

**DELIVER WITH PRODUCT
TO END USER**

EZTest[®] Steam

Geobacillus stearothermophilus

TECHNICAL REPORT

Complies with:
USP
ISO 11138
and all appropriate subsections.

Technical Data and Use of EZTest[®] Steam

SGM Part #7703
Rev. 7
11/2/06

INTRODUCTION

EZTest[®] Steam is a self-contained biological indicator to use in monitoring the efficacy of 121°C, 132°C, 134°C and 135°C steam sterilization cycles. EZTest is easy to use; no sophisticated laboratory testing or analysis is required. EZTest units consist of bacterial spores *Geobacillus stearothermophilus* (7953)⁽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 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. Place an EZTest indicator in a suitable test pack which is representative of the load (e.g., a linen pack for load of linen, a tray of instruments for metal goods).
4. Place this test pack in the most challenging areas of the sterilizer, generally on the bottom shelf near the door over the drain.

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

5. Process the load as usual.

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

6. Remove from the sterilizer and allow the pack and biological indicator to cool for a sufficient time, at least 10 minutes.
7. Retrieve the EZTest biological indicator from the test load.
8. The chemical indicator on the label changes from blue to black when exposed to steam. This distinguishes exposed from unexposed units.

NOTE: A black 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 strip.

INCUBATION CONDITIONS

Any microbiological incubator that is adjusted to 55°-60°C will satisfy the incubation conditions for EZTest Steam. 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 24 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. 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 Steam is 24 hours. SGM Biotech has performed the FDA protocol at 121°C 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 Steam product was validated according to the Center for Devices and Radiological Health, FDA protocol entitled, “Guide for Validation of Biological Indicator Incubation Time”. Six lots of EZTest Steam were prepared according to SGM’s Standard Operating Procedures. For each lot, 100 biological indicators were exposed to a steam BIER cycle. Exposure conditions were 121°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 at 121° C

Biological Indicator Lot Number	# Positive 24 Hours	# Positive 7 Days	Percent Positive ⁽¹⁾
S-234	54	54	100%
S-246	72	73	98.6%
S-269	67	67	100%
S-278	45	46	97.6%
S-296	64	64	100%
S-302	57	57	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 Steam biological indicators were exposed in a steam BIER vessel that meets the AAMI BIER standard (3/81). Exposure conditions were at 121°C ± 0.5°C, 132°C ± 0.5°C, 134°C ± 0.5°C and 135°C ± 0.5°C in saturated steam using a pre-vacuum cycle. 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.

121°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.

132°C

Crop Number	Number Positive Out of 20								Population/ Unit	D-value ⁽¹⁾ (Minutes)
	Exposure Times (in minutes)									
	1	1.5	2	2.5	3	3.5	4	4.5		
Bst 020399	20	20	20	14	7	3	7	0	2.1 x 10 ⁶	0.5
Bst 081398	20	19	2	4	0	0	0	0	1.4 x 10 ⁵	0.3
Bst 020299	N/A	20	12	5	9	0	N/A	N/A	1.3 x 10 ⁵	0.4

⁽¹⁾Calculated according to USP methods.

134°C

Crop Number	Number Positive Out of 20						Population/ Unit	D-value ⁽¹⁾ (Minutes)
	Exposure Times (in minutes)							
	1	1.5	2	2.5	3	3.5		
Bst 020399	20	19	15	2	1	0	2.1 x 10 ⁶	0.3
Bst 081398	20	17	2	0	0	0	1.4 x 10 ⁵	0.3
Bst 020299	20	20	20	0	0	0	1.3 x 10 ⁵	0.4

⁽¹⁾Calculated according to USP methods.

135°C

Crop Number	Number Positive Out of 20							Population/ Unit	D-value ⁽¹⁾ (Minutes)
	Exposure Times (in minutes)								
	0.5	1	1.5	2	2.5	3	3.5		
Bst 020399	20	20	17	11	1	2	0	2.1 x 10 ⁶	0.3
Bst 081398	20	20	18	0	0	N/A	N/A	1.4 x 10 ⁵	0.3
Bst 020299	20	0	0	0	N/A	N/A	N/A	1.3 x 10 ⁵	0.1

⁽¹⁾Calculated according to USP methods.

POPULATION DETERMINATION

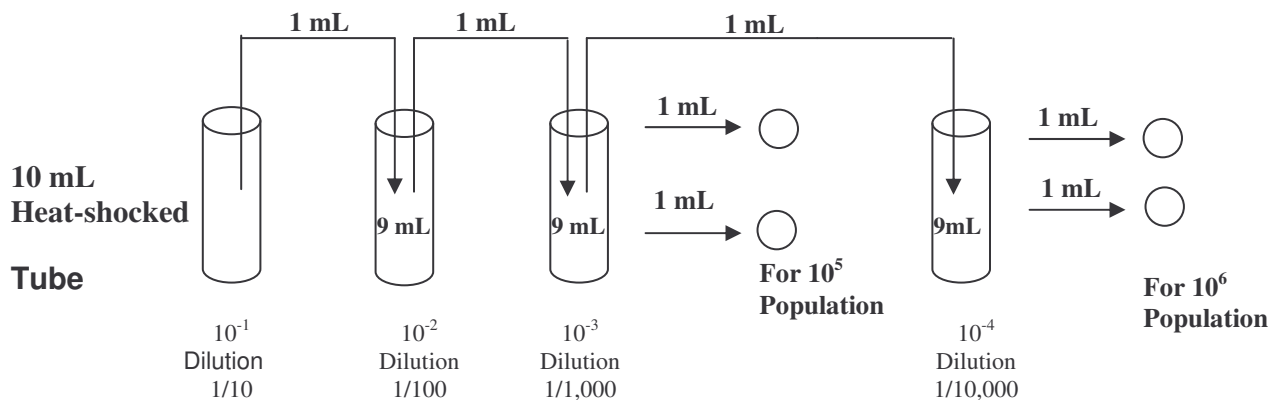
The following procedure has been provided to evaluate the spore population of EZTest Steam:

NOTE: To avoid inaccurate plate counts, it is important to perform the serial transfers using a 2 mL pipette or whichever pipette has the largest bore size. This will help avoid clogging of the pipette 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 until the paper carrier is macerated to pulp, about four to seven 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 10^5 and 10^6 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 10^6 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. EZS/

Geobacillus stearothermophilus 7953⁽¹⁾

For: Steam 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.: S-

Manufacture Date: YEAR MONTH DAY

Expiration: 24 months from Manufacture Date.

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

Assayed Resistance:

Temperature	D-value ⁽²⁾	Survival	Kill	
121°C	(3)	(3)	(3)	minutes
132°C	(4)	(4)	(4)	minutes
134°C	(4)	(4)	(4)	minutes
135°C	(4)	(4)	(4)	minutes

Z-value °C

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.

⁽⁴⁾ Empirically derived data.

Certified By: _____

Complete Quality Control testing results available upon request.