Increased Microbiological Challenge Test with Bunsen Burner Inside the Cabinet

Introduction

Bunsen Burner is the most frequently used apparatus in the laboratory as a source of heat. Typically used inside the biological safety cabinets and laminar flow hoods for sterilizing inoculating loops, test tube lips and Petri dishes lids. This barrier is designed so that gaseous fuel may be mixed with the correct amount of air to yield the maximum amount of heat. However, placing a lighted burner into a cabinet, produces a dramatic effect. In a Class II type cabinet, the hot upflow from the burner mixed the downflowing airstreams to produce turbulence and recirculation within the working area. The notion of laminar flow may be completely destroyed and any aerosols generated beneath the burner may be carried upwards to contaminate the whole of the air within the cabinet. This is why a Bunsen burner should not be used inside the cabinet; and an alternative technique should be found.

In this experiment Esco will try to find out where the Bunsen burner can be placed safe inside the cabinet. Experiment will be composed of three different tests: Cross contamination, Product protection and Operator protection test (KI Discus test). Each test will be done twice with different location of the Bunsen burner inside the cabinet.

Materials and 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: A21092004

Batch # of spores: 1PIC22072004

Vol. of Spore Suspension in Nebulizer: 55ml of $8x10^6$

Part I. Cross Contamination Test

Procedure

The LA2-4A2 with serial number 2004-7328 biosafety cabinet was used in this experiment and was set at nominal setpoint (inflow velocity of 0.53m/s and downflow velocity of 0.35m/s). A smoke tube was used to determine the location of the Bunsen burner inside the cabinet.

For the cross contamination test, the nebulizer was placed at one side of the cabinet work zone to discharge bacterial spores in the opposite direction ($8x10^4$ of *Bacillus Subtilis* spores in 5 minutes). Petri dishes were placed in the work zone in a fixed arrangement. The dishes were pre-poured with sterilized Trypticase soy agar. Two rows of dishes were placed closest to the nebulizer, and this served as the control plates. Additional rows of dishes were placed with the nearest row having a distance of 14 inches from the side wall (measured from the centre of the dish). Three replicates were performed from each sides of the cabinet.

Placement of the Bunsen burner inside the cabinet

Test Trial	*Flame Height (cm)*	*Distance from the Side wall (cm)*	*Distance from the Back wall (cm)*
Location #1	7.5	7.0	21.5
Location #2	7.5	38.5	19.0

Acceptance Criteria

The total number of CFU recovered on all agar plates from 14 inches and beyond shall not exceed 2 Colony Forming Units per test.

The control plate will be considered positive when it contains more than 300 CFU of bacteria.

Calculation for concentration of spore suspension

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 $8x10^6$.

