Technical Report

Apex® EZTest®- *Geobacillus stearothermophilus* 12980

I. Introduction

Apex EZTest, self-contained biological indicators (SCBIs) are used to monitor the efficacy of vapor hydrogen peroxide (VH_2O_2) cycles at ambient pressures.
Apex EZTest SCBIs consist of a 9 mm diameter x 0.2 mm thick stainless-steel disc inoculated with, ≥1.0 x 10^6 *Geobacillus stearothermophilus* strain 12980, 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. A Tyvek® filter paper, which is permeable to VH_2O_2, is placed over the top of the culture tube to prevent contamination, and a cap with windows is set over the top which allows VH_2O_2 to access the inoculated disc during exposure.

II. Storage

APEX EZTest units should be stored at 2-8°C and less than 50% relative humidity (RH). **Keep bag sealed between use. Do not remove desiccant.**

III. Shelf Life

Apex EZTest units have a 9-month shelf life from the date of manufacture when stored at recommended conditions.

Do not use after expiration date printed on package. Dispose of positive or 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* 12980. The sealed ampoules are of a convenient size to be placed into the plastic body with the inoculated 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 and the disc is submerged into the media) 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 decontamination process. Therefore, if the decontamination 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 Apex EZTest units from storage approximately one hour before use.

2. Identify the Apex EZTest units by labeling pertinent process or load location information on the cap.

3. Place the BIs in locations previously determined to be the most difficult to sterilize. Areas experiencing minimal gas flow or poor gas distribution include enclosure corners, areas in and around equipment, and locations around disposable materials to be used in the enclosure. Validation and mapping processes generally require multiple indicators at numerous sites in an enclosure.

   NOTE: Ideally the SCBIs should be exposed to VH_2O_2 in the upright position, as that is the intended use. However, study D1402-15 conducted with Apex EZTest units exposed to VH_2O_2 at various orientations showed that orientation of the SCBI in the system did not negatively impact product performance.

   NOTE: The SCBI cap must be in the "open" configuration during exposure to allow VH_2O_2 to penetrate into the biological indicator.

4. Conduct the decontamination and aeration cycle.

5. After processing, remove the Apex EZTest units and deliver them, plus one or more unexposed positive control indicators to the laboratory for testing.

   NOTE: Do not place the unexposed control in the same container as the processed SCBIs.

6. Culturing of exposed Apex EZTest units should be conducted as soon as possible following removal from the enclosure being tested.

   NOTE: Data collected for study D1402-17 indicated that there was no post exposure lethality due to residual VH_2O_2 and the Apex EZTest units can be activated immediately following exposure.

   NOTE: Data collected for study D1402-19 showed no significant difference when comparing immediate culturing/incubation (0 hours) to delayed culturing/incubation (24 and 72 hours).

7. To culture the disc in an Apex EZTest SCBI, place the indicator in an upright position and compress the plastic vial to break the glass ampoule. Manipulate the glass into small fragments until **the disc is completely submerged in the 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 the Apex 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, or removal from a load). Inspection of Apex EZTest units both prior to use in a decontamination process and after the process is critical because damaged units may produce inaccurate results.

   Inspect each Apex 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.
   - Missing or damaged components including cap, cap filter, disc, medium ampoule, and plastic vial. Dispose of any damaged or questionable units per site procedure. Results obtained from damaged units should be considered suspect.

8. Close the cap on the Apex EZTest by applying pressure to the top of the cap. A “click” should be heard and felt when the cap has been properly closed.

NOTE: If the cap is not completely closed, the media is subject to evaporation over the course of the 7-day incubation period.

VI. Incubation and Readout Time

The recommended incubation for Apex EZTest containing *Geobacillus stearothermophilus* 12980 is not less than 7 days at 55-60ºC.

The Apex EZTest units should be placed in the incubator immediately after the SCBIs are cultured. Placement in an optimized growth environment is necessary to gain accurate results. It is important the incubation temperature be maintained to achieve accurate results.

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’.

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 positive control is intended to assure the user that viable spores are present on the SCBI lot, and the culture medium will support the growth of the test organism. Positive controls are not intended to be a ‘color standard’ for comparing test results.

A positive control that truly has not grown is a serious problem. Fortunately, the causes are few: a grossly malfunctioning incubator; inadvertent sterilization of the positive control BI; or inadvertent “sterilization” of the entire bag of BIs due to improper storage.

A negative control (an inactivated, incubated SCBI) tests the medium for contamination. It should show no signs of growth.

VIII. Resistance Performance Characteristics

Apex SCBI resistance assessment is performed in Mesa’s test isolator in 2 mg/L VH_2O_2. D-value is determined using the Fraction Negative method and calculated using the Stumbo-Murphy-Cochran procedure. The range of exposures for the Apex SCBI are shown in Table 1.

Table 1. Test Point Intervals for Resistance Testing on Apex EZTest Units

	Exposure Time (minutes)												
BI Type (Lot Prefix)	1	2	5	8	11	14	17	20	23	26	29	32	35
AZ		x	x	x	x	x	x	x	x	x	x	x	x

IX. Population Determination

Detailed population assay instructions, TS-401 Apex Products, are available on Mesa's website.

X. Compliance

Apex EZTest is manufactured in compliance with Mesa Laboratories' quality standards and ISO 11138 guidelines and all appropriate subsections with exception of determination of resistance characteristics by verification of the survival/kill response characteristics.

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