

A New Microbiological Cross Contamination Test on Escoco CelCulture CO2 Incubator

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I. Introduction

In the laboratory setting, transferring cell growth samples in and out of incubators would be done without concern about contamination and its potential effect on samples. Escoco has designed and built its CelCulture CO2 Incubator fitted with an ULPA filter to prevent airborne contamination continuously. The one function is to eliminate cross contamination caused by airborne contaminants.

Cross contamination is the term used to describe bacterial transfer from source into high risk. Cross contamination occurs when one object becomes contaminated by either direct or indirect contact with another object which is already contaminated.

CO2 incubators have become acceptable, reliable equipment given the growth in cell culture research and the ability of incubators to grow cells in vitro. But, on the flip side of this benefit, is the constant threat of contamination to the cell culture environment.

The aim of this study was to evaluate cross contamination at inner chamber of CO2 incubator. In this study, the test was performed with both methods adopted from NSF/ANSI 49:2008 using nebulizer and another test method performed using contaminated plates.

II. Experiment and Result

1. Cross Contamination using Nebulizer

1.1 Microorganism Preparation

Spore suspension. Spore suspension of *Bacillus subtilis* var *globigii* was bought from external supplier Presque Isle Culture. Dilutions were performed to obtain suspension with concentration 104 spores/ml.

1.2 Methods

For this test, a method for BSCs (NSF/ANSI 49:2002) was modified. System was challenged with spore suspension of *B. subtilis* var *globigii* with concentration 5-8 x 104 spores/ml. Test plates were placed at 15 inches in distances apart from the nebulizer. The nebulizer was then placed at 1/3 work tray depth from the back wall of the chamber and then alternating right and left side position. Place one control plate under the outlet of the nebulizer. Nebulizer operated under 10psi for 1 minute.

A smoke generator was used to determine the airflow pattern as shown in figure 1. It is found that the air pulled point occurs at 1/3 work tray depth from the interior back wall.



Figure 1. Smoke test to checking the airflow pattern

1.3 Result

Table 1. Cross contamination test result with nebulizer

Test	Left Side			Right Side		
	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3
Control	>300	>300	>300	>300	>300	>300
Test plates	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0

2. Cross contamination test with contaminated plates

2.1 Preparation

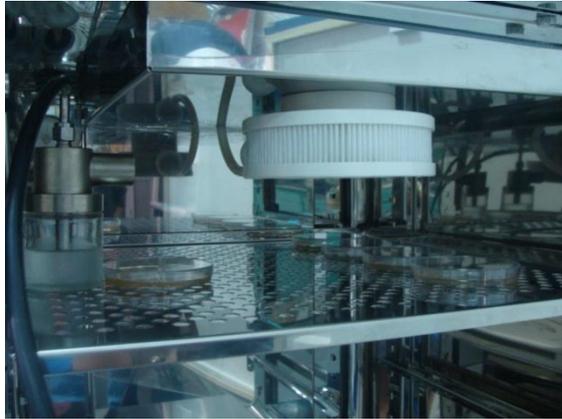
A fresh subculture of *Staphylococcus aureus* was used to prepare bacterial suspension. One oose of *Staphylococcus aureus* from agar slant was streaked into a new agar slant and incubated at 37°C for 24 hours. One oose from new slant was enriched on Trypticase Soya Broth (TSB) medium and incubated with shaker at 37°C for 24 hours, then determine the numbers of CFU/ml. Dilutions were performed to obtain suspension with concentration 10¹¹, 10¹⁰ and 10⁹ cells/ml.

2.2 Method

A 0.1 ml suspension of *Staphylococcus aureus* was spread on Trypticase Soya Agar (TSA) medium with concentration of 10¹¹, 10¹⁰, 10⁹ cells/ml, respectively, for top tray, middle tray, and bottom tray. Contaminated plates will incubate together with the test plates (clean plates) for a week.

Illustration

a. Cross Contamination with nebulizer



b. Cross contamination test with contaminated plates



2nd and 3rd column:
Clean plates

1st column:
Contaminated plates

2.3 Result

Table 2. Cross contamination test result with contaminated plates

Tray	Concentration (cells/ml)	Days of observation for clean plates						
		1	2	3	4	5	6	7
1	10 ¹¹	-	-	-	-	-	-	-
2	10 ¹⁰	-	-	-	-	-	-	-
3	10 ⁹	-	-	-	-	-	-	-

*(-) refers to no contamination from contaminated plates

3. Observation and Conclusion

The CelCulture CO₂ incubator passed the cross contamination test both using nebulizer and using contaminated plates, with **zero CFU** recovered from all tests. During the test using nebulizer the spore liberated from the nebulizer outlet was immediately pulled by ULPA filter fan system located at top of chamber. In other hand, there was no contamination the contaminated plates observe going on the clean plates during seven days. Both experiments have shown that a strategically positioned ULPA filter together with the Escoco designed air flow pattern give maximum protection to the samples in the incubator and prevent cross contamination from happening. ISO class 5 air condition with prevention of cross contamination makes this incubator an ideal environment for cell culture applications.