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

I. Introduction

For years CO₂ incubators have proved to be a reliable and user-friendly piece of equipment for researchers and laboratory operators. They are widely used for applications such as cell culturing, biochemical studies, pharmaceutical and food processing. Laboratory incubators are used to grow and maintain cell cultures and normally come in a variety of sizes and types. On the other hand, CO₂ incubators work to control three important variables related to the tissue and cell culturing mammalian environment and these are CO₂ level, temperature and relative humidity (RH).

A good controlled environment is very important in supporting cell growth. For many years, contaminants have become a serious issue in terms of “cleanliness” with CO₂ incubators. Microbial contamination caused by bacteria, bacerial spores, viruses, mycetozoa, yeast or other microorganisms often cause major risks to 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. Nowadays, a number of incubators offer a high-temperature decontamination cycle that works much like a self-cleaning oven. Using a heat-sterilization incubator will free it from any unwanted contaminants and hazardous agents.

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 0.12 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 contaminants. This test is performed by using a 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 x 10⁴ 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\textsubscript{2} incubator set up under its normal operation (37°C and 5 % CO\textsubscript{2})

b) Verify that all components of the CO\textsubscript{2} 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 CO\textsubscript{2} incubator and the operational mode under test.

c) Set up 4 evenly spaced trays inside chamber, tray 3 should be slightly below access port.

d) Cover the tray with 90 mm petri dishes. 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 was done in three replicates.

**Illustration**

![Figure 1. Agar Plates set up on tray with nebulizer](image)
IV. Result and Conclusion

Result consist of:

i. For control plates
ii. Eight test plates

Table 1. Test result Ulpa efficiency test

<table>
<thead>
<tr>
<th>Plate</th>
<th>Number of CFU captured on replicate</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
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<tr>
<td></td>
<td>1</td>
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<td>7</td>
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<td>8</td>
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<tr>
<td>Control 1</td>
<td>&gt;300</td>
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<tr>
<td>Control 2</td>
<td>&gt;300</td>
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<tr>
<td>Control 3</td>
<td>&gt;300</td>
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<tr>
<td>Control 4</td>
<td>&gt;300</td>
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</tbody>
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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 which caused the chamber to be filled with contaminants. It is described by hundreds of colonies captured by the control plates during the initial period. After a mere 13 minutes, the chamber is already back to its normal operation.

This result indicates the effectiveness of the ULPA filter in removing air contaminants inside the chamber. Thus, it guarantees that all contaminants from the room air and chamber air are filtered and only clean air is re-circulated.