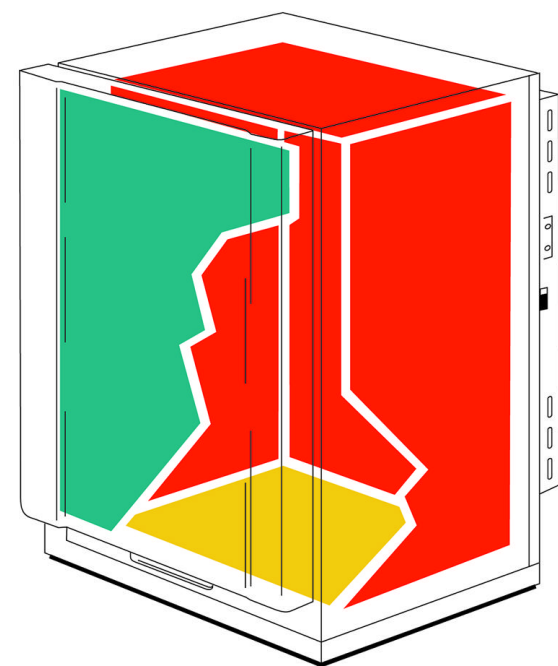


Figure 2 An air jacket with five independent heating elements arranged in three zones surrounds the interior chamber. The microprocessor control system apportions energy to heaters in response to chamber demand and ambient temperature. Side, top, and rear walls form the dominant radiant heat source. The base heater elevates the humidity reservoir water temperature to achieve 95%RH at 37 °C. The outer-door heater warms the inner glass in response to ambient conditions to eliminate condensation on the glass and around the opening, and to ensure interior uniformity.

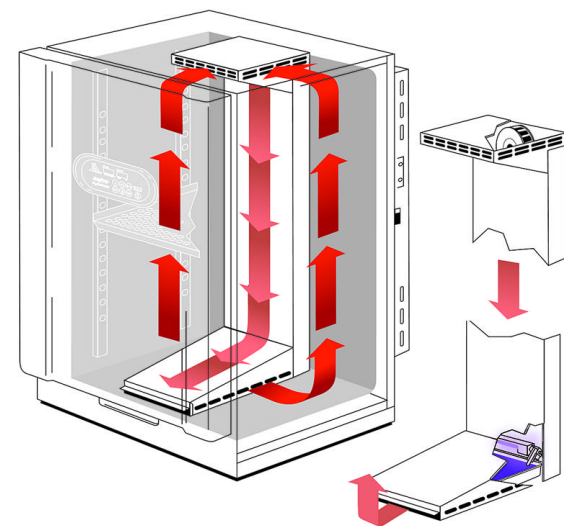


Figure 3 The airflow process within the incubator chamber reveals the relative position of the blower and UV lamp in relationship to the humidity pan and outflow ducts at the base of the chamber. The high-impact auto-clavable plastic blower wheel easily snaps off for cleaning and replacement if required.

incubators such as shelves, brackets, supports, plenums, and humidity pans are fabricated of conventional stainless steel, which can support bacterial, fungal, and other growth.

Direct Heat, Air (DHA) airflow system

The MCO-20AIC is an air-jacketed CO₂ incubator

Table 1

Test reagent	Corrosion test results at one month Incubator interior surface*		
	InCusaFe copper- enriched stainless steel	Copper (C1100)	Type 304 stainless steel
Distilled water	N	D	N
Ion-exchanged water	N	D	N
70% Ethyl alcohol	N	D	N
70% Isopropyl alcohol	N	D	N
1/500 Sodium hypochlorite	N	D	N
1/1000 Sodium hypochlorite	N	D	N
1/100 Bezalkonium chlorite	N	D	N
2% Glutaraldehyde	N	D	N
0.5% Glutaraldehyde	N	D	N
0.05% Chlorohexidine gluconate	N	D	N
Biocidal ZF disinfectant (WAK-Chemie Medical GmbH, Bad Soden/Ts., Germany)	N	D	N
1/10 Osydolum	N	D	N
1/500 Formalin	N	D	N
Eagle MEM medium (10% FBS)	N	D	N
PRM1 1640 medium (10% FBS)	N	D	N
Germicidal properties	Yes	Yes	No

*D = Discolor; N= No change.

that creates stable, uniform temperatures within the chamber through a combination of directional airflow technique and three-zone heating managed by a microprocessor controller.

The patented base heating and airflow systems are inherently related to the contamination control methodology through reduction or elimination of condensation, constant air motion through the plenum, and direct heat applied to the removable humidity pan at the base of the chamber.

