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A Comparative Analysis of Ultraviolet Light vs High-Heat Sterilization in a Cell Culture CO_2 Incubator

by Hiroki Busujima and Deepak Mistry

In 2001, **SANYO Electric Co., Ltd.** (Osaka, Japan) introduced a cell culture CO_2 incubator that employs an isolated narrow-bandwidth ultraviolet light to destroy airborne contaminants in the incubator chamber, as well as waterborne organisms in the humidity water reservoir. Comparative testing commissioned by **SANYO** in 2006 and performed by a certified independent testing laboratory (see *Table 1*) confirms that the **SANYO** ultraviolet 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 model MCO-18AIC-UV CO_2 incubator (**SANYO**) (*Figure 1*) isolates the UV light emission from cell cultures during normal operation to permit sterilization of the internal atmosphere following routine door openings without damaging cell cultures, a process that cannot be replicated with a heat sterilization technique.

The CO_2 incubator remains an essential tool for research and clinical laboratory work. The incubator performs a dynamic function that directly exposes cell cultures and culture media to an enriched yet potentially contaminated atmosphere within the chamber. Without the inherent protection of a biological safety cabinet, however, the incubator cannot inhibit the migration of airborne particulates into the chamber when the inner door is opened during routine use. Thus, by creating a humidified environment for cell culture, the CO_2 incubator poses a chronic 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.

Contamination sources

Typical incubator contaminants include bacteria, yeast, and mold. Although most cell culture work is performed in a biological safety cabinet with an optimum laboratory technique, such contaminants cannot be eliminated during transfer, nor can they be totally reduced 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 are added, and the chamber atmosphere is exposed to room air.

Heat sterilization

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 CO_2 levels. Several manufacturers offer high-temperature surface sterilization processes in incubator design. Heat decontamination appears to be effective against vegetative microorganisms and fungal spores.

- High-heat incubators require high-efficiency insulation and gaskets 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 advanced administrative planning to accommodate the culture relocation and downtime
- The CO_2 sensor, HEPA filters, and other components must be removed prior to the process, and thoroughly decontaminated or replaced prior to reassembly
- Once initiated, the complete heating and cooling cycle can extend beyond 24 hr, although the actual ramp, soak, and cool-down vary among manufacturers
- Heat sterilization is an active process independent of (and outside the parameters of) the cell culture environment generally established at 37 °C. Thus, while effective under manually initiated cycles, typically overnight, heat sterilization offers no passive benefits to protect cell cultures in situ. Therefore, the propensity for airborne contamination reoccurs at the first door opening after sterilization is complete.

Alternative to heat sterilization

The need for continued protection during the cell culture process is acute. Following years of research and testing, **SANYO Electric Co.** introduced the SafeCell™ UV sterilization system (U.S. patent no. 6,255,103), a 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.

Table 1 Independent test results*

Organism		Bacteria							Yeast	Mold
		Enterococcus faecalis ATCC 29212	*Escherichia coli ATCC 8739*	*Pseudomonas aeruginosa ATCC 9027*	*Salmonella typhimurium ATCC 13311*	*Bacillus subtilis ATTC 6633*	*Bacillus subtilis (control) ATCC 9372*	*Bacillus stearothermophilus (control) ATCC 7953*	*Candida albicans ATCC 10231*	*Aspergillus niger ATCC 12604*
Control Count		89,000 cfu/ml	290,000 cfu/ml	300,000 cfu/ml	360,000 cfu/ml	10,000 cfu/ml	44,000 cfu/ml	23,000 cfu/ml	9,000 cfu/ml	5,000 cfu/ml
Metal Substrate										
SANYO MCO-18AIC-UV, Ultraviolet Light @ 253.7nm	Bottom Recovery	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml
	Log Reduction	>3.9	>4.4	>4.4	>4.5	>3	>3.6	>3.3	>2.9	>2.7
	Top Recovery	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	20 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml
	Log Reduction	>3.9	>4.4	>4.4	>4.5	>3	>3.3	>3.3	>2.9	>2.7
Brand F, Elevated Heat @ +140°C	Bottom Recovery	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml
	Log Reduction	>3.9	>4.4	>4.4	>4.5	>3	>3.6	>3.3	>2.9	>2.7
	Top Recovery	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	250 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml
	Log Reduction	>3.9	>4.4	>4.4	>4.5	>3	>2.2	>3.3	>2.9	>2.7
Brand H, Elevated Heat @ +90°C	Bottom Recovery	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	120 cfu/ml	ND, <10 cfu/ml
	Log Reduction	>3.9	>4.4	>4.4	>4.5	>3	>3.6	>3.3	1.85	>2.7
	Top Recovery	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	30 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml
	Log Reduction	>3.9	>4.4	>4.4	>4.5	>3	>3.1	>3.3	>2.9	>2.7
Glass Substrate										
SANYO MCO-18AIC-UV, Ultraviolet Light @ 253.7nm	Bottom Recovery	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml
	Log Reduction	>3.9	>4.4	>4.4	>4.5	>3	>3.6	>3.3	>2.9	>2.7
	Top Recovery	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml
	Log Reduction	>3.9	>4.4	>4.4	>4.5	>3	>3.6	>3.3	>2.9	>2.7
Brand F, Elevated Heat @ +140°C	Bottom Recovery	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml
	Log Reduction	>3.9	>4.4	>4.4	>4.5	>3	>3.6	>3.3	>2.9	>2.7
	Top Recovery	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	20 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml
	Log Reduction	>3.9	>4.4	>4.4	>4.5	>3	>3.3	>3.3	>2.9	>2.7
Brand H, Elevated Heat @ +90°C	Bottom Recovery	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml
	Log Reduction	>3.9	>4.4	>4.4	>4.5	>3	>3.6	>3.3	>2.9	>2.7
	Top Recovery	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml
	Log Reduction	>3.9	>4.4	>4.4	>4.5	>3	>3.6	>3.3	>2.9	>2.7
Inner Door Gasket Substrate										
SANYO MCO-18AIC-UV, Ultraviolet Light @ 253.7nm	Bottom Recovery	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml
	Log Reduction	>3.9	>4.4	>4.4	>4.5	>3	>3.6	>3.3	>2.9	>2.7
	Top Recovery	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	50 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml
	Log Reduction	>3.9	>4.4	>4.4	>4.5	>3	>2.9	>3.3	>2.9	>2.7
Brand F, Elevated Heat @ 140°C	Bottom Recovery	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml
	Log Reduction	>3.9	>4.4	>4.4	>4.5	>3	>3.6	>3.3	>2.9	>2.7
	Top Recovery	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml
	Log Reduction	>3.9	>4.4	>4.4	>4.5	>3	>3.6	>3.3	>2.9	>2.7
Brand H, Elevated Heat @ +90°C	Bottom Recovery	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	120 cfu/ml	ND, <10 cfu/ml
	Log Reduction	>3.9	>4.4	>4.4	>4.5	>3	>3.6	>3.3	1.85	>2.7
	Top Recovery	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml	ND, <10 cfu/ml
	Log Reduction	>3.9	>4.4	>4.4	>4.5	>3	>3.6	>3.3	>2.9	>2.7

