

# Technical Report

## Spore Strip Dual Species

### I. Introduction

Spore Strip Dual Species is a biological indicator used in monitoring the efficacy of Steam sterilization, Chemiclave® sterilization, Ethylene Oxide sterilization or Dry Heat sterilization processes. Spore Strip consists of 10<sup>5</sup> *Geobacillus stearothermophilus* strain 7953 spores and 10<sup>6</sup> *Bacillus atrophaeus* strain 9372 spores inoculated onto a 6mm x 38 mm paper spore strip, packaged in a 25mm x 70mm glassine envelope. The glassine envelope serves as a microbial barrier which protects the spore strip from post sterilization contamination.

### II. Storage

Spore Strip should be stored cool at 15 - 27°C and 30 – 70% RH, away from sterilizing agents, direct sunlight and all other forms of UV light. Do not refrigerate.

### III. Shelf Life

Spore Strip Dual Species has an 18-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 used for Spore Strip can either be sterile Soybean Casein Digest Medium or Mesa Modified Tryptic Soy Broth with Bromocresol Purple (part TSB-BP16). A reduced incubation time (RIT) for steam and Chemiclave sterilization can only be used if Spore Strip is cultured into Mesa Modified Tryptic Soy Broth with Bromocresol Purple.

After the Spore Strip is cultured into growth medium and incubated for an appropriate amount of time, the medium will either stay clear, indicating all spores were inactivated, or become turbid, indicating surviving spores on the carrier. When using Mesa Modified Tryptic Soy Broth with Bromocresol Purple, a color change to yellow and/or turbidity indicates growth or “inadequate sterilization”, and no color change and absence of turbidity indicates a successful sterilization.

### 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.
2. Place a sufficient number of Spore Strips throughout the load to be sterilized.
3. Expose the load to the validated sterilization cycle.
4. Following the exposure, remove the spore strips and transfer them to the laboratory for culturing.

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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 culture 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 Spore Strip Dual Species is not less than 24 hours at 58 - 62°C for steam sterilization or 72 hours for Chemiclave sterilization when cultured into Mesa Modified Tryptic Soy Broth with Bromocresol Purple or not less than seven days at 55 - 60°C when using media other than Mesa Modified Tryptic Soy Broth with Bromocresol Purple.

The recommended incubation for Spore Strip Dual Species is not less than seven days at 30 - 35°C for EO or Dry Heat sterilization, regardless of media type.

Placement in an optimized growth environment which maintains the correct incubation temperature is necessary to gain accurate results.

### VII. Interpretation

The appearance of turbid medium or the formation of sediment indicates bacterial growth and a positive result. Clear medium indicates no growth and that the spores were killed in the sterilization process. When using Mesa Modified Tryptic Soy Broth with Bromocresol Purple, a color change to yellow and/or turbidity indicates growth or “inadequate sterilization”, and no color change and absence of turbidity indicates a successful sterilization.

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, 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.

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### VIII. Performance Characteristics

Steam resistance assessment testing is performed by exposing Spore Strip BIs in a Steam resistometer conforming to ANSI/AAMI/ISO 18472:2018. Exposure conditions are at  $121^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ . Additional D-value assessment at  $124^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  and  $127^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  are performed for calculation of z-value. D-value is determined using the paper carrier packaged in glassine, cultured in Mesa Modified Tryptic Soy Broth with Bromocresol Purple, and calculated using the Fraction Negative method.

Z-value is calculated using  $121^{\circ}\text{C}$ ,  $124^{\circ}\text{C}$  and  $127^{\circ}\text{C}$  D-values.

Survival and Kill times at  $121^{\circ}\text{C}$  and  $134^{\circ}\text{C}$  are calculated per the equations in ISO 11138-1, Annex E, using a population value and a D-value rounded to four decimal places.

D-value at  $134^{\circ}\text{C}$  is extrapolated data.

EO resistance assessment testing is performed by exposing MesaStrip BIs in an EO resistometer conforming to ANSI/AAMI/ISO 18472:2018. Exposure conditions are  $600\text{ mg/L} \pm 30\text{ mg/L}$  EO,  $54^{\circ}\text{C}$  and  $60\% \pm 10\%$  RH. D-value is determined using the paper carrier packaged in glassine, cultured in Mesa Modified Tryptic Soy Broth with Bromocresol Purple, 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  $160^{\circ}\text{C} \pm 2.5^{\circ}\text{C}$ . Additional D-value assessment at  $150^{\circ}\text{C} \pm 2.5^{\circ}\text{C}$  and  $170^{\circ}\text{C} \pm 2.5^{\circ}\text{C}$  are performed for calculation of z-value. D-value is determined using the paper carrier packaged in glassine, cultured in Mesa Modified Tryptic Soy Broth with Bromocresol Purple, and calculated using the Fraction Negative method.

Dry Heat z-value is calculated using  $150^{\circ}\text{C}$ ,  $160^{\circ}\text{C}$  and  $170^{\circ}\text{C}$  D-values.

Survival and Kill times at  $160^{\circ}\text{C}$  are calculated per the equations in ISO 11138-1, Annex E, using a population value and a D-value rounded to four decimal places.

### 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

Spore Strip Dual Species is manufactured in compliance with Mesa Laboratories' quality standards, USP, ISO 11138-1:2017, ISO 11138-2:2017, ISO 11138-3:2017 and ISO 11138-4 guidelines with the exceptions of Dry Heat D-value and ISO 11138-1:2017 section 4.3.1 where process is not indicated on primary labeling.