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# FAQ

## *A Comparative Analysis of Ultraviolet Light Decontamination vs. High Heat Sterilization in the Cell Culture CO<sub>2</sub> Incubator*

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## Celsis Incubator Study

### Why was this study conducted ?

- To determine the effectiveness of decontamination methods and provide an objective comparison between:
  - Sanyo MCO18AIC UV & 20AIC UV; UV Decontamination plus InCu Safe
  - Forma Stericycle – Dry Heat Sterilization
  - Heraeus HeraCell – Moist Heat Sterilization

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## Independent Testing

### Who is Celsis and why did you choose this testing laboratory ?

- Celsis laboratories are FDA registered and function under current Good Manufacturing Practices (cGMP) and have been successfully audited by regulatory agencies (FDA, EPA, DEA) and 9 out of the top 10 worldwide pharmaceutical companies
- Many Fortune 500 companies in the pharmaceutical and BioPharma industries have come to rely upon the Celsis Analytical Services divisions to fulfill their testing needs.

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## Celsis Protocol

### What is the protocol of the study ?

- Inoculated in Cu safe stainless steel, glass and silicone gasket components
- Bacteria Preparation: Three passes before use\*, then 24-hour growth. Yeast: Grown for 3-5 days. Mold: Grown for 5-7-days \*This insures the healthiest organisms are used for inoculation by growing each strain in three successive batches. Therefore the strain used will have a robust growth.
- The decontamination function was initiated on each CO2 Incubators contaminated with test organisms in accordance with each manufacturer's instructions
- After exposure the inoculated coupons (i.e. coupons are incubator interior component samples) consisting of stainless steel, glass and gasket substrates were removed from the chamber
- Results were compiled and based on organism growth after sterilization in each incubator

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## Organism Selection

### Why were these organisms selected for this study?

- Standard organisms were used as sterilization indicators plus spores from spore forming organisms common in microbiology environments:

Bacteria - *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Salmonella typhimurium*, *Bacillus subtilis*, *Bacillus stearothermophilus*.

Yeast - *Candida albicans*

Mold - *Aspergillus niger*

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## Organism Selection

### Why were these organisms selected for this study?

- Bacteria, molds and yeasts are found virtually everywhere and are able to quickly colonize and flourish in the rich and relatively undefended environment provided by cell cultures. Because of their size and fast growth rates, these microbes are the most commonly encountered cell culture contaminants. In the absence of antibiotics, microbes can usually be readily detected in a culture within a few days of becoming contaminated, either by direct microscopic observation. The species of yeast and mold used in the study are those that are can robustly thrive in harsh conditions and are difficult to detect.

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## Organism Preparation

### How were organisms prepared in the study?

- Bacteria – Three passes before use.  
Grown for 24 hours on Tryptic Soy broth
- Yeast - Grown for 3-5 days on Sabouraud Dextrose agar
- Mold – Grown for 5-7 days on Sabouraud Dextrose agar
- Dilute the stock suspension one milliliter into 500 milliliters of sterile buffered water. Inoculum concentration should be  $>1 \times 10^4$ . Confirm organism concentration by pour plate procedure.

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## Inoculation

**How were the coupons inoculated and where were they positioned in the incubator ?**

- The bacteria is tested using Tryptic Soy agar and incubated at 30-35°C for 48-72 hours. The Fungi was tested using Sabouraud Dextrose agar and incubated 5-7 days at 20-25°C.
- Incubator interior component samples (coupons) consisting of stainless steel, glass and gasket substrates used in the incubator design were inoculated and placed within the incubator interiors at the following locations: top and bottom of the chamber, water pan.

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# Results

The control count of the bacteria expressed in cfu/ml is compared to the final recovered amount in cfu/ml after each decontamination method is conducted in each incubator.

Reported initial count and log reduction in colony forming units (cfu).

Organism	Bacteria							Yeast	Mold
	<i>Enterococcus faecalis</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Salmonella typhimurium</i>	<i>Bacillus subtilis</i> (6633)	<i>Bacillus subtilis</i> (control)	<i>Bacillus stearotherophilus</i> (control)	<i>Candida albicans</i>	<i>Aspergillus niger</i>
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
<b>SANYO MCO-18AIC-UV, Ultraviolet Light @ 253.7nm</b>									
Log Reduction	>4.5							>2.9	>2.7
<b>Forma Brand SteriCycle™ 370, Elevated Heat at 90°C</b>									
Log Reduction	>4.5							>2.9	>2.7
<b>Hereaus Brand HeraCell™ 150, Elevated Heat at +140°C</b>									
Log Reduction	>4.5							>2.9	>2.7

Germ reduction capability of the decontamination process expressed in log form

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## Results – Final Count

### How was the recovery analyzed and final results reported?

- Recovery is washed from the coupon and a plate count is performed to quantify the organisms remaining.
- Compiled results based on organism growth after sterilization in each incubator.
- Reported initial count and log reduction in colony forming units (cfu).
- The control count of the bacteria expressed in cfu/ml is compared to the final recovered amount in cfu/ml after each decontamination method is conducted in each incubator.