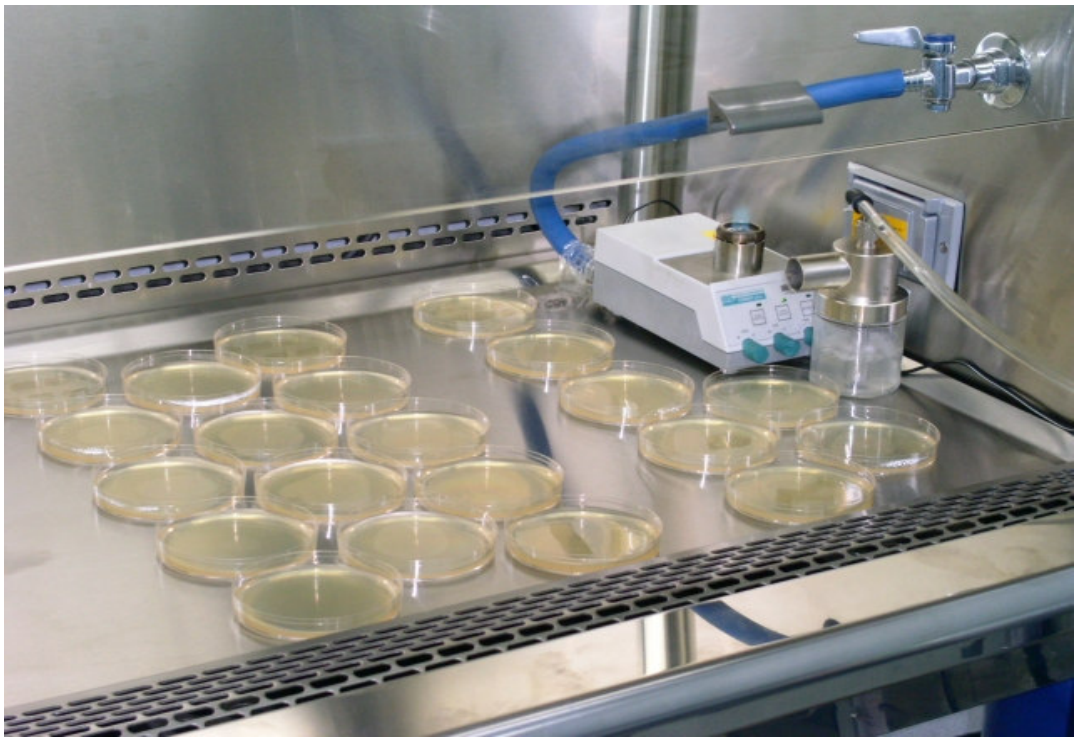
$\frac{79x10^6 \times A}{A + (60 - A)} = 8x10^6$ (target) $\Rightarrow \frac{79A}{60} = 8 \Rightarrow 79A = 480 \Rightarrow A = 6mL$

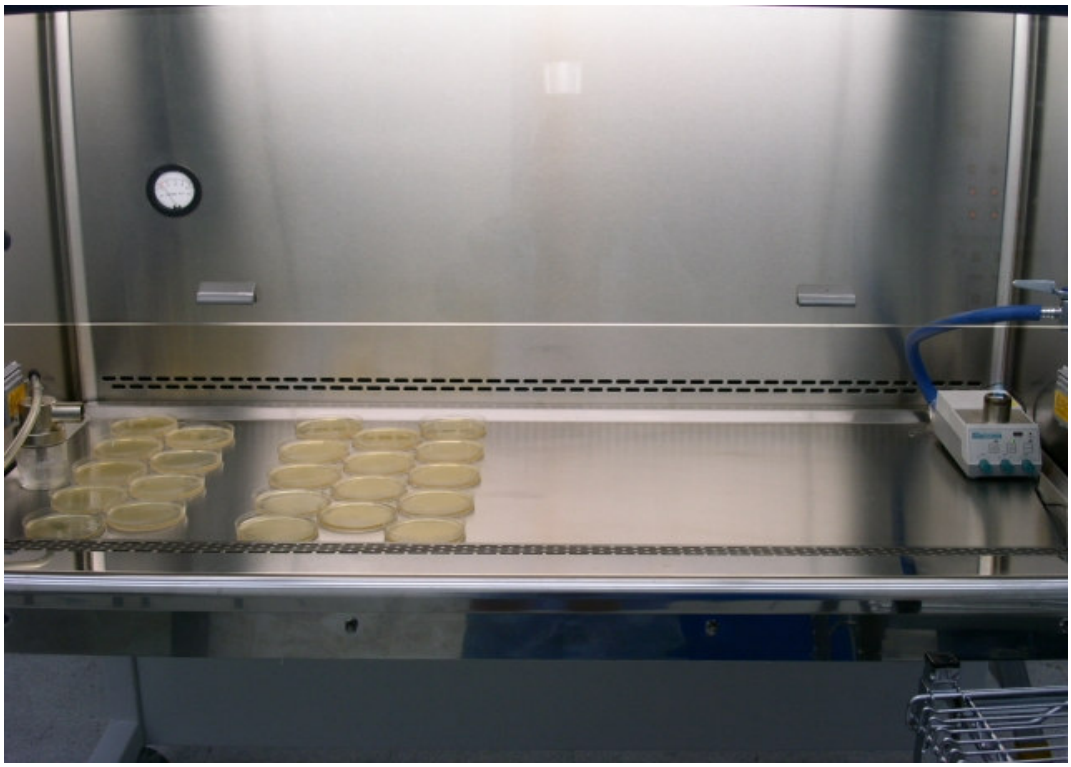
Therefore mix Spore suspension (A) : 6mL and sterile deionized water: 54 ml

