

**DELIVER WITH PRODUCT
TO END USER**

EZTest[®] Gas

Bacillus atrophaeus

TECHNICAL REPORT

Complies with:
USP
ISO 11138
and all appropriate subsections.

Technical Data and Use of EZTest[®] Gas

SGM Part #7701
Rev. 7
5/1/07

INTRODUCTION

EZTest[®] Gas is a self-contained biological indicator for use in monitoring the efficacy of ethylene oxide (EtO) gas sterilization cycles. EZTest is easy to use; no sophisticated laboratory testing or analysis is required. EZTest units consist of bacterial spores (*Bacillus atrophaeus* 9372)⁽¹⁾ inoculated onto a paper carrier, which is placed into a thermoplastic vial that serves as a culture tube. A small glass ampoule containing sterile culture medium and 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 has a 24 month shelf life. Do not dessicate.

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 (phenol red) added to it, which appears red-orange. 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 red-orange, 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 and identify the indicators by labeling with pertinent process information.
2. Place an EZTest indicator in a suitable test pack which is representative of the load.
3. Place this test pack in the most challenging area of the sterilizer, generally on the bottom shelf near the door.

NOTE: If a test pack is not being used, the EZTest unit should be oriented in a horizontal position during load processing.

4. Process the load as usual.
5. After EtO sterilization do either a or b:
 - a. Open the sterilizer door according to the manufacturer's instructions, transfer the load to the aerator and remove the test pack. Remove the biological indicators from the test pack. Return the remainder of the test pack to the load for aeration according to the health care facility's policy.

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

- b. If the sterilizer/aerator combination does not allow the test pack to be removed, and then at the end of the aeration cycle, remove the biological indicators from the test pack. Dispose of the remaining test pack as soon as the aeration is complete.
6. The process indicator on the label will turn from blue to brown when exposed to EtO gas. This distinguishes exposed from unexposed units.

NOTE: A brown color does not indicate acceptable sterilization.

INCUBATION CONDITIONS

Any microbiological incubator that is adjusted to 35°-39°C (for EtO gas units) will satisfy the incubation conditions for EZTest Gas. To culture the strip in an EZTest 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 strip. Ensure that the spore strip is completely saturated with 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 48 hours. It is best to read results routinely every 12 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 indicators throughout the test load. EZTest 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. The positive control typically turns yellow within 24 hours of activation and incubation. 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 48 hours.

Positive controls of EZTest Gas may revert to a cloudy magenta color if incubated longer than 48 hours. This reversion occurs primarily in non-sterilized control units and in grossly under-processed test units. This will occur infrequently, and then only after prolonged incubation, such as when EZTest Gas is incubated over a weekend.

The reversion is a change in the color of the media, from yellow (positive for growth) to a magenta color. This change in color will typically occur after 48 hours at 35°-39°C. It is a simple matter to distinguish between a vial that has reverted and one that represents no growth. Confirm any suspected reverted unit by aspirating the medium into a glass medicine dropper or pipet and observe for turbidity.

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 Gas is 48 hours. SGM Biotech has performed the FDA protocol for determining the incubation read-out time and the data meets the FDA criteria after 48 hours of incubation.

The incubation time of SGM’s EZTest Gas 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 Gas were prepared according to SGM’s Standard Operating Procedures. For each lot, 100 biological indicators were exposed to an ethylene oxide AAMI BIER cycle for the times indicated in Table 1. Exposure conditions were $600 \pm 30\text{mg/l}$ ethylene oxide gas, $54^\circ \pm 1^\circ\text{C}$, $60\% \pm 10\%$ relative humidity. The exposed biological indicators were activated and incubated at $35^\circ\text{-}39^\circ\text{C}$ for seven days. The results of the test were valid according to the FDA protocol (30%-80% of the tubes positive for microbial growth).

The following table of reduced incubation time (RIT) data meets the requirements of the U.S. FDA protocol for determining incubation times for biological indicators of less than seven days. Following this protocol the EZTest gas BI meets the requirements for 48 hour incubation at 35 to 39°C (FDA 510K #930683). This data validates that spores that are severely stressed by exposure to ethylene oxide gas (less than one live spore per BI) can be adequately recovered in the medium included in the EZTest gas self-contained BI when properly incubated for 48 hours.

Table 1: Results of the Reduced Incubation Time Study

Biological Indicator Lot Number	Exposure Time (Minutes)	# Positive 48 Hours	# Positive 7 Days	Percent Positive ⁽¹⁾
#1	22.0	48	49	98.0%
#2	23.0	38	39	97.4%
#3	29.0	46	46	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 48 hours as the numerator.

NOTICE TO USERS

NOTE: The above exposures were performed in an AAMI BIER vessel (Resistometer) which by design is easily purged of ethylene oxide gas allowing very little residual ethylene oxide inside the self-contained BI. The EZTest BIs should be aerated prior to activation and incubation to reduce residual ethylene oxide gas. If residual ethylene oxide gas remains inside the EZTest vial, the media may turn fuchsia and may slow the recovery of the injured spores present which could extend the incubation time.

