An Evaluation of the Decontamination Effect on the Inner Chamber of ESCO Celculture CO_2 Incubator Using the 90°C Moist Heat Decontamination Cycle

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Commercial In Confidence

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SUMMARY

The ESCO Celculture CO_2 incubator has been evaluated for the effectiveness of its 90°C moist heat decontamination cycle. The decontamination cycle was able to deactivate *Aspergillus brasiliensis* spores ATCC 16404 (formerly *Aspergillus niger*), *Bacillus atrophaeus* spores NCTC 10073, *Pseudomonas aeruginosa* vegetative cells ATCC 15442, *Staphylococcus aureus* vegetative cells ATCC 6538, *Staphylococcus epidermidis* vegetative cells ATCC 12228, *Enterobacter faecalis* vegetative cells ATCC 29212 and *Escherichia coli* vegetative cells ATCC 25922 dried on coupons (prepared at HPA).

INTRODUCTION

An ESCO Celculture CO_2 incubator (figure 1) provided by ESCO has been tested to assess whether its decontamination cycle is able to deactivate a range of micro-organisms. The decontamination cycle comprises of a 2 hour heating up period, a 9 hour hold cycle at 90°C and a 3 hour cooling down period. During each cycle 12 *Aspergillus brasiliensis* ATCC 16404 (formerly *Aspergillus niger*) coupons, 12 *Bacillus atrophaeus* NCTC 10073 coupons, 12 *Pseudomonas aeruginosa* ATCC 15442 coupons, 12 *Staphylococcus aureus* ATCC 6538 coupons, 12 *Staphylococcus epidermidis* ATCC 12228 coupons, 12 *Enterobacter faecalis* ATCC 29212 coupons and 12 *Escherichia coli* ATCC 25922 coupons were placed in four different locations within the incubator in order to fully challenge the device and to obtain precise reduction values.

Figure 1. ESCO Celculture CO_2 Incubator

MATERIALS AND METHODS

Test Micro-organisms

Bacillus atrophaeus NCTC 10073

A liquid suspension of *Bacillus atrophaeus* NCTC 10073 spores was prepared by diluting, in sterile distilled water, stock batches previously prepared by the HPA Production Division. The resultant suspension concentration was 7.70 x 10^8 colony forming units per ml (cfu/ml).

Aspergillus brasiliensis ATCC 16404

A spore suspension was prepared from 5 Tryptone Soya Agar (TSA) plates containing confluent grown of *Aspergillus brasiliensis* spores. The spores were removed from the surface of the agar by carefully spreading 10ml of a 0.1% tween suspension onto each of the plates then carefully pipetting off the liquid suspension into a sterile universal container. The number of spores in the resultant suspension was determined by spreading 0.1ml of a suitable dilution onto duplicate TSA plates. The plates were then incubated at 37°C for 48 hours. The suspension concentration was 5.75 x 10^6 colony forming units per ml (cfu/ml).

Preparation of Liquid Suspensions of Test Micro-organisms

Preparation of liquid suspensions of the following organisms:

- *Pseudomonas aeruginosa* ATCC 15442
- *Staphylococcus aureus* ATCC 6538
- *Staphylococcus epidermidis* ATCC 12228
- *Enterobacter faecalis* ATCC 29212
- *Escherichia coli* ATCC 25922

Liquid suspensions of the organisms were prepared by inoculating two universals containing 10ml of nutrient broth each. A full (generous) 10µl loop of the organism was taken from a stock plate previously prepared from fresh freeze dried vial obtained from the National Collection of Type Cultures (NCTC) and added to each of

the universals. The culture suspension was mixed thoroughly by shaking and placed in a 37°C±2°C static incubator for 24hrs.

The suspensions were assayed by plating out 0.1ml of 10^{-5}, 10^{-6} and 10^{-7} dilution in duplicate onto TSA plates and incubating the plates at 37°C±2°C for 24hrs. The colonies were counted after incubation to determine the concentration of bacteria (colony forming units (cfu)) per ml of suspension.

