H_2O_2 AS AN EFFECTIVE & FAST CO_2 INCUBATOR STERILANT

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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 the manual cleanup required for competing "wet" H_2O_2 cycles.

Introduction

Clinicians have always faced contamination control challenges within cell culture incubators. In addition to good sterile practice and continuous contamination control techniques, periodic incubator decontamination or sterilization has been needed for those labs without highly controlled air quality. In years past, manual cleaning of incubator surfaces with chemical disinfectants was required, often in conjunction with autoclaving of interior components. While fairly effective, this method is extremely time-consuming and physically exacting for clinical staff. More recently, heat decontamination entered the market as a semi-automatic option. Initially available through low-heat "wet" decontamination units, heat process has more recently expanded to include incubators with high-heat dry cycles, an approach pioneered in the late 1800's for sterilization ovens, and continuing in use for unwrapped glassware and clinical linens. These units offer the highest level biological claim, and require minimal clinician interaction during the sterilization cycle. High-heat decontamination does have its disadvantages, however, including long cycle time, typically between 9 and 14 hours, enormous unit thermal stress, and burn risk to clinicians. 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. 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 oven temperatures, 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 clinician involvement with shortest cycle duration. Cycle time is further reduced by Caron's method of integrating a dehumidification process into conditioning phase (patent pending). Existing "wet" H_2O_2 vapor cycles inject H_2O_2 into the air to achieve saturation and maintain this state. This gives the appearance of a 'fog' within the chamber. The more H_2O_2 injected into the saturated air, the more condensation forms on internal surfaces. After the cycle is over, this condensation remains. The user must then dry all surfaces post-cycle, requiring time-consuming manual cleanup and reassembly, forcing clinician contact with chemicals, and compromising cycle effectiveness. Caron's dry vapor cycle process works differently. In a dry cycle, H_2O_2 is injected into the airstream in a controlled method, keeping the amount of H_2O_2 and water vapor below saturation point, usually around 90% RH. Dry delivery systems are more sophisticated than wet cycles, requiring use of a humidity sensor to monitor the amount of H_2O_2 in the air, or open-loop controller to simulate it, and electronic control systems to throttle vapor injection and maintain it at a constant level. By keeping the humidity below saturation point, no condensation forms anywhere within the chamber. Interior reassembly, surface wipedown, and contact with chemicals are eliminated, minimizing clinician 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 reduction H_2O_2 sterilization process consists of three phases:

- Temperature / H_2O_2 increase to necessary levels (conditioning)
- Hold Temperature & H_2O_2 levels (sterilization)
- 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 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. 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 Log 6 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.

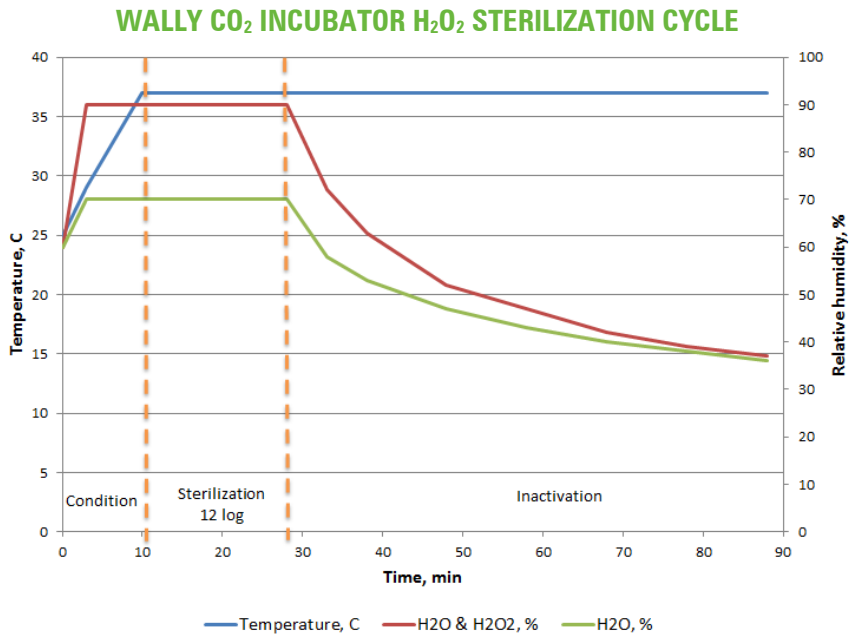
Caron uses a silver ion filter to catalyze H_2O_2 into H_2O and O_2. This long-life user-replaceable filter is located within the H_2O_2 module, and is equipped with a sensor that prevents cycle activation if the filter is either missing or exhausted. From previous Caron testing, it has been determined that an inactivation step of 60 minutes will lower the H_2O_2 to a safe level.

Materials and Methods

- 7410-5-1, Wally CO2 gel-Jacket incubator with HUMD309 (or HUMD310) options (1x)
- 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 HMV-091, Lot H1536, Population $2.1x10^6$, D-value 1.4 minutes, expiration 2017-03-31, by Mesa Laboratories, Bozeman, MT (27x)
- 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	~10 minutes	25°C to 37°C	Ambient to 90%
Sterilization	18 minutes	37°C	90%
Inactivation	60 minutes	37°C	90% to ~40%

Validation Test Procedure

1. Turn incubator off, and remove all contents
2. Remove chamber HEPA filter
3. Unclip & extend the H_2O_2 module cord
4. Remove shelves, top plenum, & side ducts.
5. Suspend a BI from the chamber ceiling on the left side
6. Attach a BI to the middle of the right side wall
7. Re-install side ducts, top plenum, water system (ultrasonic or pan), shelves, and HEPA filter frame (but not HEPA filter)
8. Attach 6 BI's on each of the six indicated interior incubator surfaces
9. Attach a control BI to the right-side exterior wall of the incubator
10. Install the three shelves in the bottom-fourth of the incubator
11. Install 35% H_2O_2 canister within H_2O_2 module
12. Install H_2O_2 module on the unit bottom shelf but do not plug in yet
13. Set the incubator temperature to 25°C.
14. Once the incubator reaches 25°C, turn the unit off & plug in the H_2O_2 module.
15. Turn the unit back 'on' and initiate a sterilization cycle.
    - Unit validation was performed with a 9 minute sterilization phase. Unit cycle on production Wally units is programmed to meet the 12-log "overkill" method, resulting in 18 minute total sterilization phase.
16. Verify unit is performing properly throughout the sterilization cycle
17. When the cycle is complete, remove all BI's & store in refrigerator at 5°C.
18. Repeat the test 2 more times, duplicating BI test locations
19. Send all exposed BI's to third-party test facility (in this case, MesaLab) for overnight processing

Note: only steps 11 – 16 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

20. Per protocol and MesaLab's internal procedures

Validation Results for Growth

Location	Test #1	Test #2	Test #3
1. Plenum left	Negative	Negative	Negative
2. Side duct right	Negative	Negative	Negative
3. Top	Negative	Negative	Negative
4. Bottom	Negative	Negative	Negative
5. Left	Negative	Negative	Negative
6. Right	Negative	Negative	Negative
7. Front	Negative	Negative	Negative
8. Back	Negative	Negative	Negative
9. 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 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 cell culture incubator, in less time and with less effort than any competing cycle or technology.

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