Application Note:
ANCO2DECON 0809

Decontamination Cycles
BBD 6220, Heracell
Incubators

Decontamination Cycles in Heraeus BBD 6220 and Heracell Incubators Completely Eliminate Mycoplasma

Abstract

Even though mycoplasma can drastically alter cell activity, lab workers often fail to detect these troublesome contaminants. Many sources harbor mycoplasma, including humans, lab animals, tissue, medium, serum and labor atory instruments. Due to mycoplasma's exceedingly small size and unique characteristics, filtration or common antibiotics may not remove or destroy these organisms. Therefore, measures must be taken to rid the laboratory of all potential contamination sources. This study proves decontamination cycles of Heraeus incubators BBD 6220 (3 hours dry heat at 180 °C) and Heracell (9 hours moist heat at 90 °C) completely eliminate mycoplasma.

Heracell CO_2 incubator

Introduction

One of the smallest free-living organism classes, mycoplasmas constitute a major contamination threat to precious cell cultures. As opposed to the true cell walls of bacteria, a lipid-rich membrane surrounds a mycoplasma. Mycoplasma lack defined nuclei and can grow in the presence or absence of oxygen, depending on the strain. As little as 0.125 µm in diameter, they can remain undetected to the naked eye, even though they may outnumber cells 10-100 fold. Furthermore they readily pass through sterile filtration devices (mean pore size 0.22 µm)

However, these contaminants adversely impact the biology and economics of basic research, disease diagnosis and production. For instance, mycoplasma can render data unreliable or result in the production of unsafe biopharmaceuticals. While rarely lethal, certain mycoplasma are also implicated in human disease. When present in contaminated cultures, mycoplasma can alter cell growth and metabolism, cause chromosomal aberrations, compete for nutrients, mimic virus infection, alter virus yields and change protein production.

Sources for mycoplasma contamination include humans and other mammals, plants and insects. In mammals, mycoplasma are especially rich in the respiratory, genital and urinogenital tracts, oral cavity and mammary glands. Cell culture medium and additive suppliers routinely screen each batch for mycoplasma, but poor lab practices can result in tainted media, trypsin, fetal calf serum or other supplements. Unless routinely disinfected, shared lab instruments like water baths, cold storage systems and cell culture incubators can also periodically harbor and spread mycoplasma.

With the advent of new and easier testing methods, worldwide reports of mycoplasma contamination have increased drastically. Experts contend that 35 % to 70 % of all cell cultures are already contaminated.

Thallium acetate, penicillin and sulfonamides are ineffective against many strains. In addition, most of these hardy contaminants resist freeze-thaw cycles and remain viable for considerable periods in aerosol form. Mycoplasma can even survive on the walls of tissue culture containers and in cell-free medium.

Therefore, a spill of mycoplasma contaminated culture medium on the surface of a cell-culture incubator may spread the organism. Certain lipid solvents, detergents and disinfectants (e.g. phenolic-based) inactivate mycoplasma, but one can never ensure that these agents reach all incubator surfaces or maintain sufficient contact time.

Formaldehyde "bombs", while effective against many contaminants, produce harmful gases, leave residue and/or demand expensive consulting fees.

The Solution
Although mycoplasma contamination presents unique challenges, the organisms are not immune to disinfection. One important weakness of mycoplasma that can be utilized is their susceptibility to heat and drying. Thus, incubator decontamination cycles should effectively and thoroughly eliminate mycoplasma on all incubator surfaces.

In this study, an independent, outside testing lab evaluated decontamination cycles of the Heraeus BBD 6220 and Heracell incubators manufactured by Thermo Scientific for their ability to eliminate mycoplasma.

Materials and methods
Mycoplasma organisms of two different strains (M.pneumoniae and M.orale) were placed on 4 locations inside the incubators. Their numbers were counted before and after the decontamination cycle was applied. Each experiment was repeated twice. Although several tests can assay for the presence of mycoplasma, the agar test employed in this study provides an extremely high level of sensitivity and can theoretically detect a single mycoplasma. This study demonstrates that the heat disinfection cycles in the BBD 6220 and Heracell incubators completely eliminate mycoplasma. This simple, effective, clean and inexpensive decontamination method is offered as a standard feature on both CO_2 incubators.

Discussion

The decontamination cycles of Heraeus BBD 6220 and Heracell incubators completely eliminated two different mycoplasma strains: Mycoplasma pneumoniae and Mycoplasma orale. This decontamination method provides a highly reliable method to rid the cell culture incubator of contaminants that could possibly infect cell cultures and influence cell behavior while going undetected. The decontamination in all four incubator chamber locations illustrates uniform mycoplasma elimination throughout the chamber. While rarely fatal to humans, some mycoplasmas can cause human and animal diseases, including pneumonia, arthritis, conjunctivitis and mastitis. For instance, Mycoplasma pneumoniae, the most clinically important mycoplasma in man, causes pneumonia and has been associated with complications including pleurisy and emphysema. Therefore, in addition to preventing unwanted changes to experimental cell cultures, the Heraeus BBD 6220 and Heracell decontamination cycles can also help prevent the spread of harmful microorganisms to human hosts.

Results

Disinfection Cycle (CFU/mL)	M.pneumoniae (CFU/mL)	M.orale
Experiment #1		
Before cycle	$2.1375x10^5$	$3.8125x10^5$
After cycle		
Vial Locations:		
Rear wall	0	0
Bottom	0	0
Door	0	0
Ceiling	0	0
Experiment #2		
Before cycle	$2.1375x10^5$	$3.8125x10^5$
After cycle		
Vial Locations:		
Rear wall	0	0
Bottom	0	0
Door	0	0
Ceiling	0	0

Table 1: Mycoplasma Count before and after BBD 6220 Decontamination Cycle

Disinfection Cycle (CFU/mL)	M.pneumoniae (CFU/mL)	M.orale
Experiment #1		
Before cycle	$2.6125x10^5$	$4.025x10^5$
After cycle		
Vial Locations:		
Rear wall	0	0
Bottom	0	0
Door	0	0
Ceiling	0	0
Experiment #2		
Before cycle	$5.4875x10^5$	$6.22x10^5$
After cycle		
Vial Locations:		
Rear wall	0	0
Bottom	0	0
Door	0	0
Ceiling	0	0

Table 2: Mycoplasma Count before and after Heracell Decontamination Cycle

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