The resultant suspension concentrations were as follows:

- *Pseudomonas aeruginosa* ATCC 15442 — 2.10×10^9 cfu/ml
- *Staphylococcus aureus* ATCC 6538 — 1.13×10^9 cfu/ml
- *Staphylococcus epidermidis* ATCC 12228 — 3.40×10^8 cfu/ml
- *Enterobacter faecalis* ATCC 29212 — 3.55×10^8 cfu/ml
- *Escherichia coli* ATCC 25922 — 1.24×10^9 cfu/ml

Preparation of Stainless Steel Coupons

Individual stainless steel coupons of 1cm diameter were prepared by pipetting 10 µl of suspension into the centre of the coupon. The coupons were dried at 37°C±2°C for 1 hour. 15 discs of each test organism were prepared for each experiment.

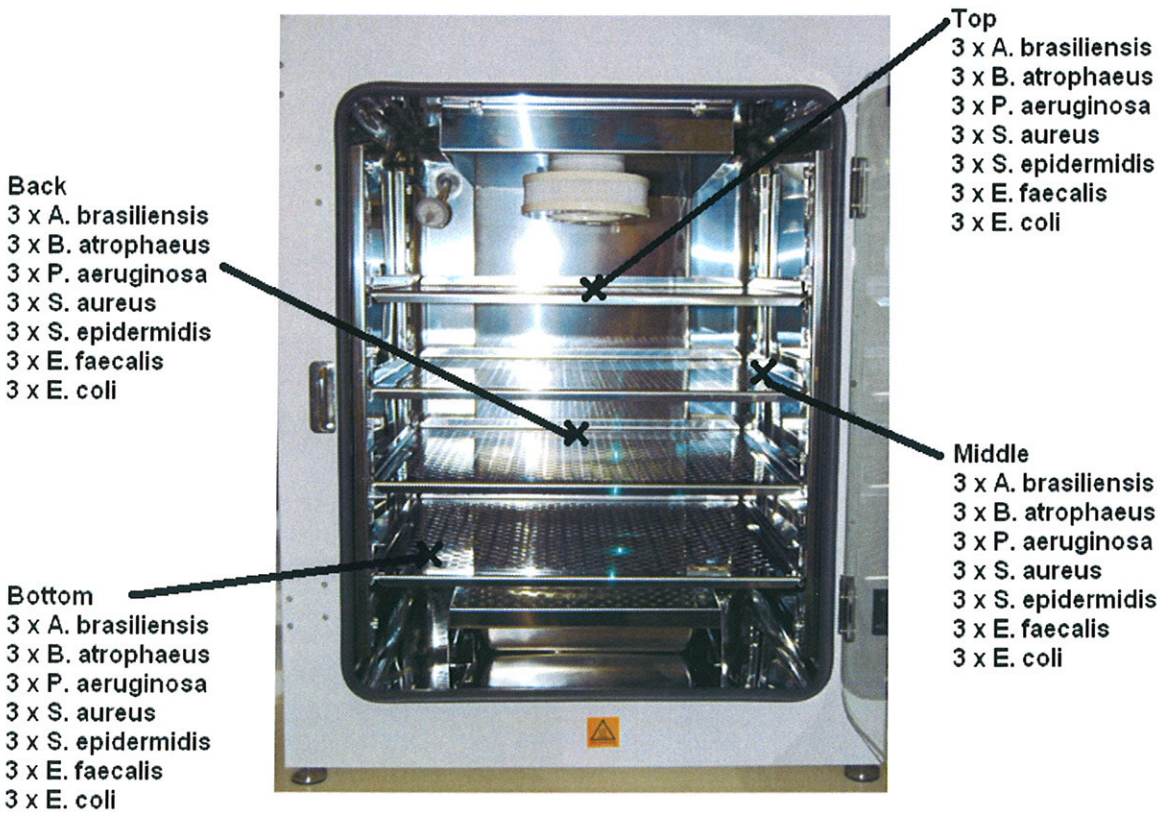
Test method

12 *Aspergillus brasiliensis* ATCC 16404 (formerly *Aspergillus niger*) coupons, 12 *Bacillus atrophaeus* NCTC 10073 coupons, 12 *Pseudomonas aeruginosa* ATCC 15442 coupons, 12 *Staphylococcus aureus* ATCC 6538 coupons, 12 *Staphylococcus epidermidis* ATCC 12228 coupons, 12 *Enterobacter faecalis* ATCC 29212 coupons and 12 *Escherichia coli* ATCC 25922 coupons were placed within the inner chamber of the ESCO Celculture CO_2 incubator at the locations indicated in Figure 2. Three coupons of each organism were placed at each location.

For each test run 3 positive control samples were carried out for each micro-organism. The positive controls were prepared using the same methods as the test samples but they were not exposed to the sterilisation cycle. All the coupons were assayed as described below.

After completion of the decontamination cycle the coupons (including negative control coupons) were placed into individual universals containing 5 ml of phosphate buffered saline (PBS). The universals were placed on a sonicator for 5 minutes. The coupons were assayed by plating out 0.1ml of the neat suspension onto duplicate TSA plates and concentrating the rest of the sample by filtration onto 0.2μm polycarbonate membrane filters (Whatman). These membrane filters were placed onto TSA plates. All the TSA plates were incubated at the appropriate growth conditions for the micro-organism and any colonies were counted.

