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## **Increased Microbiological Challenge Test with Objects Inside the Work Zone To Simulate Loaded Cabinet**

### **Purpose of Experiment**

Loading the cabinet with objects will completely disturb the airflow. The notion of linearity or laminar flow may be completely destroyed and any aerosols generated may be carried upwards to contaminate the whole of the air within the cabinet. In this experiment different glass wares and equipments were used to generate air disturbance to simulate loaded cabinet. The dimensions and distance of each objects was measured to make sure that this test can be repeatable for all Esco cabinet.

### **Experiment Method**

Cabinet Model: LA2-4A2

Serial #: 2004-7328

Motor voltage: 83.4V

Nebulizer pressure: 20 psi (pressure stable)

Nebulizer Serial Number: N2

Batch # of Agar: A08092004

Batch # of spores: 7M07062004

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

Total # plates used: 76 pcs.

### **Items Inside the Cabinet**

*Left side:*

<b>Items</b>	<b>Dimensions</b>			<b>Distance</b>	
	<i>Length</i>	<i>Height</i>	<i>Width</i>	<i>Side Wall</i>	<i>Back Wall</i>
Vortex	15.0	14.7	9.0	7.5	12.0
Color Gram Staining Kit	32.0	13.4	10.9	30.0	12.0
Test Tube Rack (with test tubes)	23.7	13.9	6.0	30.0	20.0
500 ml glass Beaker (with test tubes inside)	9.5	13.0	9.5	54.0	10.0
250 ml screw cap bottle	7.0	15.0	7.0	54.0	20.0

Right side:

Items	Dimensions			Distance	
	Length	Height	Width	Side Wall	Back Wall
Culture Media (1)	10.0	19.50	80.0	50.0	11.0
Culture Media (2)	10.0	19.5	80.0	39.0	11.0
Box of Pipette tips (with Biohit pipettor on top)	16.0	18.0	12.0	24.0	11.0
Kimwipes	12.4	8.0	12.0	7.5	11.0
250ml screw cap bottle (1)	7.0	15.0	7.0	50.0	22.0
250ml screw cap bottle (2)	7.0	15.0	7.0	39.0	22.0
250ml screw cap bottle (3)	7.0	15.0	7.0	24.0	22.0
250ml screw cap bottle (4)	7.0	15.0	7.0	7.5	22.0

Note: All measurements are in Centimetres

### Calculation for concentration of spore suspension

From serial dilution we obtain  $79 \times 10^9$  spores per ml. To obtain the required spore solution for Nebulizer:

Dilute 1mL from original spore suspension with 9mL of sterile deionized water (tube 1,  $10^8$ ) then pipette 0.5mL from tube 1 add to 9mL of sterile deionized water (tube 2,  $10^6$ ). From tube 2, pipette 6ml and add to 54 ml of sterile deionized water.

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

$$79 \times 10^6 \times A = 8 \times 10^6 \text{ (target)} \Rightarrow \frac{79A}{60} = 8 \Rightarrow 79A = 480 \Rightarrow A = 6\text{mL}$$

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

Therefore we need to mix: Spore suspension (A) : 6mL

$$\text{Water : } 60 - 6 = 54\text{mL}$$

### 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	167.07	164.93	2.14	$1.71 \times 10^7$
2	167.23	164.79	2.44	$1.95 \times 10^7$

## Procedure

The cabinet was setup with the objects/items (with respective distance measurements) and petri dishes in the work zone. The dishes are filled with sterilized Tryptone Soya Agar (TSA). A fixed amount of bacterial spores is discharged as a spray from the nebulizer in 5 minutes. The stainless steel cylinder (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 below the front air grille (supported by an empty petri dish) as a control. If the bacterial spray penetrates the workzone, it will show bacterial growth on the agar plates after incubation.

