

Sterisonic™ GxP Cell Culture CO₂ Incubator Technical Report / MCO-19AIC(UVH)

Development of the industry's fastest cell culture CO₂ incubator sterilization process using hydrogen peroxide vapor (H₂O₂) for highly regulated and general cell culture protocols that require complete, validated sterilization between processes.

New CO₂ incubator with built-in H₂O₂ sterilization system integrates UV light, copper-enriched stainless steel construction and unique cabinet design to permit frequent sterilization in less than three hours with fast return to service.

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Abstract

The value of the laboratory cell culture incubator used in highly regulated research and clinical protocols is directly related to the proportion of incubator uptime vs. downtime in applications where frequent interior chamber sterilization is required or desired. The need for interior sterilization before initiating new applications for *in vitro* fertilization, stem cell research and regenerative tissue culture is more frequent than longer-term cell culture work. The return on investment favors short, labor-saving sterilization cycles with validation of the sterilization process for GMP applications.

The use of a hydrogen peroxide vapor (H₂O₂) atomizer *in situ* to decontaminate the cell culture CO₂ incubator without the use of heat sterilization offers significant advantages in routine clinical and highly regulated research laboratories where costly downtime must be avoided. The combination of a seven-minute H₂O₂ vapor fog in the chamber, circulated by the incubator airflow blower, followed by exposure to narrow-bandwidth ultraviolet light establishes a thorough antimicrobial impact on all incubator walls, shelves, reservoirs, air plenums, sensors and other interior components without the time and expense of high heat cycles, leaving only small amounts of sterile water droplets as a residual. Because all interior components are designed to remain in the chamber for sterilization during the process, use of a separate autoclave is avoided and the incubator can be returned to service in less than three hours.

In 2009, SANYO Electric Biomedical Co. Ltd. introduced the Sterisonic™ GxP MCO-19AIC(UVH) cell culture CO₂ incubator with H₂O₂ vapor sterilization. The Sterisonic™ GxP complements the company's proactive *in situ* contamination control systems first marketed in 2001. In a layered and orchestrated approach



to cell culture incubation predicated on good laboratory technique, the addition of H₂O₂ vapor to an extensive arsenal of existing contamination control techniques, both passive and active, confronts a wide range of laboratory conditions and culture applications.

The SANYO Sterisonic™ GxP Cell Culture Solution

- Good laboratory technique
- Intelligent cabinet design
- InCu SaFe™ copper-enriched interior walls
- New single-beam, dual array infrared CO₂ sensor with passive sampling
- Patented SafeCell UV decontamination cycling, *in vitro*
- H₂O₂ vapor sterilization process, *in vitro*



The SANYO Cell Culture Solution is based on a series of mutually dependent concentric systems working together to offer the safest, most productive *in vitro* cell culture environment possible. In addition to H₂O₂ sterilization, SANYO applies the combination of structural and materials engineering, new infrared sensor technology, self-compensating narrow bandwidth ultraviolet light, multi-purpose airflow, intelligent microprocessor control and graphical monitoring into a dynamic cell culture system designed to reward good laboratory technique for the most critical and highly regulated applications.

Evolution of H₂O₂ Sterilization

The emergence of H₂O₂ vapor as a practical sterilization method has been well documented by numerous private and public agencies, and is receiving more attention at the bench level due, in part, to safety and efficacy when compared to ethylene oxide (EtO)^{2, 3}. In a review of commonly accepted sterilization techniques at the USP Annual Scientific Meeting, 2008⁴ [presentation on Sterilization and Sterility Assurance], H₂O₂ vapor was categorically added to conventional methods such as chemical, dry heat, filtration, radiation and steam sterilization for consideration in selecting the best technique for the desired application.

As a condensing vapor H₂O₂ is present in multiple phases simultaneously, requiring validation protocols to be constructed within context of a liquid and gas hybrid. While the efficacy of H₂O₂ vapor assures sterilization, the wide variation in sterilization process parameters among different products and applications requires that validation protocols associated with the cell culture incubator be ascertained from product-specific research in context with known outcomes in vastly different sterilization procedures.

Because a consensus standard for H₂O₂ remains to be established, the concepts for general sterilizing agents outlined in

ANSI/AAMI/ISO 14937 can be adopted as an appropriate validation strategy, along with other EtO standards that may apply. Additionally, several companies have obtained 510(k) clearance for the use of H₂O₂ vapor as a terminal sterilization technique for medical devices. Therefore, current practices suggest that validation for H₂O₂ vapor sterilization can be compared to EtO.

