CARON SCIENTIFIC

Setting the Standard in Environmental Control

APPLICATION NOTE

INCREASE INCUBATOR CONTAMINATION CONTROL WITH H_2O_2 STERILIZATION

Maintaining a sterile environment for cell cultures is essential in both the research and development setting and during large-scale biopharmaceutical production. The presence of bacteria and fungi can alter cell behavior and experimental results, [1,2] put valuable cell cultures at risk, lead to wasted time and resources, and compromise the research, development, and manufacturing process.[3]

Maintaining contamination control in incubator shakers and large reach-in cell culture incubators can be challenging. While consistent use of good sterile practices is essential, labs without highly controlled air quality must also rely on periodic incubator decontamination. This typically involves the manual wipe-down of incubator surfaces with chemical disinfectants such as isopropyl alcohol or ethanol, and the use of a spore cleansing agent. This approach is time-consuming, and complete sterilization is not assured. The wipe-down process has limitations due to the lack of accessibility to all areas, especially the gears within complex machinery like incubator shakers. Additionally, spore cleansing agents are highly caustic and can corrode equipment.

High heat processes are possible for smaller incubators but are not appropriate for incubator shakers due to the risk of damaging cabling and lubricated moving parts. Further, heat cycles have no real effect on spore-form bacteria and fungi, and can require up to 12 hours, effectively shutting down incubator units and cell culture suites for an entire shift.

THE ADVANTAGES OF DRY HYDROGEN PEROXIDE (H_2O_2) FOGGING

This application note describes the use of a patented dry hydrogen peroxide (H_2O_2) fogging system to achieve a 6-log reduction of bacterial units in a Caron Scientific incubator shaker.

H_2O_2 is a powerful oxidizer with well-documented antimicrobial properties. Unlike heat-based methods, H_2O_2 acts quickly without requiring extended heat-up and cool-down periods, allowing for significantly shorter cycle times. Additionally, because it does not expose the incubator chamber to extreme temperatures, it reduces stress on plastic components, sensors, and electronics.

Compared to low-temperature chemical sterilants such as ethylene oxide (EtO), peracetic acid, and paraformaldehyde, H_2O_2 offers a significant safety advantage. It naturally decomposes into oxygen and water vapor, leaving no toxic residues or harmful byproducts. As a result, H_2O_2 has become a widely adopted and highly effective technology for contamination control.

The dry H_2O_2 fogging sterilization technology, designed for use with Caron Scientific incubators, is simple to perform, requires less user involvement than other techniques, and has a short cycle time. Cycle time is further minimized through the patent-pending integration of a dehumidification process in the conditioning phase.

Unlike traditional wet H_2O_2 vapor cycles, which saturate the incubator chamber with an H_2O_2 fog that can lead to condensation and sterilant puddling, the dry process precisely injects H_2O_2 vapor into the airstream. This controlled method maintains humidity below the saturation point, typically around 90% relative humidity, preventing condensation formation. A humidity sensor, or an open-loop controller that simulates it, continuously monitors vapor levels, while electronic control systems regulate injection to maintain a stable, effective concentration.

By keeping the humidity level below saturation, the dry vapor cycle eliminates the need for interior disassembly, drying, and reassembly after sterilization. This allows for immediate use of the incubator post-cycle, significantly reducing technician time and effort. This process is also faster than even the most efficient heat-based sterilization methods.

UNDERSTANDING THE DIFFERENCES BETWEEN STERILIZATION, DISINFECTION, AND DECONTAMINATION

Decontamination is the broad process of removing microbiological contaminants from a surface. Within decontamination, there are different levels:

DISINFECTION typically removes a portion of microbes from a given surface, but it does not achieve the log reduction of sterilization. There is no strict FDA definition for what qualifies as disinfection, and even a minimal level microbial removal can be labeled as disinfection.

STERILIZATION has a strict FDA-defined standard, requiring the complete elimination of viable microorganisms, typically measured by a six-log or greater reduction.[4]

CONTAMINATION CONTROL THROUGH H_2O_2 STERILIZATION

The following study demonstrates the ability of dry H_2O_2 fogging to achieve a 6-log reduction of *Geobacillus stearothermophilus* 7953 bacterial colony forming units (CFUs)on biological indicator (BI) disks placed in an incubator shaker. *G. stearothermophilius* is a thermophilic, aerobic, spore-forming bacterium with a thick cell wall. It is frequently used in biological indicators to determine whether resistant forms of bacteria have been destroyed during the sterilization processes.[5]

Figure 1. Placement of Geobacillus stearothermophilius BI disks.

