TECHNICAL DEVELOPMENT REPORT

Use of Narrow Bandwidth Ultraviolet Light and Copper-Enriched Stainless Steel Construction To Achieve Active Background Contamination Control™[1] In A Cell Culture CO_2 Incubator

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SANYO

SANYO Electric Biomedical Co., Ltd. has developed a cell culture CO_2 incubator which employs an isolated, narrow-bandwidth ultraviolet light to destroy airborne contaminants in the incubator chamber, as well as water-borne organisms in the humidity water reservoir. Integrated with copper-enriched interior surfaces and components which inhibit the growth of organisms without surface discoloration, the SANYO incubator (Model MCO-20AIC) offers an optimum cell culture environment through a process of "Active Background Contamination Control™" which eliminates the need for high heat decontamination, frequent chamber cleaning and associated downtime.

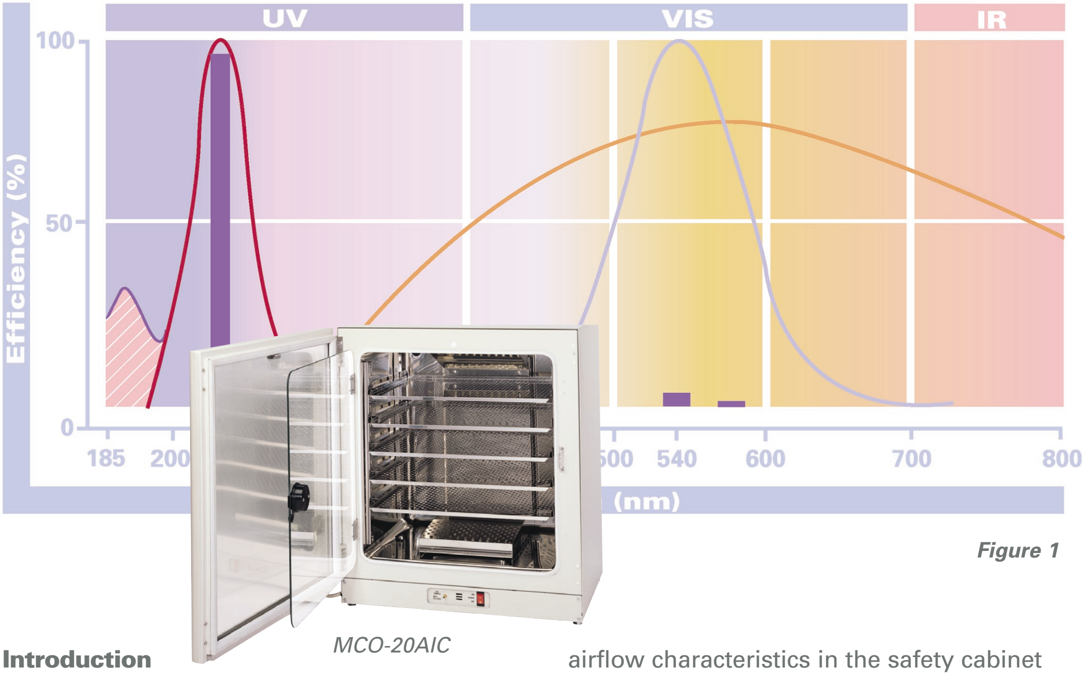

Figure 1

MCO-20AIC

Introduction

The CO_2 incubator is an essential tool in the research and clinical laboratory. Unlike basic laboratory storage and processing systems collateral to incubators such as refrigerators, ultra-low freezers and centrifuges, all of which use labware closed, capped or sealed for aseptic protection, the CO_2 incubator performs a more dynamic function which directly exposes cell cultures and culture media to the enriched atmosphere within the chamber.

Unlike a biological safety cabinet, the incubator cannot minimize the migration of airborne particulates into the chamber when the inner door is opened during routine use. Efforts to integrate basic CO_2 and humidified incubators with Class II, Type A/B3 biological safety cabinets, in fact, have been generally unsuccessful due to airflow characteristics in the safety cabinet which accelerate culture media desiccation leading to cell lysis in the *in vitro* environment. This desiccation effect is more sensitive the smaller the media vessel becomes, and practical use of multiwell plates in routine cell culture, and extended culture times require that vapor pressures on relatively low volume media plates be maintained at 37°C through aggressive humidification at or near 95%RH.

Thus, in achieving a stable, humidified environment, the use of a CO_2 incubator has traditionally posed a high risk of contamination leading to loss of cell cultures or expressed products, loss of laboratory efficiency due to downtime, compromise in reproducible results, and need for repetition of complex cell cultures.

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Part I

Conventional Contamination Control

Contamination Sources

Typical contaminants in cell culture include mycoplasma, bacteria, molds, spores, yeasts and fungi. Despite the fact that most contemporary cell culture vessels are packaged in sterile wraps and opened for plating in biological safety cabinets with optimum laboratory technique, contaminants usually cannot be eliminated altogether, nor can they be totally mitigated by adding expensive antibiotics to culture media, or chemical algaecides and fungicides to the incubator chamber surfaces and humidity reservoir.

In general, unless work is being performed in a Class III environment, laboratory investigators accept the fact that some migration of airborne contaminants into the incubator chamber is unavoidable when the chamber door is opened and shelves are extended, media plates added and chamber atmosphere exposed to room air. Because contamination sources are varied, it is necessary to restrict or eliminate risks through proper laboratory technique, and to minimize or eliminate contamination if and when it occurs *in vitro*.

Incubator Types

To understand the benefits of *Active Background Contamination Control*™, familiarization with basic CO_2 incubator construction and performance is required. CO_2 incubators are designed to achieve stable temperature throughout a setpoint range of above ambient to 50°C or 60°C, although most cell culture protocols are fixed at 37°C with a 5% CO_2 density in air. Humidification of approximately 95% helps minimize desiccation of culture media; higher humidity levels usually cause condensation.

Water-Jacketed Incubators

Water-jacketed incubators are widely used in both clinical and research applications. These double or triple-wall designs include stainless steel interior chambers surrounded by a water mass heated by an immersion or blanket heater at the base of the chamber. An interior water reservoir, usually a stainless steel pan, is placed on the chamber floor and filled periodically with distilled water. When equilibrium is achieved, these incubators can maintain temperature setpoint (37°C) with approximately 95% to 98% humidity, and with CO_2 metered through an air/gas flowmeter (constant flow) or injected on demand via a thermal or infrared sensor (automatic).

Prior to the advent of air-jacketed models, water-jacketed incubators were considered to be most stable for long-term cell culture applications with infrequent door openings.

Design challenges in water-jacket incubator construction include a variety of trade-offs to offset inherent performance inefficiencies created through repeated door openings, depletion of humidity reservoir water, top-to-bottom temperature uniformity, and contamination-rich condensation forming on door gaskets, sensors and other surfaces with even slightly disparate temperatures.

