

Effectiveness of ULPA Filter on Removing Air Contaminants on CO2 Incubator Chamber

I. Introduction

Since 1991 CO2 incubator have offered reseraches and clinical lab a reliable and user-friendly system for application including culturing cell, biochemical studies, pharmaceutical and food processing. Laboratory incubators are used to grow and maintain cell cultures and are available in a variety of sizes and types. These CO2 incubator work to control three important variable related to tissue and cell culturing mamalian environment, there are CO2 level, temperature and relative humidity (RH). Good controlling environment is the most important thing to supporting the cell growth, for example free from any contaminant.

For many years contaminant become serious issue in the term of “cleanliness” with CO2 incubator. Microbial Contamination, caused by bacteria, bacerial spores, viruses, mycetoza, yeast or other microorganisms, frequently presents a major risk in cell culture experiments. Many manufacturers are working toward addressing some of the common challenges associated with culturing cells, the most important of which is reducing aerial contamination. A number of incubators now offer a high-temperature decontamination cycle that works much like a self-cleaning oven. Using heat-sterlize incubator will free form any unwanted contaminant and hazardous agent.

Besides, instant decontamination configuration, there are also continuous contamination removal units that work all the time and do not have to be initiated manually. One technology uses Ultra Low Particulate Air (ULPA) filtration to continuously cycle the air and remove airborne particulates and contaminants. An ULPA filter (theoretically) can remove from the air at least 99.999% of dust, pollen, mold, bacteria and any airborne particle with a size larger than 0.3 µm.

The following physical tests performed are reproducible and accurate and can be used to validate the equipment's performance against accepted criteria e.g airborne particulate contaminant. This test performed by using challenge of *B. subtilis* var *globigii* spore suspension.

II. Purpose

This test was performed to verify the ULPA filter performance on Esco's CelCulture CO2 incubator.

III. Methods

Apparatus

- a) CO2 Incubator model CCL-170-B
- b) A calibrated six-jet Collison nebulizer, set to spray the spore suspension at 0.2 ± 0.02 ml/min, complete with cone
- c) Retord stand and clamp to hold the nebulizer
- d) *Bacillus subtilis* var *globigii* spore suspension, with concentration of 5 to 8×10^4 spores/ml
- e) 90 mm (3.5”) petri dishes, filled with Trypticase Soya Agar
- f) A compressor, connected to dryer with pressure regulator to power the nebulizer
- g) A calibrated 0 to 28 psi pressure gauge

Test Procedure

- a) The CO2 incubator set up under its normal operation (37°C and 5 % CO2)

- b) Verify that all components of the CO₂ incubator system which contributes to its operations (air handling, filtration system, etc) are complete and functioning in accordance with the requirements of the type of CO₂ incubator and the operational mode under test.
- c) Set up 4 evenly spaced tray inside chamber, tray 3 should be slightly below access port.
- d) Cover the tray with 90 mm petri dish. See Figure 1
- e) Open the cover for control plates.
- f) Place the nebulizer with mounted next to the access port.
- g) Generate the nebulizer for 1 minute, 20 Psi.
- h) After 13 minutes, open all agar plates.
- i) After 5 minutes cover all agar plates, then incubate at 37°C for 48 hours.
- j) Observe and count the colony on agar plates.
- k) Test were done in three replicates.

Illustration

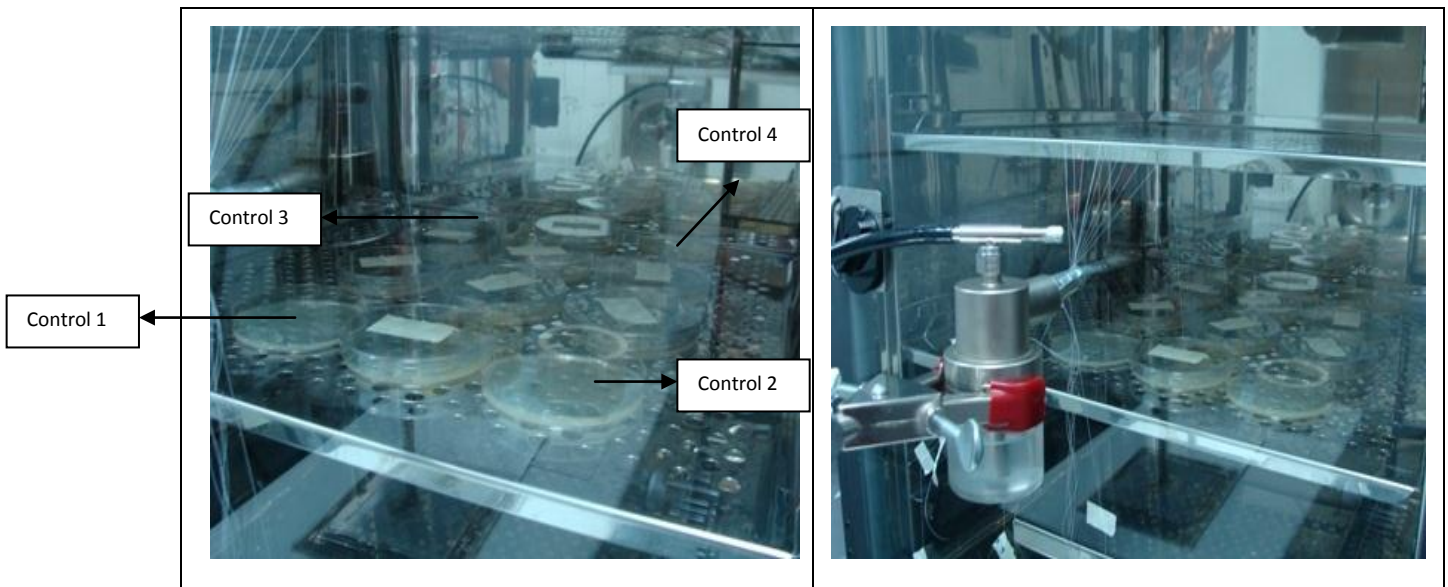


Figure 1. Agar Plates set up on tray with nebulizer

IV. Result and Conclusion

Result consist of:

- i. For control plates
- ii. Eight test plates

Table 1. Test result Ulpa efficiency test

Plate	Number of CFU captured on replicate		
	1	2	3
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
6	0	0	0
7	0	0	0
8	0	0	0
Control 1	>300	>300	>300
Control 2	>300	>300	>300
Control 3	>300	>300	>300
Control 4	>300	>300	>300

Table 1 shows that there is no single colony found on the test plates on the whole replicates, while the control plates captured more than 300 CFU *B. subtilis var globigii*.

The aerosol was sprayed into the chamber, caused the chamber to be filled with contaminants. It is describe by hundreds of colonies captured by the control plates at during initial time. After mere 13 minutes, the chamber is already back to its normal operation.

This result indicates the effectiveness of ULPA filter on removing air contaminants inside the chamber. This ensures all contaminants from the room air and chamber air are filtered and only clean air is re-circulated.