STERILIZATION OF REACH-IN INCUBATORS USING H_2O_2

Bob Dotterer, Engineering Director; Alan Campbell, VP Marketing

Abstract

Caron's patent-pending "dry" vaporized hydrogen peroxide chamber 12-log reduction sterilization system avoids the long wait time, unit stresses, high power consumption, and environmental (heat) issues of high heat sterilization and saves hours of manual unit interior disassembly and decontamination.

Introduction

Users of large reach-in cell culture incubators have always faced contamination control challenges. In addition to good sterile practice, periodic incubator decontamination or sterilization has been needed for those labs without highly controlled air quality. Manual cleaning of incubator surfaces with chemical disinfectants is often required, often in conjunction with autoclaving of large, bulky interior components. While fairly effective, this method is extremely time-consuming and physically exacting for facility staff. More recently, heat-based processes entered the market as a semi-automatic option, often packaged as a parasite or vegetative bacterial "decontamination" cycle. While reasonably effective in these limited roles, heat-decon cycles have no real effect on spore-form bacteria and fungi. Additional heat decon disadvantages include long cycle times, often up to 12 hours, high power requirements, enormous unit thermal stress, and user burn risk. Fortunately, alternative automatic chemical sterilization methods have become available, foremost among which is Hydrogen Peroxide (H_2O_2). This powerful oxidizer has well documented and accepted anti-microbial properties, permitting a true Sterilization claim. Since it is fast-acting and doesn't require lengthy heat up and cool-down times to be effective, it offers extremely short cycle times. Without the need to heat a chamber up to its maximum temperature, it also minimizes stress on plastic components, sensors, and electronics. Unlike previous generations of low temperature chemical sterilants, such as Ethylene Oxide (EtO), Peracetic Acid, and Paraformaldahyde, H_2O_2 decomposes into oxygen and water vapor after use, producing no lingering toxic chemicals or byproducts. As a result, H_2O_2 has become a major contamination elimination technology, not only within clinical Sterile Processing Departments, but also for Life Science research.

Caron has advanced the application of vaporized H_2O_2 sterilization technology within incubators by simplifying usage, pairing reduced user involvement with shortest cycle duration. Cycle time is further reduced by Caron's method of integrating a dehumidification process into the conditioning phase (patent pending). Unlike existing "wet" H_2O_2 vapor cycles, which saturate the chamber with an H_2O_2 "fog", creating condensation and sterilant puddling, Caron's dry process injects hydrogen peroxide vapor into the airstream in a controlled method, keeping the amount of H_2O_2 and water vapor below saturation point, usually around 90% RH. A humidity sensor is used to monitor the amount of H_2O_2 in the air, or open-loop controller is used to simulate it, and electronic control systems throttle vapor injection and maintain it at a constant level. By keeping the humidity below saturation point, no condensation forms anywhere within the chamber. Instead of a process that requires interior disassembly, drying, and reassembly after use, Caron's dry vapor cycle process permits immediate unit use post-cycle, minimizing technician time and effort. Total cycle time is faster than even the quickest heat-based processes, permitting full sterilization cycles to be run within a single workday.

Background

Caron's 12-log (10^{12}) reduction H_2O_2 sterilization process consists of three phases:

1. Temperature (dehumidification)/ H_2O_2 increase to necessary levels (conditioning)
2. Hold Temperature & H_2O_2 levels (sterilization)
3. H_2O_2 removal (inactivation)

Caron's sterilization cycle duration parameters were determined through both theoretical and empirical test data:

- A 35% H_2O_2 concentration was selected for two reasons:
    - A higher concentration sterilant solution decreases the total volume of liquid that must be injected into the chamber, and reduces condensation potential
    - It is the highest commonly used & commercially available concentration that is still under the US Department of Transportation threshold for passenger, cargo air freight, and rail shipment
- Theoretically, a 6-Log (10^6) reduction at 37°C should take 6 minutes. Typical D-value of H_2O_2 with 3-4 mg/L concentration at 37C is 0.5 to 1 minutes. Multiply the D-value by 6 to get a 6-log reduction and a time duration of 3 to 6 minutes.
- Early tests showed that ramping temperature from 25°C to 37°C while injecting H_2O_2 to 90% (about 10 minutes) had a 66% kill rate (2 of 3) at Log 6 reduction level. Biological Indicators (BI's) were removed without the sterilization phase but did process through the inactivation phase. This shows the effectiveness of combining the dehumidification step with the conditioning step *(patent pending)* and continued potency of kill effects taking place during the beginning of the inactivation step.
- Starting out at 37°C and injecting H_2O_2 to 90% for 5 minutes resulted in a 100% kill rate at Log 5 reduction level and 33% kill rate (2 of 6) at 6-Log reduction level. BI's were removed immediately and without having gone through either the conditioning or inactivation phases.
- Bacillus stearothermophilus is the most prevalent organism BI for validating H_2O_2 because it is one of the most resistant to hydrogen peroxide.