Advantages in GMP and GLP Applications

Systems and design of the Sterisonic™ GxP incubator support both clinical and non-clinical applications, starting with research and leading into development, manufacturing and quality control. As laboratories work to maintain contemporary tools and technologies in advance of new demands for both commercial and clinical success, selection of the laboratory incubator must include consideration for scalability and compliance. When retrofitting or building a new laboratory, lab planners must anticipate reporting and data logging performance of laboratory incubators heretofore classified as commodity equipment, but now recognized a critical link in the chain of custody for quality management and validation⁵.

The Sterisonic™ GxP incubator offers significant advantages in complying with GMP and GLP criteria imposed by outside and internal regulatory agencies or process manuals.

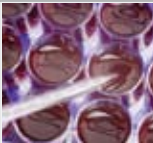



- With respect to GMP, the incubator includes relational operating systems and safeguards designed to protect the cell culture or cell expressed product, particularly when associated with direct human application such as IVF, stem cells, regenerative tissue processes or autologous cell culture⁶.
- GLP criteria promoting continuity in technique and preserving the acquisition and integrity of performance data associated with the typical incubator performance as well as the sterilization cycle is accommodated through the integral control and monitoring system, complete with data point logging and archiving, and optional communications for remote or offsite monitoring.

In developing the Sterisonic™ contamination control model, SANYO engineers based their H₂O₂ design on well-documented efficacy⁷ of the increasingly popular hydrogen peroxide vapor sterilization technique often used in decontamination of biological safety cabinets, environmental chambers and other enclosures. When H₂O₂ vapor is deployed in association with the narrow bandwidth ultraviolet light decontamination system already designed into the SANYO incubator, the complete sterilization process is safe, effective and significantly faster than conventional high-heat decontamination solutions.

The SANYO Sterisonic™ GxP Cell Culture Incubator System

Model and Standard Features	MCO-19AIC	MCO-19AIC(UV)	MCO-19AIC(UVH)
Sterisonic™ H ₂ O ₂ Sterilization System	Optional	Optional	Standard
SafeCell™ UV System	Optional	Standard	Standard
IR2™ Single Beam, Dual Array Infrared CO ₂ Sensor	Standard	Standard	Standard
InCu SaFe™ Copper Enriched Stainless Steel Interior	Standard	Standard	Standard
Intelligent Cabinet Design and Graphical Control/Monitor	Standard	Standard	Standard
Stackable	Standard	Standard	Standard



Typical Applications for Sterisonic™ GxP			
Protocol		Requirements	Sterisonic™ GxP Advantages
Stem cell culture		<ul style="list-style-type: none"> Highly stable temperature and CO₂ control with elevated relative humidity to minimize small sample media desiccation. 	<ul style="list-style-type: none"> Precise temperature control at all shelf levels established through microprocessor controlled Direct Heat and Air™ air-jacket heating system¹⁰. Precise CO₂ control, impervious to short-term humidity shifts following door openings. Safe, hydrogen peroxide vapor 3-hour sterilization <i>in situ</i> without heat. Constant scrubbing of chamber air to reduce potential for mycoplasma and other contaminants. Scalable for use in routine research or for cell cultures highly sensitive to environmental stability and contamination.
IVF		<ul style="list-style-type: none"> Complete sterilization between batch processes. Continuous mitigation of airborne contaminants following door openings. 	
Regenerative tissue culture		<ul style="list-style-type: none"> Elimination of cross-contamination. Flexibility for a broad range of cell culture applications. 	
Conventional cell culture			

The Sterisonic™ GxP Contamination Control System

The H₂O₂ incubator sterilization system *in vitro* is an extension of the SANYO Active Background Contamination Control™ technique introduced by SANYO Electric Biomedical Co., Ltd. in 2001. Now part of the new MCO-19AIC(UVH) incubator series, the new cell culture CO₂ incubator employs an isolated narrow-bandwidth ultraviolet (UV) light⁸ 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 offers an optimum cell culture environment which protects cultures *in vitro*, and minimizes frequent chamber cleaning and downtime.

In 2006, comparative testing commissioned by SANYO and performed by a certified independent testing laboratory⁹ confirmed that the SANYO UV light sterilization process is as effective against bacteria, yeasts and molds as high heat sterilization at sustained temperatures ranging from 90°C to 140°C offered in competitive products. Additionally, the SANYO incubator isolates the UV emission from cell cultures during normal operation to permit sterilization of the internal atmosphere following routine door openings without damaging cell cultures, a process which a heat sterilization technique cannot replicate.

The SANYO Productivity Advantage

Automatically coordinated processes within the SANYO Sterisonic™ GxP cell culture incubator work together to maintain optimum *in vitro* conditions of temperature, humidity and CO₂ control while arresting contamination. When complete sterilization is required, the Sterisonic™ H₂O₂ sequence offers an important uptime advantage over competitive models using high heat or conventional decontamination. The three-hour *in*

situ sterilization sequence returns the SANYO Sterisonic™ GxP incubator to service more quickly and with greater efficiency than competitive models using high heat or other decontamination protocols. In applications that require frequent sterilization between processes, the SANYO Sterisonic™ GxP yields a significant advantage in productivity.