In this particular test, $79x10^9$ spores per ml were obtained.

Experiment Set-up

Location #1





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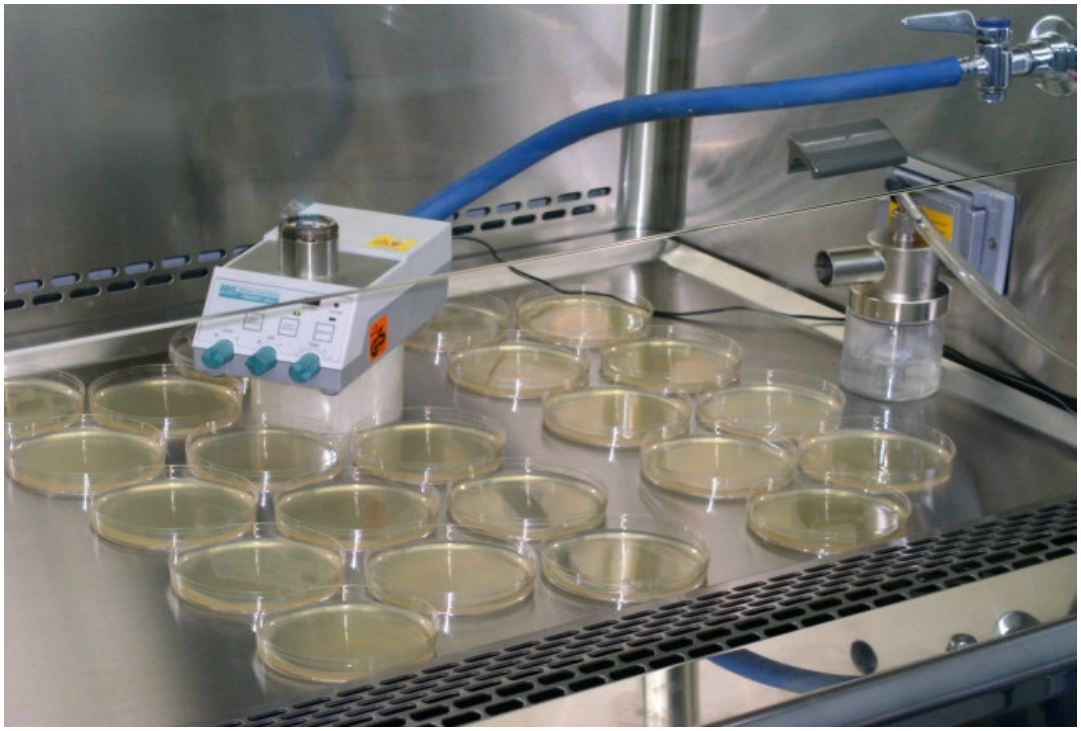
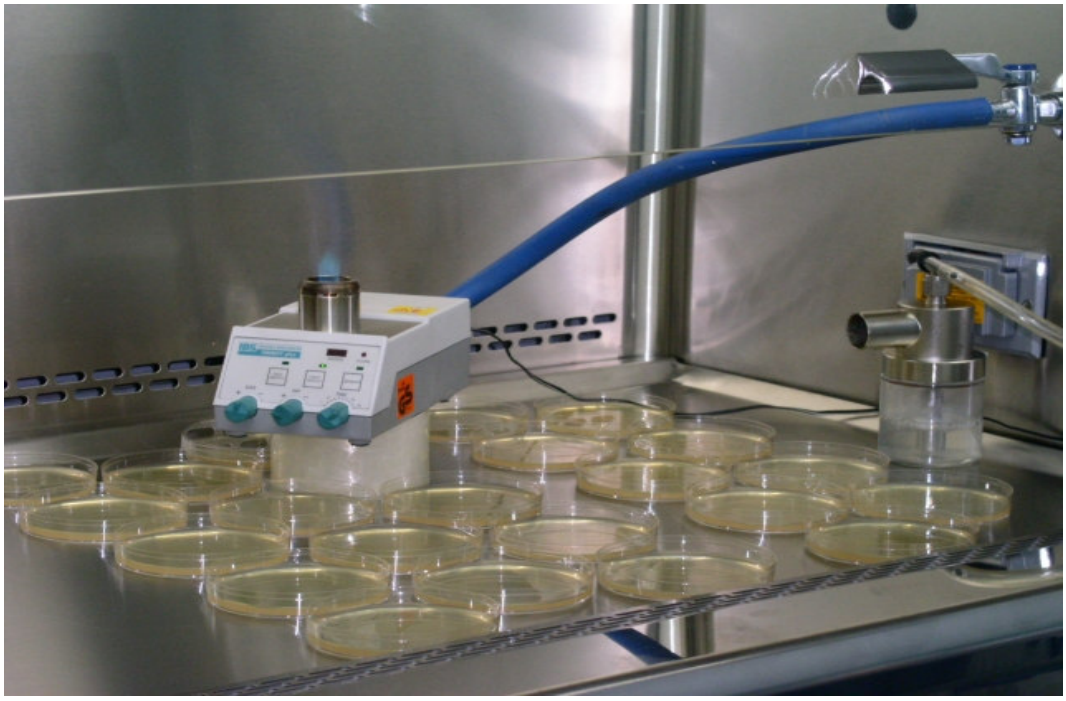
Location #2





