Eliminate Incubator Contamination with the Thermo Scientific Heracell CO_2 incubator

Key Words
Incubator Contamination, Heracell, Decontamination, Microorganisms, Cell Culture, Sterility

The Thermo Scientific™ 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 more 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 chronic contamination problems. The Heracell CO_2 incubator offers the Thermo Scientific ContraCon 90 °C moist heat decontamination cycle, proven by CAMR to eliminate microbial contamination from all internal surfaces of the incubator.

Additionally, the Heracell CO_2 incubator 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.

The ContraCon™ cycle, unlike manual cleaning, is a proven automated decontamination program at 90 °C using moist heat. To use this feature simply add 350 mL of water to generate the moist heat and press one button to begin the process.

The advantage over other automated methods utilized in incubators is that moist heat is more lethal and more penetrating than dry heat, so it can be used at lower temperatures while retaining lethal effects on microorganisms, especially spores that would be resistant to dry heat. This is because the moisture causes spores to germinate, eliminating the natural resistance of the endospore form. Another benefit of the moisture and the lower decontamination temperature is that all parts and sensors remain in the incubator, so there is no manual handling at all.

Thermo Scientific Heracell CO_2 Incubator

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Materials and Methods

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

- *Aspergillus niger* ATCC 16404, a mold, was prepared as a spore suspension from malt extract agar (MEA) plates that were overgrown and used at concentrations of 10^7 CFU/mL in phosphate buffered saline solution (PBS).
- *Bacillus subtilis var niger*, a typical gram-positive bacterium stored in sterile distilled water, was prepared as a spore suspension in PBS at concentrations of 10^9 spores per mL.
- *Saccharomyces cerevisiae* ATCC 9763, a baker's yeast, was prepared from MEA plates that were overgrown, and used at a concentration of 10^8 per mL in PBS.

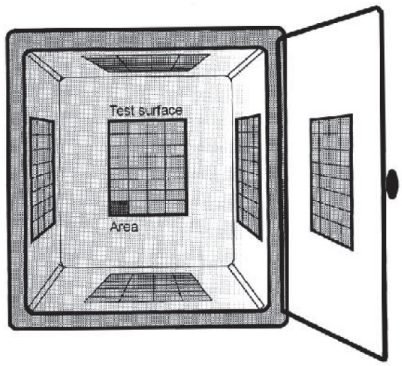

Figure 1. Illustration shows test surfaces and distribution of the individual areas for artificial contamination with various germ species.

1) 10 µL drops of each organism were applied to the six interior walls of the Heracell 240i CO_2 incubator (right, left, top, bottom wall and the glass door).
2) One extra 10 µL drop of each microorganism was placed on the floor to be used as a positive control.
3) For the application of the samples in the center of the incubator, steel 1 cm^2 coupons were used. Each was inoculated with 10 µL drops of different microbial suspensions. An extra inoculated coupon for each microorganism was used as a positive control.
4) After the drops dried, the positive control swabs and coupons were removed from the incubator and resuspended in 2 mL PBS for the duration of the disinfection cycle.
5) After all the samples were placed in the incubator, the unit was shut off and 350 mL of water was added to the water reservoir.
6) The unit was turned back on and the ContraCon routine was initiated.
7) After the cycle was complete the soiled areas were swabbed with clinical swabs wetted with sterile PBS then either:
   - mixed in universal containers containing 2 mL PBS, or
   - placed in bottles containing 10 mL of nutrient broth for bacteria or malt extract broth for fungi to confirm both positive and negative results. The test coupons were treated in the same way.

Assays

1) The swabs of the soils and the coupons in 2 mL PBS were vortexed for 1 minute and assayed as follows:
   - The *B. subtilis var niger* isolates were serially diluted (for positive controls) then plated on Tryptone Soya Broth Agar (TSBA) plates and incubated at 37 °C for 24 hours.
   - The *A. niger and S. cerevisiae* isolates were serially diluted (for positive controls) then plated on Malt Extract Agar (MEA) plates and incubated at 30 °C for 48 hours.
2) The bottles containing 10 mL nutrient broth or malt extract broth were incubated as above for 7 days. They were checked visually for growth each day (excluding the weekend). Any bottles showing growth were assayed to identify the test microorganism. 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, S. cerevisiase* and *B. subtilis* var. *niger*. The data in Tables 1, 2, 3 and 4 illustrate the measured reduction rate of these organisms in duplicate tests on different days. Fungi are physically larger than bacteria, making it difficult to deposit as many fungal spores, resulting in overall lower numbers for fungi. While it is technically correct to score cells recovered as <5 (fungi) or <2 (bacteria), these results indicate that zero growth was recovered. Further dilutions would have allowed scores to be recorded as <1, thus resulting in an even greater log reduction score. Except in a few samples in the second test that were shown to be contaminated, likely due to technical error (see below), the ContraCon eliminated all test microorganisms.

