

**DELIVER WITH PRODUCT
TO END USER**

EZTest[®] for H₂O₂ Plasma Sterilization

Geobacillus stearothermophilus

TECHNICAL REPORT

Complies with:
USP
ISO 11138
and all appropriate subsections.

Technical Data and Use of EZTest[®] for H₂O₂ Plasma Sterilization

SGM Part #7717
Rev.1
13AUG08

INTRODUCTION

EZTest[®] for H₂O₂ Plasma Sterilization is a self-contained biological indicator to use in monitoring the efficacy of H₂O₂ Plasma sterilization cycles. EZTest H₂O₂ is easy to use; no sophisticated laboratory testing or analysis is required. EZTest H₂O₂ units consist of bacterial spores of *Geobacillus stearothermophilus*, 7953⁽¹⁾, inoculated onto a stainless steel carrier, which is placed into a thermoplastic vial that serves as a culture tube. A small glass ampoule containing sterile culture medium and pH color indicator is also contained in the vial.

STORAGE

EZTest indicators should be stored at room temperature. The indicators should not be stored near sterilants or other chemicals. EZTest H₂O₂ has an 18 month shelf-life. Do not desiccate.

MEDIUM

The culture medium, consisting of a proprietary formulated soybean casein digest base, is filled into glass ampoules and flame sealed. Following manufacture, the ampoules are exposed to a steam processing cycle to render them sterile. The sealed ampoules are of a convenient size to be placed into the plastic body with the spore paper. The ampoule is an "onion skin" glass that allows it to be easily crushed when the plastic body is compressed. This provides the spores with a nutrient medium for growth.

The culture medium has a pH indicator (bromocresol purple) added to it, which appears purple. After activation (when the plastic body is compressed), if the spores grow, the medium changes to yellow which means viable spores were present and acid is being produced. If the medium remains purple, the spores did not grow, indicating they were killed in the sterilization process. Therefore, if the sterilization process was not effective, the spores will grow and turn the medium cloudy and yellow. If any ampoules show signs of a visual color change or turbidity prior to use, they should be autoclaved and discarded.

USE

Exposure:

1. Remove an appropriate number of EZTest units from the box.
2. Identify the indicators by labeling pertinent process information.
3. It is recommended that duplicate BIs be used per cycle. Place EZTest indicators in a horizontal position with representative materials to be sterilized and in the "worst case" (least lethal) location in the load.
4. The EZTest H₂O₂ biological indicator is available in a 10⁵ population for use in lumens or process challenge devices (PCD), and a 10⁶ population is used for stand alone testing.

⁽¹⁾ Culture is traceable to a recognized culture collection identified in USP and ISO 11138.

5. Process the load as usual.
6. Remove from the sterilizer and retrieve the EZTest biological indicator from the load.
7. The chemical indicator on the cap filter changes from blue to pink when exposed to hydrogen peroxide. This distinguishes exposed from unexposed units.

NOTE: A pink color does not indicate acceptable sterilization.

9. To activate the media, place the indicator in an upright position in a plastic crusher. Gently squeeze the crusher to break the glass ampoule. This will allow the growth media to come in contact with the spore disc.

INCUBATION CONDITIONS

Any microbiological incubator that is adjusted to $60^{\circ}\text{C} \pm 2.0^{\circ}\text{C}$ will satisfy the incubation conditions for EZTest for Hydrogen Peroxide. To culture the disc in an EZTest H₂O₂ biological indicator, compress the plastic vial with a crushing device and break the glass ampoule. This will allow the growth medium to come in contact with the spore disc. Ensure that the spore disc is completely immersed in the culture medium. Do not allow the culture medium to come in contact with the filter in the cap at any time. Place the activated indicator in the incubator rack and incubate immediately. Placement in an optimized growth environment is necessary to achieve accurate results.

The medium in the plastic tube should be observed for color change for 24 hours. It is best to read results routinely every 6 hours from 12 to 24 hours.

INTERPRETATION

The appearance of a yellow color indicates bacterial growth. No color change indicates the spores were killed in the sterilization process.

Act on a positive test (a color change to yellow) as soon as the color change is noted. Color change is to be interpreted as "inadequate sterilization". Always retest the sterilizer with several EZTest H₂O₂ indicators throughout the test load. EZTest H₂O₂ indicators can be subcultured if identification of positive growth is desired.