Condensation and Temperature Control In The Water-Jacketed Design

Condensation within the incubator chamber is a serious problem, aggravated by changes in ambient temperature within the laboratory due to air-conditioning or heating fluctuations which overwhelm the ability of temperature control sensors and heaters to respond through improperly selected insulation or water mass. Too much or too little cabinet insulation leads to loss of temperature control and, ultimately, the paradox of accuracy over repeatability exaggerates problems with cell culture protocols.

Water-jacketed incubators exhibit slightly more temperature stability during a loss of power, but are slower to recover temperature (and humidity) following routine door openings or power restoration. Also, when shared by several users requiring multiple door openings during the day, the average temperature may be offset by several degrees.

Water-Jacket Construction Attributes

The stainless steel interior surface, when properly welded during assembly and manually cleaned with approved disinfectants, can resist corrosion and discoloration. Stainless steel, however, can create a collateral environment for contamination growth from airborne particulates entering the chamber during normal door openings. This situation is aggravated if fingerprints or media spills are not properly cleaned. The propensity for contamination on stainless steel applies to shelves and shelf supports, as well as removable humidity pans.

Recently introduced water-jacketed models offer copper-bonding options to create contamination resistant interior walls. To be effective, however, the copper bonding, which discolors easily, must apply to all shelves, shelf supports and other interior components.

Since the filled water jacket applies direct pressure to the interior walls, the stainless steel chamber must be of a sufficient thickness or reinforced to prevent bowing. Although some newer models include "deep drawn" interior inserts with coved corners, most water-jacket models are constructed from a butt-welding technique which creates sharp interior corners. Furthermore, if welding technique is poor, the stainless steel oxidizes at the weld points creating rust faults which eventually cause irreparable leaks from the jacket. In some cases, the seams are "back filled" by welding, but the propensity for weld faults remains.

Water-jacketed incubators use the principle of natural air convection through and around perforated shelves to establish uniform atmospheres within the chamber. Automatic incubators, however, require an air sample passing over an internal or externally mounted sensor to compare actual CO_2 density with a known reference. Thus, an air blower within the chamber can improve air circulation while serving the CO_2 sensor as well. Because aggressive air movement can create opportunities for desiccation and cross contamination from surface to culture, air circulation technique must be managed with careful engineering consideration.

Recent amendments to water-jacketed incubators with air circulation systems include the addition of 0.3 micron HEPA filters adjacent to the circulating blower.

Forced Draft Incubators

While water-jacketed incubators are limited in size due to practical constraints on jacket construction, usually 5 cu.ft. to 7 cu.ft., forced-draft (or forced-air) incubators offer large capacity alternatives. Benchtop models of 10 cu.ft. and reach-in models as large as 30 cu.ft. can be used with laboratory roller apparatus and other instrumentation because air-flow and heating systems are more responsive in a positive circulation environment.

When used with roller bottles or other cell culture vessels with relatively high liquid media content, desiccation of culture media is less of a concern. When humidified, however, forced-draft incubators typically have direct-dial or direct-set humidification systems which require water feed reservoirs, float valves, and humidity sensors configured around lithium chloride, wet/dry bulb or other technology. Direct humidity systems require care and attention and, as in all CO_2 incubators, pose a chronic contamination risk.

In order to assure uniform airflow across solid, non-perforated shelves, forced-draft incubators typically have interior plenums with supply and return air holes or slots. Contamination control requires a complete disassembly to expose all interior surfaces for cleaning.

Air Jacket Incubators

Improvements in microprocessor control, sensors and surface heating technology have led to the introduction of newer air jacket incubators which avoid the structural and performance limitations of water-jacketed models, while offering significant benefits over forced-draft cabinets.

By avoiding the need for a filled water-jacket surrounding the interior chamber, engineers have more flexibility in interior design, resulting in improved radius corners for easier cleaning, and more efficient front gasket temperature transitions which minimize condensation around the opening.

Sampling for the CO_2 sensor requires air circulation created by a blower wheel mounted in the chamber. As in water-jacketed incubators, some manufacturers have attached 0.3 micron HEPA filter assemblies to the blower system in an effort to trap airborne particulates which enter the chamber.

Heating configurations in air jacketed incubators vary widely, but most manufacturers include an independently controlled outer door heater to warm the inner door glass in an attempt to minimize condensation. The sensitivity of the door heater varies widely among manufacturers.

Conventional Contamination Control Approaches

In recent years, manufacturers of laboratory incubators have attempted to solve contamination problems with various approaches to incubator design. These operational techniques have been moderately successful, but limited in terms of long-term efficacy and convenience, with most requiring extended periods of downtime during which cultures must be removed and placed in other incubators to maintain temperature, humidity and CO_2 levels.

Active efforts to achieve contamination control are classified as:

- Manual cleaning
- HEPA filtration of incubator air
- Elevated temperature decontamination
- Copper bonded interior

Manual Cleaning

Many manufacturers recommend a periodic wipedown of the incubator interior with a solution of 70% ethanol in distilled water or other non-volatile disinfectant such as iodine or bleached based solutions.[3] The frequency of service depends on many variables, including user preference, shared users, adherence to good laboratory practice, lab air quality and culture protocol.

Successful decontamination through a manual wipedown requires complete removal of all interior components and ductwork, including blower wheels, shelf brackets and falsework inherent to the design. Oftentimes, these components are autoclaved while the manual work continues.

Despite the effort, manual cleaning requires labor and downtime, and is often incomplete. Cell cultures must be removed and protected. Gaskets, seals, pass-thru port plugs, humidity reservoirs and other exposed surfaces can quickly contaminate the chamber following reassembly. Contaminants within shelf perforations are often missed. Replacement of cell culture vessels in the freshly cleaned incubator can transfer contaminants from the staging area. Fumes and aerosols from cleaning agents can linger, and efforts to “air out” the chamber invite additional migration.

If the incubator has a copper interior, discoloration is typical.

HEPA Filtration

Unlike a biological safety cabinet with sophisticated airflow in and around the work area, the laboratory incubator has no provision for complete protection from airborne contamination during door openings. This virtually neutralizes the effect of Class 100 air directed from plenum outflows fitted with HEPA filters within the incubator airflow system, which have demonstrated some practical advantage in trapping contaminants.

Some incubator designs, however, permit the circulation blower to operate during door openings, a practice which exaggerates the migration of contaminants from ambient air. Once trapped in the HEPA filter, contaminants can remain viable, although copper lacing within some filters promotes a limited germicidal affect.

Heat Decontamination

Several manufacturers have developed high temperature surface decontamination capabilities in incubator design. Heat decontamination under certain conditions appears to be effective against vegetative microorganisms (e.g. *E. coli, Pueudomonas* species, staph, strep and mycoplasma species) as well as fungal spores (e.g. *Aspergillus* and *Penicillium* species).[4]

- These incubators require high efficiency insulation and gasketing to withstand cyclical decontamination procedures.
- All cell cultures must be removed prior to the process, effectively suspending the productivity of the incubator
- Initiation of the heat decontamination sequence requires a measure of advance administrative planning to accommodate the culture relocation and downtime.
- The CO_2 sensor and any HEPA filters must be removed prior to the process, and thoroughly decontaminated or replaced prior to reassembly.

