

## Increased Microbiological Challenge Cabinet side-by-side

### Introduction

The air curtain of the front of the cabinet is fragile and can easily be disrupted by people walking parallel to it, by open windows, air supply registers or laboratory equipment that creates air movement, and even another cabinet placed beside it. This is why biosafety cabinets should be located away from high traffic areas, doors and air supply/exhaust grilles that may interrupt airflow patterns. Whenever possible, a 30 cm clearance should be provided on each side and behind the cabinet to allow for maintenance access and for undisrupted air supply.

In this experiment Escoc will check the cabinet performance whether it can still aid protection to the operator as well as to the products and processes being handle inside the cabinet. Biosafety cabinet model LA2-4A2 will be used instead of the AC2 because it has stronger inflow thus allowing greater challenge to the cabinet.

### Materials

Cabinet Model: LA2-4A2 (cabinet tested)

Serial #: 2004-7328

Motor voltage: 83.4V

Cabinet Model: LA2-4A2

Serial #: 2003-6217

Nebulizer pressure: 20 psi (pressure stable)

Nebulizer Serial Number: N2

Batch # of spores: 1PIC22072004

Vol. of Spore Suspension in Nebulizer: 55ml of  $8 \times 10^6$

Total # plates used: 189 pcs.

### Calculation of Spore Suspension concentration

To obtain the required spore solution for nebulizer (product protection test):

1 mL of the original suspension was transferred to the first dilution tube containing 9 mL of sterile deionized water (tube 1,  $10^8$ ). Another 1 ml from the first tube was transferred to the second dilution tube with 9 ml sterile deionized water (tube 2,  $10^6$ ). From tube 2, 6 ml of the diluted suspension was obtained and added to the third dilution tube containing 54 ml of sterile deionized water.

To get the target concentration of  $8 \times 10^6$ .

$$79 \times 10^6 \times A = 8 \times 10^6 \text{ (target)} \Rightarrow \frac{79A}{79} = 8 \Rightarrow 79A = 480 \Rightarrow A = 6 \text{ mL}$$

$$A + (60 - A) = 60$$

Therefore mix Spore suspension (A) : 6mL and sterile deionized water: 54 ml

In this particular test,  $79 \times 10^9$  spores per ml were obtained.

### Computation on Spores Output (from the Nebulizer)

Nebulizer weight before 5 minutes test spray

Nebulizer weight after 5 minutes test spray

Fluid loss over 5 minutes test

Spore out-put = Fluid loss in grams x spore concentration in CFU/ml

Spore Concentration in CFU/ml :  $8 \times 10^6$

Test No.	Weight before	Weight after	Fluid loss	Spore output
1	493.42	490.49	2.93	$2.34 \times 10^7$
2	488.97	486.36	2.61	$2.09 \times 10^7$
3	488.87	486.73	2.14	$1.71 \times 10^7$

### Procedure

Biosafety cabinet model LA2-4A2 with serial number 2004-7328 cabinet was used in this experiment and was placed beside the LA2-4A2 2003-6217. One side was touching the side of the LA2-4A2 cabinet under test, and set at nominal setpoint (inflow velocity of 0.53m/s and downflow velocity of 0.35m/s).

For the product protection test, the cabinet was set-up with Petri dishes in the work zone. The dishes were filled with sterilized Trypticase soy agar. A fixed amount of bacterial spores were discharged from the nebulizer ( $8 \times 10^6$  of *Bacillus Subtilis* spores for 5 minutes). The stainless steel cylinder (this acts as an artificial arm to simulate normal operating conditions, airflow disturbance) was placed at the centre of the working area. A single Petri dish was placed beneath the front air grille (supported by an empty Petri dish), this serve as the control. If the bacterial spray penetrates the workzone, bacterial growth will be visible on the agar plates after hours of incubation.

### Acceptance Criteria

The maximum number of Colony Forming Units (CFU) recovered from all agar plates inside the work zone shall not exceed 5.

