TECHNICAL BULLETIN
GENERAL INFORMATION

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NUAIRE INCUBATOR
STERILITY TEST

Introduction

Incubator contamination is inherently a potential for all incubator manufacturers. Typical laboratories have thousands of airborne contaminants that may enter a tissue cell culture incubator during a door opening, and enter the perfect growth environment to survive and reproduce. Elimination of these airborne contaminants as they enter the incubator would be the best solution, but not feasible given today's technology. However, other potential solutions to the contamination problem are available today. High Efficiency Particulate Air (HEPA) filters are available to remove airborne contaminants thus providing a solution to the problem.

NuAire incorporates a HEPA large capacity capsule filter into the NU-2000, 4000 and 8000 Series Autoflow CO_2 Water-jacketed Incubator to remove airborne contaminants that enter the chamber during door openings. The HEPA filter is incorporated into the recirculation system (See Figure 1). The chamber air is drawn into the inlet tube, to the pump, through a 0.3 micron HEPA inline capsule filter, through a 0.3 micron Hydrophobic HEPA filter to the IR Sensor and returned to the chamber. The recirculation provides the filtration necessary to remove these airborne contaminants and reduce the possibility of chamber contamination.

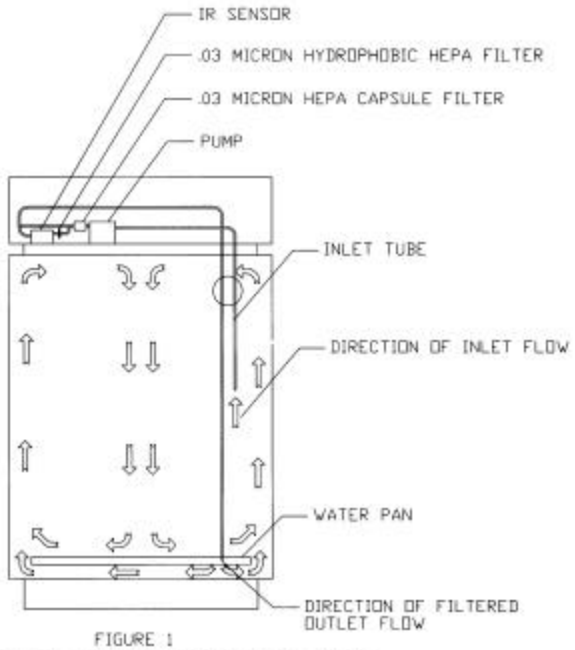

FIGURE 1
WATER-JACKET HEPA FILTERED RECIRCULATION SYSTEM

To test the incubator's ability to filter the airborne contaminants, a biological test was developed.

Materials & Methods

A NuAire CO_2 Water-Jacketed Incubator with setpoint parameters of 5% CO_2 , 96% humidity and 37°C was tested. The incubator was set and stabilized for 24 hours. The incubator shelf placement was kept standard with four shelves equally spaced in the chamber, but not causing interference to the side access port.

On the middle shelf, covered soy agar plates were placed on the center plane from the side access port (See Figure 2).

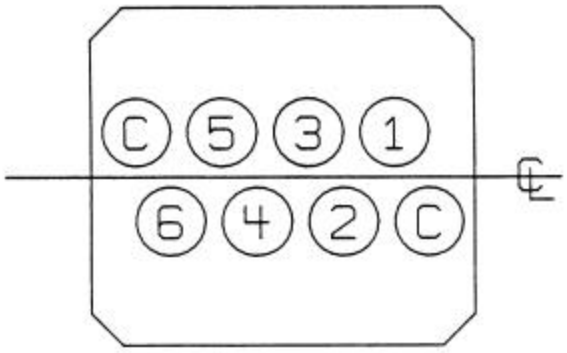

FIGURE 2
SOY AGAR PLATE DISTRIBUTION ON SHELF

A 316 upgrade stainless steel 6-jet collision refluxing nebulizer was used to deliver B. subtilis var. Niger spores prepared to a concentration of 1.0×10^4. The nebulizer was mounted next to the side access port as to distribute the B. subtilis spore suspension to the incubator chamber (See Figure 3).