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Results

Location #1

Nebulizer on Left Side of Work Space

Test	Recovery of CFU 14" mark and beyond	Control Plates
1	14 plates 0 CFU **Total 0 CFU**	3 plates TNTC 6 plates 16 to 300 CFU
2	14 plates 0 CFU **Total 0 CFU**	3 plates TNTC 6 plates 5 to 300 CFU
3	14 plates 0 CFU **Total 0 CFU**	3 plates TNTC 6 plates 3 to 300 CFU

Nebulizer on Right Side of Work Space

Test	Recovery of CFU 14" mark and beyond	Control Plates
1	14 plates 0 CFU **Total 0 CFU**	1 plate TNTC 6 plates 1 to 200 CFU
2	14 plates 0 CFU **Total 0 CFU**	1 plate TNTC 6 plates 0 to 200 CFU
3	14 plates 0 CFU **Total 0 CFU**	1 plate TNTC 6 plates 0 to 200 CFU

Location #2

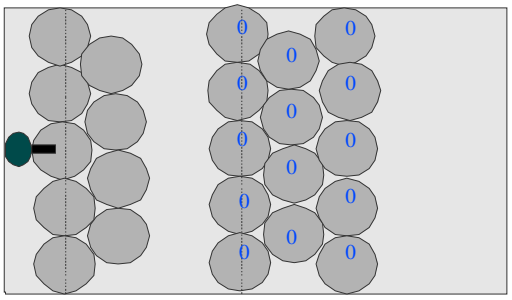
Nebulizer on Right Side of Work Space

Test	Recovery of CFU 14" mark and beyond	Control Plates
1	7 plates have >100CFU 5 plates have 11 to 18 CFU **Total >300 CFU (Fail)**	9 plates TNTC
2	7 plates have >100CFU 5 plates have 10 to 21 CFU **Total >300 CFU (Fail)**	9 plates TNTC
3	7 plates have >100CFU 5 plates have 11 to 23 CFU **Total >300 CFU (Fail)**	9 plates TNTC

