# Effectiveness of ULPA Filter on Removing Air Contaminants on $CO_2$ Incubator Chamber

by
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## I. Introduction

Since 1991 $CO_2$ incubator had offered research and clinical lab a reliable and user-friendly system for application including culturing cell, biochemical studies, pharmaceutical, and food processing. $CO_2$ incubators are used to grow and maintain cell cultures and are available in a variety of sizes and types. These $CO_2$ incubators work to control different parameters related to tissue and cell culturing mammalian environment– $CO_2$ and $O_2$ 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 $CO_2$ incubator. Microbial Contamination caused by bacteria, bacterial spores, viruses, Mycetozoa, 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 incubator with high heat sterilization 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 performed to verify the ULPA filter performance on $CO_2$ incubator

## III. Methods

### Apparatus

a) $CO_2$ Incubator model CCL-170B

b) A calibrated six-jet Collison nebulizer, set to spray the spore suspension at 0.2 $\pm$ 0.02 ml/min, complete with cone

c) Retort stand and clamp to hold the nebulizer

d) *Bacillus subtilis* var *globigii* spore suspension, with concentration of 5 to 8 x $10^4$ 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 $CO_2$ incubator set up under its normal operation (37°C and 5 % $CO_2$)
b) Verify that all component of the $CO_2$ incubators system which contributes to its operations (air handling, filtration system, etc) are complete and functioning in accordance with the requirements of the type of $CO_2$ 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

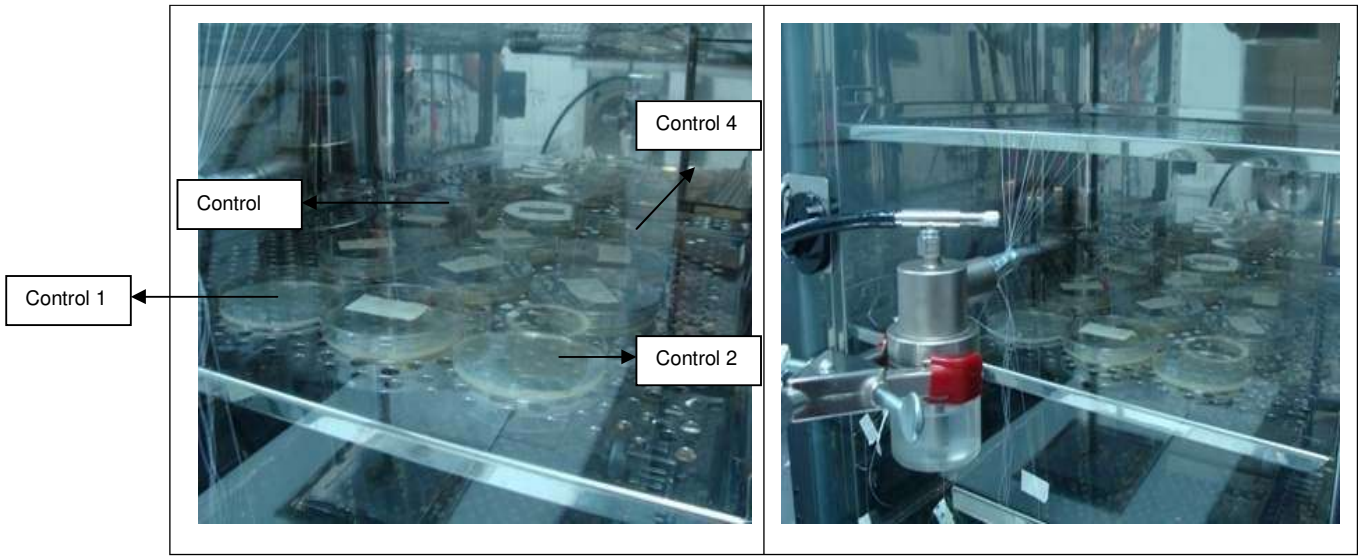


*Figure 1. Agar Plates set up on tray with nebulizer*

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 shown that there is no single colony found captured by test plates on whole replicates. While the control plates captured more than 300 CFU *B. subtilis* var *globigii*. The aerosol spraying from nebulizer into the chamber, caused the chamber full of contaminant. It's described by an hundred colony captured by the control plates at initial time. After 13 minutes with circulating system provide by ULPA filter, the chamber re-back at normal condition, describe by zero colony on all test plates for 5 minutes. This result indicating that ULPA filter recirculating system contribute high efficiency on airborne contaminant removal inside the chamber through airflow system. This condition contributes cleanliness for $CO_2$ incubator especially and cell culture laboratories in general.