A New Microbiological Cross Contamination Test on Esco CelCulture® CO_2 Incubator

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I. Introduction

In the laboratory setting, transferring cell growth samples in and out of incubators can be done without concerns about contamination and its potential effect on samples. Esco has designed and built its CelCulture® CO_2 Incubator fitted with an ULPA filter to prevent airborne contamination. It eliminates cross-contamination caused by airborne contaminants.

Cross-contamination occurs when a sample becomes contaminated by either direct or indirect contact with another sample which is already contaminated.

CO_2 incubators have become acceptable and reliable equipment in the development and growth of cell cultures. But the threat of contamination to the cell culture environment remains a problem.

The aim of this study is to evaluate cross-contamination in the inner chamber of CO_2 incubator. Test was performed with both methods adopted from NSF/ANSI 49:2008 using a 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 of 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 positions. Place one control plate under the outlet of the nebulizer. The 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 check 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

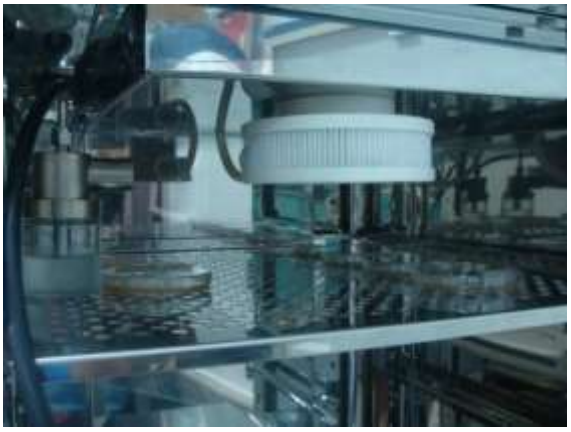
A fresh subculture of *Staphylococcus aureus* was used to prepare bacterial suspension. One ooze of *Staphylococcus aureus* from agar slant was streaked into a new agar slant and incubated at 37°C for 24 hours. One ooze from new slant was enriched on Trypticase Soya Broth (TSB) medium and incubated with 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 were incubated together with the test plates (clean plates) for a week.

Illustration

a. Cross Contamination with nebulizer



b. Cross contamination test with 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® CO_2 incubator passed the cross-contamination test both using nebulizer and contaminated plates, with **zero CFU** recovered from all tests. During the test using the nebulizer, the spore liberated from the nebulizer outlet was immediately pulled by ULPA filter fan system located at the top of the chamber. On the other hand, there was no contamination from the contaminated plates that were observed going on the clean plates for seven days. Both experiments have shown that a strategically positioned ULPA filter together with the Esco designed air flow patterns can give maximum protection to the samples in the incubator and prevent cross-contamination from happening. ISO class 5 air condition with the prevention of cross-contamination makes this incubator an ideal environment for cell culture applications.