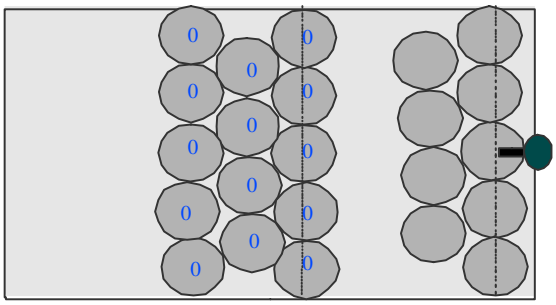
Illustration of Results

Location #1

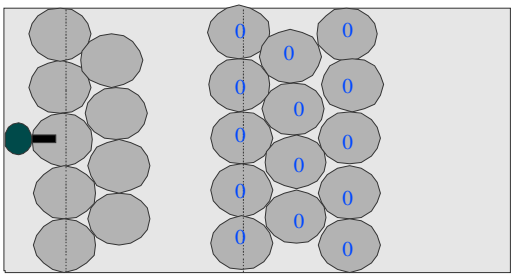
Test I. Left Side of Work Space



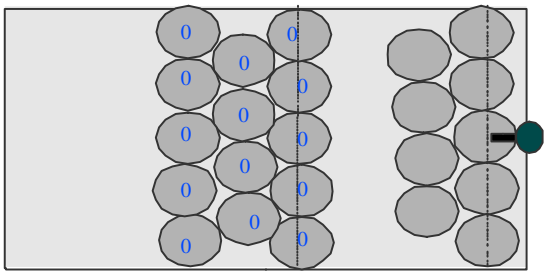
Right Side of Work Space



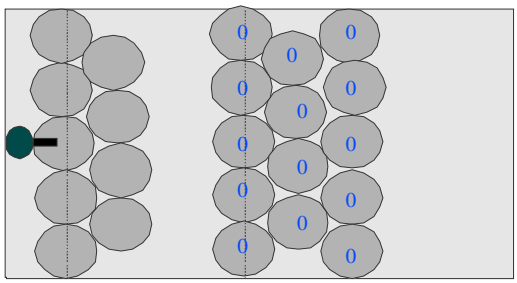
Test II. Left Side of Work Space



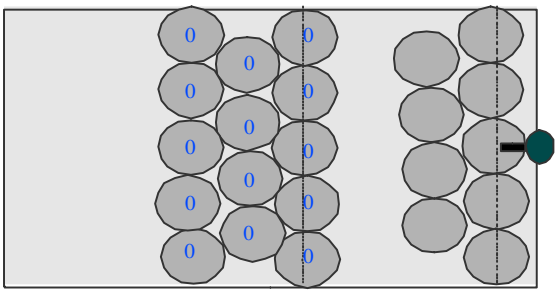
Right Side of Work Space



Test III. Left Side of Work Space



Right Side of Work Space



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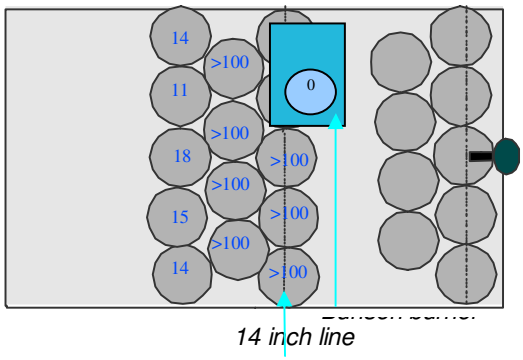
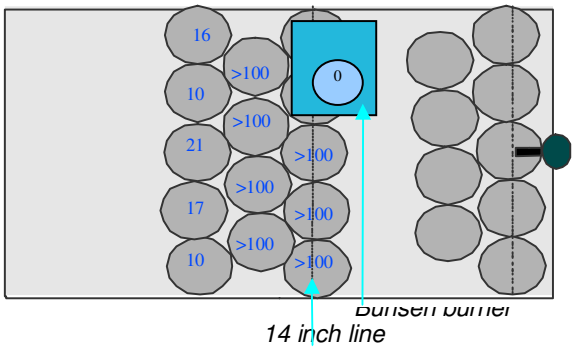
Location #2