- Once initiated, the complete heat and cooling cycle can extend up to two days, although the actual dwell time at the specified decontamination temperature, excluding ramp up and cooling time, ranges from one to two hours.
- Despite exposure to high heat ranging from 90°C to 140°C, it is suggested that latent thermophilic or hyperthermophilic organisms can remain in the incubator chamber, assuming an even more aggressive expression than before the cycle.
- Heat decontamination is an active process independent of and outside the parameters of the cell culture environment generally established at 37°C, Thus, while effective under controlled testing conditions, there are no passive benefits from heat decontamination alone to protect cell cultures when the cycle is completed and interior components are replaced; the propensity for airborne contamination re-occurs through normal door openings.
- The SANYO Model MCO-20AIC cell culture CO_2 incubator does not require a heat sterilization function, yet the incubator has been proven effective against thermophilic organisms such as *B. stearothermophilus*. See Part III, Laboratory Testing and Performance Evaluations, Pages 12-13, Photo 11, Graph 11A.

Ozone, Ethylene Oxide and Ultraviolet Light

The use of ozone and ethylene oxide for incubator contamination control is impractical. Unlike decontamination protocols associated with biological safety cabinets, incubators lack the airflow and cabinet seal provisions required for safe and effective use of these media.

Broad-spectrum ultraviolet light, also used in routine decontamination of biological safety cabinet work surfaces, cannot penetrate concealed interior surfaces of the incubator chamber. Ozone, ethylene oxide and conventional ultraviolet light also require complete removal of active cell cultures, and result in added downtime and loss of productivity.

Part II

SANYO Contamination Control

Technical Development Report: MCO-20AIC

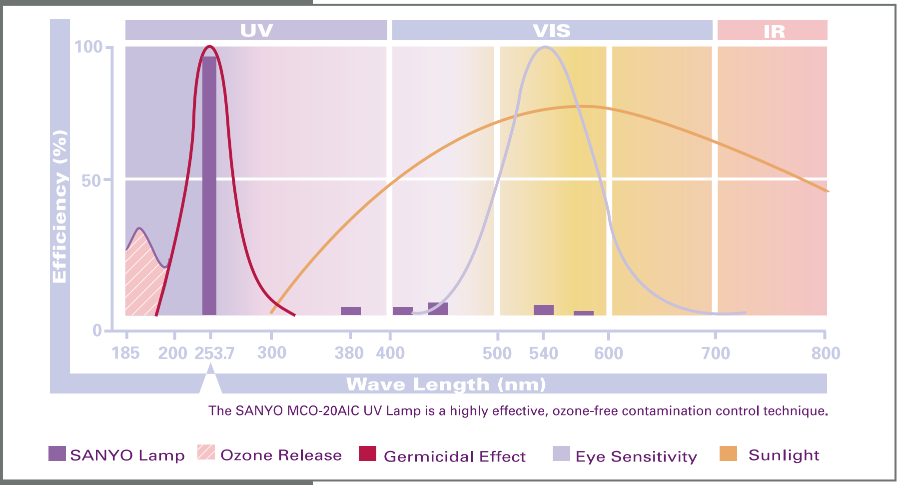
Since incubator contamination can manifest itself in many forms, and because contaminants can migrate into the chamber from a variety of sources, the need for a continued protection during the cell culture process is acute.

Manual cleaning prior to first use is always a prerequisite to good laboratory practice. Once cell cultures protocols are in process, however, an integrated approach to contamination control can offer protection without affecting culture growth and without downtime.

Following years of research and testing, the SANYO Electric Biomedical Co., Ltd. applied a composite approach to development of the MCO-20AIC cell culture incubator in a successful effort to solve chronic problems of contamination control.

Through a unique process described as *Active Background Contamination Control*™, the SANYO MCO-20AIC offers a totally integrated process which combines several proven techniques in arresting and destroying particulates in the chamber.
These include:

- A patented isolated, narrow bandwidth (253.7nm) ozone-free ultraviolet lamp with door interlock[5]
- A copper-enriched stainless steel interior chamber[6] with copper-enriched stainless steel shelves, brackets and plenum components
- A patented direct heat (air) heating system with three independent heating zones[7]
- A directional air-flow and containment plenum surrounding a UV exposed[8] humidity reservoir in a removable, stainless steel pan



The SANYO MCO-20AIC UV Lamp is a highly effective, ozone-free contamination control technique.

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

The multi-faceted approach to contamination control is designed to destroy airborne particulates introduced during door openings, as well as contaminants that typically grow in the water reservoir. With active and passive systems working together in the SANYO model, contaminants that inevitably enter the chamber through routine door openings or other means are intercepted and destroyed while cell culture continues uninterrupted.

Ultraviolet Protection

Because cell cultures are incubated in clear media vessels, the use of conventional ultraviolet 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 build-up, combined with unprotected UV exposure, can create user and environmental hazards in the laboratory. And UV light cannot reach hidden interior surfaces.

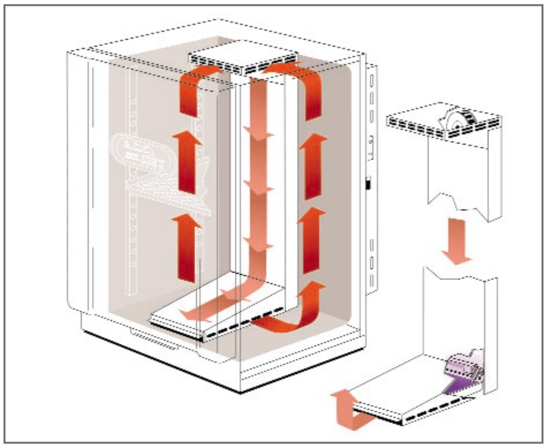
The SANYO MCO-20AIC is the first cell culture incubator to incorporate an active cycle ultraviolet function within the chamber for contamination control purposes. Marketed as SafeCell™[9], the SANYO ultraviolet lamp generates a narrow bandwidth emission of 253.7nm which is toxic when directly applied to microorganisms in plenum air and humidity pan water. Because this emission is outside the 185nm bandwidth that generates ozone, no ozone toxicity is present anywhere in the incubator and cell cultures remain unaffected.

Ultraviolet 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 be synthesized, usually.[10]

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 UV cycle is factory set to glow for 5 minutes following each door opening, which is sufficient to destroy contaminants during normal operation. The lamp ON time is programmable from 0 to 30 minutes depending on user preference. (See Part III.)

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 MCO-20AIC.

Figure 2
The airflow process within the SANYO MCO-20AIC 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 autoclavable plastic blower wheel easily snaps off for cleaning and replacement if required.



