

Technical Report

EZTest® Steam

I. Introduction

EZTest Steam is a self-contained biological indicator (SCBI) for use in monitoring the efficacy of 121°C, 132°C, 134°C and 135°C steam sterilization cycles. EZTest Steam is easy to use; no sophisticated laboratory testing or analysis is required. EZTest Steam units consist of 10⁵ or 10⁶ *Geobacillus stearothermophilus* strain 7953 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 holes 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 steam.

II. Storage

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

III. Shelf Life

EZTest Steam 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 *Geobacillus stearothermophilus* 7953. 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) 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 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.

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.

Technical Report

EZTest® Steam

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. Remove from the sterilizer and allow the pack and biological indicator to cool for a sufficient time, at least 10 minutes.
6. Retrieve the EZTest SCBI from the test load.
7. The chemical indicator on the unit label changes from blue to a green/gray color when exposed to steam. Extended exposure will result in further change to a black color. The purpose of the chemical indicator is to distinguish exposed from unexposed units. A black color does not indicate acceptable sterilization.
8. 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.

VI. Incubation and Readout Time

The recommended incubation for EZTest Steam is not less than 24 hours at 55° – 60°C.

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

Technical Report

EZTest® Steam

NOTE: If incubation will be longer than 24 hours, the growth medium must be protected from evaporation by sealing the holes 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 Steam 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). Six lots of EZTest Steam were prepared according to internal Standard Operating Procedures. For each lot, 100 biological indicators were exposed to a steam BIER. Exposure conditions were 121°C ± 0.5°C. The exposed biological indicators were incubated at 55°C - 60°C for seven days. Table 1 displays the results were 30% to 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 | 97.8% |
| 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. 7 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.

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. 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 24 hours.

Technical Report

EZTest® Steam

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

Steam resistance assessment testing is performed by exposing EZTest Steam SCBIs in a steam resistometer conforming to ANSI/AAMI/ISO 18472:2018. Exposure conditions are 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. Additional D-value assessment at 124°C ± 0.5°C and 127°C ± 0.5°C are performed for calculation of z-value. D-value is determined using the Fraction Negative method.

Sample performance data is presented in below.

| Crop Number | 121°C | | | | | | | | | | Population / Unit | D-value ⁽¹⁾ (Minutes) |
|-------------|--------------------------------|----|----|----|----|----|----|----|----|----|-----------------------|-------------------------------------|
| | Number Positive Out of 20 | | | | | | | | | | | |
| | 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.

Technical Report

EZTest® Steam

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.

IX. Population Determination

Detailed population assay instructions, TS-403 Paper, Quartz, & Cotton Thread Carrier Products, are available on Mesa's website.

X. Compliance

EZTest Steam is manufactured in compliance with Mesa Laboratories' quality standards, USP, ISO 11138-1:2017 and ISO 11138-3:2017 guidelines.