# Moist Heat Decontamination Effectiveness of Esco CelCulture® with High-Temperature $CO_2$ Sensor (HITEMP) $CO_2$ Incubator

by Siti Rahmania Sari


## Summary

Esco CelCulture® with High-Temperature $CO_2$ Sensor (HITEMP) $CO_2$ Incubator is equipped with a 90°C moist heat decontamination feature. This feature has been evaluated for its effectiveness against bacteria. The bacteria tested were *Bacillus atrophaeus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis*. All tested bacteria were placed on the middle of the top, middle, and bottom tray. The decontamination temperature reached 90°C while relative humidity is about 35 - 60%. The study result shows that 6 logs of vegetative cells, and 4 logs of bacterial spore are totally killed after 14 hours decontamination cycle.


## Introduction

A $CO_2$ incubator is an essential tool for maintaining a constant and suitable temperature of growth and cultivation cells[1]. Different from the biological safety cabinet, the incubator cannot minimize the movement of airborne particles into the chamber when the inner door is opened during routine use. The microorganism can be transported through the air, attached to particles, or via direct contact with humans. Cross-contamination may result in irreversible damage to experimental results that a repeated experimental procedure is needed. The cleanliness of the a$CO_2$ incubator must be maintained, either by decontamination or disinfection.

Moist heat decontamination is a method which combined heat and humidity[3]. Moist heat in the form of saturated steam under pressure is the most widely used and the most dependable because it is non-toxic, inexpensive, rapidly microbicidal, sporicidal, and rapidly heats and penetrates fabrics[1]. The ideal steam for decontamination is dry saturated steam and entrained water (dryness fraction ≥97%)[4]. Moist heat or steam sterilizers are also used in healthcare facilities to decontaminate microbiological waste and sharps containers. Esco CelCulture® $CO_2$ Incubator with moist heat decontamination feature will help the user to decontaminate the $CO_2$ incubators.

## Materials and Method

Materials used in this experiment were:

1. Esco CelCulture® $CO_2$ Incubator (CCL-170B/T-8-HITEMP SN-148590)
2. Biosafety Cabinet Class II (Esco Airstream)
3. Vortex (Labnet)
4. Pipette volume 10µL
5. Pipette volume 100µL
6. Pipette volume 1000µL
7. Pipette tips
8. Tryptone Soya Broth medium (Neogen)
9. Tryptone Soya Agar medium (Neogen)
10. Sterile DI water
11. Sterile 0.9% NaCl solution (produced in house and sterilized)
12. 10mm stainless steel disc (produced in house and sterilized)

In this study, the following test strain on the recommendation of cell culture specialist due their resistant natures as shown below:

1. *Bacillus atrophaeus*, a typical gram-positive bacterium
2. *Enterococcus faecalis*, a typical gram-positive bacterium

3. *Pseudomonas aeruginosa*, a typical gram-negative bacterium
4. *Staphylococcus epidermidis*, a typical gram-positive bacterium

Table 1. Test Bacteria

| Test Bacteria | Type of Bacteria | Initial Population |
|---|---|---|
| *Bacillus atrophaeus* | Bacterial spore | $9.60 \times 10^4$ |
| *Enterococcus faecalis* | Vegetative bacteria | $2.85 \times 10^6$ |
| *Pseudomonas aeruginosa* | Vegetative bacteria | $3.40 \times 10^6$ |
| *Staphylococcus epidermidis* | Vegetative bacteria | $4.85 \times 10^6$ |

**Preparation of bacterial vegetative and spore suspension**

The bacteria were inoculated from slant agar to 10 mL 0.9% (NaCl) sodium chloride solution. Enumeration was done with Total Plate Count method on Tryptone Soya Agar (TSA). The number of bacteria should reach $10^8$ CFU/ml.

**Preparation of test carrier**

Stainless steel disc carriers (d=10mm) were prepared from the same material as the Esco CelCulture® $CO_2$ Incubator chamber to imitate contaminated incubator chamber surface. The discs were inoculated with 10µl of bacteria inoculums and spore suspension then let it dry in the BSC. Place the discs in the top, middle, and bottom of incubator. Additional discs were prepared in the same manner to serve as positive controls and initial number of populations. Positive control discs were put into TSB medium and the initial number discs were aseptically transferred into 0.9% NaCl solution then enumerated. Other additional discs were also prepared as a negative control.