Test I. Right Side of Work Space



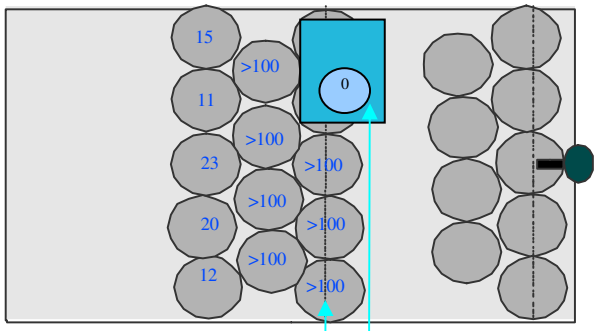
14 inch line

Test II. Right Side of Work Space



14 inch line

Test III. Right Side of Work Zone



Bunsen burner

14 inch line

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Part II. Product Protection Test

Procedure

The LA2-4A2 with serial number 2004-7328 biosafety cabinet was used in this experiment and was set at nominal setpoint (inflow velocity of 0.53m/s and downflow velocity of 0.35m/s). A smoke tube was used to determine the location of the Bunsen burner inside the cabinet.

For the product protection test, the cabinet was set-up with Petri dishes in the work zone. The dishes were pre-poured with sterilized Trypticase soy agar. A fixed amount of bacterial spores was discharged from the nebulizer ($8x10^6$ of *Bacillus Subtilis* spores for 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 beneath the front air grille (supported by an empty Petri dish) and serve as a control.

Placement of Bunsen burner inside the cabinet

Test Trial	*Flame Height (cm)*	*Distance from the Side wall (cm)*	*Distance from the Back wall (cm)*
Location #1	9.0	7.0	21.5
Location #2	9.0	53.5	35.0

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 will be considered “positive” when it contains more than 300 CFU of bacteria.

Calculation for concentration of spore suspension

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 $8x10^6$.

$$\frac{79x10^6 \times A}{A + (60 - A)} = 8x10^6 \text{ (target)} \Rightarrow \frac{79A}{60} = 8 \Rightarrow 79A = 480 \Rightarrow A = 6mL$$

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

In this particular test, $79x10^9$ spores per ml were obtained.

Results

Location #1

Test	No. of CFU from work area	Positive Control Plates
1	All plates has 0CFU **Total 0 CFU (Pass)**	TNTC
2	All plates has 0CFU **Total 0 CFU**	TNTC
3	All plates has 0CFU **Total 0 CFU**	TNTC

