Effectiveness of Moist Heat Decontamination in Esco CelCulture® Incubator Touch Screen

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Summary

Esco CelCulture® CO_2 Incubator with Touchscreen Controller features a 90°C moist heat decontamination cycle. The decontamination was tested using *Bacillus atrophaeus*, *Bacillus subtilis*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Staphylococcus aureus*. Bacterial suspensions were placed on stainless steel discs in different chamber areas. After a 14-hour cycle the system successfully achieved a 6-log reduction of vegetative cells, proving its effective decontamination performance.

Key Words: Esco CelCulture® Incubator Touch Screen, moist heat decontamination, log reduction

Introduction

When performing cell culture experiments outside the human body, it requires specific and stable conditions to support and maintain cell growth. These essential conditions are provided by a CO_2 incubator. CO_2 incubators are designed to create and maintain ideal conditions for optimal cell growth by controlling all critical environmental factors and keeping them stable throughout the culture process[8]. Maintaining these conditions consistently is crucial, as any deviation can lead to contamination in cell cultures, disrupting research and causing wasted time due to repeated experiments. To prevent this, keeping the CO_2 incubator clean is essential for maintaining culture quality and enhancing research efficiency, with regular decontamination being one of the most effective methods[2].

The Esco CelCulture® CO_2 Incubator with Touchscreen Controller is equipped with a Moist Heat Decontamination feature, designed to maintain a contaminant-free environment and ensure consistent culture quality. Moist heat decontamination is processing combined heat and humidity to eliminate microorganisms. Moist heat, delivered as saturated steam under pressure, is the most used and reliable sterilization method. It is non-toxic, cost-effective, quickly destroys microorganisms and spores, and efficiently heats and penetrates materials[7]. This feature will help the user to decontaminate their CO_2 Incubator.

Materials and Method

Materials used in this experiment were:

1. Esco CelCulture® CO_2 Incubator (CCL-050B-8-TS SN-200840)
2. Esco CelCulture® CO_2 Incubator (CCL-170B-8-TS SN-207067)
3. Biosafety Cabinet Class II (Esco Airstream)
4. Vortex (Labnet)
5. Pipette volume 10µL
6. Pipette volume 100µL
7. Pipette volume 1000µL
8. Pipette tips
9. Tryptone Soya Broth medium (Neogen)
10. Tryptone Soya Agar medium (Neogen)
11. Sterile deionized (DI) water
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 to their resistant natures as shown below:

1. *Bacillus atrophaeus*, a typical gram-positive bacterium
2. *Bacillus subtilis*, a typical gram-positive bacterium
3. *Staphylococcus epidermidis*, a typical gram-positive bacterium
4. *Enterococcus faecalis*, a typical gram-positive bacterium
5. *Staphylococcus aureus,* a typical gram-positive bacterium

Table 1. Test Bacteria

Test Bacteria	***Type of Bacteria***	***Initial Population***
Bacillus atrophaeus	*Vegetative bacteria*	*6.62 x 10^6*
Bacillus subtilis	*Vegetative bacteria*	*5.33 x 10^6*
Staphylococcus epidermidis	*Vegetative bacteria*	*3.67 x 10^6*
Enterococcus faecalis	*Vegetative bacteria*	*1.30 x 10^6*
Staphylococcus aureus	*Vegetative bacteria*	*4.57 x 10^6*

Preparation of bacterial vegetative suspension

The bacterial culture was transferred from agar plate into 30 mL of sterile deionized (DI) water. Enumeration was carried out using the Total Plate Count method on Tryptone Soya Agar (TSA). The target concentration was 10^8 CFU/mL.

Preparation of bacterial vegetative suspension

Stainless steel disc carriers, made from the same material as the Esco CelCulture® CO_2 Incubator chamber, were used to simulate a contaminated surface inside the incubator. Each disc was inoculated with 10 µL of a bacterial suspension and air-dried in a biosafety cabinet. To test decontamination performance in a challenging area, the discs were placed at the bottom tray. Additional discs served as positive controls (placed in TSB), initial count controls (enumerated in sterile DI water), and negative controls to ensure test reliability.

Results and Discussion

Moist heat decontamination is driven by two critical factors: heat and moisture. Heat induces protein denaturation, membrane disruption, and nucleic acid degradation, while moisture enhances heat penetration into microbial structures, thereby accelerating inactivation. The Health Protection Agency (HPA), United Kingdom, has validated that a CO_2 incubator equipped with a 90 °C moist heat decontamination cycle is capable of effectively inactivating resistant microorganisms. This 90 °C moist heat cycle has also been shown to inactivate a wide range of microorganisms, achieving an A_o value of ≥600, which corresponds to a ≥6-log reduction of common bacteria[4,5].