Figure 2. Position of the coupons



Microbial Assay of Positive Controls

The positive control coupons of *Bacillus atrophaeus* NCTC 10073, *Pseudomonas aeruginosa* ATCC 15442, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Enterobacter faecalis* ATCC 29212 and *Escherichia coli* ATCC 25922 were assayed by carrying out a 10 fold serial dilution and plating out 0.1ml of 10^{-2}, 10^{-3} and 10^{-4} in duplicate onto TSA plates. The plates were incubated at 37 °C±2°C for 24 hrs and any colonies counted.

The coupons of *Aspergillus brasiliensis* ATCC 16404 were serially diluted in sterile distilled water and 0.1ml of neat, 10^{-1} and 10^{-2} was plated out onto duplicate TSA plates. The plates were incubated at 30°C±2°C for 48hrs and any colonies counted.

Calculations

$$\text{Total colony forming units (cfu)} = n \times df \times v$$

n = number of colonies per millilitre
df = dilution factor
v = volume of sample

$$\text{Log}_{10}\ \text{Reduction} = \left| \frac{\text{Total cfu of positive control}}{\text{Total cfu of test sample}} \right|_{\text{Log}}$$

RESULTS

Table 1a Run 1 carried out on 14/12/10

Location	Coupon no.	Average total colony forming units (cfu)			
		Bacillus atrophaeus	*Aspergillus brasiliensis*	*Pseudomonas aeruginosa*	*Staphylococcus epidermidis*
Top	1	0 (NG)	0 (NG)	0 (NG)	0 (NG)
	2	0 (NG)	0 (NG)	0 (NG)	0 (NG)
	3	0 (NG)	0 (NG)	0 (NG)	0 (NG)
Middle	1	0 (NG)	0 (NG)	0 (NG)	0 (NG)
	2	0 (NG)	0 (NG)	0 (NG)	0 (NG)
	3	0 (NG)	0 (NG)	0 (NG)	0 (NG)
Bottom	1	0 (NG)	0 (NG)	0 (NG)	0 (NG)
	2	0 (NG)	0 (NG)	0 (NG)	0 (NG)
	3	0 (NG)	0 (NG)	0 (NG)	0 (NG)
Back	1	0 (NG)	0 (NG)	0 (NG)	0 (NG)
	2	0 (NG)	0 (NG)	0 (NG)	0 (NG)
	3	0 (NG)	0 (NG)	0 (NG)	0 (NG)
+ ve control (not exposed)	1	1.59×10^6	1.52×10^4	2.38×10^6	2.33×10^6
	2				
	3				