MCO-20AIC UV Light Uniformity Index

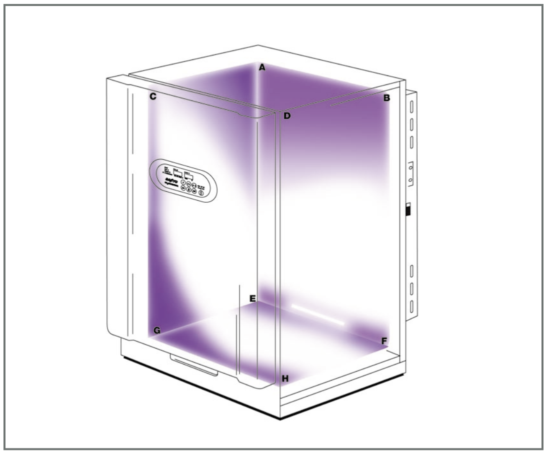


Figure 3
With interior shelves, brackets and plenum components removed, MCO-20AIC interior chamber surfaces are exposed to narrow bandwidth ultraviolet light in a timed decontamination cycle whenever desired. Light intensity varies according to location and distance from the source as measured in Chart 1.

UV Intensity (microwatt seconds per square centimeter)

UV light intensity against interior surfaces as measured by a UV sensor (Minolta Model UV-250/2A) positioned in each corner of the chamber (see chart Key and Measuring Point Location). Incubator inner and outer doors were closed and UV intensity was logged after one minute.

Key	Measuring Point Location	UV Intensity Index*	
		Single Unit Test Data	Typical Test Data (Single Unit Results X .07)
A	top left rear	0.9	.6
B	top right rear	1.0	.7
C	top left front	4.0	2.8
D	top right front	3.4	2.4
E	lower left rear	4.3	3.0
F	lower right rear	3.9	2.7
G	lower left front	41.0	28.7
H	lower right front	32.0	22.4

Chart 1
**Data is for informational reference only and is not a UV uniformity emission performance guarantee of the MCO-20AIC.*

Example: Legionella pneumophila (Legionaire's Disease) effective UV dose is 12,300 microwatt seconds/cm2. Typical test data at measuring point C, top left front is index 2.8, or 12,300/2.8 = 4393 seconds, 73 minutes, or 1 hour, 13 minutes exposure for neutralization. See Incident Energies Chart 3 for specific organism and UV dosage.

Safety Characteristics of SANYO Ultraviolet Lamp Emission

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

Chart 2

Safe For Cell Cultures and Laboratory Personnel

While the 253.7nm emission from the SANYO ultraviolet lamp is effective against airborne 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.*

For Cell Cultures

- Ozone release free, <0.01ppm (under detectable dose) after 1 week with lamp continuous ON.
- UV emission does not penetrate culture vessel, <0.001J/m2 (under detectable dose) inside of culture bottle.

For Incubator Users

- The ultraviolet lamp cut-off switch is automatically OFF during door openings.
- In worst case scenario, failure of auto cut-off function, ultraviolet emission from front of the chamber without airflow plenum (pan cover) in place is <0.03W/m2.

Chart 3 ▶

Microorganisms Deactivated By Ultraviolet Germicidal Light (A Partial Listing)

Chart 3 illustrates the incident energies at 253.7nm necessary to inhibit colony formation in greater than 99.9% of microorganisms (measured in microwatt seconds/cm2).

Bacteria	UV Dose
Agrobacterium lumefaciens [5]	8,500
Bacillus anthracis [1,4,5,7,9]	8,700
Bacillus anthracis Spores	46,200
Bacillus megatherium Sp. (veg) [4,5,9]	2,500
Bacillus megatherium Sp. (spores) [4,9]	5,200
Bacillus paratyphosus [4,9]	6,100
Bacillus subtilis [3,4,5,6,9]	11,000
Bacillus subtilis Spores [2,3,4,6,9]	22,000
Clostridium tetani	23,100
Clostridium botulinum	11,200
Corynebacterium diphtheriae [1,4,5,7,8,9]	6,500
Dysentery bacilli [3,4,7,9]	4,20
Eberthella typhosa [1,4,9]	4,100
Escherichia coli [1,2,3,4,9]	6,600
Legionella bozemanii [5]	3,500
Legionella dumoffill [5]	5,500
Legionella gormanil [5]	4,900
Legionella micdadei [5]	3,100
Legionella longbeachae [5]	2,900
Legionella pneumophila (Legionnaire's Disease)	12,300
Leptospiracanicola-Infectious Jaundice[1,9]	6,000
Leptospira interrogans [1,5,9]	6,000
Micrococcus candidus [4,9]	12,300
Micrococcus sphaeroides [1,4,6,9]	15,400
Mycobacterium tuberculosis [1,3,4,5,7,8,9]	10,000
Neisseria catarrhalis [1,4,5,9]	8,500
Phytomonas tumefaciens [1,4,9]	8,500
Proteus vulgaris [1,4,5,9]	6,600
Pseudomonasaeruginosa (Environ. Strain) [1,2,3,4,5,9]	10,500
Pseudomonas aeruginosa (Lab. Strain) [5,7]	3,900
Pseudomonas fluorescens [4,9]	6,600
Rhodospirillum rubrum [5]	6,200
Salmonella enteritidis [3,4,5,9]	7,600
Salmonella paratyphi (Enteric Fever) [5,7]	6,100
Salmonella Species [4,7,9]	10,000
Salmonella typhimurium [4,5,9]	15,200
Salmonella typhi (Typhoid Fever) [7]	7,000
Salmonella	10,500
Sarcina lutea [1,4,5,6,9]	26,400
Serratia marcescens [1,4,6,9]	6,160
Shigella dysenteriae - Dysentery [1,5,7,9]	4,200
Shigella flexneri - Dysentery [5,7]	3,400
Shigella paradysenteriae [4,9]	3,400
Shigella sonnei [5]	7,000
Spirillum rubrum [1,4,6,9]	6,160
Staphylococcus albus [1,6,9]	5,720
Staphylococcus aureus [3,4,6,9]	6,600
Staphylococcus epidermidis [5,7]	5,800
Streptococcus faecaila [5,7,8]	10,000
Streptococcus hemolyticus [1,3,4,5,6,9]	5,500
Streptococcus lactis [1,3,4,5,6]	8,800
Streptococcus pyrogenes	4,200
Streptococcus salivarius	4,200
Streptococcus viridans [3,4,5,9]	3,800
Vibrio comma (Cholera) [3,7]	6,500
Vibrio cholerae [1,5,8,9]	6,500

Molds	UV Dose
Aspergillus amstelodami	77,000
Aspergillus flavus [1,4,5,6,9]	99,000
Aspergillus glaucus [4,5,6,9]	88,000
Aspergillus niger (breed mold) [2,3,4,5,6,9]	330,000
Mucor mucedo	77,000
Mucor racemosus (A & B) [1,3,4,6,9]	35,200
Oospora lactis [1,3,4,6,9]	11,000
Penicillium chrysogenum	56,000
Penicillium digitatum [4,5,6,9]	88,000
Penicillium expansum [1,4,5,6,9]	22,000
Penicillium roqueforti [1,2,3,4,5,6]	26,400
Rhizopus nigricans (cheese mold) [3,4,5,6,9]	220,000

