

**Personnel Protection Test on  
Standard Width (4ft) Biosafety Cabinet**  
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 cabinets 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, and as a member of the Joint Committee, Esco is seeking to contribute on this study.

The authors recently conducted a study on operator protection test on Esco Airstream Class II BSC with 2 ft width by varying the distance of slit air sampler to the side walls, and found out that the most challenging test condition occurs when the slit air samplers are placed at 5" (127 mm) from the side walls. Any further distance than that would make the slit air samplers collide with the impingers.

The purpose of this study is to compare the findings on the 2ft width cabinet to standard 4ft cabinet of identical model and airflow setting, specifically to see which slit air sampler distance from the walls that would pose the greatest challenge for the cabinet, and to compare the test result if similar 5" (127 mm) distance is used on this cabinet.

## 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 Collison nebulizer, set to spray the spore suspension at  $0.2 \pm 0.02$  ml/min
9. *Bacillus subtilis* var. *globigii* spore suspension, with concentration of  $5$  to  $8 \times 10^8$  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:

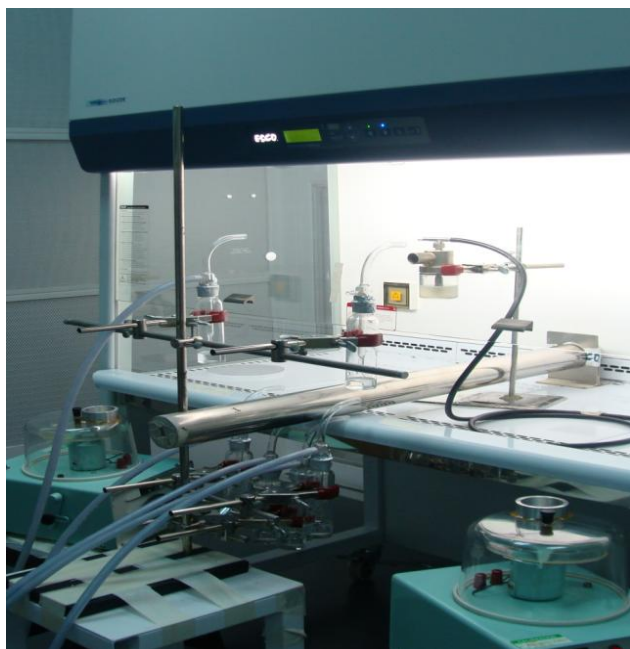
Impingers	Height	Distance from sash	Separation from each other
2 x Top	355 mm (14") above work tray	51 mm (2")	152 mm (6") from BSC centerline
2 x Middle	Same as top of the cylinder	63.5 mm (2.5")	152 mm (6") apart
2 x Bottom	Same as bottom of the cylinder	63.5 mm (2.5")	51 mm (2") apart

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	203 mm (8")
2	127 mm (5")
3	101 mm (4")
4	76 mm (3")
5	51 mm (2")

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 AC2-4E1 Class II Biosafety Cabinet (**4 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 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 samplers.

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 2 slit air samplers 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 203 mm (8”) 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	2	0	0	> 300
2	0	0	0	> 300
3	0	0	0	> 300
4	0	0	0	> 300
5	0	0	0	> 300
<b>Conclusion:</b> Since all Impingers < 10 CFU and all slit samplers < 5 CFU, this test is: <b>Pass</b>				

**Test #2: 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	0	> 300
2	0	0	0	> 300
3	0	1	0	> 300
4	0	2	0	> 300
5	1	0	0	> 300
<b>Conclusion:</b> Since all Impingers < 10 CFU and all slit samplers < 5 CFU, this test is: <b>Pass</b>				

**Test #3: 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	1	1	0	> 300
2	0	0	0	> 300
3	0	1	0	> 300
4	0	0	0	> 300
5	0	0	0	> 300
<b>Conclusion:</b> Since all Impingers < 10 CFU and all slit samplers < 5 CFU, this test is: <b>Pass</b>				