NG – No Growth

Table 1b Run 1 carried out on 14/12/10

Location	Coupon no.	Average total colony forming units (cfu)		
		Escherichia coli	*Staphylococcus aureus*	*Enterobacter faecalis*
Top	1	0 (NG)	0 (NG)	0 (NG)
	2	0 (NG)	0 (NG)	0 (NG)
	3	0 (NG)	0 (NG)	0 (NG)
Middle	1	0 (NG)	0 (NG)	0 (NG)
	2	0 (NG)	0 (NG)	0 (NG)
	3	0 (NG)	0 (NG)	0 (NG)
Bottom	1	0 (NG)	0 (NG)	0 (NG)
	2	0 (NG)	0 (NG)	0 (NG)
	3	0 (NG)	0 (NG)	0 (NG)
Back	1	0 (NG)	0 (NG)	0 (NG)
	2	0 (NG)	0 (NG)	0 (NG)
	3	0 (NG)	0 (NG)	0 (NG)
+ ve control (not exposed)	1	1.57×10^6	5.72×10^6	2.15×10^6
	2			
	3			

NG – No Growth

Table 2a Run 2 carried out on 04/01/11

Location	Coupon no.	Average total colony forming units (cfu)			
		Bacillus atrophaeus	*Aspergillus brasiliensis*	*Pseudomonas aeruginosa*	*Staphylococcus epidermidis*
Top	1	0 (NG)	0 (NG)	0 (NG)	0 (NG)
	2	0 (NG)	0 (NG)	0 (NG)	0 (NG)
	3	0 (NG)	0 (NG)	0 (NG)	0 (NG)
Middle	1	0 (NG)	0 (NG)	0 (NG)	0 (NG)
	2	0 (NG)	0 (NG)	0 (NG)	0 (NG)
	3	0 (NG)	0 (NG)	0 (NG)	0 (NG)
Bottom	1	0 (NG)	0 (NG)	0 (NG)	0 (NG)
	2	0 (NG)	0 (NG)	0 (NG)	0 (NG)
	3	0 (NG)	0 (NG)	0 (NG)	0 (NG)
Back	1	0 (NG)	0 (NG)	0 (NG)	0 (NG)
	2	0 (NG)	0 (NG)	0 (NG)	0 (NG)
	3	0 (NG)	0 (NG)	0 (NG)	0 (NG)
+ve control (not exposed)	1	1.14×10^6	1.66×10^4	1.96×10^6	1.30×10^6
	2				
	3				

NG – No Growth

Table 2b Run 1 carried out on 04/01/11

Location	Coupon no.	Average total colony forming units (cfu)		
		Escherichia coli	*Staphylococcus aureus*	*Enterobacter faecalis*
Top	1	0 (NG)	0 (NG)	0 (NG)
	2	0 (NG)	0 (NG)	0 (NG)
	3	0 (NG)	0 (NG)	0 (NG)
Middle	1	0 (NG)	0 (NG)	0 (NG)
	2	0 (NG)	0 (NG)	0 (NG)
	3	0 (NG)	0 (NG)	0 (NG)
Bottom	1	0 (NG)	0 (NG)	0 (NG)
	2	0 (NG)	0 (NG)	0 (NG)
	3	0 (NG)	0 (NG)	0 (NG)
Back	1	0 (NG)	0 (NG)	0 (NG)
	2	0 (NG)	0 (NG)	0 (NG)
	3	0 (NG)	0 (NG)	0 (NG)
+ ve control (not exposed)	1	1.28×10^6	2.23×10^6	1.48×10^6
	2			
	3			