The three-zone approach with independent heaters offers greater control sensitivity than standard blanket heaters or water jackets can provide, and is particularly effective in minimizing surface temperature variations on interior walls, which would otherwise cause condensation in the high humidity atmosphere. A base heater allows humidity levels to remain elevated through direct heat applied to a removable humidity pan. Because cell cultures are particularly susceptible to drying out or desiccating, especially those plated in 96-well or smaller SBS (Standard of the Society for Biomolecular Sciences) plates, the water level in the humidity pan is a critical consideration. An optical water level sensor warns of low water level in the humidity pan. When water evaporates and the surface reaches a minimum depth, the optical sensor activates a flashing lamp on the main control panel to prompt refilling with distilled water. For refilling, the plenum lifts and the humidity pan slides forward. The optical sensor lifts automatically. When the pan is inserted, the sensor returns to position and the plenum lowers.

The position of the humidity pan, which is exposed directly to the UV lamp under the plenum, is important. Although many airborne microorganisms are attracted to the copper-enriched walls and other germicidal interior surfaces, migration of contaminants into the humidity water reservoir is inevitable. In conventional incubators, the humidity reservoir is a leading source of contamination.

However, in the MCO-20AIC, the SafeCell UV lamp glows directly over the humidity pan and effectively destroys contaminants in the water. As warm air passes through the plenum, it passes over the UV lamp, then across the water reservoir where moisture vapor is brought through the front and sides for gentle circulation through and around the perforated shelves. Because water in the humidity pan is directly exposed

Table 2

Material	Thickness (mm)	Transmit rate (%)	Typical use
Glass	1.0	0	Petri dish
Polystyrol	0.05	0	Petri dish, culture flask, microplate
Distilled water	3000	10	Humidity water in pan

Table 3

Species	Contamination test results after 24 hr Kill rate by surface type (%)*	
	InCusaFe copper-enriched stainless steel and conventional copper	Type 304 stainless steel
<i>E. coli</i> (ATCC8739)	99.928	0
<i>E. coli</i> (IFO3301)	99.847	0
<i>S. Aureus</i> (ATCC6538P)	99.998	0
<i>B. subtilis</i> (ATCC6633)	99.997	0
<i>B. stearothermophilus</i> (ATCC7953)	99.870	—

*Bacteria killing rate = (1 test sample colony no./control colony no.) × 100 (n = 3).

to the UV light, there is no need to add potentially toxic germicidal agents to the distilled water. Germicidal agents can increase surface tension and lower relative humidity potential at 37 °C. Their effect on active cell cultures must be considered as well.

Copper-enriched stainless steel

Interior components of the MCO-20AIC are fabricated from a copper-enriched stainless steel that exhibits the same appearance and resistance to discoloration as conventional stainless steel but has inherent germicidal characteristics found in the copper bonded or copper insert cabinets.

Marketed as inCusaFe™, this material extends to all interior surfaces including perforated shelves, shelf brackets, and plenum components.

CO₂ sampling and inject system

The incubator uses a ceramic-based infrared sensor system to maintain precise CO₂ control regardless of temperature and relative humidity changes within the incubator chamber. The CO₂ sensor externally samples both ambient air (for automatic calibration) and interior air (for CO₂ density measurement). Both ambient and chamber air samples are filtered through 0.3-µm HEPA in-line filters in advance of the infrared sensor to protect the sensor and returned air to the chamber from contamination. In addition, CO₂ inputs to the incubator from dual-stage regulator(s) are filtered through 0.3-µm HEPA in-line filters. A CO₂ sampling port is located on the front of the chamber.

Laboratory testing and performance evaluations

Numerous performance tests on the MCO-20AIC incubator were conducted to corroborate the efficacy of SafeCell UV and inCusaFe contamination control methodology. Incubator interior samples shown in Table 1 were exposed to distilled water, phosphate buffer, and DMEM (dulbecco) medium for one month. No corrosion was present on inCusaFe copper-enriched stainless steel and conventional Type 304 stainless steel samples tested; conventional copper surfaces discolored under all tested reagents. Table 2 shows the safety characteristics of UV lamp emission. The bacterial kill rate following a 24-hr test (Table 3) illustrates the germicidal prop-

erties of inCusaFe copper-enriched stainless steel vs conventional Type 304 stainless steel at normal operating temperatures

Safety

While the 253.7-nm emission from the UV lamp is effective against air-borne pathogens as well as those in the humidity water pan, the ozone-free emission at narrow bandwidth does not penetrate glass or plastic cell culture vessels, does not affect cell cultures in vitro, and does not affect incubator users.

In cell cultures it is ozone release free, <0.01 ppm (under detectable dose), after one week with the lamp continuously "on." UV emission does not penetrate the culture vessel, <0.001J/m² (under detectable dose), inside of the culture bottle. For incubator users the UV lamp cut-off switch is automatically "off" during door openings. In the worst case scenario, failure of auto cut-off function, UV emission from the front of the chamber without airflow plenum (pan cover) in place is <0.03W/m².

Conclusion

Together with the passive resistance of copper-enriched stainless steel, the active effort to destroy airborne contaminants in vitro forms effective contamination control unique to the MCO-20AIC incubator. As the cell culture process continues in the incubator chamber, the work of germicidal protection from thermophilic and airborne organisms including bacteria, mycoplasma, molds, yeasts, spores, and fungi continues unabated without costly downtime.

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