The effectiveness of moist heat is also strongly influenced by relative humidity (RH). RH levels above 50% create optimal conditions for microbial inactivation by facilitating the deep penetration of heat into fungal spores, bacterial spores, and vegetative cells. In addition, higher RH levels promote viral inactivation through mechanisms such as desiccation, lipid oxidation, and disruption at the air–water interface[3]. To address these requirements, the Esco CelCulture® CO_2 Incubator with Touchscreen Controller integrates an advanced Moist Heat Decontamination system. Its decontamination cycle consists of three phases: heating, soaking (with simultaneous drying), and cooling, ensuring effective and reliable contamination control for laboratory applications.

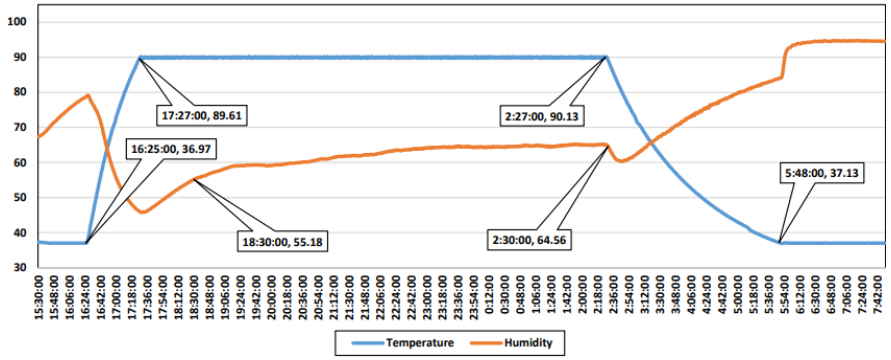

Figure 1. Graph of temperature and humidity during full cycle decontamination (CCL-050B-8-TS)

The decontamination cycles on CCL-050B-8-TS a heating phase that raises the chamber temperature to 90°C in approximately 62 minutes, a soaking phase that maintains 90°C with 55–65% relative humidity for 9 hours to ensure thorough microbial inactivation while drying occurs simultaneously, and a cooling phase that gradually reduces the temperature to 37°C over 4 hours, during which the humidity briefly drops to 60% then rising to 90%.

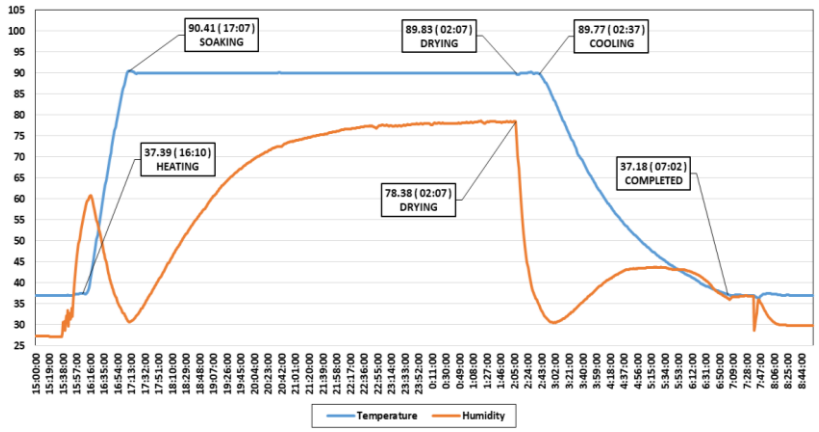

Figure 2. Graph of temperature and humidity during full cycle decontamination (CCL-170B-8-TS)

The decontamination cycle on the CCL-170B-8-TS begins with a heating phase that raises the chamber temperature to 90 °C in approximately 57 minutes, followed by a soaking phase. During soaking, the chamber is maintained at 90 °C with 75–80% relative humidity for 9 hours to ensure effective microbial inactivation, while simultaneous drying takes place as the relative humidity gradually decreases from around 78% to below 40%, ensuring the chamber is thoroughly dried. The cycle then enters a cooling phase, gradually reducing the temperature to 37 °C over 5 hours while humidity remains relatively low, before reaching the completed phase.

Both graphs show differences in humidity behavior during the cooling phase, with the first cycle exhibiting a sharp humidity rise while the second maintains relatively low levels. This indicates condensation occurs, which limited moisture removal during the drying process on CCL-050B-8-TS.

To evaluate the effectiveness of the decontamination cycle, a bacterial suspension was used to simulate microbial contamination inside the incubator chamber. The table below demonstrates the results of triplicate decontamination test runs.

Table 1. Result of Decontamination Test on CelCulture® CO_2 Incubator with Touchscreen Controller for 7 days Repetition 1

Test Bacteria	Days of Incubation						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
CCL-050B-8-TS							
Negative Control	-	-	-	-	-	-	-
Positive Control	+	+	+	+	+	+	+
Bacillus atrophaeus	-	-	-	-	-	-	-
Bacillus subtilis	-	-	-	-	-	-	-
Staphylococcus epidermidis	-	-	-	-	-	-	-
Enterococcus faecalis	-	-	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	-	-	-
CCL-170B-8-TS							
Negative Control	-	-	-	-	-	-	-
Positive Control	+	+	+	+	+	+	+
Bacillus atrophaeus	-	-	-	-	-	-	-
Bacillus subtilis	-	-	-	-	-	-	-
Staphylococcus epidermidis	-	-	-	-	-	-	-
Enterococcus faecalis	-	-	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	-	-	-

