Worldwide Headquarters • Esco Micro Pte Ltd • 21 Changi South Street 1 • Singapore 486777
Phone +65 6542 0833 • Fax +65 6542 6920 • mail@escoglobal.com • www.escoglobal.com

ROC No. 198400165W

Nebulizer Placement for Cross contamination Test on BSC

by
Dian Susanti and Alexander Atmadi

Introduction

NSF JointComittee is a discussion group consisting of representatives from NSF International, academia, leading NSF-certified biosafety cabinet manufacturers (including Esco), certifying companies, and filter manufacturers, tasked to maintain and improve the ANSI/NSF 49 standard on Class II Biosafety Cabinets.

The NSF Joint Committee meets annually, and one of the topics discussed during the 2010 meeting was the possibility of revising the NSF/ANSI 49 standard to perform cross contamination testing, specifically on the nebulizer placement for conducting this test. As a member of the NSF Joint Committee, Esco would like to contribute on this study.

The existing ANSI/NSF 49 standard specifies that the nebulizershould be placed at the midpoint(front to back)of the interior side wall, pointing towards the opposite wall. As comparison, the European Biosafety Standard EN 12469 specifies that the nebulizer should be placed at the air split point, to create the most challenging condition to pass the test.The air split point should be determined by a smoke generator, and the nebulizer should be placed accordingly. This European approach is being considered by the NSF Joint Committee.

The purpose of this study is to compare the cross contamination test result, between placing the nebulizer at the midpoint of the work surface per existing NSF/ANSI 49, versus at the actual air split point as determined by a smoke generator per EN 12469.

Apparatus

1. A calibrated six-jet Collison nebulizer, set to spray the spore suspension at 0.2 $\pm$ 0.02 ml/min
2. Retort stand, rod, and clamp to hold the nebulizer
3. *Bacillus subtilis var globigii* spore suspension, with concentration of 5 to 8 x 10^4 spores/ml
4. 90 mm (3.5”) petri dishes, filled with Trypticase Soya Agar
5. A compressor, connected to dryer and HEPA filter, with pressure regulator to power the nebulizer
6. A calibrated 0 to 28 psi pressure gauge
7. A smoke generator to determine the air split point

Test Procedure

1. An Esco Airstream AC2-4E1 Class II Biosafety Cabinet (**4ftwide**) was selected for this testing. The cabinet airflow was set at Nominal point (normal operation) at:

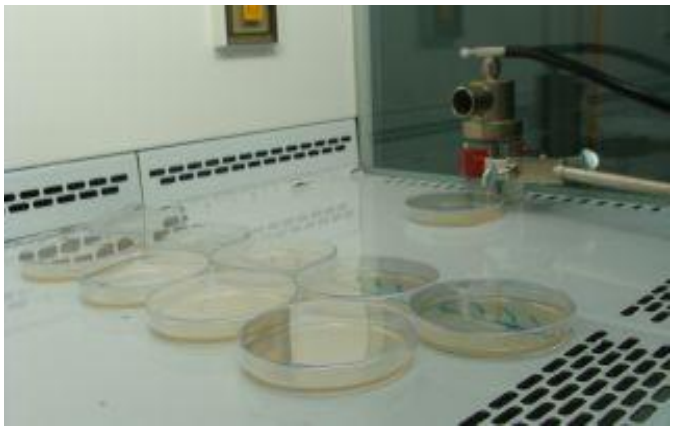
Airflow	**Nominal**
Inflow	0.45 m/s (90 fpm)
Downflow	0.30 m/s (60 fpm)

2. The nebulizer was positionedwith the back of the nebulizer located against the midpoint of the left interior side wall, with the spray axis parallel to the work surface and directed toward the opposite sidewall. Because this cabinet has side air grille, the back of nebulizer was placed against the side grille, instead of the side wall.