Inherent Factors Assure Maximum Productivity

When complete sterilization is required, the Sterisonic™ H₂O₂ sequence offers a cost-effective time-saving advantage over competitive models using high heat or conventional decontamination.

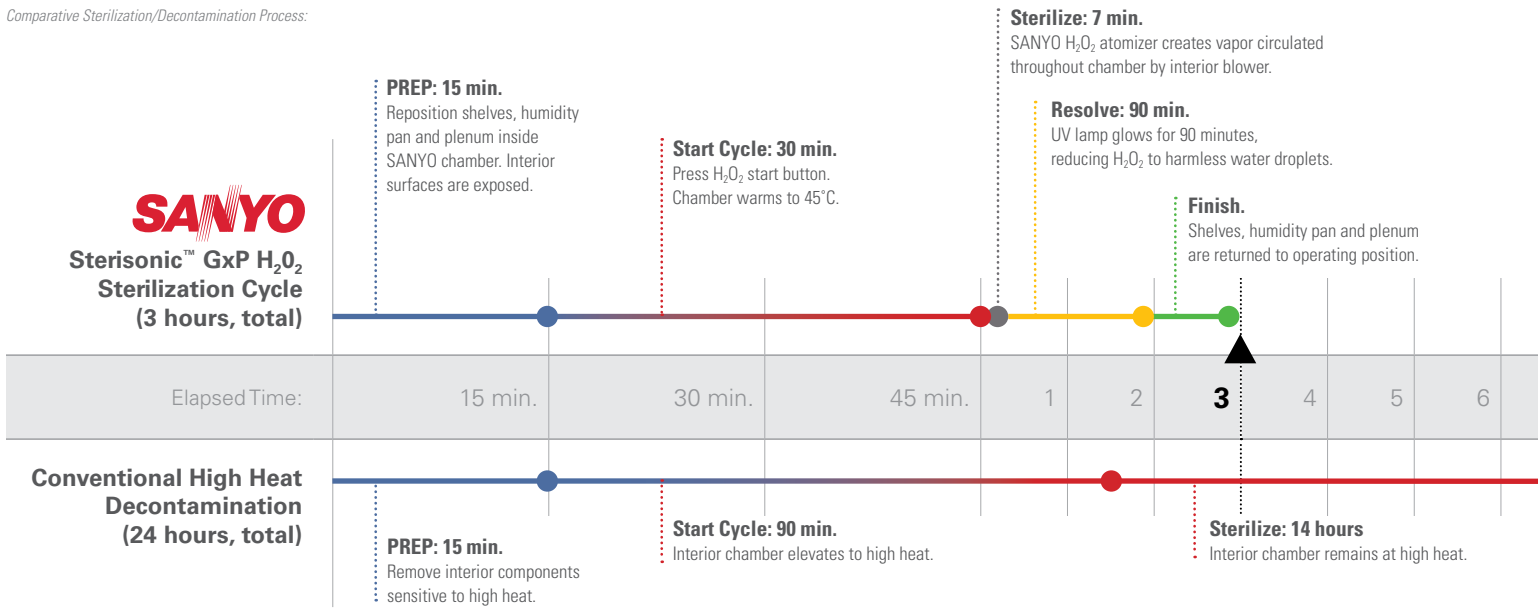


SANYO Sterisonic™
(H₂O₂ sterilization)



Brand X
(high heat sterilization)

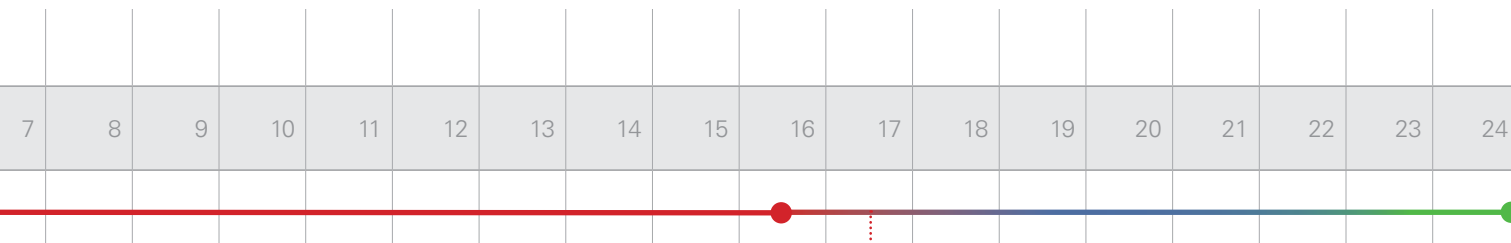
Comparative Sterilization/Decontamination Process:



H ₂ O ₂ vs. Heat Sterilization			
	SANYO Sterisonic™	High Heat Models	SANYO Benefit
Speed	Minimal planning required. Entire process can be completed in less than three hours.	Significant downtime expected. Process can take up to 24 hours from start to finish.	Sterisonic™ allows sterilization anytime and permits frequent sterilization with validation for high value GMP protocols.
Construction	No special requirements for materials such as metal surfaces, gaskets, outlets, sensors or other interior components.	Requires high-efficiency insulation and gaskets to withstand cyclical decontamination procedures.	SANYO components are not subjected to stress beyond typical operating conditions.
Convenience	All interior components remain inside the incubator to be sterilized concurrently with the interior surfaces.	Interior components must be removed and sent to an autoclave for sterilization.	Sterisonic™ reduces preparation time and labor for sterilization process; returns incubator to service faster.
Adjacency	No effect on adjacent incubators or other laboratory appliances, instrumentation or equipment.	Adjacent incubator chamber must be vacated or carefully monitored for temperature increases during high heat cycle.	No need to vacate adjacent incubator or other equipment above, below or aside Sterisonic™ incubator during sterilization process.
CO₂ Sensor	Remains inside chamber. Sensor sampling system is completely sterilized during cycle.	The CO ₂ sensor, HEPA filters and other components must be removed prior to the process, and thoroughly decontaminated or replaced prior to reassembly.	Sterisonic™ CO ₂ sensor uses no moving parts and requires no recalibration following sterilization process.
In Situ Protection	Active Background Contamination Control remains in operation, continuously scouring the incubator of airborne and waterborne pathogens that can cause contamination or cross-contamination among cultures.	Heat sterilization offers no passive benefits to protect cell cultures <i>in situ</i> .	Sterisonic™ continues to mitigate contamination during normal operation.

Hydrogen peroxide vapor is more efficient than heat sterilization and requires a fraction of the downtime. Manufacturers of laboratory incubators claim to solve contamination problems with various approaches to incubator design. Some of these operational techniques are moderately successful but limited in terms of long-term efficacy and convenience. Most require periods of downtime during which cultures must be removed and placed in other incubators to maintain temperature, humidity and CO₂ levels. Several manufacturers offer high temperature surface sterilization processes in incubator design. Heat decontamination appears to be effective against vegetative microorganisms and fungal spores.

Where it Matters: Sterisonic™ GxP Value Increases with Sterilization Frequency: Beyond conventional clinical and research applications, the advantages of using the Sterisonic™ GxP increase in direct proportion to the critical sensitivity of the cell lines in process, or the frequency of complete sterilization procedures required to separate one lot from another under GMP or other regulatory criteria. The more often sterilization is required, the more comparative availability of the Sterisonic™ GxP vs. conventional incubators that use time-consuming high heat sterilization systems, and thus, the greater the value in the laboratory.



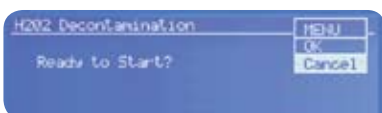
Finish.

Incubator must cool from high heat temperatures to near ambient.



The integrated microprocessor controller includes a simple membrane touch switch to initiate the sterilization process. When pressed the LCD display will prompt the user to confirm START. When confirmed, the electric door interlock will engage to seal the door; the air circulation blower will move air and atomized H₂O₂ through the chamber and infrared sensor sampling circuit; the LED display will indicate the H₂O₂ cycle status and remaining time; the UV lamp ON status and remaining time. The controller will provide an audible and visual notification of cycle completion.

The SANYO Sterisonic GxP graphical display indicates sterilization sequence status throughout the process which typically takes less than three hours after shelves, humidity pan and plenum components are repositioned within the chamber for proper exposure.



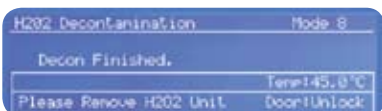
After inner and outer door locking is confirmed, the operator initiates the sterilization sequence.



The H₂O₂ solution is vaporized and circulated throughout the interior chamber for 7 minutes, exposing all interior surfaces and components.



When the vaporization sequence is completed the UV lamp is energized for up to 90 minutes, reducing the H₂O₂ aerosol to sterile water droplets.



The control panel indicates the cycle is completed. Once interior shelves, humidity pan and plenum components are replaced in their operating position the incubator is ready for service.

