Technical Report

ProSpore

I. Introduction

ProSpore is a biological indicator (BI) produced for the manufacturers of sterile solutions. The bacterial spores in this unit respond predictably to specific F_0 exposures measured inside the product container by certified thermocouples. ProSpore is a self-contained unit, making it easy to use with no sophisticated laboratory testing or analysis required. ProSpore consist of either 10^4, 10^5 or 10^6 *Geobacillus stearothermophilus* strain 7953 spores suspended in a specially formulated culture medium.

ProSpore contains 3.5 - 3.9 mL of a spore/medium suspension sealed inside a 4 mL (14.75 x 55 mm) pharmaceutical-grade glass ampoule. The shape and fill level of the ampoule allows for easy placement into containers of liquid being sterilized. Because of the need to have the ampoule inside the liquid rather than floating, the ampoule was designed so that it would sink in aqueous solutions. The shape also allows the ampoule to be suspended using a fine gauge wire, in the location of the liquid that is most difficult to sterilize, which is typically 1/3 to 1/2 the way up from the bottom of the container or the geometric center of the liquid. This is especially useful for larger volumes of liquids greater than 100 mL. The flat bottom of the ampoule also allows for the ampoule to be positioned among containers of similar size containing product with similar resistance characteristics as ProSpore.

II. Storage

ProSpore should be stored refrigerated at 2° - 8°C.

Geobacillus stearothermophilus is a thermophile and has a recommended growth temperature of 55°C - 60°C (131°F - 140°F). The spores are dormant at room temperature (18°C - 24°C/65°F - 75°F). Since some areas of the world can reach ambient temperatures above 38°C (100°F), refrigeration is recommended to assure stable indicators.

III. Shelf Life

ProSpore 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 growth medium has a pH color indicator to aid in the early detection of growth. The pH indicator is purple when the ampoules are manufactured. Spores that have survived the sterilization process will then turn the media inside the ampoule from purple to yellow upon incubation. If any ampoules show signs of a visual color change or turbidity prior to use, they should be autoclaved and discarded.

V. Use

Place ProSpore inside identical product containers as the product being sterilized. If more than one size container is used, then each different size should be monitored.

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The product containers should be filled to the same level or fill volume used for the product. Each ProSpore ampoule displaces approximately 6.0 mL of liquid and weighs approximately 6.3 grams. The liquid may be the product or simulated product. If a simulated product is used, it should have similar heat transfer characteristics. This most often varies with viscosity. The 'product packages' should be closed in a similar manner as the actual product being sterilized.

The positions of the BI in the load should be based on thermocouple profiling of the loaded chamber to ensure that the 'most difficult to sterilize' locations are being monitored. Generally, locations consist of placing BIs top to bottom, front to back and in the geometric center of the load.

Following sterilization, the BIs should be removed from the load, cooled at least to incubation temperature 55°C - 60°C and then placed into the incubator. The ProSpore may remain inside the product container if the color change can be easily observed.

VI. Incubation and Readout Time

The recommended incubation for ProSpore is not less than 48 hours at 55° – 60°C. Placement in an optimized growth environment which maintains the incubation temperature is necessary to gain accurate results.

Since ProSpore is a totally self-contained system, it can be incubated in either a water bath or standard bacteriological incubator. If the ProSpore is incubated inside the product container, the time to reach incubation temperature will vary based on the mass of the product container and solution, as well as the start temperature of the container and contents.

Mesa Labs' I1480 dry bath incubator is a small, convenient, tabletop incubator capable of maintaining the correct incubation temperature for ProSpore BIs.

The incubation time of ProSpore 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 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.

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 in the BI lot prior to testing the

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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 48 hours.

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 ProSpore ampoules in a steam resistometer conforming to ANSI/AAMI/ISO 18472:2018. Exposure conditions are at 121°C ± 0.5 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.

Z-value is calculated using 121°C, 124°C and 127°C D-values.

Survival and Kill times at 121°C and 132°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 132°C is extrapolated data.

IX. Population Determination

Detailed population assay instructions, TS-407 ProSpore 4mL Ampoules, are available on Mesa's website.

X. Compliance

ProSpore is manufactured in compliance with Mesa Laboratories' quality standards, USP, ISO 11138-1:2017 and ISO 11138-3:2017 guidelines, with the exception of the 10^4 ProSpore population.

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