Table 2. Result of Decontamination Test on CelCulture® CO_2 Incubator with Touchscreen Controller for 7 days Repetition 2

Test Bacteria	Days of Incubation						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
CCL-050B-8-TS							
Negative Control	-	-	-	-	-	-	-
Positive Control	+	+	+	+	+	+	+
Bacillus atrophaeus	-	-	-	-	-	-	-
Bacillus subtilis	-	-	-	-	-	-	-
Staphylococcus epidermidis	-	-	-	-	-	-	-
Enterococcus faecalis	-	-	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	-	-	-
CCL-170B-8-TS							
Negative Control	-	-	-	-	-	-	-
Positive Control	+	+	+	+	+	+	+
Bacillus atrophaeus	-	-	-	-	-	-	-
Bacillus subtilis	-	-	-	-	-	-	-
Staphylococcus epidermidis	-	-	-	-	-	-	-
Enterococcus faecalis	-	-	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	-	-	-

Table 3. Result of Decontamination Test on CelCulture® CO_2 Incubator with Touchscreen Controller for 7 days Repetition 3

Test Bacteria	Days of Incubation						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
CCL-050B-8-TS							
Negative Control	-	-	-	-	-	-	-
Positive Control	+	+	+	+	+	+	+
Bacillus atrophaeus	-	-	-	-	-	-	-
Bacillus subtilis	-	-	-	-	-	-	-
Staphylococcus epidermidis	-	-	-	-	-	-	-
Enterococcus faecalis	-	-	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	-	-	-
CCL-170B-8-TS							
Negative Control	-	-	-	-	-	-	-
Positive Control	+	+	+	+	+	+	+
Bacillus atrophaeus	-	-	-	-	-	-	-
Bacillus subtilis	-	-	-	-	-	-	-
Staphylococcus epidermidis	-	-	-	-	-	-	-
Enterococcus faecalis	-	-	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	-	-	-

Based on the data presented above, bacterial suspensions with concentrations of 6 logs used to assess decontamination effectiveness showed no growth after 7 days of incubation. Moist heat inactivates bacteria by denaturing proteins, damaging nucleic acids, and disrupting cell membrane integrity, resulting in irreversible loss of cellular function[6]. These findings confirm that the moist heat decontamination cycle remains highly effective in eliminating bacteria at such concentrations.

Conclusion

The Esco CelCulture® CO_2 Incubator with Touchscreen Controller features a 90°C moist heat decontamination has been proven highly effective in eliminating high concentrations of bacterial contamination. The system successfully achieved complete inactivation of bacterial suspensions up to 6-log CFU/mL with no regrowth observed after 7 days of incubation. These results confirm the reliability and performance of Esco's decontamination system in maintaining a contamination-free environment, ensuring the safety and integrity of your cell culture work.

References

1. Almatuari, S.A.H, Zainab E.A.S., Abeer E.A.S., Zainab S.A., Zainab A.H.A., Alsheri, F.H.M. Al Meshaal M.A.A., Badriah J.E.A., Alhajhouj, Z.T.M., Alsaedi, A.O.A. 2023. The Role of Heat, Moisture, and Time in Achieving Effective Sterilization. Letters in High Energy Physics 2: 1148-1156.
2. Bekti.T.S. 2013. Whitepaper. Decontamination Effectiveness of Esco CelMate 170L CO_2 Incubator
3. Casanova L.M., Jeon S, Rutala WA, Weber DJ, Sobsey MD. 2010. Effects of air temperature and relative humidity on coronavirus survival on surfaces. Appl Environ Microbiol 76(9):2712-7.
4. Health Protection Agency. (2011, January 26). An evaluation of the decontamination effect on the inner chamber of ESCO CelCulture CO_2 incubator using the 90°C moist heat decontamination cycle (Report No. 55/10). Health Protection Agency, Microbiology Services, Porton Down.
5. Kremer, T. A., McDonnell, G., Mitzel, E., Jain, N., Hubert, H., Roth, K., ... & Villella, A. 2021. Thermal disinfection validation: the relationship between A0 and microbial reduction. Biomedical Instrumentation & Technology 55(3): 85-90.
6. Rashed, A. M., Hetta, A., Hashem, Z. S., & El-Katatny, M. M. H. 2020. Validation of moist and dry heat processes used for sterilization and depyrogenation during ampoules manufacturing. Journal of Advanced Biomedical and Pharmaceutical Sciences 3(3): 177-183.
7. Rutala,W.A, Weber D.J., and Healthcare Infection Control Practices Advisory Committee (HICPAC). 2008. CDC: Guideline for Disinfection and Decontamination in Healthcare Facilities
8. Tayebi-Khorami M, Chegeni N, Birgani MT, Danyaei A, Fardid R, Zafari J. 2022. Construction a CO_2 Incubator for Cell Culture with Capability of Transmitting Microwave Radiation. Journal Medical Signals Sens 12(2):127-132.