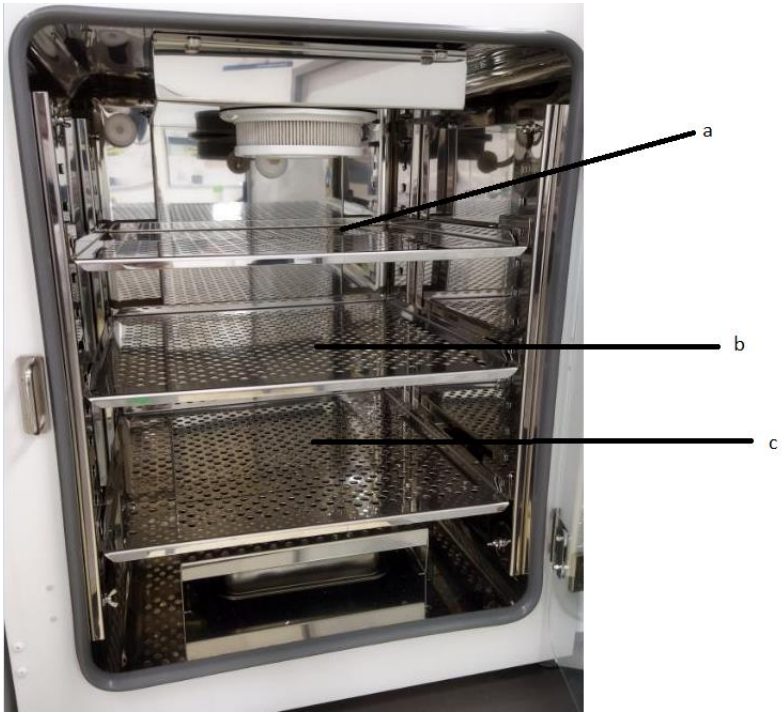


Figure 1. Set up for decontamination effectiveness test on Esco CelCulture® $CO_2$ Incubator.
a. top tray; b. middle tray; c. bottom tray

**Result and Discussion**

The table below summarizes the results of triplicate test runs. Decontamination cycle in Esco CelCulture® with HITEMP $CO_2$ Incubator consists of three phases: Heating phase when the temperature increase to 90°C, which take approximately 60 minutes; soaking or decontamination phase when the temperature 90°C is held for 9 hours, drying also occurs in this phase, during decontamination the temperature reached 90°C while relative humidity is about 35 - 60%, relative humidity value is different for each incubator; cooling phase which takes around 4 hours for the temperature to cool down to 37°C, while the humidity which already decreases to 30%, then increase again until 70%.

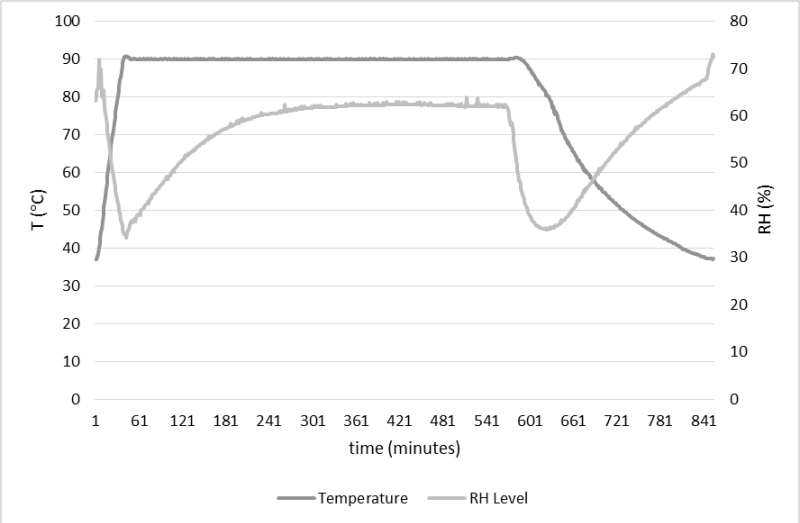


Figure 2. Graph of temperature during full cycle decontamination

The Health Protection Agency (HPA) in the United Kingdom has proved that a $CO_2$ incubator with a 90°C moist heat decontamination cycle could deactivate ordinarily resistant fungus, bacterial spores, and vegetative cells. At the completion of the 14 hour decontamination cycle, the chamber is cold and dry. The challenge of the decontamination feature was to reduce microorganisms 6 logs. Microorganisms tested were a vegetative cell of bacteria and spore of bacteria.