**Test #4: Slit air sampler at 76 mm (3") from interior side walls**

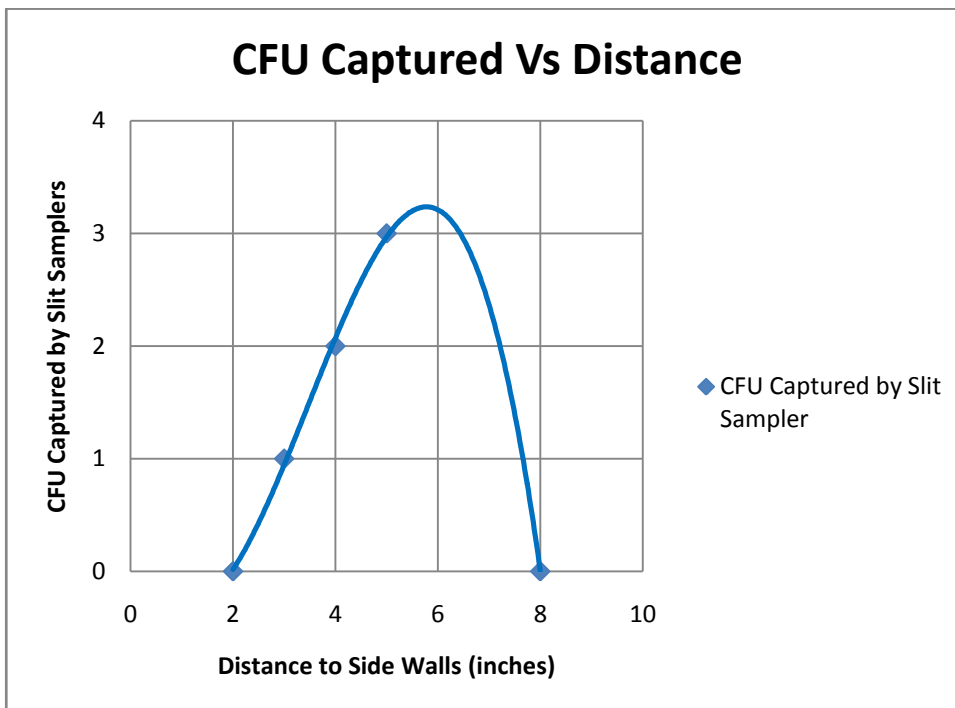
Run	Number of <i>Bacillus Subtilis</i> CFU captured by:			
	Six Impingers	Left Slit Sampler	Right Slit Sampler	Positive Control
1	0	1	0	> 300
2	0	0	0	> 300
3	0	0	0	> 300
4	1	0	0	> 300
5	0	0	0	> 300
<b>Conclusion:</b> Since all Impingers < 10 CFU and all slit samplers < 5 CFU, this test is: <b>Pass</b>				

**Test #5: 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	1	0	0	> 300
<b>Conclusion:</b> Since all Impingers < 10 CFU and all slit samplers < 5 CFU, this test is: <b>Pass</b>				

**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	203 mm (8")	0
2	127 mm (5")	3
3	101 mm (4")	2
4	76 mm (3")	1
5	51 mm (2")	0



**Observation**

1. The cabinet passed operator protection test at varied distance of slit air sampler to the cabinet inner walls.
2. There is zero CFU captured by slit air samplers at the standard distance of 8" (203 mm), which is also the furthest distance used on this study, and also zero CFU captured when the slit air samplers were placed at 2" (51 mm), which is the closest distance used on this study.
3. The CFU count gradually increases towards the midpoint between 8" (203 mm) to 2" (51 mm) slit sampler distance to side walls.
4. The cabinet has higher CFU count on the slit air samplers, when they are placed at 5" (127 mm) from the inner walls.

**Conclusion**

It is interesting to observe that there is incremental increase in captured CFU in the midpoint between minimum slit air sampler distance of 2" (51 mm) and the ANSI / NSF 49 prescribed distance of 8" (203 mm). The recovered CFU reached the peak at 5" (127 mm) distance from the side walls, which coincidentally is the same peak CFU distance observed on previous study on 2ft wide biosafety cabinet. A further study is suggested to investigate this phenomenon on other cabinets, to see if there is an certain distance that would pose a greater challenge in conducting operator protection test on the biosafety cabinets, to ensure better operator safety.