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ROC No. 198400165W

Personnel Protection Test on Biosafety Cabinet With Width Less Than 3 ft

by
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Introduction

NSF Joint Committee is a discussion group consisting of representatives from NSF International, academics, leading NSF-certified biosafety cabinet manufacturers (including Esco), certifying companies, and filter manufacturer, 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 establishing the protocol in NSF/ANSI 49 standard to perform operator protection test on small-sized biosafety cabinets (BSC), with width less than 3ft.

Operator protection test is done by using a nebulizer to spray the challenge spores from inside the cabinet work zone, and sampling the air outside the cabinet sash opening to check for potential spore escape. Air sampling is at the center of sash opening is taken by 6x impingers, and at the right & left side of sash opening is taken by slit air samplers.

The existing ANSI/NSF 49 standard specifies that the slit air samplers should be placed at 203 mm (8") from the cabinet interior side walls. However, given the size limitations of smaller than 3 ft, such 8" distance is not possible. Therefore, the Joint Committee is seeking to establish a new measurement distance or method to test cabinets smaller than 3ft.

As a member of NSF Joint Committee, Esco is committed to provide recommendations on the revised testing protocol, and thus the purpose of this study is to establish and validate the recommended test protocol.

Apparatus

Operator protection test is performed to verify the ability of the biosafety cabinet to provide containment against a large amount of microorganisms released just behind the sash window, using test apparatus below:

1. Six impingers, each filled with 20 ml sterile distilled water, and connected to a manifold
2. Retort stand, bars, and clamps, to hold the impingers at the center of the cabinet front opening
3. Vacuum pump that provides suction for the impingers via manifold and controlled via individual valves
4. BIOS Flow Definer flow meter, to verify the flow rate into the impingers to be 12.5 l/minute
5. Two Barramundi slit air samplers, with 28.3 l/min suction and 1 rotation / 30 min speed
6. 140 mm Petri dishes, filled with Trypticase soy agar, for the slit air samplers
7. Stands to support the slit air samplers
8. A calibrated six-jet Collision nebulizer, set to spray the spore suspension at 0.2 ± 0.02 ml/min
9. *Bacillus subtilis var. globigii* spore suspension, with concentration of 5 to 8×10^8 spores / ml
10. A compressor, connected to dryer and HEPA filter, to power the nebulizer
11. Pressure gauge and valve, to adjust the pressure from compressor to the nebulizer to be 20 psi
12. A metal cylinder with 63 mm (2.5") diameter, to simulate the blocking caused by the operator's arm
13. A glass funnel, connected to vacuum pump
14. Millipore HA 0.45 micron membrane paper, to capture the spores from the impingers to the paper
15. An incubator, set to 37°C

Test Setup

1. The metal cylinder was placed at the left to right center of the work tray, protruding outside.
2. Nebulizer containing 60 ml suspension *B.subtilis globigii* was placed inside the cabinet at 101 mm (4") behind the sash window and 355 mm (14") above the work surface, directly above the stainless steel cylinder.
3. The glass impingers were placed outside the sash window at following locations:

Impingers	Height	Distance from sash	Separation from each other
2 x Top	355 mm (14") above work tray	51 mm (2")	152 mm (6") from BSC centerline
2 x Middle	Same as top of the cylinder	63.5 mm (2.5")	152 mm (6") apart
2 x Bottom	Same as bottom of the cylinder	63.5 mm (2.5")	51 mm (2") apart

4. The slit air samplers were installed with the inlet cylinder located at same height as the work tray, and the vertical axes of the inlets were at 150 mm (6") in front of the cabinet and placed at **varied distance from BSC inner side wall** as follows:

Test	Distance
1	127 mm (5")
2	101 mm (4")
3	76 mm (3")
4	51 mm (2")

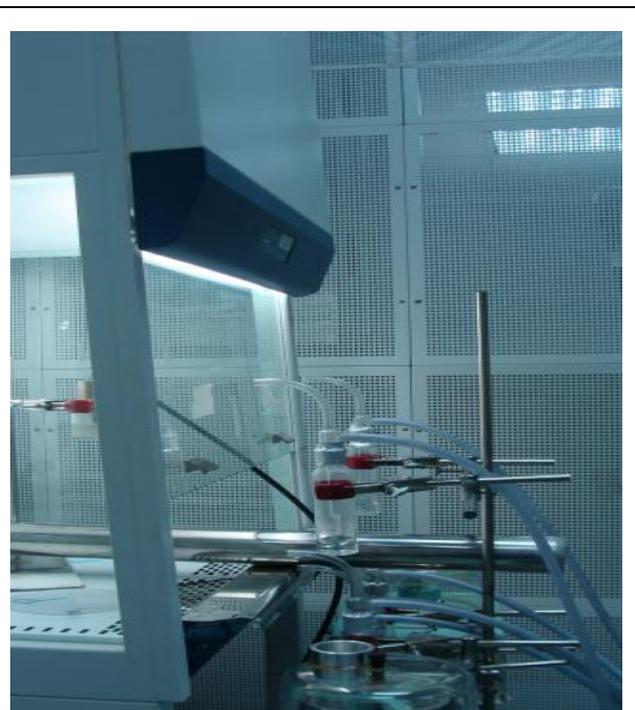
Note: Due to small cabinet size, slit air sampler distance of more than 127 mm (5") is not possible, because the slit air samplers will hit the impingers

5. The positive control agar plate was placed below the front intake grill to minimize the obstruction of airflow into the grill.