Protozoa	UV Dose
Chlorella vulgaris (algae) [1,2,3,4,5,9]	22,000
E. hystolytica	84,000
Giardia lamblia (cysts) [3]	100,000
Nematode Eggs [6]	40,000
Paramecium [1,2,3,4,5,6,9]	200,000

Virus	UV Dose
Adeno Virus Type III [3]	4,500
Bacteriophage [1,3,4,5,6,9]	6,600
Coxsackie	6,300
Infectious Hepatitis [1,5,7,9]	8,000
Influenza [1,2,3,4,5,7,9]	6,600
Rotavirus [5]	24,000

Yeasts	UV Dose
Baker's Yeast [1,3,4,5,6,7,9]	8,800
Brewer's Yeast [1,2,3,4,5,6,9]	6,600
Common Yeast Cake [1,4,5,6,9]	13,200
Saccharomyces cerevisiae [4,6,9]	13,200
Saccharomyces ellipsoideus [4,5,6,9]	13,200
Saccharomyces sp. [2,3,4,5,6,9]	17,600

Chart 3 References

1. *"The Use of Ultraviolet Light for Microbial Control", Ultrapure Water, April 1989.*
2. *William V. Collentro, "Treatment of Water with Ultraviolet Light - Part I", Ultrapure Water, July/August 1986.*
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4. *Dr. Robert W. Legan, "Alternative Disinfection Methods - A Comparison of UV and Ozone", Industrial Water Engineering, March/April 1982.*
5. *Unknown*
6. *Rudolph Nagy, Research Report BL-R-6-1059-3023-1, Westinghouse Electric Corporation.*
7. *Myron Lupal, "UV Offers Reliable Disinfection", Water Conditioning & Purification, November 1993.*
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9. *Bak Srikanth, "The Basic Benefits of Ultraviolet Technology", Water Conditioning & Purification, December 1995.*

Lamp Life

The SANYO 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 minutes following each door opening. The cycle is programmable, and may be set to 100% ON for overnight decontamination of an empty chamber if desired.

UV Lamp Program Options

Mode	Function
After door opening	UV lamp automatically ON for five minutes after door is closed. Time is factory set, user programmable from 0-30 minutes.
OFF	If UV protection is not desired.
Continuous ON (24-hour process)	Useful for overnight decontamination prior to first use or following total chamber wipe-out after maintenance or service.
Continuous ON (repeated 24-hour process)	Useful for extended decontamination for small percentage of organisms that require greater UV dose (incident energy) beyond standard 24-hour cycle. See Chart 3.

Chart 4

inCusaFe™[11] Copper-Enriched Stainless Steel

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

Interior components of the SANYO MCO-20AIC are fabricated from a copper-enriched stainless steel which 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 SANYO material extends to all interior surfaces, including perforated shelves, shelf brackets and plenum components.

Patented DHA Airflow System

The SANYO MCO-20AIC is an air-jacketed CO_2 incubator. The air-jacket design 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 SANYO 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[12] plates, the water level in the humidity pan is a critical consideration.
- SANYO uses an optical water level sensor to warn 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.

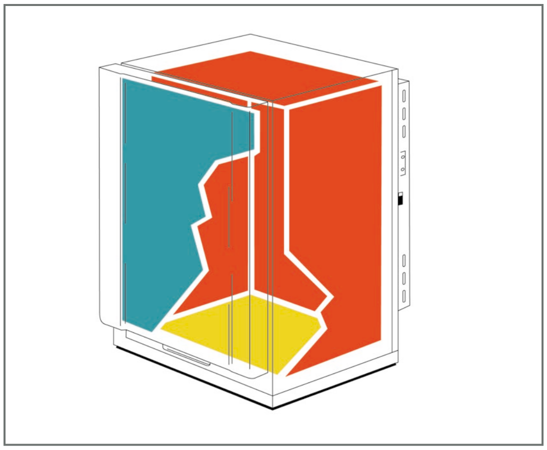


Figure 4

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 assure interior uniformity.
- 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, in direct proximity of the SafeCell™ UV lamp, 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.

- In the SANYO MCO-20AIC, however, 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 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.

CO_2 Sampling and Inject System

The SANYO MCO-20AIC uses a unique ceramic-based infrared sensor system to maintain precise CO_2 control regardless of temperature and relative humidity changes within the incubator chamber. The CO_2 sensor externally samples both ambient air (for automatic calibration), and interior air (for CO_2 density measurement).

Both ambient and chamber air samples are filtered through 0.3 micron HEPA in-line filters in advance of the infrared sensor to protect the sensor and returned air to the chamber from contamination. In addition, CO_2 inputs to the incubator from dual-stage regulator(s) are filtered through 0.3 micron HEPA in-line filters. A CO_2 sampling port is located on the front of the chamber.

Part III

Laboratory Testing and Performance Evaluations

SANYO Electric Biomedical Co., Ltd. has conducted numerous performance tests on the MCO-20AIC in an effort to corroborate the efficacy of SafeCell™ UV and inCusaFe™ contamination control methodology.

Rust and Corrosion Characteristics

Accelerated salt spray testing illustrates rust and corrosion differences between SANYO inCusaFe™ copper enriched stainless steel (copper alloy), conventional copper interior construction and conventional welded and polished Type 304/Type 430 stainless steel.

Photo 1A ▲

Photo 1B ▲

Photo 1C ▲

Photo 1D ▲

Photo 1A-1D — Neutral Salt Spray Test

During testing, selected testing surfaces are exposed to salt spray at 35°C, 99%RH for 8 hours spray, 16 hours stop, and repeated through 26 consecutive test cycles. At completion of testing, visible rust and corrosion appear in welded and polished surfaces of Type 430 and Type 304 stainless steel as well as throughout C1100 copper. See Chart 5.

Reagent Corrosion Test Results (1 Month)

Incubator interior samples 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.

Chart 6 ▼

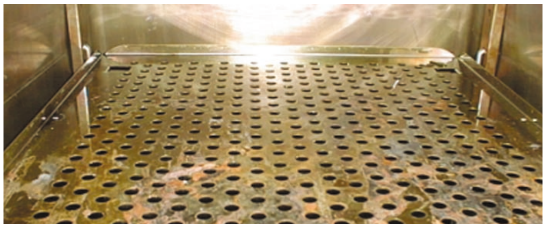
Test Reagent	Incubator Interior Surface		
	inCusaFe™ Copper Enriched Stainless Steel	Copper (C1100)	Type 304 Stainless Steel
Distilled Water	N	D	N
Ion Exchanged Water	N	D	N
70% Ethyl Alcohol	N	D	N
70% Isopropyl Alcohol	N	D	N
1/500 Sodium Hypochlorite	N	D	N
1/1000 Sodium Hypochlorite	N	D	N
1/100 Bezalkonium 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	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

Field Corrosion Observations

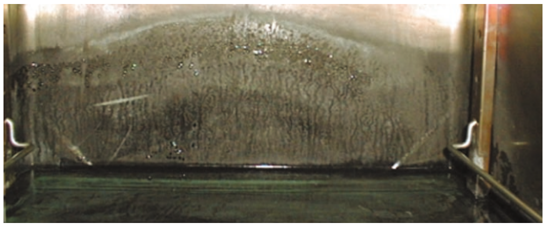
Conventional C1100 Copper Corrosion Performance Standard laboratory conditions.