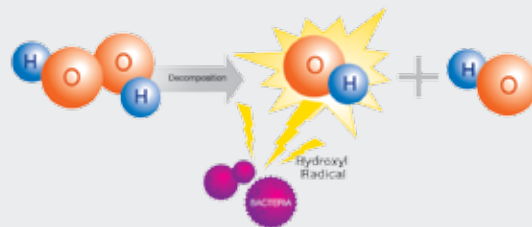
H₂O₂ and Ultraviolet Light: The Fastest Combination

The H₂O₂ sterilization process permits quick turn-around of the cell culture incubator from process to process where a complete sterilization is required. Applications include *in vitro* fertilization, tissue regeneration and other highly specific protocols subject to intense scrutiny or regulation.

Removing an incubator from service is a costly distraction that requires significant downtime for the decontamination process, prep before and after, and additional time for the chamber to reach a measured equilibrium suitable for cell culture.

While H₂O₂ is effective for a complete sterilization required separating protocols, the need for a continued protection during the cell culture process is acute. Following years of research and testing, the SANYO Electric Co., Ltd. introduced the SafeCell™ UV sterilization system. SafeCell™ is a unique sterilization technology described as Active Background Contamination Control™. This process arrests and destroys contaminants within the incubator chamber, and also compares favorably to high heat sterilization offered by leading industry competitors at 90°C and 140°C.

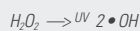
Ultraviolet Light Neutralization of H₂O₂¹¹



When hydrogen peroxide is added to an aqueous solution that is simultaneously irradiated with ultraviolet light (UV) the result is that the hydrogen peroxide more readily breaks down into •OH free radicals than when UV is not present, as illustrated in equation



There are, therefore, significantly more hydroxyl free radicals to enter into chain initiation steps than is the case without UV. UV light thus greatly increases the oxidative power of hydrogen peroxide in a manner similar to that of metal activation (Fenton's reagent). Although it has not been made clear how the reaction proceeds, it seems likely that the ultraviolet energy enables hydrogen peroxide to either separate into two hydroxyl free radicals, each having nine protons and nine electrons, as suggested by equation



or to obtain an electron from some source, probably the target organic compounds, and thus dissociate into one hydroxide ion (nine protons and ten electrons [OH⁻]) and one hydroxyl free radical (nine protons and nine electrons [•OH]) as shown in equation



The hydroxyl free radicals then go on to enter or perpetuate a chain reaction.

Independent Test Results Document the Efficacy of SANYO H₂O₂ Technique

Independent testing commissioned by SANYO supports the efficacy of the concentric contamination control technique based on H₂O₂ vapor followed by ultraviolet light exposure to render the H₂O₂ to trace amounts of sterile water and oxygen. The decontamination of the inner chamber of the incubator by hydrogen peroxide gas was verified with no BI (biological indicator) growing as observed in every BI collected from all setting locations inside the chamber. While a proposed ISO standard 11138-6¹² is under consideration by the association for the Advancement of Medical Instrumentation, standards for the use of EtO have been suggested for H₂O₂ protocols.

Test Protocol and Results

Objective: To certify the decontamination effect to the inner chamber of an incubator by hydrogen peroxide gas.

Client: SANYO Electric Co., Ltd. 370-0596 1-1-1 Sakata Oizumi Oura-gun Gunma, Japan

Product Identification: CO₂ incubator Model MCO-19AIC(UVH) with H₂O₂ decontamination kit and H₂O₂ atomizer.

Test Microorganism: *Geobacillus stearothermophilus* ATCC 12980 (spore) selected by SANYO. This microorganism is used as an index microorganism in verification of H₂O₂ vapor technologies for decontaminating indoor surfaces contaminated with biological or chemical agents issued by the United States Environmental Protection Agency.

Biological Indicator (BI) for H₂O₂ gas made by Apex Laboratories, Inc., Lot H1838.

Test Method: The test method is conducted as following the decontamination effect validation protocol that is attached to the product. Following the protocol, biological indicators were positioned at strategic locations in the inner chamber of the incubator. The inner chamber was decontaminated using the product H₂O₂ decontamination mode. After decontamination, biological indicators were put into Tryptic Soy Broth (BBL) and cultured at 55°C for one week. Location map detailed in report. Contact SANYO for detailed test results.

Test Result: With no growth exhibited in any of the biological indicators the sterilization of the inner chamber by hydrogen peroxide gas was certified. See Table 1.

Residual H₂O₂ concentration is indicated in Table 2. Residual H₂O₂ gas level in the inner chamber after the H₂O₂ vapor sterilization process followed by the programmed ultraviolet light exposure suggests that concentration was below 0.1ppm, below the detection limit.