A positive control should be run for each cycle tested or at least once per week. As soon as a control turns yellow, it should be appropriately recorded and then autoclaved and discarded. It should not be held any longer than necessary because of the possibility of contaminating your work area with organisms resistant to sterilization. The control is intended to assure you that viable spores are present on the BI lot prior to testing the sterilizer. Positive controls are not intended to be a "color standard" for comparing test results. It is not recommended to incubate these positive controls more than 24 hours. A true negative or no growth in a positive control is a serious problem. Fortunately the causes are few: a grossly malfunctioning incubator; inadvertent sterilization of the control vial; or inadvertent sterilization of the box of indicators - due to improper storage.

INCUBATION READ-OUT TIME

The recommended incubation time for EZTest Hydrogen Peroxide is 24 hours. SGM Biotech has performed the FDA protocol for determining the incubation read-out time and the data meets the FDA criteria after 24 hours of incubation.

The incubation time of SGM’s EZTest Hydrogen Peroxide product was validated according to the Center for Devices and Radiological Health, FDA protocol entitled, “Guide for Validation of Biological Indicator Incubation Time”. Three lots of EZTest Hydrogen Peroxide were prepared according to SGM’s Standard Operating Procedures. For each lot, 100 biological indicators were exposed to an H₂O₂ plasma sterilization cycle. Exposure conditions were 3.8 mg/L at 45°C ± 0.5°C. The exposed biological indicators were activated and incubated at 55°-60°C for seven days. Table 1 displays the results where 30%-80% of the tubes positive for microbial growth.

Table 1: Results of the Reduced Incubation Time Study

Biological Indicator Lot Number	# Positive 24 Hours	# Positive 7 Days	Percent Positive ⁽¹⁾
H-104	40	41	97.6
H-106	45	45	100
H-109	76	76	100

⁽¹⁾Acceptable protocol results require greater than 97% of the base number of biological indicators to test positive. This % is calculated by using the number of positive biological indicators on day 7 as the base number (denominator data) and using the number of positive biological indicators at 24 hours as the numerator.

This data shows that the 24 hour incubation time claim was valid (ratio of positives at 24 hours vs. seven days greater than 97%). A 24 hour incubation time provides users with a rapid release of sterilized product. It should be emphasized that incubator performance is critical to achieve these incubation times.

RESISTANCE PERFORMANCE TESTING

D-value determination was performed by fraction negative analysis and a population assay was performed on the biological indicators. EZTest for H₂O₂ plasma sterilization biological indicators were exposed in a Sterrad 100 HPV sterilizer. Exposure conditions were at 3.8 mg/L injected at 45°C ± 0.5°C. Twenty units per exposure were used. Following exposure, samples were activated and incubated at 55° to 60°C for 24 hours. Performance data is presented below.

3.8 mg/L ,45°C

Crop Number	Number Positive Out of 20										Population/ Unit	D-value ⁽¹⁾ (Minutes)
	Exposure Times (in minutes)											
	7	8	9	10	11	12	13	14	15	16		
Bst 020399	20	20	18	20	14	11	2	2	3	0	2.1 x 10 ⁶	1.8
Bst 081398	20	18	12	15	6	1	1	2	0	0	1.4 x 10 ⁵	1.9
Bst 020299	20	18	17	11	11	13	7	0	0	0	1.3 x 10 ⁵	2.1

⁽¹⁾Calculated according to USP methods.