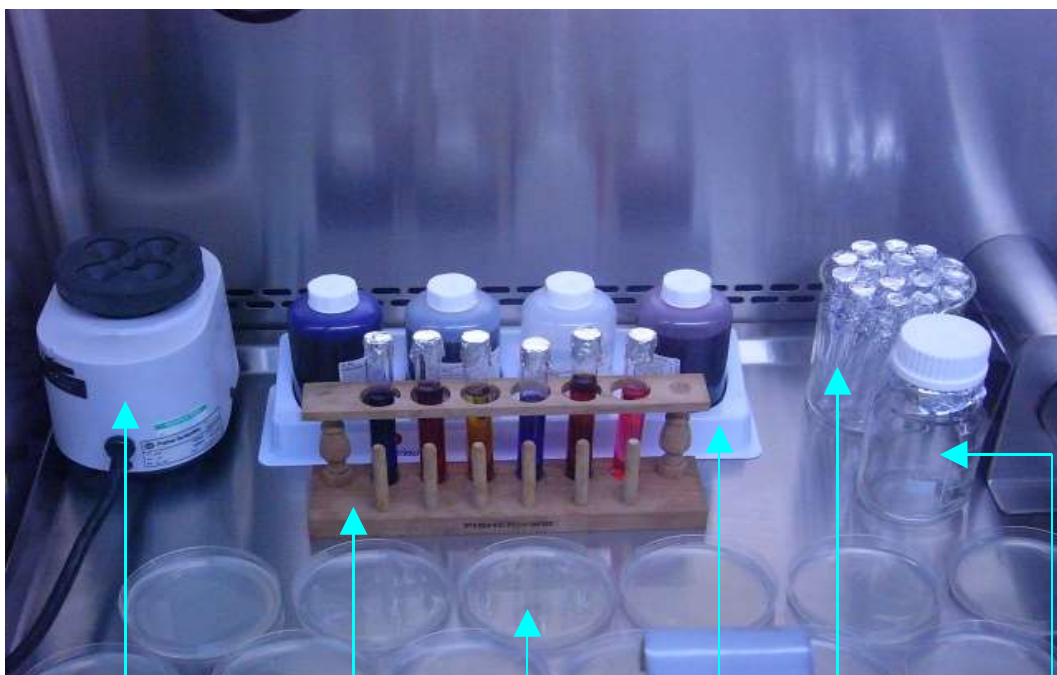
## Acceptance Criteria

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

The control plate is positive when it contains more than 300 CFU of bacteria.