Photo 2 ▼



Conventional incubator copper interior shelf with extreme corrosion.

Photo 3 ▼



Conventional incubator copper interior with wall and humidity reservoir corrosion.

Chart 5 ▼

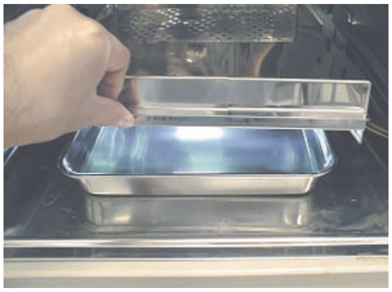
Efficacy after 26 Days*	Photo 1A Stainless Steel. SUS 430	Photo 1B C1100 Copper	Photo 1C SANYO inCusaFe™ Copper Alloy Stainless Steel	Photo 1D Stainless Steel, SUS 304
Rust	moderate at weld seam	minimul overall	none	minimal at wels seam
Discoloration	moderate at weld seam	severe overall	minimal	moderate at weld seam

**Conditions: 35°C, 99% RH, 8 hours salt spray, 16 hours stop, repeat 26 cycles.*

UV Exposure in Humidity Water Reservoir

The position of the ultraviolet lamp, as well as the relationship between the lamp, plenum, humidity pan and airflow system is integral to the performance of the MCO-20AIC.

Photo 4 ▼



The humidity pan and UV lamp are contained within a base plenum (Photo 4) which lifts easily without tools for addition of water or removal of the pan. The UV lamp is exposed to the humidity water at all times, while UV emissions are contained within the plenum to protect active cell cultures.

Photo 5 ▼

With the plenum cover removed (Photo 5), the ultraviolet lamp is exposed and can be viewed in relationship to the humidity pan.

Efficacy of UV Exposure On Humidity Water

SafeCell™ UV tests on humidity pan water demonstrate how periodic exposure to narrow bandwidth ultraviolet light destroys bacterial and fungal contaminants, including thermophilic organisms, which migrate to the humidity pan water during routine door openings.

Humidity Water Test Methodology

Organisms were suspended into humidity pan water at cabinet base (Photo 6), then exposed to SafeCell™ UV emission for determined period (see Graphs 9A, 10A, 11A). Sample water solutions of 0.2ml were plated on nutrient agar plates (Photos 7, 8) and cultured prior to observation of colonies.

Photo 6 ▼

Photo 7 ▼

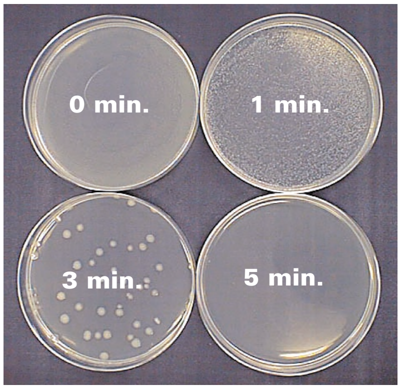
Photo 8 ▼

Results, 48 Hours *E. Coli*

3 x 10^9 cells, 3 liters = 1 x 10^6ml

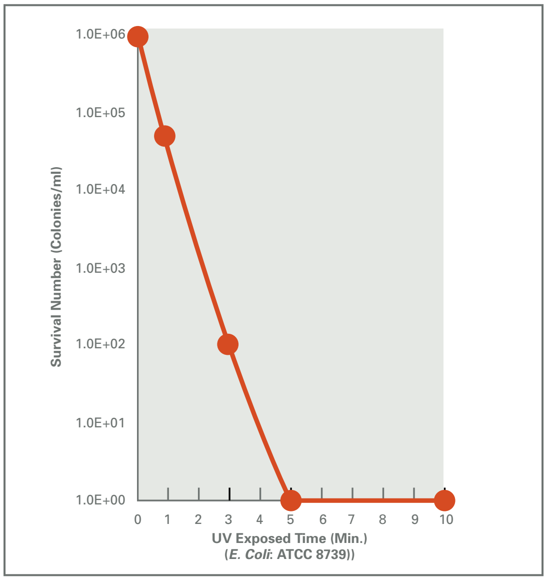
Culture plate array (Photo 9) shows 48 hour 37°C cultures of humidity pan water with *E. Coli*, bacteria (Source ATCC8739) following exposure to SafeCell™ UV light for 0, 1, 3 and 5 minutes. See Graph 9A.

Photo 9 ▼



UV Exposed time (0,1,3,5 min,)

Graph 9A ▼

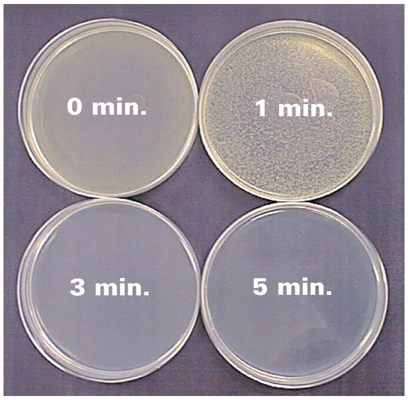


Results, 48 Hours, *S. Aureus*

3 x 10^9 cells, 3 liters = 1 x 10^6ml

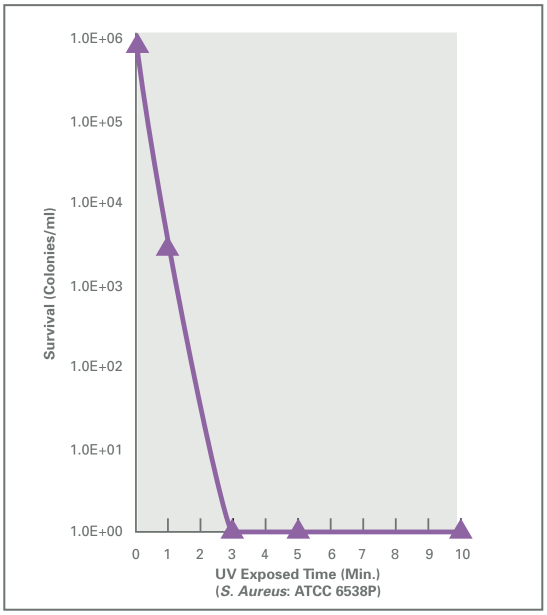
Culture plate array (Photo 10) shows 48 hour 37°C cultures of humidity pan water with *S. Aureus* bacteria (Source ATCC6538P) following exposure to SafeCell™ UV light for 0, 1, 3 and 5 minutes. See Graph 10A.