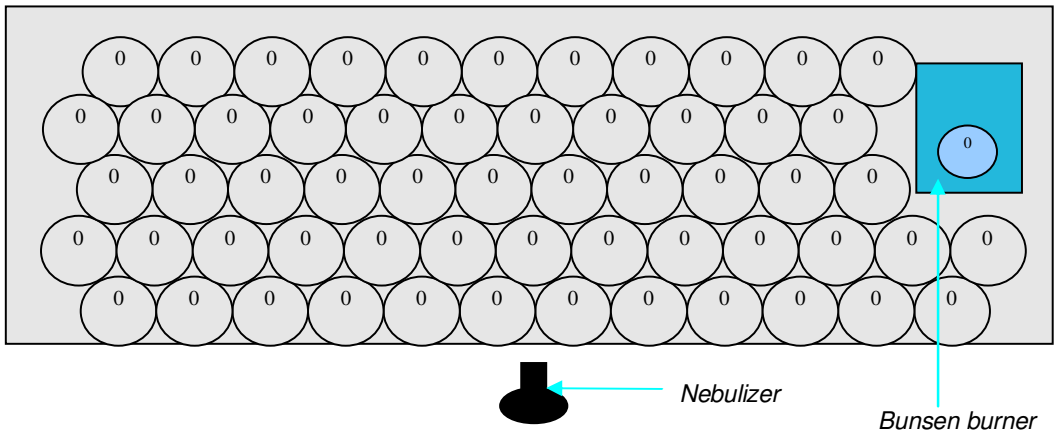
Location #2

Test	No. of CFU from work area	Positive Control Plates
1	1 plate has 5 CFU 2 plates has 4 CFU 1 plate has 3 CFU 7 plates has 2 CFU 18 plates has 1 CFU **Total 48 CFU (Failed)**	TNTC