NG – No Growth

Table 3a Run 3 carried out on 06/01/11

Location	Coupon no.	Average total colony forming units (cfu)			
		Bacillus atrophaeus	*Aspergillus brasiliensis*	*Pseudomonas aeruginosa*	*Staphylococcus epidermidis*
Top	1	0 (NG)	0 (NG)	0 (NG)	0 (NG)
	2	0 (NG)	0 (NG)	0 (NG)	0 (NG)
	3	0 (NG)	0 (NG)	0 (NG)	0 (NG)
Middle	1	0 (NG)	0 (NG)	0 (NG)	0 (NG)
	2	0 (NG)	0 (NG)	0 (NG)	0 (NG)
	3	0 (NG)	0 (NG)	0 (NG)	0 (NG)
Bottom	1	0 (NG)	0 (NG)	0 (NG)	0 (NG)
	2	0 (NG)	0 (NG)	0 (NG)	0 (NG)
	3	0 (NG)	0 (NG)	0 (NG)	0 (NG)
Back	1	0 (NG)	0 (NG)	0 (NG)	0 (NG)
	2	0 (NG)	0 (NG)	0 (NG)	0 (NG)
	3	0 (NG)	0 (NG)	0 (NG)	0 (NG)
+ ve control (not exposed)	1	1.29×10^6	1.29×10^4	1.26×10^6	1.16×10^6
	2				
	3				

NG – No Growth

Table 3b Run 3 carried out on 06/01/11

Location	Coupon no.	Average total colony forming units (cfu)		
		Escherichia coli	*Staphylococcus aureus*	*Enterobacter faecalis*
Top	1	0 (NG)	0 (NG)	0 (NG)
	2	0 (NG)	0 (NG)	0 (NG)
	3	0 (NG)	0 (NG)	0 (NG)
Middle	1	0 (NG)	0 (NG)	0 (NG)
	2	0 (NG)	0 (NG)	0 (NG)
	3	0 (NG)	0 (NG)	0 (NG)
Bottom	1	0 (NG)	0 (NG)	0 (NG)
	2	0 (NG)	0 (NG)	0 (NG)
	3	0 (NG)	0 (NG)	0 (NG)
Back	1	0 (NG)	0 (NG)	0 (NG)
	2	0 (NG)	0 (NG)	0 (NG)
	3	0 (NG)	0 (NG)	0 (NG)
+ ve control (not exposed)	1	1.32×10^6	2.34×10^6	1.31×10^6
	2			
	3			

NG – No Growth

Table 4 Results of the Negative Controls (Sterile Coupons)

	Coupon no.	Run 1	Run 2	Run 3
Negative control	1	0 (NG)	0 (NG)	0 (NG)
	2	0 (NG)	0 (NG)	0 (NG)
	3	0 (NG)	0 (NG)	0 (NG)

NG – No Growth

Table 5 Average Log_{10} Reductions for each of the 7 micro-organisms

Micro-organism	Average Log_{10} Reduction		
	Test Run 1	Test Run 2	Test Run 3
Bacillus atrophaeus	>6.20	>6.05	>6.11
Pseudomonas aeruginosa	>6.37	>6.29	>6.10
Staphylococcus epidermidis	>6.36	>6.11	>6.06
Escherichia coli	>6.19	>6.10	>6.12
Staphylococcus aureus	>6.75	>6.34	>6.36
Enterobacter faecalis	>6.33	>6.17	>6.11
Aspergillus brasiliensis	>4.18*	>4.22*	>4.11*

*Due to the size of *Aspergillus brasiliensis* spores 10^4 cfu is the highest concentration that can be achieved on the test coupons in 10µl.

CONCLUSIONS

The ESCO Celculture CO_2 incubator 90°C decontamination cycle has been shown to be an effective method for deactivation of the normally resistant fungi and bacterial spores *Aspergillus brasiliensis* and *Bacillus atrophaeus*, and the vegetative cells *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterobacter faecalis* and *Escherichia coli.* For each of the three test runs carried out no growth was observed from any of the seven test micro-organisms indicating that the 90°C decontamination cycle of the incubator is capable of deactivating high levels of biological contamination.