Photo 10



UV Exposed time (0,1,3,5 min,)

Graph 10A

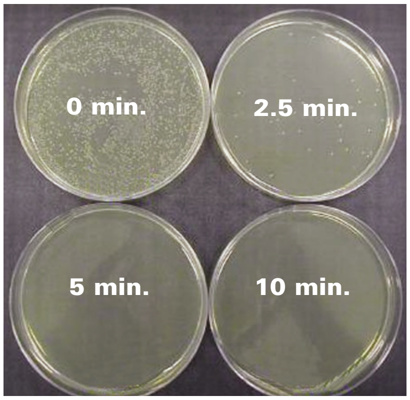


Results, 24 Hours, *B. Stearothermophilus*

5 x 10^7 cells, 2 liters +2.5 x 10^4ml

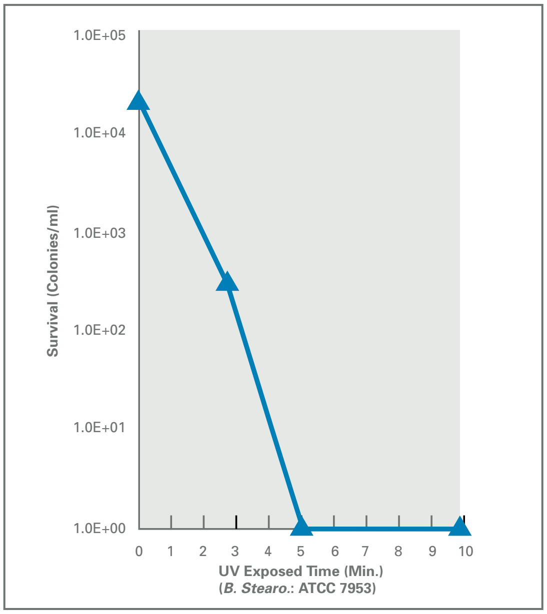
Culture plate array (Photo 11) shows 24 hour 55°C cultures of humidity pan water with *B. Stearothermophilus* bacteria (Source IFO13737, equivalent to ATCC7953) following exposure to SafeCell™ UV light for 0, 1, 3 and 5 minutes. See Graph 11A.

Photo 11



UV Exposed time (0,2.5,5,10 min,)

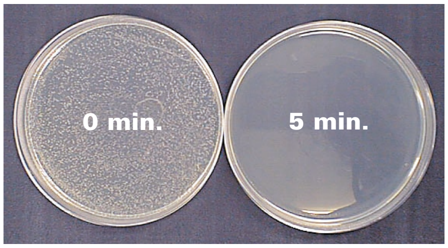
Graph 11A



Results, Three Months, Airborne Exposure

Humidity pan water cultures (Photo 12) following three months of incubation illustrate the comparison between water exposed to narrow bandwidth ultraviolet light for 5 minutes (right), and no exposure (left). Test results showing the effect of narrow bandwidth ultraviolet light on fungal contaminants *A. Niger* and *P. Chrysogenum* demonstrate similar efficacy.

Photo 12



UV Exposed time

Passive Contamination Control Benefits of SANYO inCusaFe™ Copper Enriched Stainless Steel

Resists Mycoplasma Contamination

Test results comparing SANYO inCusaFe™ copper-enriched stainless steel with conventional copper construction illustrate the passive resistance of inCusaFe™ interior surfaces against common *mycoplasma* contamination.

Mycoplasma Affect on Media pH and Color

Chart 7

Mycoplasma Strain	ATCC Equivalent	Medium Only pH	*Mycoplasma* Induced pH
Mycoplasma fermentans (PG 18)	ATCC19989	pH 7.0	6.7
Mycoplasma orale (CH19299)	ATCC23714		8.0
Mycoplasma arginini (G230)	ATCC23838		7.8
Mycoplasma hominis (PG21)	ATCC23114		7.7

Changes in media color indicate pH change induced by *mycoplasma* contamination.

Mycoplasma Test Methodology

1. *Mycoplasma* suspension (10^5-10^6 is dropped on inCusaFe™ or copper test piece. (Photo 13)
2. The suspension is incubated at 37°C, 5% CO$_2$ for 24 hours.
3. After 24 hours the sample is resuspended in fresh media. (Photo 14)
4. The media is incubated at 37°C for 14 days.
5. *Mycoplasma* contamination is revealed by color variance. (Photos 15a, 15b, 15c, 15d)

Photo 13

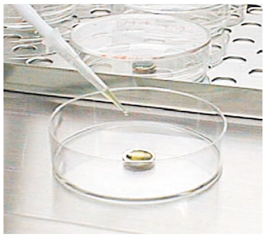


Photo 14

Mycoplasma Survival Results

Test results with four strains of *mycoplasma* summarized in Chart 8 demonstrate how SANYO inCusaFe™ copper-enriched stainless steel offers germicidal properties of conventional C1100 copper while maintaining both corrosion-proof and discoloration-resistant properties of conventional Type 304 stainless steel.

Chart 8

Mycoplasma Strain	Negative Control	Conventional Type 304 Stainless Steel	SANYO inCusaFe™ Copper-Enriched Stainless Steel	Conventional Copper C1100
Mycoplasma fermentans (PG 18)	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

Results

Chart 9

Negative Control	Positive Control	inCusaFe™	Copper
NA	survival	no survival	no survival

Photo 15A, *Mycoplasma fermentans*

Photo 15B, *Mycoplasma orale*

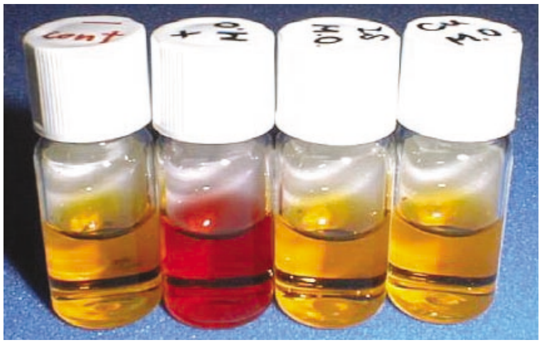
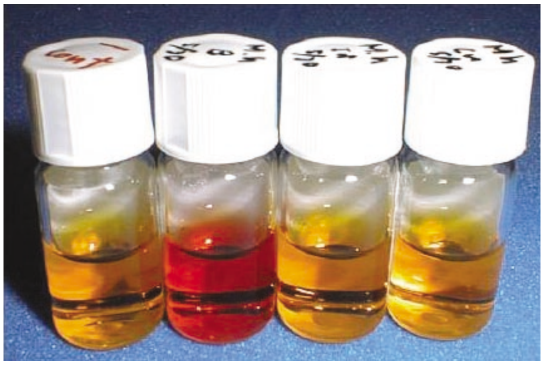


Photo 15C, *Mycoplasma arginini*

Photo 15D, *Mycoplasma hominis*



Comparative Antibacterial Characteristics of SANYO inCusaFe™ Copper-Enriched Stainless Steel

The inherent germicidal efficacy of SANYO inCusaFe™ copper-enriched stainless steel (copper alloy) versus conventional C1100 copper and conventional Type 304 stainless steel is demonstrated through both film cover and drop methodology, and summarized in Chart 10.

