

# A Comparative Analysis of Ultraviolet Light vs High-Heat Sterilization in a Cell Culture CO<sub>2</sub> Incubator

by Hiroki Busujima and Deepak Mistry

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In 2001, SANYO Electric Biomedical Co., Ltd. (Osaka, Japan) introduced a cell culture CO<sub>2</sub> incubator that employs an isolated narrow-bandwidth ultraviolet light to destroy airborne contaminants in the chamber and water-borne organisms in the humidity reservoir. In 2006, comparative testing commissioned by SANYO and performed by a certified independent testing laboratory (see Appendix

suggests that the ultraviolet light sterilization process is as effective against bacteria, yeasts, and molds as high-heat sterilization

however, the incubator cannot inhibit the migration of airborne particulates into the chamber when the inner door is opened

**Testing suggests that the ultraviolet light sterilization process is as effective against bacteria, yeasts, and molds as high-heat sterilization at sustained temperatures.**



**Figure 1.** Model MCO-18AIC-UV CO<sub>2</sub> incubator, 6.0 ft<sup>3</sup> (170 L) with integrated UV light decontamination system and copper-enriched interior surfaces.

at sustained temperatures ranging from +90 to +140. The company's MCO-18AIC-UV CO<sub>2</sub> incubator (Figure 1) isolates 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<sub>2</sub> incubator remains an essential tool for research and clinical work. The incubator performs a dynamic function that directly exposes cell cultures and culture media to an enriched atmosphere within the chamber. Without the inherent protection of a biological safety cabinet,

during routine use. Thus, by creating a humidified environment for cell culture, the CO<sub>2</sub> incubator poses a chronic risk of contamination leading to loss of cell cultures or expressed products, loss of efficiency due to downtime, compromise in reproducible results, and the 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 technique, such contaminants cannot be eliminated during transfer, nor can they be totally reduced by adding antibiotics to culture media, or chemical algacides 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 migra-

tion 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

Various approaches to incubator design are utilized to ameliorate contamination problems. 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<sub>2</sub> 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 planning to accommodate culture relocation and downtime
- The CO<sub>2</sub> 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 hours, 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 Biomedical Co. introduced the SafeCell UV sterilization system, a sterilization technology described as Active Background Contamination Control. This process arrests and destroys contaminants within the incubator chamber, and compares favorably to high-heat sterilization.

### 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 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

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. As the cell culture process proceeds in the incubator chamber, the work of germicidal protection from airborne

## Ultraviolet light affects DNA by causing pyrimidine dimers to form when adjacent pyrimidine bases on the DNA strand become covalently linked. The dimer disrupts the normal replication of the DNA and destroys contaminants.

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

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. Lamp "on" time is programmable from 0 to 30 min. The position of the UV lamp, as well as

organisms continues unabated without downtime. This protection extends to thermophilic organisms as well.

### About the authors

Mr. Busujima is Chief Researcher, SANYO Electric Biomedical Co., Ltd., in Osaka, Japan. Mr. Mistry is Marketing Manager, SANYO E&E America Co., in Bensenville, IL. Independent test results. Independent testing funded by SANYO E&E America Co. and performed by Celsis Analytical Services, St. Louis, MO. Detailed test results are available at [www.sanyobiomedical.com/beattheheat](http://www.sanyobiomedical.com/beattheheat). More information is available by sending email to [dmistry@sss.sanyo.com](mailto:dmistry@sss.sanyo.com) or from:

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