Aspergillus niger: The Heracell ContraCon cycle eliminated more than 4 logs of *A. niger* spores. CAMR says that the swab results combined with zero growth in the malt extract broth over 7 days in the first test (results not shown) means the results were "nearer a 5 log reduction"[3]. The first test showed elimination of all *A. niger* spores by the ContraCon cycle. The second test showed some growth in a few samples. However, further testing by CAMR indicated that this was likely due to contamination of the samples through technical error.[3] Ideally they would have repeated the test.

Saccharomyces cerevisiae: The ContraCon cycle eliminated all the yeast tested, greater than 2 logs of this common cell culture contaminant. Since some of the yeast died just in the process of drying them on the surfaces, CAMR says "the total reduction" approached 5 log.

Bacillus subtilis var. *niger:* The *B. subtilis* var. *niger* tests demonstrated an average greater than 6 log reduction over two independent tests, eliminating all of these heat resistant bacterial spores. These reduction rates are

certainly sufficient to ensure the decontamination of any normal background contamination levels.[3] In the parallel nutrient broth tests of the swabs exposed to the ContraCon, none of the samples showed any growth (results not shown), so, as noted by CAMR, this clearly indicates a greater than 6 log reduction. This > 6 log reduction in *Bacillus subtilis* spores is important because these spores are an accepted biological indicator (BI) organism for the U.S. Pharmacopeia and pharmacopeias from several other nations. A BI, by definition, "provides a defined and stable resistance to a specific sterilization process."[4] And spore-forming bacteria such as *B. subtilis* "are significantly more resistant than other microflora."[4] In other words, a BI is much more resistant to high temperature than organisms that are likely to be encountered in a typical laboratory setting. So eliminating more than 6 logs of *B. subtilis* spores is significant because it indicates a less than 1 in 1 million chance that any would survive the ContraCon process, and that any other microorganisms would also be eliminated.

The ContraCon decontamination program provides total thermal disinfection of the CO_2 incubator, proven by the detailed results shown here. Periodic application of the fully automated decontamination program eliminates microbial contamination, even in those difficult to reach areas of the incubator that are often missed during manual cleaning.

The moist heat decontamination routine leaves no residue, is environmentally friendly, fully automated and requires little preparatory or subsequent manual work. All the built-in components, fans and sensors remain intact during decontamination so no additional disinfection or autoclaving is required. Since manual handling is eliminated, there is no chance of reintroducing contamination when replacing autoclaved parts.

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.
3. Efficacy of the Heracell 240 CO_2 Incubator Thermal Disinfection Cycle on Dried Microbes, Test Report from the Biosafety Investigation Unit of the CAMR, 2001.
4. United States Pharmacopeia and The National Formulary (USP-NF). USP 5-NF 30, 2012.

Table 1: Recovery of *Aspergillus niger* from assayed swabs.

Location of Swab	Cells Recovered		Minimum Log Reduction	
	Test 1	Test 2	Test 1	Test 2
Positive Control	8.9×10^4	7.9×10^4	0	0
Floor	<5	<5	>4.3	>4.2
Left Side	<5	<5	>4.3	>4.2
Right Side	<5	10	>4.3	3.9 *
Back	<5	<5	>4.3	>4.2
Door	<5	<5	>4.3	>4.2
Ceiling	<5	<5	>4.3	>4.2

Table 2: Recovery of *Saccarymyces cerevisiae* from assayed swabs.

Location of Swab	Cells Recovered		Minimum Log Reduction	
	Test 1	Test 2	Test 1	Test 2
Positive Control	4.7×10^2	2.14×10^3	0	0
Floor	<5	<5	>2.0	>2.6
Left Side	<5	<5	>2.0	>2.6
Right Side	<5	<5	>2.0	>2.6
Back	<5	<5	>2.0	>2.6
Door	<5	<5	>2.0	>2.6
Ceiling	<5	<5	>2.0	>2.6

Table 3: Recovery of *Bacillus subtilis* from assayed swabs.

Location of Swab	Cells Recovered		Minimum Log Reduction	
	Test 1	Test 2	Test 1	Test 2
Positive Control	2.46×10^6	1.52×10^6	0	0
Floor	<2	<2	>6.1	>5.9
Left Side	<2	<2	>6.1	>5.9
Right Side	<2	<2	>6.1	>5.9
Back	<2	<2	>6.1	>5.9
Door	<2	<2	>6.1	>5.9
Ceiling	<2	<2	>6.1	>5.9

Table 4: Recovery of microorganisms from assayed coupons.

Coupons	Cells Recovered		Minimum Log Reduction	
	Test 1	Test 2	Test 1	Test 2
A. niger positive control	1.45×10^5	1.48×10^5	0	0
A. niger test	<5	<5	>4.5	>4.5
S. cerevisiae positive control	1.64×10^3	4.83×10^4	0	0
S. cerevisiae test	<5	<5	>2.5	>4.0
B. subtilis positive control	2.31×10^6	2.36×10^6	0	0
B. subtilis test	<2	<2	>6.1	>6.1

* Contaminated through technical error

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Australia +61 39757 4300
Austria +43 1 801 40 0
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China +800 810 5118 or +400 650 5118
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