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## Results – log reduction

### How are results measured in log reductions?

- The germ reduction capability of the decontamination process is 3 orders of magnitude ( $=10^3$  or "log 3"), which means the survival probability of the germs is  $10^{-3}$ . If such items are sterilized the average number of "surviving germs" or, correctly spoken, cfu's which are found on the items after sterilization.
- A lot of items which have to be sterilized are carrying a contamination of 10 germs each prior to sterilization. The germ reduction capability of the sterilization process is 6 orders of magnitude ( $=10^6$  or "log 6"), which means the survival probability of the germs is  $10^{-6}$ . If one sterilises a statistically significant number of these items the average number of cfu's which is found on the items after sterilisation is:  $10 / 10^6 = 10^{-5}$  or  $10 * 10^{-6} = 10^{-5}$ . This means that, in average 1 cfu is found per  $10^5 = 100.000$  items.

## Results – log reduction

### Why are the majority of the log reductions equivalent for each decontamination method ?

- The majority of the log reductions for recovery after each decontamination method were ND (Non-detectable), less than 10 cfu/ml which demonstrates a minimum level of detection for an organism (i.e. ND) in microbiology. The equivalent log reductions in organism growth showed that each decontamination method are equally effective against selected organisms.

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## Results

What is the position of the independent testing facility ? How is their position unbiased and objective ?

- Celsis found no significant difference between incubators; The UV sterilization technique employed by the Sanyo incubator is equally effective against contamination as conventional high-heat sterilization over a range of +90 °C to +140 °C.
- "Celsis Laboratory Group performed this testing solely on its own at the St. Louis, MO Celsis facility without any supervision, direction, input or other source of bias from either Sanyo or any third party. As for the results, Celsis Laboratory Group determined that Sanyo's ultraviolet light sterilization process is as effective against bacteria, yeasts and molds as high heat sterilization, when measured in competitive products at sustained temperatures ranging from +90°C to +140°C."

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## Conclusions / Implications

### What was the implications of the study ?

- Independent testing confirms the efficacy of our patented SANYO SafeCell™ ultraviolet decontamination system compared to high-heat methods
- Heat sterilization offers no passive benefits to protect cell cultures *in situ* from airborne contamination
- UV sterilization technique employed by the SANYO incubator is equally effective against contamination as conventional high heat sterilization over a range of +90°C to +140°C.

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## UV Decontamination

### What types of organisms can UV decontaminate ?

- SANYO ultraviolet light sterilization process is as effective against bacteria, yeasts and molds.

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## Heat Sterilization

What are disadvantages of heat sterilization ?

- 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 sterilization sequence requires planning to accommodate the 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 heat and cooling cycle can extend beyond 24 hours, although the actual ramp, soak and cool-down vary among manufacturers.
- While effective under manually initiated cycles, typically overnight, heat sterilization offers no passive benefits to protect cell cultures in situ. Thus, the propensity for airborne contamination re-occurs at the first door opening after sterilization is complete.

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## Passive Benefits

**Why does heat sterilization offer no passive benefits to protect cell cultures *in situ* from airborne contamination?**

- 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.
- The need for a continued protection during the cell culture process is acute and is not addressed by event heat sterilization process.

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## UV Decontamination

### How can UV decontaminate and mitigate the growth of organisms?

- Microorganisms are deactivated by ultraviolet light, by specific incident energies.
- The efficacy of ultraviolet light at 253.7nm is a function of exposure over time for selected organisms. Some organisms require more exposure than others. The SafeCell™ UV system is designed to destroy many common organisms known to impact the cell culture environment.
- 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. (i.e. Primarily UV light causes alterations in the structure of DNA that inhibits DNA replication and therefore an increase in the number of organisms).

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# UV Efficacy – Mechanism of SANYO SafeCell UV System

## What is the mechanism by which UV effectively decontaminates in the Sanyo SafeCell system ?

- A directional air-flow and containment plenum surrounds the UV exposed humidity reservoir in a removable, stainless steel pan.
- The mechanism employs 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.
- 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.

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## Effect on cell cultures

### How does the Sanyo Safecell UV system not affect cell cultures during incubation?

- 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 decontamination of circulated, humidified air and humidity pan surface water to remain in process without damaging the cells.

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# Active Background Contamination Control

**What is the mechanism of active background contamination control? How does SANYO Active Background Contamination Control™ ?**

- Mechanism: Active reduction of microbial contamination already existing in an area.
- 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 decontamination 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.

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