To minimize this variability it is recommended that EZTest gas BIs be removed from the load as soon as it is safe to do so and aerated for two to three hours prior to activation and incubation.

Incubator conditions can vary from laboratory to laboratory. Therefore, it is recommended that each user verify that their incubator maintains the temperature range recommended by SGM for the EZTest gas BIs.

RESISTANCE PERFORMANCE TESTING

D-value determination was performed by fraction negative analysis and a population assay was performed on the biological indicators. EZTest Gas biological indicators were exposed in an EtO gas BIER vessel conforming to AAMI standards. Exposure conditions were as follows: 600 ± 30mg/l ethylene oxide gas, 54° ± 1°C, 60% ± 10% relative humidity. Twenty units per exposure were used. Following exposure, samples were activated and incubated at 35° to 39°C for 48 hours. Performance data is presented in Table 2.

Table 2: Resistance Performance Data

Crop Number	Number Positive Out of 20							Population/Unit	D-value ⁽¹⁾ (Minutes)
	Exposure Times (in minutes)								
	18	20	22	24	26	28	30		
Bsub 071596	20	16	6	6	0	1	0	1.6 x 10 ⁶	3.7
Bsub 032398	20	17	16	2	0	0	0	1.1 x 10 ⁶	3.6
Bsub 071100	20	17	4	0	1	0	0	2.4 x 10 ⁶	3.2

⁽¹⁾Calculated according to USP methods.

POPULATION DETERMINATION

SGM offers a Population Assay Kit with all the materials necessary to perform the population determination of SGM Biological Indicator products. This kit includes the same materials used at SGM for the population stated on the Certificate of Analysis. The reorder number for this kit is PAK/1.

The following procedure has been provided to evaluate the spore population of EZTest Gas.

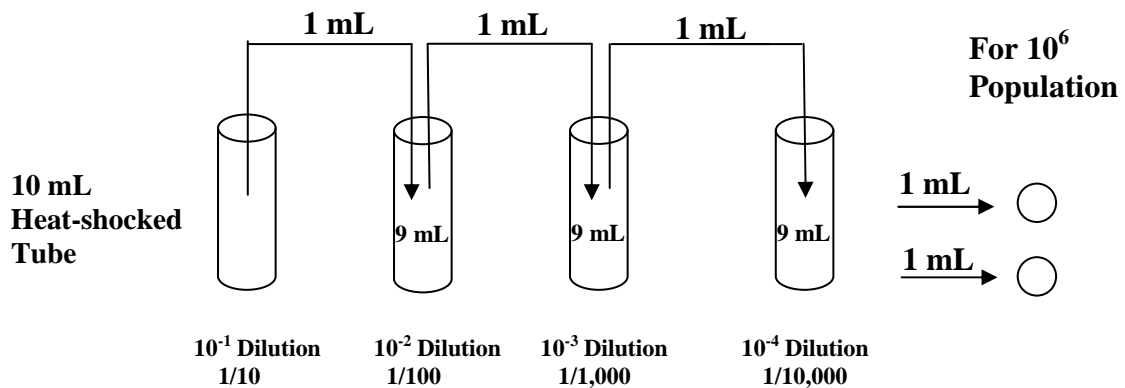
Glass Bead Method (For inoculated paper carriers):

NOTE: To avoid inaccurate plate counts, it is important to perform the serial transfers using a 2 mL pipette or whichever pipet has the largest bore size. This will help avoid clogging of the pipet tip with cotton fibers.

1. Randomly select four inoculated paper 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 purified water.
3. Vortex four to seven minutes until the paper carrier is macerated to pulp.
4. Add 5 mL sterile purified water. Vortex again.
5. Heat shock procedure:

- a. Place the desired dilution tube in a preheated bath at 80°-85°C for 10 minutes.
 - b. Remove tubes and cool rapidly in ice bath (0° to 4°C).
6. Dilution Series:

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. Pipet 1 mL from this dilution tube into two 15 x 100 mm petri dishes. Pour approximately 20 mL of melted TSA 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 30°-35°C.
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.

EZTEST[®]
BIOLOGICAL INDICATOR
CERTIFICATE OF ANALYSIS

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Reorder No. EZG/6

Bacillus atropheus

9372⁽¹⁾

For: Ethylene Oxide Sterilization.

Culture: EZTest Media, 35-39°C. The supplied bacteriological medium will meet requirements for growth promoting ability.

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

Lot No.: G-

Manufacture Date: YEAR MONTH DAY

Expiration: 24 months from Manufacture Date.

Heat Shocked Population: $x 10^6$ Spores/Unit

Assayed Resistance:

Ethylene Oxide (600 ± 30 mg/l, 60% ± 10% RH, 54 ± 1°C)

D-value⁽²⁾ Survival⁽³⁾ Kill⁽³⁾
minutes

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 Limited-Holcomb-Spearman-Karber method.

⁽³⁾ Survival/Kill values are calculated according to USP and ISO 11138.

Certified By: _____

Complete Quality Control testing results available upon request.