The control plate will be considered as "positive" when it contains more than 300 CFU of bacterial growth.





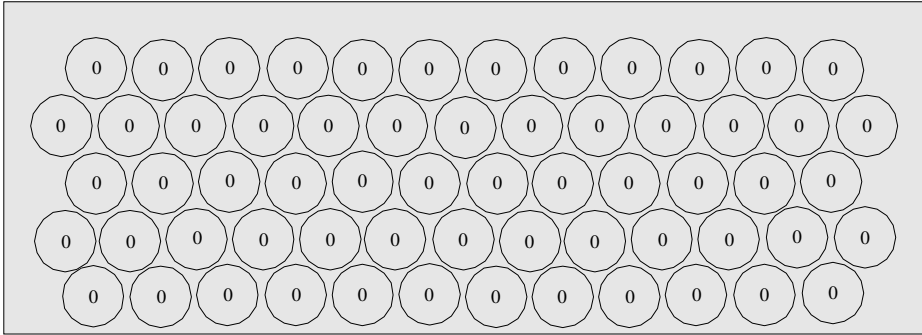
*Product Protection test*

## Results

Test	No. of CFU from work area	Positive Control Plates
1	All plates has 0 CFU <b>Total 0 CFU</b>	TNTC
2	All plates has 0 CFU <b>Total 0 CFU</b>	TNTC
3	All plates has 0 CFU <b>Total 0 CFU</b>	TNTC

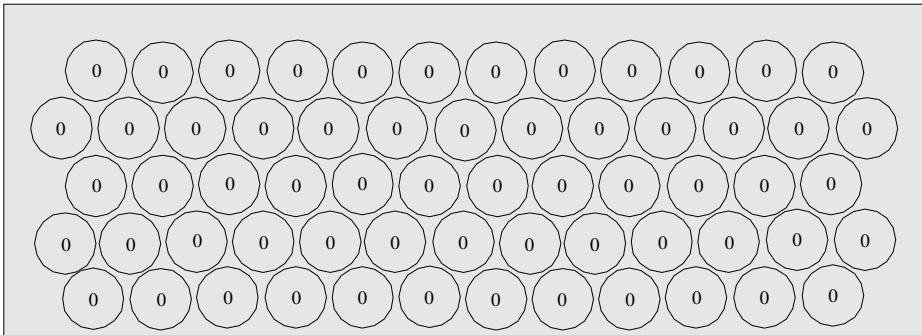
## Illustration of results

### Test 1

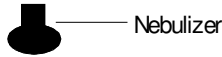
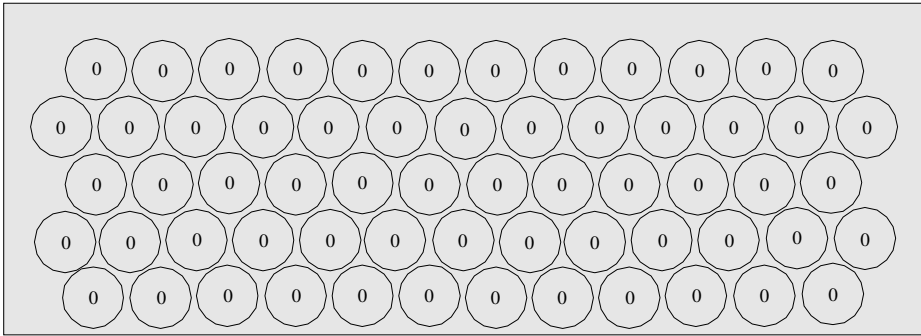


Total CFU: 0  
Result: Pass

### Test 2



Total CFU: 0  
Result: Pass



Total CFU: 0  
Result: Pass

## Conclusion

Result showed that no bacterial colony forming unit was recovered from all of the agar plates in 3 rounds of testing. This showed that the cabinet can maintain its perfect containment even if the 'laminar flow' may be disrupted. Thus, only demonstrates that Esco biosafety cabinets can still maintain and render product protection as well as operator protection even if air disturbance is present.