Technical Report

EZTest® Gas

I. Introduction

EZTest Gas is a self-contained biological indicator (SCBI) for use in monitoring the efficacy of ethylene oxide (EO) gas sterilization cycles. EZTest Gas is easy to use; no sophisticated laboratory testing or analysis is required. EZTest Gas units consist of 10^6 *Bacillus atrophaeus* strain 9372 spores 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 pH indicator is also contained in the vial. Filter paper is placed over the top of the culture tube to prevent contamination, and a cap with a hole is set over the top which allows sterilant penetration into the EZTest. A label on the outside of the vial includes a chemical indicator that serves as a visual indicator of whether or not the unit has been exposed to EO gas.

II. Storage

EZTest Gas should be stored at room temperature. The indicators should not be stored near sterilants or other chemicals. Do not desiccate.

III. Shelf Life

EZTest Gas has a 24-month shelf life from the date of manufacture when stored at recommended conditions.

Do not use after expiration date printed on package. Dispose of expired indicators by autoclaving at 121°C for not less than 30 minutes or per site procedures.

IV. 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 autoclaved to render them sterile and growth promotion is performed using less than 100 spores of *Bacillus atrophaeus* 9372. 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) and an appropriate incubation period, 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.

V. Use

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.

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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 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 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 EO. Extended exposure will result in further change to a brown color. The purpose of the chemical indicator is to distinguish exposed from unexposed units. A brown color does not indicate acceptable sterilization

7. To culture the strip in an EZTest SCBI, place the indicator in an upright position and compress the plastic vial with a crushing device to 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

   NOTE: The medium ampoule contained in EZTest is made from thin walled glass that is designed to break easily during culturing/activation. For this reason, the ampoule can be damaged in shipping or in handling (placement in a load, product or process challenge device, or removal from a load, product or process challenge device). Inspection of EZTest units both prior to use in a sterilization process and after the process is critical because damaged units may produce inaccurate results.

   Inspect each EZTest unit for:

   - Indication of a damaged ampoule including low medium fill volume, wet or dried medium inside vial, cap filter appearing wet or discolored or spore strip appearing wet or discolored.

   - Missing or damaged components including cap, cap filter, spore strip, medium ampoule and plastic vial.

   Dispose of any damaged or questionable units per site procedure. Results obtained from damaged units should be considered suspect.

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VI. Incubation and Readout Time

The recommended incubation for EZTest Gas is not less than 48 hours at 35° – 39°C.

EZTest should be placed in the incubator immediately after activation. Placement in an optimized growth environment which maintains the correct incubation temperature is necessary to gain accurate results.

NOTE: If incubation will be longer than 48 hours, the growth medium must be protected from evaporation by sealing the hole in the cap. Mesa's Clear Sealing Caps (part #758001) serve this purpose.

Mesa Labs' I1410 dry bath incubator is a small, convenient, tabletop incubator capable of maintaining the correct incubation temperature for all EZTest SCBIs.

The incubation time of EZTest Gas was validated according to the guidelines set forth in Attachment II of the *Guidance for Industry and FDA Staff: Biological Indicator (BI) Premarket Notification [510(k)] Submissions*, issued October 4, 2007 by the Food and Drug Administration (FDA) Center of Devices and Radiological Health (CDRH). 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 BIER cycle for the times indicated in Table 1. Exposure conditions were 600mg/L ± 30 mg/L ethylene oxide gas, 54°C ± 1°C, 60% ± 10% relative humidity. The exposed biological indicators were activated and incubated at 35 – 39°C for seven days.

Table 1: Results of the Reduced Incubation Time Study

BI Lot #	Exposure Time (Minutes)	# Positive 48 Hours	# Positive 7 Days	Percent Positive [1]
G-196	14.7	45	46	97.8%
G-203	14.5	70	71	98.6%
G-204	14.0	71	71	100%

[1] Acceptable protocol results require greater than 97% of the base number of biological indicators to test positive. This perc entage 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.

The results of the test were valid according to the FDA protocol (30% to 80% of the tubes positive for microbial growth). 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.

The CDRH reduced incubation time (RIT) protocol for validation of RIT may or may not meet each user's requirements for regulatory compliance. Users should therefore confirm regulatory requirements for reduced incubation time or incubate for 7 days.

NOTE: The above exposures were performed in an AAMI BIER vessel (Resistometer) which, by design, is easily purged of ethylene oxide gas allowing for 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 recovery of the injured spores present which could extend incubation time.

To minimize 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

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conditions may vary from laboratory to laboratory. Therefore, it is recommended that each user verify that their incubator maintains the temperature recommended in the instructions for use.

VII. 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'. Carefully review sterilizer process records to ensure that all physical process parameters are within specification. Always ensure that loading configuration and product and package specifications are in agreement with the sterilization validation process. 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. The control is intended to assure you that viable spores are present on the SCBI 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 – 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 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.

VIII. Resistance Performance Characteristics

EO resistance assessment testing is performed by exposing EZTest Gas SCBIs in an EO resistometer conforming to ANSI/AAMI/ISO 18472:2018. BIER exposure conditions are 600 mg/L ± 30 mg/L EO, 54°C and 60% ± 10% RH. D-value is determined using the Fraction Negative method.

Sample performance data is provided in Table 2.

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Table 2: Resistance Performance Data

Lot #	Number Positive Out of 25 Exposure Time in Minutes							Population / Unit	D-value [1] (Minutes)
	12	14	16	18	20	22			
G-199	25	16	8	2	0	0		3.4×10^6	2.2
G-204	25	15	3	2	0	0		3.1×10^6	2.2
G-205	25	24	3	1	0	0		1.9×10^6	2.3

[1] Calculated according to USP methods.

IX. Population Determination

Detailed population assay instructions, TS-403 Paper, Quartz, & Cotton Thread Carrier Products, are available on Mesa's website: https://biologicalindicators.mesalabs.com/documents-manuals/

X. Compliance

EZTest Gas is manufactured in compliance with Mesa Laboratories' quality standards, USP, ISO 11138-1:2017 and ISO 11138-2:2017 guidelines.

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