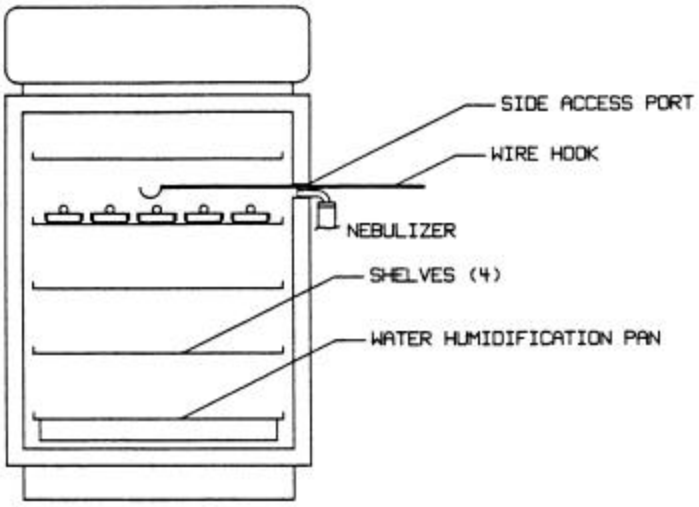

FIGURE 3
INCUBATOR TEST SET UP

A wire hook was also present at the side access port to remove the agar plate covers during the test.

Once all the above materials were in place, the following procedure was initiated:

Procedure

A. Remove the (2) control agar plate covers through the side access port.

B. Place the nebulizer over the side access port. Connect 20 PSIG air source and run nebulizer for one minute.

C. Remove nebulizer from side access port and remove agar plate covers per the following schedule:

Plate 1 - 5 minutes
Plate 2 - 10 minutes
Plate 3 - 15 minutes
Plate 4 - 20 minutes
Plate 5 - 25 minutes
Plate 6 - 30 minutes

D. Allow agar plates to incubator for 24 hours. Remove agar plates and record results.

E. Three replicate tests shall be performed.

Results

The test results were as indicated below. Each plate was analyzed for colony forming units (CFU) of the B. subtilis var. Niger spore.

Test #1:
Plate 1 - 122 CFU
Plate 2 - 69 CFU
Plate 3 - 27 CFU
Plate 4 - 19 CFU
Plate 5 - 16 CFU
Plate 6 - 4 CFU
Control Plates - TNTC

Test #2:
Plate 1 - 67 CFU
Plate 2 - 35 CFU
Plate 3 - 20 CFU
Plate 4 - 7 CFU
Plate 5 - 3 CFU
Plate 6 - 1 CFU
Control Plates - TNTC

Test #3:	Plate	1 - 200 CFU
	Plate	2 - 150 CFU
	Plate	3 - 75 CFU
	Plate	4 - 16 CFU
	Plate	5 - 7 CFU
	Plate	6 - 1 CFU
	Control Plates - TNTC	

The control plates were also evaluated to be sure the spore concentration was an acceptable challenge providing a valid test. The control plate should contain greater than 300 CFU's to be considered valid.

Conclusion

The testing results indicate a sizable reduction in the chamber spore concentration as a function of time. The reduction of spores can be directly attributed to filtration through the HEPA filtered recirculation system that includes the 0.3 micron HEPA inline capsule filter.

The results also indicate the general cleanliness level of being class 100 or better 15 minutes after the chamber has been exposed to airborne contaminants through a door opening. The filtration system being this effective will reduce the chance for contamination on a daily basis. However, good laboratory practice including periodic decontamination of the incubator chamber is recommended to further reduce potential chamber contamination. Additionally, adding a small amount of copper sulfate to the water humidification pan will also reduce contamination.

The NuAire CO_2 Water-Jacketed Incubator offers a substantial reduction in contamination potential. Today's laboratory technicians can assure NuAire is bringing dependability for the most demanding environments.