Application Note:
ANCO2ELCON 0809

Eliminate Incubator Contamination with Thermo Scientific Heracell

Eliminate
Incubator Contamination
Heracell

The Heracell® CO_2 incubator decontamination cycle, ContraCon, is proven to eliminate contamination problems. The 90°C moist heat decontamination program has been tested by the Centre for Applied Microbiology and Research (CAMR, UK) for its ability to inactivate resistant bacterial and fungal strains.

Introduction

Cell culture contamination continues to be a potential threat, as cell culture technology has expanded into research laboratories and biopharmaceutical production facilities around the world.[1] Bacteria and fungi are the most common forms of contamination in cell culture. Typically, contamination arises from improper sterilization, improper storage of reagents and materials, and poor aseptic technique.[2]

Contamination problems with bacteria, fungi and their spores can cause the loss of years of work. Even after routine UV or liquid disinfection, residual spores or cells can potentially remain, leading to continued contamination problems. The Heracell CO_2 incubator offers the ContraCon 90°C moist heat decontamination cycle, proven by CAMR in eliminating contamination problems from all internal surfaces of the incubator.

Additionally, the Heracell does not have a water pan; this eliminates a potential contamination source. The water pan in some incubators can remain contaminated, even after manual cleaning, and therefore contribute to contamination of the entire incubator and its contents.

ContraCon, unlike manual cleaning, is a proven automated decontamination program at 90°C using moist heat. To use this feature simply add 300 ml of water to generate the moist heat and press one button to begin the process.

The advantage over other methods utilized in incubators is that moist heat is able to penetrate all surfaces.

Materials and Methods

CAMR selected the following test strains on the recommendation of animal cell culture specialists due to their resistant natures:

- *Aspergillus niger*, a mold, was prepared as a spore suspension from malt plates that were overgrown.
- *Bacillus subtilis var niger*, a typical gram-positive bacterium, was prepared as a spore suspension in both PBS (phosphorous buffer solution) and distilled water at concentrations of 10^9 spores per ml.
- *Saccharomyces cerevisiae NCYC 91,* a baker's yeast, was prepared in PBS and distilled water.

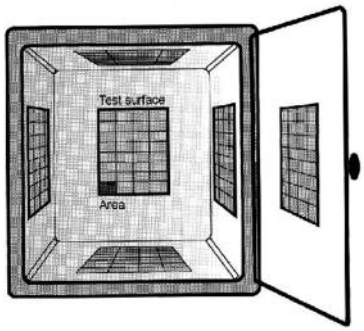

Figure 1.
Incubator sample chamber and glass door
Distribution of the test surfaces and individual areas for artificial contamination with various germ species

1) 10 microliter drops of each organism were applied to the six interior walls of the incubator (right, left, top, bottom wall and the glass door)
2) An extra ten drops were placed on the floor to be used for control swabs.
3) For the application of the samples in the center of the incubator, steel 1 cm^2 plates were used which were inoculated with 10 drops of the each organism.
4) After the drops dried, two control plates were inoculated with swipes from the extra drops on the floor.
5) Once all the samples were in the incubator, the unit was shut off and 300 ml of water was added to the water reservoir.
6) The unit was turned back on and the door was left open for 30 seconds before pressing the 90 °C button to initiate the ContraCon routine.
7) The entire cycle took 25 hours.
8) After the cycle was complete the soiled areas were swabbed with clinical swabs wetted with PBS then either:
   - mixed in universals containing 5 ml PBS, or
   - placed in bottles containing 10 ml of nutrient broth for bacteria or malt extract broth for fungus to get both positive and negative results.
9) The control plates were also removed and one plate for each micro-organism was added to a universal container containing 2 ml of PBS.
10) The other three plates from each microorganism were added to bottles containing 10 ml tryptone soya broth for the bacteria and malt extract broth for the fungus and yeast. These were incubated at the appropriate temperatures.