Caron assumes a unit temperature start value of 25°C, prior to cycle start:

- If the incubator has been 'off' for hours, the internal temperature will be ambient, typically 18°C-22°C. In this case, 25°C is also a conservative cycle start value number
- If the incubator had been 'on' recently and running at 37°C, the walls may be 37°C, but the air temperature will be much less than 37°C because the doors must be opened to insert the H_2O_2 module and initiate the test.

Prior testing proved that a unit sterilization cycle could achieve a complete 6-log reduction of all BI's by ramping from 25°C back up to 37°C and holding that temperature for 6 minutes while injecting H_2O_2 to 90% RH. By introducing a 50% safety factor, and then doubling the standard cycle sterilization time to 18 minutes, Caron provided a robust 12-log biological reduction within its cycle.

Caron's H_2O_2 injection takes place via an ultrasonic nebulizer, which wicks liquid sterilant out of the disposable container. All H_2O_2 module functions are directed by the incubator's microprocessor controller and powered through the incubator's low voltage power supply. H_2O_2 injection can only be initiated through the designated process, eliminating the potential for H_2O_2 release outside of the sealed incubator environment.

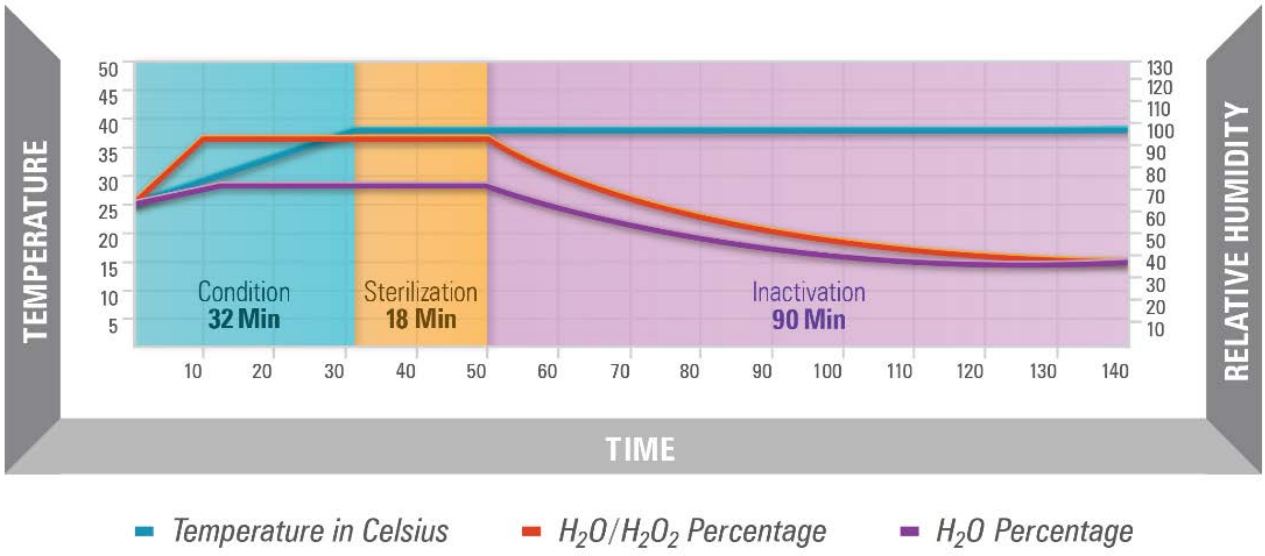
Caron uses a silver ion filter to catalyze H_2O_2 into H_2O and O_2. This long-life user-replaceable filter is located within the H_2O_2 module and is equipped with a sensor that prevents cycle activation if the filter is either missing or exhausted. It has been determined that an inactivation step of 90 minutes (25 ft3) or 110 minutes (33 ft3) will lower the H_2O_2 to a safe level.

Materials and Methods

- 7400-33, Reach-in CO_2 incubator with HUMD311 option (note: results would apply to all Caron units built using the 25 & 33 cu.ft. architecture).
- STER305/306, Sterilization prep with electronic chamber door lock
- STER301, Sterilization module, generates vaporized Hydrogen Peroxide (1x)
- STER302, 35% Hydrogen Peroxide, container of 35ml (3x)
- Biological Indicator (BI), Apex Discs, Geobacillus stearothermophilus spores, Catalog number APEX-456, Lot P1955, by Mesa Laboratories, Bozeman, MT
- Data logger, Keysight model 34972A LXI
- Basic refrigerator, maintain temperature 2-8°C

BI Test Locations Within the Chamber

Summary of H_2O_2 Cycle Steps



Stage	Time	Temperature	Humidity (H_2O_2 + H_2O)
Conditioning	~32 minutes	25°C to 37°C	Ambient to 90%
Sterilization	18 minutes	37°C	90%
Inactivation	90 minutes (25 ft3) 110 minutes (33 ft3)	37°C	90% to ~40%