Methodology

Primary Incubation

- **Film Cover Method**
  Photos 16-19 illustrate bacterial suspension (Photo 16a) applied to test piece (Photo 16b) of conventional Type 304 stainless steel, SANYO inCusaFe™ copper-enriched stainless steel and conventional C1100 copper. The piece is covered with polyethylene film (Photo 17c, Photo 18d, Photo 19e) and incubated for 24 hours at 37°C in 5% CO_2.
- **Drop Method**
  Photo 20 illustrates bacterial suspension (Photo 20a) applied to test piece (Photo 20b, 20c and 20d) of conventional Type 304 stainless steel, SANYO inCusaFe™ copper-enriched stainless steel and conventional C1100 copper and incubated for 24 hours at 37°C in 5% CO_2.

Photo 16 ▼

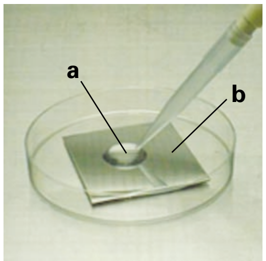


Photo 17 ▼

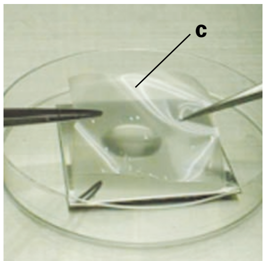


Photo 18 ▼

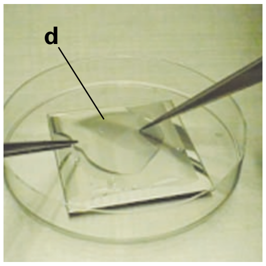


Photo 19 ▼

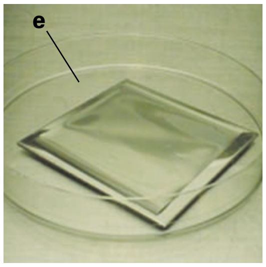
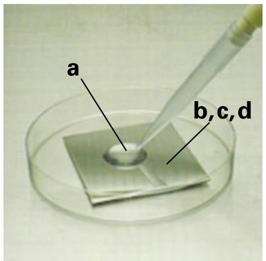


Photo 20 ▼



Secondary Incubation

- **Film Cover and Drop Method**
  After primary incubation, the bacterial suspension is removed from the test piece and spread on normal agar plates (Photo 21), then cultured at 37°C in a secondary incubation prior to observation of colonies.

Photo 21 ▲

Results

Typical results are shown in Photos 22, 23 and 24, and laboratory test results are shown in Chart 10.

Photo 22 ▲
inCusaFe™ Copper Enriched Stainless Steel

Photo 23 ▲
Conventional Copper C1100

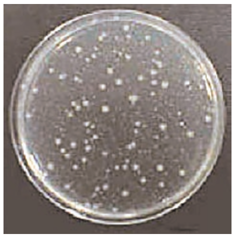


Photo 24 ▲
Conventional T304 Stainless Steel

Chart 10 ▼ **Efficacy After 24 Hours Incubation at 37°C**

Species	inCusaFe™ Copper Enriched Stainless Steel	Conventional Stainless Steel
E. coli (ATCC8739)	99.928 %	0 %
E. coli (IFO3301)	99.847 %	0%
S. aureus (ATCC6538P)	99.998 %	0 %
B. subtilis (ATCC6633)	99.997 %	0 %
B. stearothermophilus (ATCC7953)	99.870 %	—

*(N=3) *Bacteria killing rate = (1-Test Sample Colony No./ Control Colony No.) x 100*

Note: Additional performance test results may be available. Visit the SANYO web site for the latest information on the complete product line from SANYO Electric Biomedical, Co. Ltd.

Part IV

Conclusions

SANYO *Active Background Contamination Control™*

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

General References

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- Cell culture contamination: sources, consequences, prevention and elimination, by C.K. Lincoln, and M.G. Gabridge. In Animal Cell Culture Methods (1998), J. P. Mather and D. Barnes, eds., pp. 49-65 (Academic Press, San Diego).
- Antibiotic treatment of mycoplasma-infected cultures. Molecular and Diagnostic Procedures in Mycoplasmology Vol. II (1996), S. Razin and J.G. Tully, eds., p. 439 (Academic Press, San Diego).

On-Line References

- American Type Culture Collection www.atcc.org
- American Type Culture Collection www.atcc.org/searchcatalogs/faqcellbiology
- Association for the Advancement of Medical Instrumentation www.aami.org
- U.S. Pharmacopoeia www.usp.org
- SANYO Electric Biomedical Co., Ltd. www.sanyobiomedical.com

FDA Clears MCO-20AIC For *In Vitro* Fertilization Applications

SANYO has received U.S. Food and Drug Administration (FDA) 510(k) clearance to market the MCO-20AIC for *in vitro* fertilization applications. Following formal submission to the FDA in accordance with the Safe Medical Devices Act of 1990 and the Medical Device Amendments of 1992, the clearance extends to all SANYO MCO Series CO_2 and MCO-175M "tri-gas" laboratory incubators as "assisted reproduction accessories" required to establish and maintain optimum temperature, CO_2 and/or O_2, and relative humidity critical to *in vitro* protocols. Details of the FDA 510(k) clearance referenced at: http://www.fda.gov/cdrh/510k/sumdec01.html

- Number: K013703
- Trade/Device Name: SANYO CO_2 Incubators
- Regulation Number: 21 CFR 884-6120
- Regulation Name: Assisted Reproduction Accessories
- Regulatory Class: II
- Product Code: 85 MQG
- Dated: October 30, 2001

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[1] *Active Background Contamination Control™ is a trademark of SANYO Electric Biomedical Co., Ltd.*

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[3] *"Tissue Culture Techniques. An Introduction" Bernice M. Martin, Birkhouse 1994*

[4] *Heat Sterilization and Thermo Forma Steri-Cycle™ CO_2 Incubators*

[5] *U.S. Patent 6255103*

[6] *SANYO Patent Pending*

[7] *U.S. Patent 5519188*

[8] *SANYO Patent Pending*

[9] *SafeCell™ is a trademark of SANYO Electric Biomedical Co., Ltd.*

[10] *STRYER, L. (1988). Biochemistry. 3rd Edition. WH Freeman and Co., New York.*

[11] *inCusaFe™ is a trademark of SANYO Electric Biomedical Co., Ltd.*

[12] *Society for Biomolecular Sciences*

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