MATERIALS AND METHODS

Nine G. stearothermophilius BI disks were placed in a Caron Scientific incubator shaker (model # 7406-33-1) and two to four control disks were placed outside the incubator as shown in Figure 1. Three replicates of the test were performed.

The incubator shaker was subjected to a nine-minute cycle of fogging with vaporized 35% H_2O_2 at 90 °C (Figure 2). At the end of the cycle, all BI disks were individually packaged and transferred overnight to an analytical testing lab to detect the presence of any remaining CFUs.

Exposed BI disks and unexposed control disks were macerated in an appropriate fluid as per the manufacturer's instructions; heat shock was then applied to the control disks. The resulting solutions were plated in agar and incubated. Following the incubation period, the plates were counted. To calculate the log reduction of colony forming units, the log of the population of the exposed BI disks was subtracted from the log of the control disks.

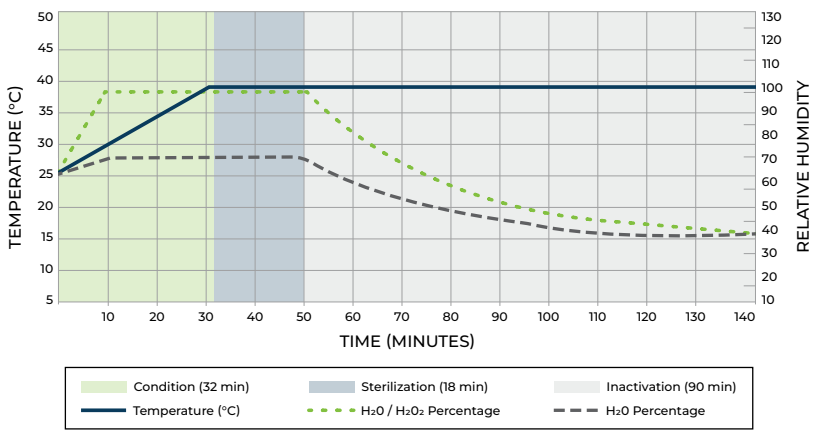

Figure 2. Steps in the dry H_2O_2 fogging cycle.

RESULTS AND DISCUSSION

The vaporized H_2O_2 cycle achieved a 6-log reduction (99.9999%) in bacterial units across the series of three tests, effectively meeting the defined standard for sterilization (Table 1). Additionally, no viable colonies were observed on the BI disks. These results meet the defined sterilization benchmark, which requires a 6+ log reduction of specific organisms with no detectable growth or viable survivors.

		Log Reduction					
BI Disk Number	**BI Disk Location**	**Test1**	**Test 2**	**Test 3**	**Average**	**Average Starting Population**	**Average Ending Population**
1	Top right ceiling	6.02	6.13	6.18	6.11	6.11	0
2	Left side, on top of top shaker platform	6.02	6.13	6.18	6.11	6.11	0
3	Right side, on underside of top shaker platform	6.02	6.13	6.18	6.11	6.11	0
4	Left side, on top of middle shaker	6.02	6.13	6.18	6.11	6.11	0
5	Left side, on underside of middle shaker platform	6.02	6.13	6.18	6.11	6.11	0
6	Right side, on top of bottom shaker platform	6.02	6.13	6.18	6.11	6.11	0
7	Top Shaking Platform Gear Box	6.02	6.13	6.18	6.11	6.11	0
8	Mid Shaking Platform Gear Box	6.02	6.13	6.18	6.11	6.11	0
9	Bottom Shaking Platform Gear Box	6.02	6.13	6.18	6.11	6.11	0
10	Control: Outside incubator shaker on top right back	0	0	0	0	6.11	6.11
11	Control: Outside incubator shaker on top right front	0	0	0	0	6.11	6.11
12	Control: Outside of incubator shaker attached to glass door top	n/a*	0	0	0	6.11	6.11
13	Control: Outside of incubator shaker attached to glass door bottom	n/a*	0	0	0	6.11	6.11
* two controls were used in Test 1							

Table 1. Log reduction in colony forming units across three separate tests.

CONCLUSION

Use the dry vaporized H_2O_2 process developed by Caron Scientific for the company's incubators provides cell culture labs with a powerful and efficient solution for contamination control. By achieving a 6-log reduction of bacterial units, this sterilization protocol significantly outperforms traditional disinfection methods while reducing processing time to just two hours.

This technology supports research and production-scale cell culture processes requiring the use of an incubator with several benefits including minimized downtime, reduced operational costs, and decreased waste from culture contamination. By integrating this sterilization cycle, laboratories can enhance efficiency, improve reliability, and maintain a contamination-free environment with greater confidence.

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