Technical Report

Releasat® Biological Indicator Culturing Set

I. Introduction

Releasat Biological Indicator Culturing Set is used in monitoring the efficacy of Ethylene Oxide (EO) or Dry Heat sterilization processes. The set consists of MesaStrip for EO and Dry Heat and tubes of Mesa Labs' Releasat culture medium.

MesaStrip consist of 10^6 *Bacillus atrophaeus* strain 9372 spores inoculated onto a 6mm x 25mm paper spore strip, packaged in a 27mm x 73mm glassine envelope. The glassine envelope serves as a microbial barrier which protects the spore strip from post sterilization contamination.

Mesa's Releasat medium is specially formulated for rapid outgrowth of *Bacillus atrophaeus* spores that may have survived the EO or Dry Heat process. The 16mm x 100mmxculture tubes are filled with 3.6 ± 0.2 mL of Releasat medium.

II. Storage

Releasat Biological Indicator Culturing Set should be stored at room temperature. The strips should not be stored near sterilants or other chemicals. Do not desiccate.

III. Shelf Life

Releasat Biological Indicator Culturing Set has a 12-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 Releasat culture medium, consisting of a proprietary formulated soybean casein digest base, provides the spores with a nutrient medium for growth. Following manufacture, the tubes of medium are autoclaved to render them sterile and growth promotion is performed using less than 100 spores of *Bacillus atrophaeus* 9372.

The culture medium has a pH indicator added to it, which appears red-orange color. If viable spores are added, the medium changes to yellow as the acidic metabolic products of the growing bacteria accumulate. If the medium remains red-orange and clear after the spore strip is added, no microbial growth occurred, indicating that the spores were killed in the sterilization process. If the sterilization process was not effective, the spores will grow, and the medium will turn yellow and/or turbid. If a media tube shows signs of a visual color change or turbidity prior to use, it should be autoclaved and discarded.

V. Use

1. Identify the spore strips by labeling pertinent process or load location information. Place inside the product or product package and place in the most difficult location to sterilize. Refer to the manufacturer's operating manual for guidelines.

NOTE: Inspect each strip prior to use for the following:

- Damage that has left an opening in the glassine envelope
- Separation along the edge of the glassine envelope
- The presence of a strip in the glassine envelope
- Two strips in one glassine envelope

Dispose of any damaged or questionable units per site procedure.

2. Place a sufficient number of MesaStrips throughout the load to be sterilized.

3. Expose the load to the validated sterilization cycle.

4. Following the exposure and appropriate aeration, remove the spore strips and transfer them to the laboratory for culturing.

5. In the laboratory, using strict aseptic technique and working in a clean, dust free room and within confines of a laminar flow hood, transfer each spore strip into a tube containing Releasat medium.

6. The tubes should be placed in the incubator immediately after the strips are cultured.

VI. Incubation and Readout Time

The recommended incubation for the Releasat BI Culturing Set is not less than 72 hours at 36 - 38°C. Placement in an optimized growth environment which maintains the correct incubation temperature is necessary to gain accurate results.

The incubation time of the Releasat BI Culturing Set 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). 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.

VII. Interpretation

The appearance of a yellow color and/or turbidity indicates bacterial growth. No color change indicates that the spores were killed in the sterilization process.

Act on a positive test as soon as it is noted. Carefully review sterilizer process records to ensure that all physical process parameters are within specifications. Always ensure that loading configuration and product and package specifications are in agreement with the sterilization validation process. Positive units may be subcultured if identification of positive growth is desired.

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 within 24 to 48 hours of incubation. As soon as the control turns positive, it should be appropriately recorded,

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autoclaved and discarded. The positive control is intended to confirm viable spores are present on the spore strip and the culture media will support growth of the test organism.

A positive control that has not grown is a serious problem. Fortunately, the causes are few: a grossly malfunctioning incubator; inadvertent sterilization of the positive control strip; or inadvertent sterilization of the entire box of indicators due to improper storage.

A negative control (a tube incubated without a spore strip) tests the medium for contamination. It should show no signs of growth.

VIII. Performance Characteristics

EO resistance assessment testing is performed by exposing MesaStrip BIs in an EO resistometer conforming to ANSI/AAMI/ISO 18472:2018. Exposure conditions are 600 mg/L ± 30 mg/L EO, 54°C and 60% ± 10% RH. D-value is determined using the paper carrier packaged in glassine, cultured into Releasat medium, and calculated using the Fraction Negative method.

Survival and Kill times are calculated per the equations in ISO 11138-1, Annex E, using a population value and a D-value rounded to four decimal places.

Dry Heat resistance assessment testing is performed by exposing MesaStrip BIs in a Dry Heat resistometer conforming to ANSI/AAMI/ISO 18472:2018. Exposure conditions are 160°C ± 2.5°C. Additional D-value assessment at 150°C ± 2.5°C and 170°C ± 2.5°C are performed for calculation of z-value. D-value is determined using the paper carrier packaged in glassine, cultured in Releasat medium, and calculated using the Fraction Negative method.

Dry Heat z-value is calculated using 150°C, 160°C and 170°C D-values.

Survival and Kill times at 160°C are empirically derived data.

IX. Population Determination

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

X. Compliance

MesaStrip for EO and Dry Heat is manufactured in compliance with Mesa Laboratories' quality standards, USP, ISO 11138-1:2017, ISO 11138-2:2017 and ISO 11138-4:2017 guidelines, with the exception of Dry Heat D-value.

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