Test Protocol

Incubator Preparation: Comparative tests performed under identical conditions in a SANYO MCO-18AIC-UV and two competitive incubators of the same nominal interior size. UV sterilization (SANYO) and high heat sterilization processes (competitive incubators) were initiated in accordance with the manufacturers' operating manual.

Bacteria Preparation: Three passes before use, then 24-hour growth. Yeast: Grown for 3-5 days. Mold: Grown for 5-7-days.

Inoculation: Incubator interior component samples (coupons) consisting of stainless steel, glass and gasket substrates used in incubator design were inoculated and placed within the incubator interiors at the following locations: ceiling, floor, water pan.

Inoculum Concentration: 1 x 10^6, volume 0.1ml, confirmed by plate count protocol.

Process: With contaminated test organisms on coupons placed inside the chamber, the decontamination function was initiated on each CO2 incubator in accordance with manufacturers' instructions. After exposure to the decontamination process, each inoculated coupon was removed from the chamber. Recovery was washed from the coupon and a plate count was performed to quantify remaining organisms. Bacteria was tested using Tryptic Soy agar and Dextrose agar incubated 5-7 days at 20-25°C.

Results: Compilation of results was based on organism growth following the manufacturer's recommended decontamination process (sterilization). Results were expressed by comparing the initial count and log reduction.

Conclusions: Based on the results, the independent investigation concludes that each sterilization method, including UV active background contamination control and high heat cycle, are equally effective in sterilzation of the empty incubator interior chamber against selected organisms.

Testing Organization: Independent testing funded by SANYO E&E America Company 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.

*Independent testing funded by **SANYO E&E America Co.** (Bensenville, IL) and performed by **Celsis Analytical Services** (St. Louis, MO). Detailed test results are available at www.sanyobiomedical.com/beattheheat.

Figure 1 Model MCO-18AIC-UV CO_2 incubator, 6.0 ft^3 (170 L) with integrated UV light decontamination system and copper-enriched interior surfaces.

UV sterilization efficacy

The UV system is based on an isolated, narrow-bandwidth (253.7-nm) ozone-free ultraviolet lamp interlocked with the incubator door. The interior comprises 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 multifaceted 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 system, contaminants that inevitably enter the chamber through routine door openings or other means are intercepted and destroyed while cell culture continues uninterrupted.

Overnight or event UV sterilization

Independent testing confirms that the UV sterilization technique employed by the incubator is equally effective against contamination as conventional high-heat sterilization over a range of +90 °C to +140 °C. Whenever overnight or event sterilization of the incubator chamber is desired, all interior components are removed for autoclaving, exposing all interior surfaces to ultraviolet light. 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 proteins and destroys contaminants.

Safety and efficiency of UV 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 5 min following each door opening. 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, are integral to the performance of the incubator.

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.

*Mr. Busujima is Chief Researcher, **SANYO Electric Co., Ltd.,** Osaka, Japan. Mr. Mistry is Marketing Manager, **SANYO E&E America Co.,** 1062 Thorndale Ave., Bensenville, IL 60106, U.S.A.; tel.: 800-858-8442; fax: 630-238-0074; e-mail: dmistry@sss.sanyo.com. For a detailed copy of the **SANYO** White Paper, visit www.sanyobiomedical.com/beattheheat.*