Testing Institution: Kitasato Research Center of Environmental Sciences, Judicial Foundation, 1-15-1 Kitasato Sagami-hara-city Kanagawa, Microbiology Division Test dates, October 27-28, 2008

Validating Report of Decontamination Effect on Incubator by Hydrogen Peroxide Gas (Abstract)

Tested Product:
SANYO CO₂ Incubator, MCO-19AIC(UV)
with H₂O₂ decontamination kit, MCO-HL and H₂O₂ generator, MCO-HP

Test Result:

- With no BI (Biological Indicator) growing was observed in every BI collected from all setting locations inside chamber, the decontamination of the inner chamber of the incubator by hydrogen peroxide gas was verified.
- Residual H₂O₂ gas level in the inner chamber after the decontamination process / UV decomposition process was below 0.1 ppm which is the lower detection limit.

Abstract of Test Report: No20_0289 issued on 5th November 2008

KITASATO Research Center of Environmental Sciences
1-15-1 Kitasoto Sagami-hara city Kanagawa, Japan

Approved by: *Toshihiro Itoh*
President, Toshihiro Itoh

Table 1 Biological Indicator Culture Results, H₂O₂ Decontamination with UV Cycle, Biological Indicator (BI) *geobacillus stearothermophilus* ATCC 12908 (spore), by Apex Laboratories, Inc., Lot H1838.

Biological Indicator Growth			
Biological Indicator	Test 1	Test 2	Test 3
Interior	-	-	-
Control	+	+	+

Table 2 Residue, H₂O₂ gas concentration, measured by hydrogen peroxide detector tube, Gastech, Co. Ltd., No. 32. Valid until February 2011.

Test Condition Growth	Measured Site	Measured Value
Decontamination process, UV decomposition of H ₂ O ₂	Suction from access port, chamber rear. H ₂ O ₂	<0.1 ppm (lower than detection limit)

UV Sterilization Efficacy

The SANYO UV system is based upon an isolated, narrow bandwidth (253.7nm) ozone-free ultraviolet lamp interlocked with the incubator door. The interior is comprised of copper-enriched stainless steel with copper-enriched stainless steel shelves, brackets and plenum components. A directional airflow and containment plenum surrounds the UV exposed humidity reservoir in a removable, stainless steel pan. The multi-faceted approach to contamination control is designed to destroy airborne particulates introduced during door openings, as well as contaminants that grow in the water reservoir. With active and passive systems working together in the SANYO performance model, contaminants that inevitably enter the chamber through routine door openings or other means are intercepted and destroyed while cell culture continues uninterrupted.

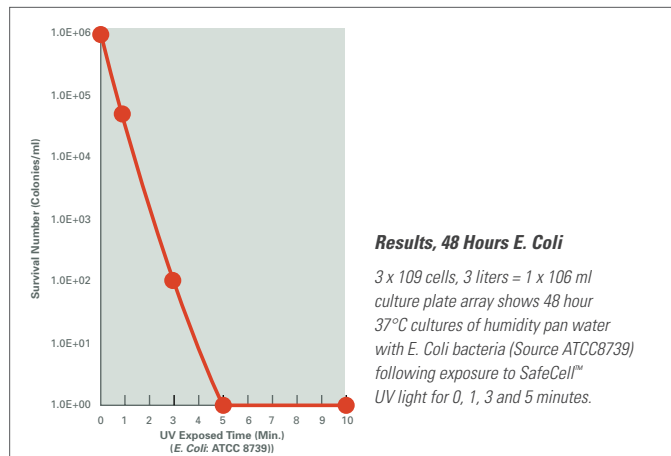
METHOD	UV	HIGH HEAT	
	SANYO	Brand F (140°C)	Brand H (90°C)
TEST RESULTS, MAXIMUM LOG REDUCTIONS			
Bacteria	> 4.5	> 4.5	> 4.5
Yeast	> 2.9	> 2.9	> 2.9
Mold	> 2.7	> 2.7	> 2.7
DECONTAMINATION OPTIONS			
Overnight	✓	✓	✓
Active Background Contamination Control™	✓	⊘	⊘