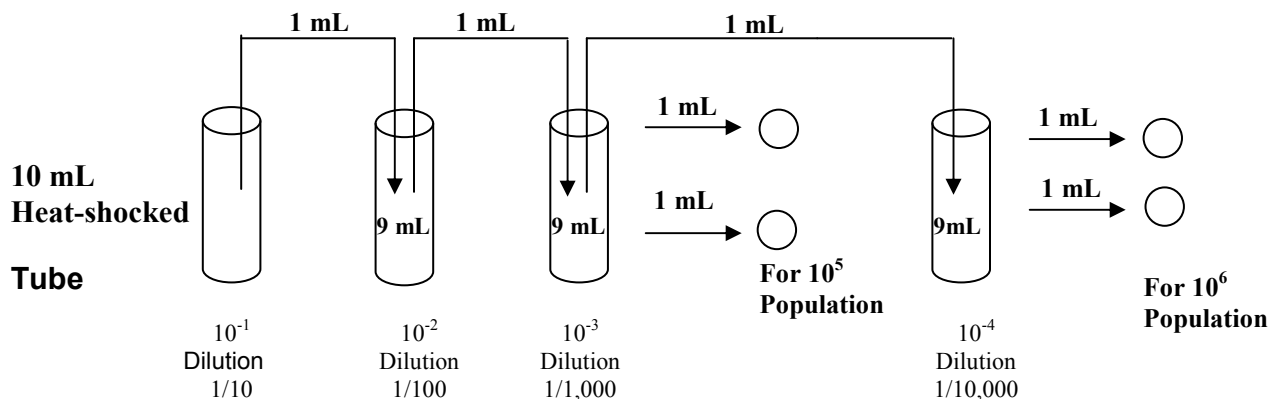
POPULATION DETERMINATION

The following procedure has been provided to evaluate the spore population of EZTest Hydrogen Peroxide:

1. Randomly select four inoculated stainless steel carriers from the lot to be assayed.
2. Place each carrier in a sterile, screw cap 19.5 x 145 mm, flat-bottom tube with four 6 mm glass beads, and 5 mL of sterile 0.1 % Tween 80.
3. Sonicate for not less than (NLT) three minutes using 45-60 kHz.
3. Vortex for NLT five minutes.
4. Add 5 mL sterile purified water. Vortex again.
5. Heat shock procedure:
 - 5.1. Place the desired dilution tube in a preheated bath at 95°-100°C for 15 minutes.
 - 5.2. Remove tubes and cool rapidly in ice bath (0° to 4°C).
6. Dilution Series:

For a 10⁵ and 10⁶ population:

A dilution series will be made from each tube. Vortex each heat-shocked tube for at least 10 seconds. From each tube, transfer a 1 mL aliquot to a dilution tube containing 9 mL of sterile purified water. Vortex the dilution tube for at least 10 seconds. Transfer 1 mL to a second dilution tube containing 9 mL of sterile purified water (**repeat this step one more time for a 10⁶ population**). Vortex this tube for at least 10 seconds. Pipette 1 mL each from this dilution tube into two 15 x 100 mm Petri dishes. Pour approximately 20 mL of melted TSA Difco agar cooled to 45° to 50°C into the Petri dishes. Swirl to assure adequate mixing and allow the agar to solidify. Do not use agar that has been melted and held longer than eight hours.



NOTE: It is extremely important to make each serial transfer immediately after vortexing.

7. Invert and incubate plates at 55°-60°C for 48 hours.

8. After 48 hours of incubation count plates. Preferably plates with counts between 30 and 300 CFUs should be used, but not less than six per USP.
9. Average counts and then multiply by the dilution factor to calculate population per original unit.
10. Document all information.

CERTIFICATE

Units are manufactured in compliance with SGM Biotech's quality standards, USP, and ISO 11138 guidelines and all appropriate subsections.



BIOLOGICAL INDICATOR CERTIFICATE OF ANALYSIS



Reorder No. EZH/

Geobacillus stearothermophilus 7953⁽¹⁾

For: Hydrogen Peroxide Plasma Sterilization

Culture: EZTest Media, 55-60°C. The supplied bacteriological medium will meet requirements for growth promoting ability.

Purity: No evidence of contaminants using standard plate count techniques.

Lot No.: H-

Manufacture Date: YEAR MONTH DAY

Expiration: 24 months from Manufacture Date.

Heat Shocked Population: 0.0×10^0 Spores/Unit

Assayed Resistance: Hydrogen Peroxide Vapor at 45°C, 3.8 mg/L

D-value⁽²⁾
min

D-value reproducible only when exposed in an AAMI BIER vessel and cultured under the exact conditions used to obtain results reported here. MPN method used.

Units are manufactured in compliance with SGM Biotech's quality standards, USP, and ISO 11138 guidelines and all appropriate subsections.

⁽¹⁾ Culture is traceable to a recognized culture collection identified in USP and ISO 11138.

⁽²⁾ D-value calculated using the Stumbo-Murphy-Cochran method.

Certified By: _____

Complete Quality Control testing results available upon request.