Table 1. Result of Decontamination Test on Esco CelCulture® with HITEMP $CO_2$ Incubator for 7 Days Repetition 1

| Test Bacteria | Carrier placed | Day | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Negative control | | - | - | - | - | - | - | - |
| Positive control | | + | + | + | + | + | + | + |
| *Bacillus atrophaeus* | Top Tray | - | - | - | - | - | - | - |
| | Middle Tray | - | - | - | - | - | - | - |
| | Bottom Tray | - | - | - | - | - | - | - |
| *Enterococcus faecalis* | Top Tray | - | - | - | - | - | - | - |
| | Middle Tray | - | - | - | - | - | - | - |
| | Bottom Tray | - | - | - | - | - | - | - |
| *Pseudomonas aeruginosa* | Top Tray | - | - | - | - | - | - | - |
| | Middle Tray | - | - | - | - | - | - | - |
| | Bottom Tray | - | - | - | - | - | - | - |
| *Staphylococcus epidermidis* | Top Tray | - | - | - | - | - | - | - |
| | Middle Tray | - | - | - | - | - | - | - |
| | Bottom Tray | - | - | - | - | - | - | - |

Table 2. Result of Decontamination Test on Esco CelCulture® with HITEMP $CO_2$ Incubator for 7 Days Repetition 2

| Test Bacteria | Carrier placed | Day | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Negative control | | - | - | - | - | - | - | - |
| Positive control | | + | + | + | + | + | + | + |
| *Bacillus atrophaeus* | Top Tray | - | - | - | - | - | - | - |
| | Middle Tray | - | - | - | - | - | - | - |
| | Bottom Tray | - | - | - | - | - | - | - |
| *Enterococcus faecalis* | Top Tray | - | - | - | - | - | - | - |
| | Middle Tray | - | - | - | - | - | - | - |
| | Bottom Tray | - | - | - | - | - | - | - |
| *Pseudomonas aeruginosa* | Top Tray | - | - | - | - | - | - | - |
| | Middle Tray | - | - | - | - | - | - | - |
| | Bottom Tray | - | - | - | - | - | - | - |
| *Staphylococcus epidermidis* | Top Tray | - | - | - | - | - | - | - |
| | Middle Tray | - | - | - | - | - | - | - |
| | Bottom Tray | - | - | - | - | - | - | - |

Table 3. Result of Decontamination Test on Esco CelCulture® with HITEMP $CO_2$ Incubator for 7 Days Repetition 3

| Test Bacteria | Carrier placed | Day | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Negative control | | - | - | - | - | - | - | - |
| Positive control | | + | + | + | + | + | + | + |
| *Bacillus atrophaeus* | Top Tray | - | - | - | - | - | - | - |
| | Middle Tray | - | - | - | - | - | - | - |
| | Bottom Tray | - | - | - | - | - | - | - |
| *Enterococcus faecalis* | Top Tray | - | - | - | - | - | - | - |
| | Middle Tray | - | - | - | - | - | - | - |
| | Bottom Tray | - | - | - | - | - | - | - |
| *Pseudomonas aeruginosa* | Top Tray | - | - | - | - | - | - | - |
| | Middle Tray | - | - | - | - | - | - | - |
| | Bottom Tray | - | - | - | - | - | - | - |
| *Staphylococcus epidermidis* | Top Tray | - | - | - | - | - | - | - |
| | Middle Tray | - | - | - | - | - | - | - |
| | Bottom Tray | - | - | - | - | - | - | - |

*Enterococcus faecalis, Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* vegetative cell log reduction were 6 logs. Spore of *Bacillus atrophaeus* log reduction is 4 logs. Moist heat decontamination destroys microorganisms by the irreversible coagulation and denaturation of enzymes and structural proteins. In support of this fact, it has been found that the presence of moisture significantly affects the coagulation temperature of proteins and the temperature at which microorganisms are destroyed[1].

Recent studies of moist heat decontamination of Esco CelCulture® $CO_2$ Incubator have shown that 5 logs of *Escherichia coli* and *Bacillus subtilis* are killed[2].

**Conclusion**

With the validation of a decontamination cycle to Esco CelCulture® with HITEMP $CO_2$ Incubator, the user can be assured of complete contamination elimination. The decontamination feature of Esco CelCulture® with HITEMP $CO_2$ Incubator successfully reduces 6 logs of bacteria, and 4 logs of bacterial spore.

**References**


1. Rutala,W.A, D.J. Weber, and Healthcare Infection Control Practices Advisory Committee (HICPAC).2008.CDC: Guideline for Disinfection and Decontamination in Healthcare Facilities
2. Nuryanti. 2009. Whitepaper: Effective Heat Decontamination on Esco CelCulture® $CO_2$ Incubator.
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4. Association for the Advancement of Medical Instrumentation. Steam decontamination and sterility assurance in health care facilities. ANSI/AAMI ST46. Arlington, VA, 2002:ANSI/AAMI ST46:2002.