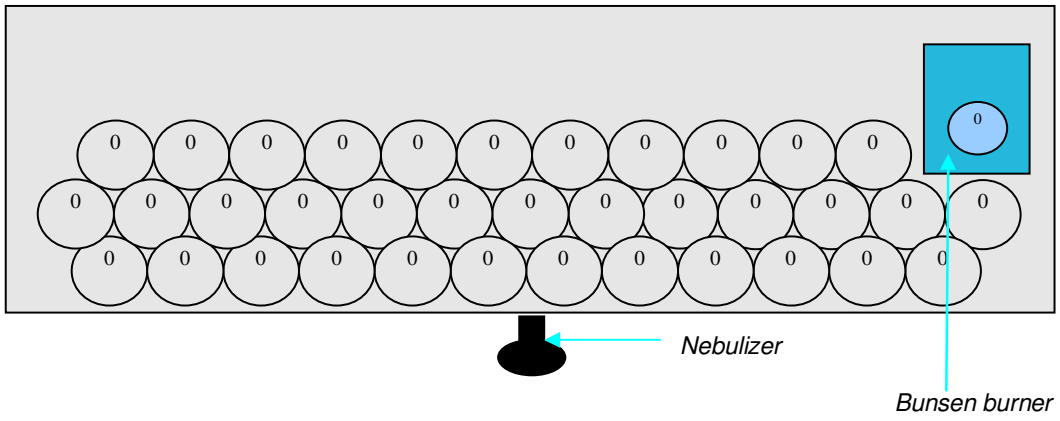
Illustration of Results

Location #1

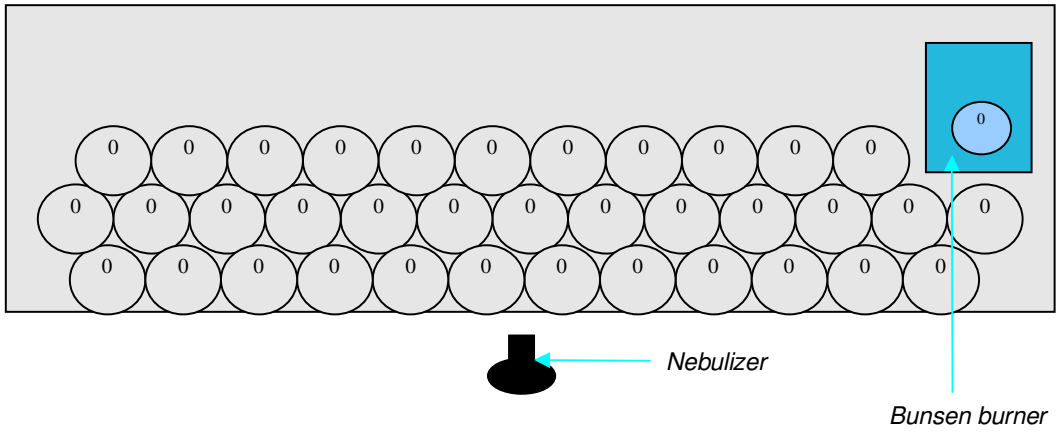
Test I



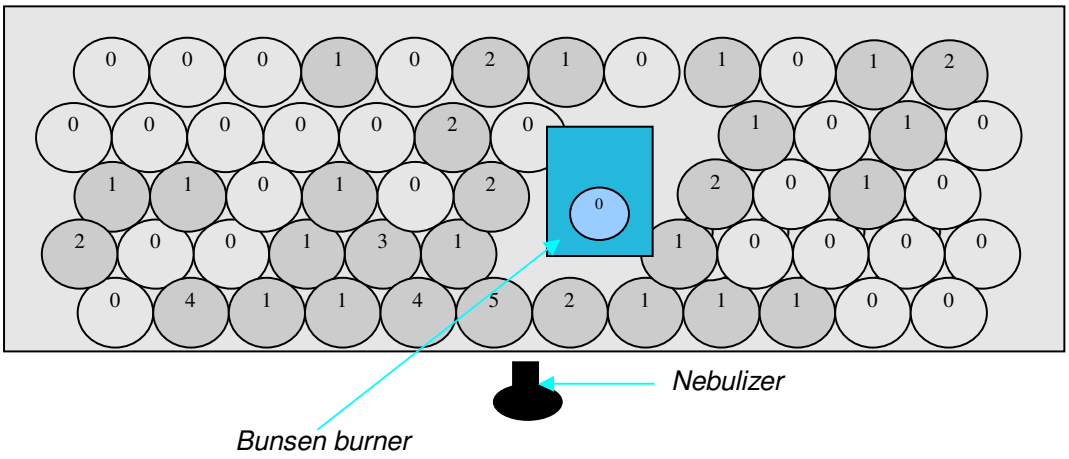
Test II



Test III



Location #2



Part III. Operator Protection Test (KI Discus Test)

Procedure

The LA2-4A2 with serial number 2004-7328 biosafety cabinet was used in this experiment and set at nominal setpoint (inflow velocity of 0.53m/s and downflow velocity of 0.35m/s). A smoke tube was used to determine the location of the Bunsen burner inside the cabinet.

For the personnel/operator protection test, KI discus was used, according to study, this is more stringent than the microbiological test method, moreover, more difficult to pass. The KI Discus was positioned in front of the cabinet and the stainless steel cylinder was placed in the centre of the work tray to simulate normal operating conditions (the airflow disturbance of operator's arm). The disc was placed inside the work zone to discharge the Potassium iodide spray outwards. Four (4) suction cones were placed outside the cabinet as it simulate the breathing of the operator working on the cabinet. The suction cone has a filter membrane inside to suck the Potassium iodide released by the disc.

Placement of Bunsen burner inside the cabinet

Test Trial	*Flame Height (cm)*	*Distance from the Side wall (cm)*	*Distance from the Back wall (cm)*
Location #1	9.5	7.0	21.5
Location #2	9.5	35.5	45.5

Acceptance Criteria
The total number of brown dots recovered from each filter membrane shall not exceed more than 62. This will correspond to aperture protection factor of 100,000 meaning that for every 100,000 particles liberated behind the sash window, only 1 manages to escape. If there are zero brown dots captures, this translates to aperture protection factor of greater than 6,200,000.

Results

Location #1

X1	0	**Y1**	0
X	0	**Y**	0

Location #2

X1	2	**Y1**	9
X	14	**Y**	23

Conclusion

Bunsen burner, alcohol lamp, or any other apparatus which requires the consumption of flames, should not be used inside biosafety cabinets. Flame creates turbulence in airflow and will compromise sterility and heat build-up may damage the filters. When seemed absolutely necessary, Bunsen burner should be place at the rear of the workplace where resulting air turbulence has a minimal effect. In this particular experiment, result showed that the safest location of the Bunsen burner or any fire-requiring material inside the cabinet would be at the further right side of the cabinet: 7.0 cm from the right side wall and 21.5 cm from the back wall. Microbiological test result showed no bacterial growth on the agar plates. Hence, this location in the cabinet passed the three (3) microbiological tests which are the Cross contamination, Product protection and Operator protection test.