Assays

The swabs of the soils in the 5 ml PBS and the plates in 2 ml PBS were vortexed for 1 minute before serially diluting and assaying on Tryptone Soya Broth agar plates:

- *The B. subtilis var niger* isolates were incubated on TSBA plates at 37 °C for 24 hours.
- The fungus and yeast isolates were incubated on Malt Extract Agar (MEA) at 30 °C for 48 hours.

The media bottles were incubated and any bottles showing growth were assayed to identify the test micro-organism. The plates were counted for number of colony forming units.

Results and Discussion

The Heracell ContraCon decontamination cycle was shown to be effective in inactivating the normally resistant fungal and bacterial spores, *A. niger and B. subtilis var niger*. (Tables 1 and 2) illustrate the reduction rate of these organisms. As there was an average of 1.6 x 10^5 on the swabbed surfaces, the lack of growth in both suggests that the log reduction obtained was greater than 5.2. These reduction rates should be sufficient to ensure the decontamination of any normal background contamination levels. The greater than 5.2 log reduction for A. niger shows that realistic levels of this common cell culture contaminant present in an incubator should be inactivated by the normal cycle. The average reduction of the *B. subtilis var niger spores* should be sufficient to ensure the decontamination of any normal background contamination levels.[3]

The ContraCon decontamination program provides total thermal disinfection of the CO_2 incubator. Periodic application of the fully automated decontamination program prevents the formation and spread of contamination sources, even in those more difficult to reach areas of the incubator during manual cleaning. The moist heat decontamination routine leaves no residue, is environmentally friendly, fully automated and requires little preparatory or subsequent work. All the built-in components, fans and sensors remain intact during decontamination so no additional autoclaving is required. There are neither UV lamps nor HEPA filters to check or replace.

References

1) Cell Culture Contamination: Sources, Consequences, Prevention and Elimination C.K. Lincoln and M. G. Gabridge, *Methods Cell Biol* – 1998; 57: 49-65.

2) Tissue Culture Techniques, An Introduction, Bernice M. Martin, Birkhauser, 1994, p.72.

3) CO_2 Incubator Disfinfection Validation, Test Report from the Biosafety investigation unit of the CAMR, 1998.

Location of swab	Cells recovered	Log reduction
Control	1.6 x 105	0
Front	<50	>3.51
Left side	<50	>3.51
Right side	<50	>3.51
Back	<50	>3.51
Door	<50	>3.51
Top of unit	<50	>3.51
Average Counter Control	6.3 x 104	0
Counter 1	<20	>3.50
Counter 2	<20	>3.50

Table 1: Recovery of *A. niger* suspended in PBS from assayed swabs and coupons.

Location of swab	Cells recovered	Log reduction
Control	1.99 x 107	0
Front	200	5.0
Left side	<50	>5.6
Right side	<50	>5.6
Back	<50	>5.6
Door	<50	>5.6
Top of unit	<50	>5.6
Average Counter Control	1.07 x 107	0
Counter 1	84	5.11
Counter 2	46	5.37

Table 2: Recovery of *B. subtillis* suspended in PBS from assayed swabs and coupons.

Product information

Type	Description	Catalog No.
Heracell	stainless steel, inner casing, 120 V, 50/60 Hz	51013668
Heracell	all copper inner casing, 120 V, 50/60 Hz	51013669
Heracell	twin chamber incubator, stainless steel inner casing, complete with support frame, 120 V, 50/60 Hz	500051907
Heracell	twin chamber incubator, all copper inner casing, complete with support frame, 120 V, 50/60 Hz	50051908
Heracell	stainless steel inner casing, 230 V, 50/60 Hz	51013568
Heracell	all copper inner casing, 230 V, 50/60 Hz	51013569
Heracell	twin chamber incubator, stainless steel inner casing, complete with support frame, 230 V, 50/60 Hz	50051905
Heracell	twin chamber incubator, all copper inner casing, complete with support frame, 230 V, 50/60 Hz	50051906

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North America:
USA/Canada
+1 866 984 3766 (866-9-THERMO)
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Austria
+43 1 801 40 0
Belgium
+32 2 482 30 30
France +33 2 2803 2180
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