EZTest® Gas

Bacillus atrophaeus

TECHNICAL REPORT

Complies with:

USP

ISO 11138

And all appropriate subsections

Technical Data and Use of EZTest® Gas

Rev.1

TR-002

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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 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 (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:

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

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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 healthcare facility's policy.

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 chemical indicator on the unit label changes from blue to a green color upon exposure to ethylene oxide. Extended exposure will result in further change to a brown color. The purpose of the chemical indicator is to distinguish exposed to 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 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 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 the 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 in 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 – 39C. It is a simple matter to distinguish between a vial that has

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reverted and one that represents no growth. Confirm any suspected reverted unit by aspirating the medium into a glass medicine dropper or pipette 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. 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 48 hours of incubation.

The incubation time of the 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 internal 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 ± 30 mg/L ethylene oxide gas, 54° ± 1°C, 60% ± 10% relative humidity. The exposed biological indicators were activated and incubated at 35 – 39C 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 biological indicator meets the requirements for 48 hour incubation at 35° – 39°C [FDA 510(k) 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

BI Lot #	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 percentage is calculated by using the number of positive biological indicators on day seven as the base number (denominator data) and using the number of positive biological indicators as the numerator.

NOTICE TO USERS

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 BI 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 may vary from laboratory to laboratory. Therefore, it is recommended that each user verify that their incubator maintains the temperature range recommended by Mesa Laboratories, Bozeman Manufacturing Facility for the EZTest Gas BI.

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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 ± 30 mg/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° – 39°C for 48 hours. Performance data is presented in Table 2.

Table 2: Resistance Performance Data

Crop #	Number Positive Out of 20 Exposure Time in Minutes							Population / Unit	D-value[1] (Minutes)
	18	20	22	24	26	28	30		
BSUB-071596	20	16	6	6	0	1	0	1.6×10^6	3.7
BSUB-032398	20	17	16	2	0	0	0	1.1×10^6	3.6
BSUB-071100	20	17	4	0	1	0	0	2.4×10^6	3.2

[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 Laboratories, Bozeman Manufacturing Facility's quality standards, USP and ISO 11138 guidelines and all appropriate subsections. Sample Certificate of Analysis can be found in Figure 1.

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Figure 1: Sample Certificate of Analysis

EZ-TEST®

BIOLOGICAL INDICATOR

CERTIFICATE OF ANALYSIS

Reorder No.: EZG/6

Bacillus atrophaeus 9372[1]

Biological Indicator 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-000** Manufacture Date: YEAR MONTH DAY

Expiration: 24 months from manufacture date.

Heat Shocked Population: 0.0 x 10^6 Spores / Unit

Carrier size: ¼" x ¾" (6 mm x 19 mm)

Assayed Resistance:

	D-value[2]	Survival[3]	Kill[3]	
Ethylene Oxide (600 ± 30 mg/L, 60 ± 10% RH, 54 ± 1°C) Oxyfume 2000[4]	0.0	00.00	00.00	min
Ethylene Oxide (600 ± 30 mg/L, 60 ± 10% RH, 54 ± 1°C) 100% EtO	0.0	00.00	00.00	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 Mesa Laboratories, Bozeman Manufacturing Facility'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 the formula in USP and ISO 11138. Mesa Labs uses a D-value rounded to four decimal places in this calculation.
[4] Oxyfume 2000 is a registered trademark of Honeywell.

Certified By: _____
Quality Representative

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