## Experiment Set-up

### *Left side of the cabinet*



*Vortex*

*Test tube rack*

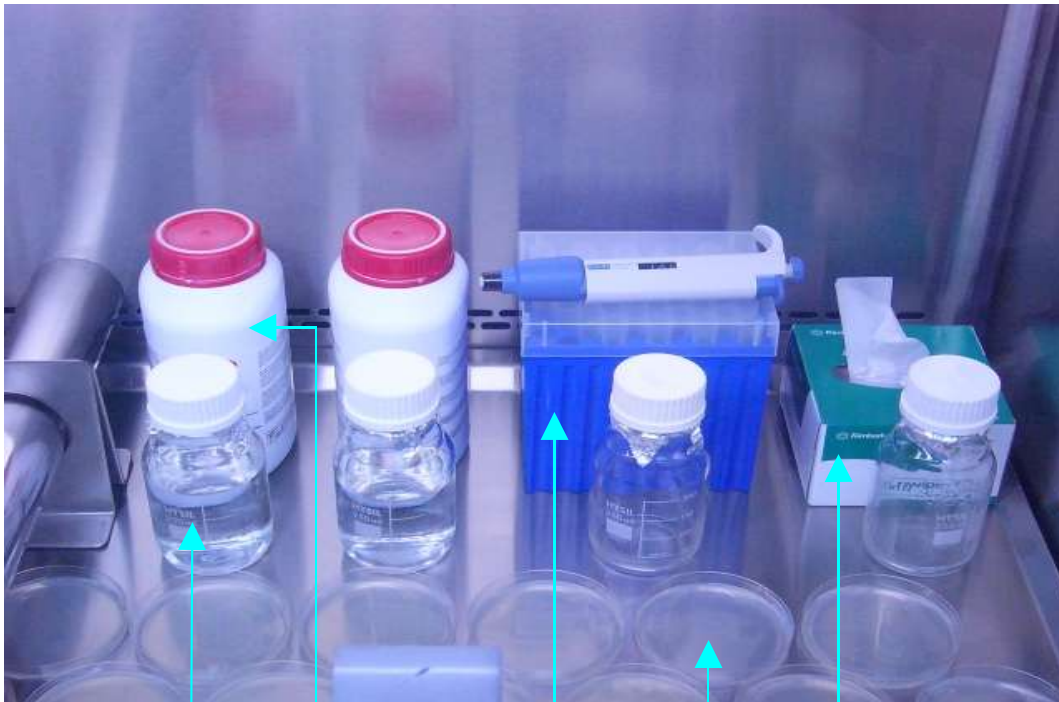
*Agar plates*

*Gram staining kit*

*Beaker with test tubes*

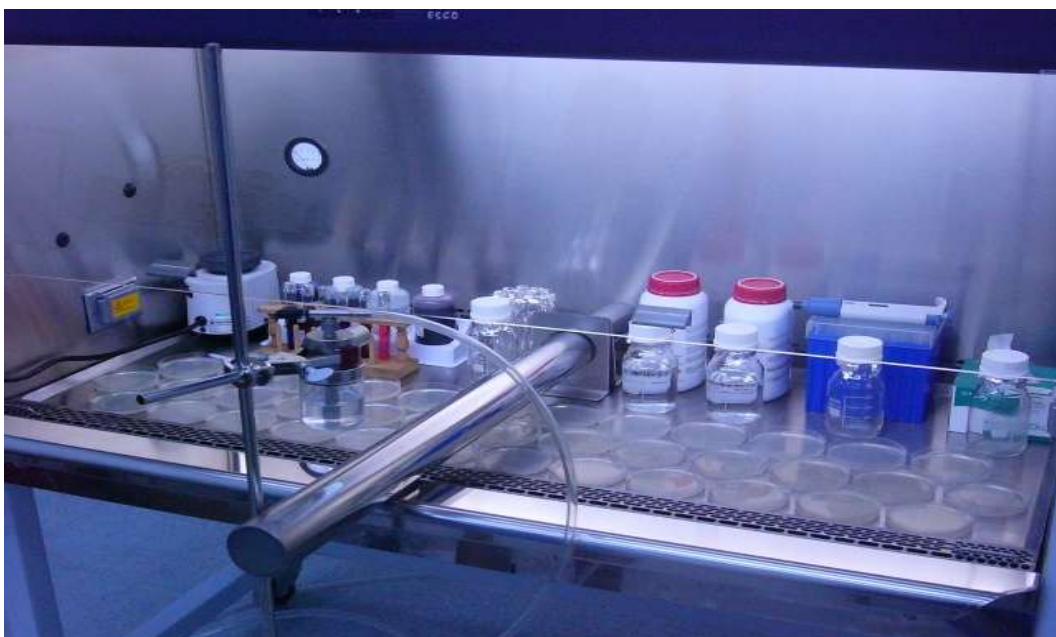
*Screw cap bottle*

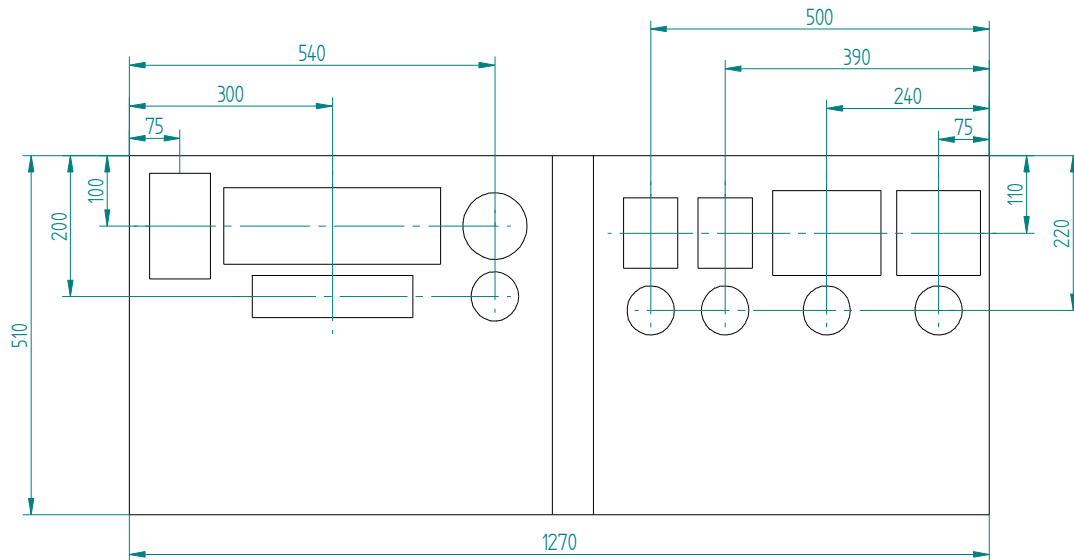
**Right side of the cabinet**



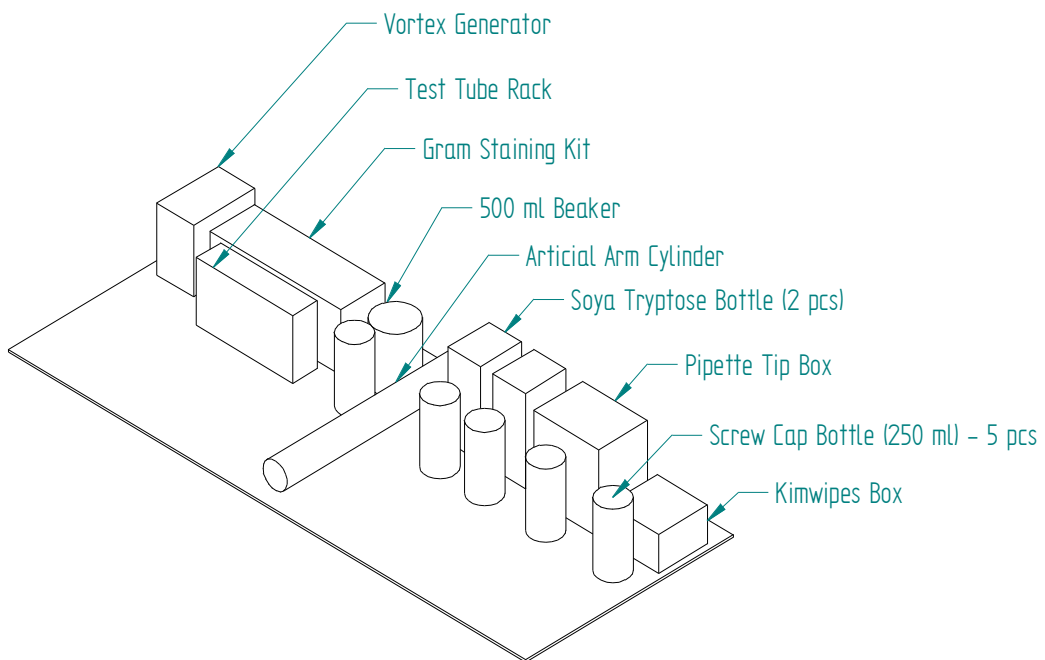
- Culture media**
- Screw cap bottle**
- Box of pipette tips with pipettor**
- Agar plates**
- Kimwipes**

**Whole set up**





### 3 Dimensional Illustration of the Setup



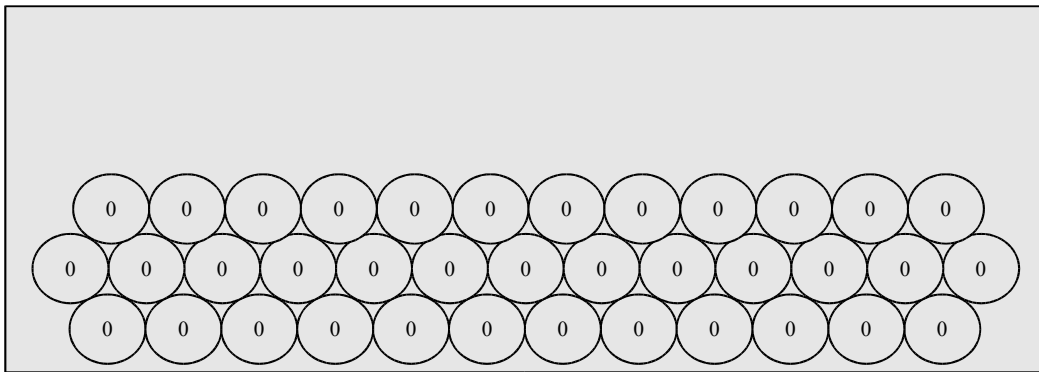
## Results

The results is shown in table below:

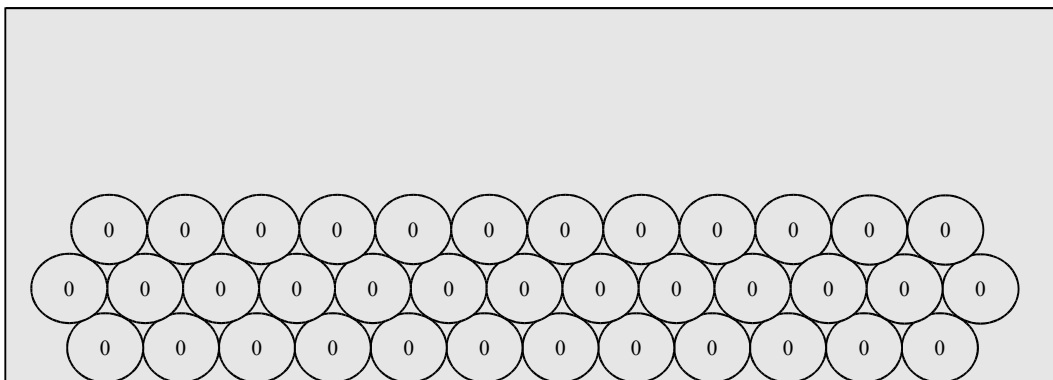
<i>Test</i>	<i>Number of CFU from work area</i>	<i>Positive control plate (CFU)</i>
1	0	>300
2	0	>300

## Illustration of results

### Test 1



### Test 2



## **Conclusion**

This test demonstrate that the cabinet will maintain product protection even with an internal air disturbance such as loading the cabinet with equipments and glasswares used in microbiological testing. From the data above, the results indicate that there is no Colony Forming Unit recovered from the entire agar plate layed inside the cabinet. This shows that the cabinet still maintain its perfect containment even the 'laminar flow' may be completely destroyed.

Elmie M. Antonio 09/09/2004

L: Physical Performance Testing/Microbiological Testing/Esco Microbiology Laboratory/Tech report/Objects inside the work zone.doc