Worldwide Headquarters • Esco Micro Pte Ltd • 21 Changi South Street 1 • Singapore 486777
Phone +65 6542 0833 • Fax +65 6542 6920 • mail@escoglobal.com • www.escoglobal.com
ROC No. 198400165W

3. Place the agar plates on the work surface in the following manner (see Figure 1)
   - 1 control plate under the outlet of the nebulizer
   - 1 row of target plates with their centers at 14 inches (36 cm) from the side wall being tested
   - 1 more row of target plates beyond the 14 inches (36 cm) row

Left side



Right side

Figure 1. Cross contamination test with nebulizerat midpoint of interior side wall

4. The agar plate covers were removed, the the nebulizer was run for 5 minutes. After that, the agar plate covers were replaced, and the agar plates were placed in incubator at 37°C (90°F) for 48 hours. This test was performed three times on the left wall, then another three times on the right wall.

5. A Smoke generator was used to determine the air split point as shown in Figure 2. It was found that for this cabinet, the air split point occurs at 1/3 work surface depth from the interior back wall.

Figure 2. Smoke test to verify the air 'split" pattern

Worldwide Headquarters • Esco Micro Pte Ltd • 21 Changi South Street 1 • Singapore 486777
Phone +65 6542 0833 • Fax +65 6542 6920 • mail@escoglobal.com • www.escoglobal.com
ROC No. 198400165W

6. The nebulizer was placed at the 1/3 work surface depth from the back wall, and the agar plates were placed at the same location as in previous test, as shown in Figure 3.

Left side

Right side

Figure 3. Cross contamination test with nebulizer placed at the actual air split point (1/3 work surface depth from back wall)

Acceptance Criteria

The control plate placed under the nebulizer nozzle should recover more than 300 CFU of *Bacillus subtilis to* indicate sufficient amount of spores discharged. The total number of CFU recovered on target plates with centers greater than 14 inches (36 cm) shall not exceed 2 CFU per test.

Result

Test #1. Nebulizer at midpoint of interior side wall

Run	Number of *Bacillus Subtilis*CFU captured by:					
	Left Side			Right side		
	Front Row	Back row	Control	Front row	Back row	Control
1	**1**	0	>300	**1**	0	>300
2	0	0	>300	0	0	>300
3	0	0	>300	0	0	>300
Since all plates < 2 CFU, this test is**: Pass**						

Test #2. Nebulizer at air split point

Run	Number of *Bacillus Subtilis*CFU captured by:					
	Left Side			Right side		
	Front Row	Back row	Control	Front row	Back row	Control
1	0	0	>300	0	0	>300
2	0	0	>300	0	0	>300
3	0	0	>300	0	0	>300
Since all plates < 2 CFU, this test is**: Pass**						

Worldwide Headquarters • Esco Micro Pte Ltd • 21 Changi South Street 1 • Singapore 486777
Phone +65 6542 0833 • Fax +65 6542 6920 • mail@escoglobal.com • www.escoglobal.com

ROC No. 198400165W

Observation

1. The cabinet passed the cross contamination test at both midpoint and air split point, with zero CFU recovered from all tests, except the first test where the nebulizer was placed at the midpoint.

2.One CFU was recovered on the first left and right test when the nebulizer was placed at midpoint of the interior side walls.

3. During this test, the spore stream from the nebulizer outlet was immediately pulled by the side air grille located just behind the nebulizer. There was no spore stream observed going into the front of back air grille.

Conclusion

In contrast to the EN 12469 perception, placing the nebulizer at the midpoint may pose slightly more challenge to the cabinet compared to placing the nebulizer at the air split point. This argument would be more substantial if the cabinet only has front and back air grilles. However, this cabinet has additional novel side air grille, that removes the spores more effectively compared to the front and back air grille. When the NSF/ANSI 49 and EN 12469 standards were established, it's likely that only the front and back air grilles were present / taken into account on existing biosafety cabinets.

Based on this preliminary study, to pose a greater challenge to the cabinet, it is suggested that the NSF Joint Committee sticks to the current standard of placing the nebulizer at the midpoint of the interior side wall. A further study is suggested for cabinets that have side air grilles, to investigate the interaction between the side air grille and the front & back grilles.