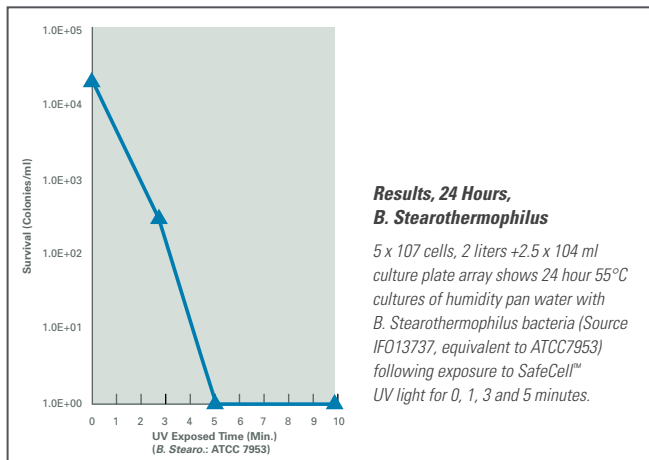
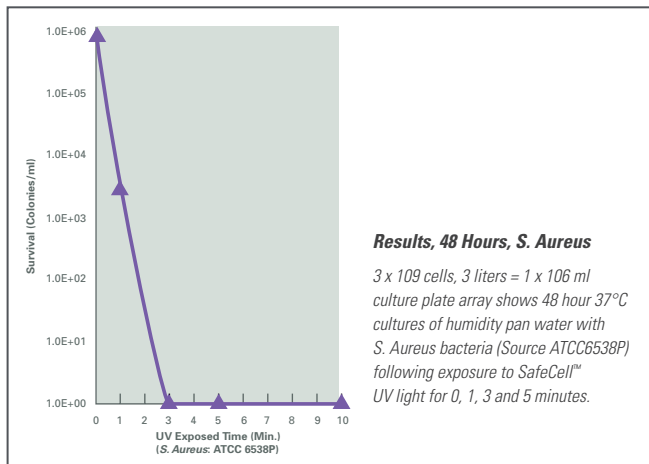
Efficacy of UV Exposure On Humidity Water

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, 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 and cultured prior to observation of colonies.



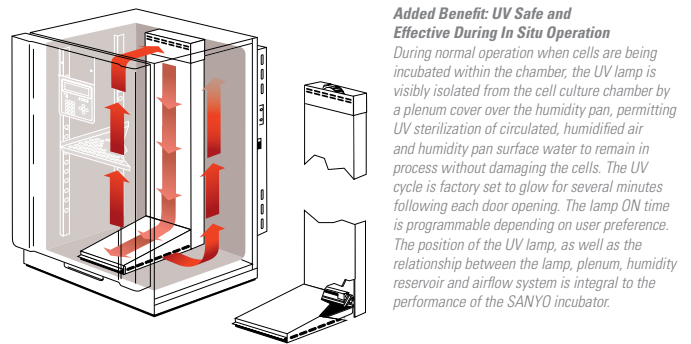


Results, Three Months, Airborne Exposure

Humidity pan water cultures 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.

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 incubator with UV sterilization function. As the cell culture process proceeds in the incubator chamber, the work of germicidal protection from airborne organisms continues unabated without costly downtime. This protection extends to thermophilic organisms as well.



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 over the humidity pan, permitting UV sterilization of circulated, humidified air and humidity pan surface water to remain in process without damaging the cells. The UV cycle is factory set to glow for several minutes following each door opening. The lamp ON time is programmable 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 SANYO incubator.

InCuSaFe™ Construction for Germicidal Protection

SANYO offers exclusive use of InCu SaFe™ copper-enriched stainless steel alloy interior surfaces within a technical design created to eliminate contamination sources and to mitigate the effect of airborne contaminants introduced through normal use.

- Selected to provide natural germicidal protection without rust or corrosion, InCu SaFe™ expresses a natural germicidal attribute to inhibit the growth of molds, fungi, mycoplasma and bacteria when exposed to humidity and CO₂.
- All interior components, including the air management plenum, shelf supports, humidity pan and blower wheel assembly are easily removable without tools if required.
- During the H₂O₂ sterilization cycle interior components can be repositioned within the chamber for *in situ* sterilization.
- All interior surfaces are exposed for conventional wipe down.
- Large curve corners and electropolished surfaces are easy to clean.
- Pass-thru ports accommodate probes or instrumentation leads as required for specialized cell culture protocols. Each chamber includes a port positioned in the rear wall, upper left, with dual silicone stoppers inside and outside the cabinet for added protection.

Mycoplasma Strain

	Positive Control	Conventional Type 304 Stainless Steel	SANYO InCu SaFe™	Conventional Copper C1100
Mycoplasma fermentans PG18	YES	YES	NO	NO
Mycoplasma orale CH19299				
Mycoplasma arginini G230				
Mycoplasma hominis PG21				

How SANYO InCu SaFe™ Inhibits Mycoplasma: Survival Results

Chart summarizes test results with four strains of mycoplasma. Results demonstrate how SANYO InCu SaFe™ copper-enriched stainless steel alloy offers germicidal properties of conventional C1100 copper while maintaining both corrosion-proof and discoloration-resistant properties of conventional Type 304 stainless steel. Detailed test results are available from SANYO.



Passive Contamination Control Benefits of SANYO InCu SaFe™ Copper Enriched Stainless Steel

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

Comparative Antibacterial Characteristics of SANYO InCu SaFe™ Copper-Enriched Stainless Steel

The inherent germicidal efficacy of SANYO InCu SaFe™ 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 below.