Validation Test Procedure

1. Turn incubator off, and remove all contents
2. Open sensor access panel, located on the rear air duct
3. Unclip & extend the H_2O_2 module cord
4. Suspend a BI from the chamber ceiling
5. Attach a BI to the left and right-side walls
6. Suspend a BI above the chamber floor
7. Attach a control BI to the exterior wall of the incubator
8. Install the one shelf in the center and the rest of the shelves beneath it
9. Install 35% H_2O_2 canister within H_2O_2 module
10. Install H_2O_2 module on the unit middle shelf
11. Turn the unit off & plug in the H_2O_2 module.
12. Turn the unit back 'on' and initiate a sterilization cycle.
    - Unit validation was performed with a 9 minute sterilization phase. Unit cycle on production reach-in units is programmed to meet the 12-log "overkill" method, resulting in 18 minute total sterilization phase.
13. Verify unit is performing properly throughout the sterilization cycle
14. When the cycle is complete, remove all BI's & store in refrigerator at 5°C.
15. Send all exposed BI's to third-party test facility (in this case, MesaLab) for overnight processing

Note: only steps 9 – 13 are required for a standard (non-validation) sterilization cycle.

In normal operation, the sterilization cycle requires no repositioning of unit internal components. The no-touch H_2O_2 canister doesn't require measuring or pouring and can be disposed of after use. Estimated cumulative user time to initiate and conclude a cycle is less than 5 minutes.

BI Processing

16. Per protocol and MesaLab's internal procedures

Validation Results for Growth

Location	Test #1	Test #2	Test #3
1. Top	Negative	Negative	Negative
2. Bottom	Negative	Negative	Negative
3. Left	Negative	Negative	Negative
4. Right	Negative	Negative	Negative
5. Outside (Control)	Positive	Positive	Positive

Full third-party test report available by request.

Conclusions

Caron's H_2O_2 cycle achieves fast, documented, and highly reproducible low temperature chemical sterilization for CO2 incubators. This cycle was developed using established and recognized methods and test materials and employs Caron's uniform directed airflow to ensure consistent heat, humidity, and H_2O_2 distribution throughout the chamber. The six-log kill factor with 50% safety factor and 2X overkill (12 log total) process employed exceeds clinical test standards.

Vaporized H_2O_2's ability to rapidly sterilize surfaces and materials make it an ideal choice for use in this, as well as many other, applications.

Caron provides true sterilization within a large reach-in chamber, in less time and with less effort than any competing cycle or technology.

Bibliography and References

Kokubo, M., T. Inoue, and J. Akers.
Resistance of common environmental spores of the
genus Bacillus to vapor hydrogen peroxide vapor.
PDA J. Pharm. Sci. Technol. 52: 228-231, 1998.

ANSI/AAMI/ISO 11138-1
Sterilization of health care products – Biological
Indicators-Part 1: General requirements
Association for the Advancement of Medical Instrumentation, 2006.

S. Radl, S. Ortner, R. Sungkorn, and J. Khinast.
The Engineering of Hydrogen Peroxide Decontamination Systems
J Pharm Innov, 4: 51-62, 2009.

C. Hultman, A. Hill, and G. McDonnell.
The Physical Chemistry of Decontamination with Gaseous Hydrogen Peroxide
Pharmaceutical Engineering, Jan/Feb: 22-32, 2007.

Vaisala Oyj.
Humidity Conversion Formulas
B210973EN-F, 2013.

B. Unger-Bimczok, V. Kottke, C. Hertel.
The Influence of Humidity, Hydrogen Peroxide Concentration, and Condensation on the Inactivation of Geobacillus stearothermophilus Spores with Hydrogen Peroxide Vapor
J Pharm Innov, 3:123-133, 2008.

D. Watling, C. Ryle, M. Parks, and M. Christopher.
Theoretical Analysis of the Condensation of Hydrogen peroxide Gas and Water Vapour as used in Surface Decontamination
Bioquell Pharma, 2006.

R. Jones, J. Drake, and D. Eagleson
Using H_2O_2 to decontaminate biological safety cabinets
The Baker Company, Acumen Volume 1, No 1, 1993

J. Agalloco, J Akers.
Overcoming Limitations of Vaporized Hydrogen Peroxide
Pharmaceutical Technology, Volume 37, Issue 9, 2013.

J. Spiegelman and D. Alvarez.
Cheating Rault's Law to Enable Delivery of Hydrogen Peroxide as a Stable Vapor
Gases & Instrumentation: 14-19, Jan/Feb 2015.

US Department of Transportation.
49CFR 172.101, Hazardous Materials Table
US Code of Federal Regulations, amended 12/21, 1990.

CARON PRODUCTS & SERVICES, INC. PO Box 715 / Marietta OH 45750 / PHONE 740 373 6809 / 800 648 3042 / FAX 740 374 3760