EZTest® for H_2O_2 Plasma Sterilization

Geobacillus stearothermophilus

TECHNICAL REPORT

Complies with:
USP
ISO 11138
and all appropriate subsections.

Technical Data and Use of EZTest® for H_2O_2 Plasma Sterilization

Rev.1
TR-016

INTRODUCTION

EZTest® for H_2O_2 Plasma Sterilization is a self-contained biological indicator (BI) to use in monitoring the efficacy of H_2O_2 Plasma sterilization cycles. EZTest H_2O_2 is easy to use; no sophisticated laboratory testing or analysis is required. EZTest H_2O_2 units consist of *Geobacillus stearothermophilus*, 7953[(1)], bacterial spores, inoculated onto a stainless steel disc, and 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 H_2O_2 should be stored at room temperature. Do not desiccate. The BIs should not be stored near sterilants or other chemicals. EZTest H_2O_2 has an 18 month shelf life.

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 disc. 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 during 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 BIs from the box.
2. Identify the BIs by labeling pertinent process information.
3. It is recommended that duplicate BIs be used per cycle. Place EZTest BIs in a horizontal position with representative materials to be sterilized and in the “worst case” (least lethal) location in the load.
4. The EZTest H_2O_2 BI is available in a 10^5 population for use in lumens or process challenge devices (PCD), and a 10^6 population for standalone testing.
5. Process the load as usual.
6. Retrieve the EZTest BIs from the sterilizer load.

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

7. The chemical indicator on the cap filter changes from pink to blue when exposed to hydrogen peroxide. This distinguishes exposed from unexposed units.

   NOTE: A blue color does not indicate acceptable sterilization.

8. 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 55-60C will satisfy the incubation conditions for the EZTest H_2O_2 BI. To culture the disc in an EZTest H_2O_2 BI, 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 into 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 at 24 hours. It is best to read results routinely every six 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 H_2O_2 BIs throughout the test load. EZTest H_2O_2 BIs 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 the EZTest H_2O_2 BI is 24 hours. Mesa Laboratories, Bozeman Manufacturing Facility 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 the EZTest H_2O_2 BI 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 H_2O_2 BI were prepared according to Mesa's standard operating procedures. For each lot, 100 BIs were exposed to an H_2O_2 plasma sterilization cycle. Exposure conditions were 3.8 mg/L at 45°C ± 0.5°C. The exposed BIs 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

BI 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 percentage 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 is 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 BIs. EZTest for H_2O_2 BIs 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 — Exposure Times (in minutes)										Population/ Unit	D-value[(1)] (Minutes)
	7	8	9	10	11	12	13	14	15	16		
Bst 020399	20	20	18	20	14	11	2	2	3	0	2.1×10^6	1.8
Bst 081398	20	18	12	15	6	1	1	2	0	0	1.4×10^5	1.9
Bst 020299	20	18	17	11	11	13	7	0	0	0	1.3×10^5	2.1

[(1)]Calculated according to USP methods.

POPULATION DETERMINATION

Detailed population assay instructions are available in PDF format on the company website (www.mesalabs.com).

CERTIFICATE

Units are manufactured in compliance with Mesa Labs', quality standards, USP, and ISO 11138 guidelines and all appropriate subsections.

EZ-TEST®

BIOLOGICAL INDICATOR
For Industrial Use Only

CERTIFICATE OF ANALYSIS

Reorder No: EZH/0

Geobacillus stearothermophilus 7953[1]

Indicator for: Hydrogen Peroxide Sterilization.

Culture: 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-000** Manufacture Date: YEAR MONTH DAY

Expiration Date: YEAR MONTH DAY

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

Carrier size: Approximately 7.16 mm diameter

Assayed Resistance: Hydrogen Peroxide Vapor at 45°C, 2.0 mg/L

D-Value[2]

0.0 min

D-value reproducible only when exposed to the exact sterilization conditions and cultured under the exact conditions used to obtain results reported here.

Units are manufactured in compliance with Mesa Laboratories, Bozeman Manufacturing Facility's quality standards.

[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: _____
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