APEX Biological Indicators for H_2O_2 Vapor

Geobacillus stearothermophilus & Bacillus atrophaeus

TECHNICAL REPORT

Complies with:
ISO 11138

Technical Data and Use of APEX BIs for H_2O_2 Vapor

Rev 1
TR-001

INTRODUCTION

Apex Biologoical Indicators (BIs) are used to monitor the efficacy of H_2O_2 Vapor cycles at ambient pressures.

Apex BIs for H_2O_2 Vapor are a stainless steel carrier inoculated with varying populations of *Geobacillus stearothermophilus* strains 12980[(1)] and 7953[(1)] and *Bacillus atrophaeus* 9372[(1)]. These carriers are packaged in a medical grade Tyvek/Tyvek pouch, which is permeable to H_2O_2 Vapor.

STORAGE

APEX BIs should be stored at 2-8°C and less than 50% RH. During shipping, ambient temperatures and below 50% RH are acceptable. **(Note:** Cold packs and desiccant may be used to moderate conditions during shipping.)

The BIs should not be stored near sterilants or other chemicals. APEX BIs have a 9 month shelf-life.

MEDIUM

The culture media used for APEX BIs can either be sterile Soybean Casein Digest Medium (SCDM or Mesa Releasat Medium (a proprietary formulated soybean casein digest base). Once the APEX BI has been placed in the medium, the medium will either stay clear, indicating all spores were inactivated, or become turbid, indicating surving spores on the carrier. When using Mesa Releasat Medium, a color change to yellow indicates growth or "inadequate sterilization". No color change and absence of turbidity indicates a successful sterilization.

USE

Exposure:

1. Remove an appropriate number of Apex BIs from storage at least one hour before use.

2. Identify the Apex BIs by labeling pertinent process or load location information. Do not write on the face of the packaging over the indicator. Place BI 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.

   Note: The inoculated side of the carrier faces the printed label on the Tyvek pouch, therefore the printed side should face outward during a process cycle.

3. Validation and mapping processes generally require multiple indicators at numerous sites in an enclosure.

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

4. Remove the BIs and deliver them, plus one or more unexposed control indicators to the laboratory for sterility testing.

5. Culturing of exposed BIs should be conducted as soon as possible following removal from the enclosure being tested.

6. Culture in a laminar flow hood using strict aseptic procedures.

7. Any microbiological incubator that is adjusted to the appropriate incubation temperature (55ºC-60ºC)will satisfy the incubation conditions for the APEX BIs. **NOTE: It is important that this temperature be maintained to achieve accurate results.** The tubes should be placed in the incubator immediately after the BIs are cultured. Their placement in an optimized growth environment is necessary to gain accurate results. The medium should be observed for growth for no less than seven days.

INTERPRETATION

The appearance of color change and/or turbid medium indicates bacterial growth. Clear medium or no color change indicates no growth and the spores were killed in the sterilization process.

Turbidity in mediumis to be interpreted as "inadequate sterilization". Act on a positive test as soon as it is noted. The medium can be subcultured if identification of positive growth is desired. When using Mesa Releasat Medium, a color change to yellow and/or turbidity indicates growth or "inadequate sterilization".

A positive control should be prepared periodically or at least weekly. Many users perform a positive and negative control for each cycle tested. The positive control typically turns turbid (or changes color if using Relesat medium) within 24 to 48 hours of incubation. As soon as the control turns positive, it should be appropriately recorded, autoclaved and discarded. The positive control is intended to assure the user that viable spores are present on the BI and the culture medium will support the growth of the test organism.

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 (a tube incubated without a BI) tests the medium for contamination. It should show no signs of growth.

INCUBATION CONDITIONS/INCUBATION READ-OUT TIME

The recommended incubation temperature and time for Apex *Geobacillus stearothermophilus* 12980 & 7953 BIs is 55-60°C for 7 days. Apex *Bacillus atrophaeus* 9372 BIs recommended incubation temperature and time are 30-35°C for 7 days. Placement in an optimized growth environment for 7 days is necessary to achieve accurate results When using Mesa's Releasat Medium 7 days of incubation is also necessary for accurate results.

RESISTANCE PERFORMANCE TESTING

Apex BIs are tested for resistance in the VPHP test isolator.

The resistance of Apex BIs is measured in terms of a D-value. The D-value represents the time in minutes at a specified set of conditions to decrease the viable spore population by one log, or 90%. D-values are determined on Apex BIs using the Most Probable Number (MPN) method, and calculated using the Stumbo-Murphy-Cochran (SMC) equation.

10 BI units per exposure time are used. The range of exposures shall be from 1-35 minutes, in 3 minute increments (see Table 1).

Table 1. Test Point Intervals for Resistance Testing on Apex BIs

	Minutes of Exposure												
BI Type	1	2	5	8	11	14	17	20	23	26	29	32	35
H		x	x	x	x	x	x	x	x	x	x	x	x
N	x	x	x	x	x	x	x	x	x	x	x	x	
G	x	x	x	x	x	x	x	x	x	x	x	x	
P	x	x	x	x	x	x	x	x	x	x	x	x	
K	x	x	x	x	x	x	x	x	x	x	x	x	
S		x	x	x	x	x	x	x	x	x	x	x	x

POPULATION DETERMINATION

Detailed population assay instructions are available in PDF format on the company's website (www.mesalabs.com) at the following link:

http://biologicalindicators.mesalabs.com/wp-content/uploads/sites/31/2014/02/PopAssayIFU.v14.pdf

CERTIFICATE

Certificate of Analysis

Apex Biological Indicator (Reorder # HMV-091)
for Gaseous Hydrogen Peroxide

Lot #: **H0000**
Manufacture: YEAR MONTH DAY Expiration: YEAR MONTH DAY
Indicator: *Geobacillus stearothermophilus* 12980[(1)]
Mean population: 0.0 x 10^6 CFU per stainless steel carrier[(2)]
Storage conditions: 2 - 8°C; less than 50% RH; move to ambient conditions ≥ 1 hr before use.
Shipping conditions: Ambient temperatures; cold pack and desiccant may be used to moderate conditions during shipping.

Resistance Characteristics:

D-value[(3)]: 0.0 minutes in 2mg/L gaseous H_2O_2

D-value is reproducible only when exposed and cultured under identical conditions used to obtain results reported here. MPN method used. Units are manufactured in compliance with Mesa Laboratory, Bozeman Manufacturing Facility's quality standards and ISO 11138-1 guidelines and all appropriate subsections.

Purity: No evidence of contaminants using standard plate count techniques.

Incubate at 55 – 60˚C for 7 days. The recommended growth medium is Soybean Casein Digest Medium (SCDM), Tryptic Soy Broth (TSB) or Mesa Releasat Medium (PM/100).

This product is for Industrial Use Only.

Disposal: Treat as non-pathogenic material and sterilize (steam, EtO, etc) or incinerate before discarding.

(1) Culture is traceable to a recognized culture collection identified in USP and ISO 11138.
(2) Heat shock population determined at 95-100°C for 15 minutes
(3) D-value calculated using the Stumbo-Murphy-Cochran method

Certified By:_____
Quality Representative

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