Species	InCu SaFe™ Copper-Enriched Stainless Steel	Conventional Stainless Steel
<i>E. Coli</i> (ATCC8739)	99.928%	0%
<i>E. Coli</i> (IFO3301)	99.847%	0%
<i>S. Aureaus</i> (ATCC6538P)	99.998%	0%
<i>B. Subtilis</i> (ATCC6633)	99.997%	—
<i>B. Stearothermophilus</i> (ATCC7953)	99.870%	0%

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

Conclusion

The SANYO Sterisonic™ GxP Model MCO-19AIC(UVH) incorporates a series of internal systems, processes and design factors that work together to maintain a multi-layered defense against contamination in the *in vitro* environment. Integration of a safe and effective two-hour sterilization process, the fastest in the industry, using an H₂O₂ vapor atomizer offers total sterilization of all interior surfaces and return to service more quickly than conventional incubators that use high heat sterilization. As a result, the SANYO incubator can be used for a broader range of cell culture applications, including the industry's most highly regulated protocols.

For additional product details visit www.sanyobiomedical.com/sterisonic

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- DeSorbo, Mark A.; March 3, 2009, *Contamination Control for the Life Sciences; Vaporized Hydrogen Peroxide*, "... a never-ending quest for sterility, safety and quality assurance."
- Caputo, Ph.D., Ross A.; Robert Reich, Jim Fisher, Robert E. Byrnes, Ph.D.; March 3, 2009; Contamination Control for the Life Sciences; *VHP: The Sterilant of Choice, Characterization, Properties and Biological Effects of Vapor Phase Hydrogen Peroxide*.
- Agalocco, James, 2008; Member, USP Microbiology and Sterility Assurance Expert Committee: Quality of Manufactured Medicines, General Session II, Wednesday, September 24, 2008; Performance Testing, *Microbiology Topics - A Look to the Future: USP Activities Impacting Sterilization & Sterility Assurance* [71, Sterility Testing; 1211, Sterilization/Sterility Assurance; 1229 Sterilization Methods].
- Aldridge, Ph.D., Susan; February 15, 2007; Genetic Engineering News; *Techniques for Cell Culture Improvement*.
- Typical applications such as *in vitro* fertilization, stem cell culture, regenerative tissue culture, autologous cell culture or proprietary pharmaceutical processes require the CO₂ incubator to be vacated, completely sterilized and validated at the conclusion of one process or batch and preceding the next. The speed and efficacy of the SANYO H₂O₂ system permits frequent sterilization with validation under these mandates with the benefit of short lead time, minimal preparation, quick cycle and resolve and fast return to service, usually within three hours.
- Validating Report of Decontamination Effect on Incubator by Hydrogen Peroxide Gas* (Abstract) Tested Product SANYO CO₂ incubator, MCO-19AICUVH with H₂O₂ decontamination kit, MCO-HL and H₂O₂ generator, MCO-HP Test Result: With no BI (Biomedical Indicator) growing was observed in every BI collected from all setting locations inside chamber, the decontamination of the inner chamber of the incubator by hydrogen peroxide gas was verified. Residual H₂O₂ gas level in the inner chamber after the decontamination process / UV decomposition process was below 0.1ppm which is the lower detection limit. Abstract of Test Report : No. 20-0289 issued on 5th November 2008 KITASATO Research Center of Environmental Sciences 1-15-1 Kitasato Sagami-hara city Kanagawa, Japan.
- Marketed as SafeCell UV, US Patent 6,255,103.
- Where indicated, independent testing funded by SANYO Commercial Solutions and performed by Celsis Analytical Services, 6200 S. Lindbergh Blvd., St. Louis, MO, 63123 USA, Celsis is an FDA registered cGMP analytical services laboratory and functions under current Good Manufacturing Practices (cGMP) and applicable Good Laboratory Practices (GLP). Celsis has been successfully audited by regulatory agencies (FDA, EPA, DEA). www.celsis.com/lab. Detailed test results are available from SANYO, toll-free (800) 858-8442.
- Direct Heat and Air Jacket™ U.S. Patent 5519188.
- Industrial Waste Treatment Handbook. Woodard & Curran, Frank Woodard, Woodard & Curran, Inc.; Edition: 2, illustrated, revised; Published by Butterworth-Heinemann, 2006; ISBN 0750679638, 9780750679633; Page 182.
- Association for the Advancement of Medical Instrumentation, Working Group, ISO/DIS 14161, *Sterilization of Healthcare Products, Biological Indicators, Guidance for the Selection, Use and Interpretation of Results*; December 3, 2007. Resolution to develop a new ISO standard, ISO 11138-6 for biological indicators (BIs) for processes using vapor phase hydrogen peroxide.

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