Pictures of Test Setup



Front View



Side View

Test Procedure

1. An Esco Airstream AC2-2E1 Class II Biosafety Cabinet (**2 ft wide**) was selected for this testing. The cabinet airflow was set at Low Inflow – High Downflow, which is the most difficult airflow combination from ANSI/NSF 49 microbiology performance envelope test to pass this operator protection test. For this cabinet:

Airflow	Nominal	➔	Low Inflow – High Downflow
Inflow	0.45 m/s (90 fpm)		0.40 m/s (80 fpm)
Downflow	0.30 m/s (60 fpm)		0.35 m/s (70 fpm)

2. The nebulizer, impinger suction, and slit air sampler were operated according to the cycle table below

Time lapse (min)	Operation procedure
0.0	Slit sampler start
5.0	Nebulizer start
6.0	Impinger start
11.0	Impinger end
11.5	Nebulizer end
30.0	Slit sampler end

3. At the end of each cycle, the content liquid of all impingers were poured into vacuum-powered the glass funnel to capture the potential spores by the membrane filter, which is then placed on agar plates without generating bubbles.

4. This test was repeated five times for each slit air sampler distance to the cabinet inner wall.

5. The agar plates with the filter, the agar plates from slit air sampler, and the positive control plates were incubated at 37°C (98°F) temperature for 48 hours.

Results

The result consists of:

- The total Colony Forming Unit (CFU) recovered per test retrieved by all six impingers.
- The total CFU recovered per test retrieved by all slit air sampler.

While the acceptance criteria are:

- The total CFU recovered per test retrieved by all 6 impingers should not exceed 10 CFU.
- The total CFU recovered per test retrieved by all slit air sampler should not exceed 5 CFU.
- The control plate shall be positive when it contains greater than 300 CFU of B.subtilis.

Test #1: Slit air sampler at 127 mm (5") from interior side walls

Run	Number of <i>Bacillus Subtilis</i> CFU captured by:			
	Six Impingers	Left Slit Sampler	Right Slit Sampler	Positive Control
1	0	0	1	> 300
2	0	0	0	> 300
3	0	0	2	> 300
4	0	0	0	> 300
5	2	0	0	> 300
Conclusion: Since all Impingers < 10 CFU and all slit samplers < 5 CFU, this test is: Pass				

Test #2: Slit air sampler at 101 mm (4") from interior side walls

Run	Number of <i>Bacillus Subtilis</i> CFU captured by:			
	Six Impingers	Left Slit Sampler	Right Slit Sampler	Positive Control
1	0	0	0	> 300
2	1	0	1	> 300
3	0	0	0	> 300
4	0	0	0	> 300
5	1	0	0	> 300
Conclusion: Since all Impingers < 10 CFU and all slit samplers < 5 CFU, this test is: Pass				

Test #3: Slit air sampler at 76 mm (3") from interior side walls

Run	Number of <i>Bacillus Subtilis</i> CFU captured by:			
	Six Impingers	Left Slit Sampler	Right Slit Sampler	Positive Control
1	1	0	1	> 300
2	0	0	0	> 300
3	2	0	0	> 300
4	0	0	0	> 300
5	0	0	0	> 300
Conclusion: Since all Impingers < 10 CFU and all slit samplers < 5 CFU, this test is: Pass				

Test #4: Slit air sampler at 51 mm (2") from interior side walls

Run	Number of <i>Bacillus Subtilis</i> CFU captured by:			
	Six Impingers	Left Slit Sampler	Right Slit Sampler	Positive Control
1	0	0	0	> 300
2	0	0	0	> 300
3	0	0	0	> 300
4	0	0	0	> 300
5	0	0	0	> 300
Conclusion: Since all Impingers < 10 CFU and all slit samplers < 5 CFU, this test is: Pass				

Summary of CFU captures from both left and right slit air samplers from 4 tests:

Test	Slit sampler distance to walls	Total CFU from 5 runs
1	127 mm (5")	3
2	101 mm (4")	1
3	76 mm (3")	1
4	51 mm (2")	0

Observation

1. The cabinet passed the operator protection test at varied distance of slit air sampler to the cabinet inner walls.
2. The cabinet has higher CFU count on the slit air samplers, when they are place furthest from the inner walls.
3. When the slit air samplers are furthest from the inner wall, they become closer to the nebulizer, the source of the spores. This effect is especially pronounced since this is a 2 ft (60 cm) wide cabinet, where the nebulizer becomes very close to the slit air samplers, as compared to the standard 4 ft (120 cm) cabinet.

Conclusion

To increase the challenge for operator protection test on biosafety cabinets with less than 3 ft width, the slit air samplers should be placed **as far away** from the cabinet inner side walls as possible, closer to the nebulizer. A **5 inches (127 mm)** distance between center of slit inlet and the cabinet side walls is suggested by this study.