Technical Report

SterilAmp® II

I. Introduction

SterilAmp II 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. SterilAmp II is a self-contained unit, making it easy to use with no sophisticated laboratory testing or analysis required. SterilAmp II consist of either 10^4, 10^5 or 10^6 *Geobacillus stearothermophilus* strain 7953 spores suspended in a specially formulated culture medium.

SterilAmp II contains 0.3 mL of a spore/medium suspension sealed inside a small, thin-walled, pharmaceutical-grade glass ampoule. These ampoules are approximately 6.75 mm diameter and 27 mm long. This size allows them to be placed inside small product vials or ampoules. These units can also be placed inside thermowells to effectively monitor Sterilization-in-Place (SIP) of product transport lines and filling machines. In some cases, a user may need a smaller ampoule size such as one that allows for the ampoule to be placed inside the smallest of medical devices, such as plastic trays containing liquid used for packaging contact lenses. For this purpose, Mesa Labs also manufactures a SterilAmp II product that contains 0.13 mL of the spore/suspension medium sealed inside a small, thin-walled, pharmaceutical-grade glass ampoule that measures 6.75 mm in diameter and 18 mm long.

II. Storage

SterilAmp II should be stored refrigerated at 2° - 8°.

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

SterilAmp II 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 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 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

The SterilAmp II biological indicators should be removed from the refrigerator and allowed to warm to room temperature. The ampoules should then be placed inside identical product containers as

MesaLabs ph: 877.746.8883 • 625 Zoot Way, Bozeman, MT 59718 • mesalabs.com

the product being sterilized. If more than one size container is used, then each different size should be monitored.

The product containers should be filled to the same level or fill volume used for the product. If extremely small volumes are used, 1 to 2 mL, the volume displacement and mass of the SterilAmp II must be considered. Each SterilAmp II (27 mm size) displaces approximately 0.8 mL of liquid and weighs approximately 0.7 g. The SterilAmp II (18 mm size) displaces approximately 0.4 mL of liquid and weighs approximately 0.35 g. 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 assure 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 SterilAmp II may remain inside the product container if the color change can be easily observed.

VI. Incubation and Readout Time

The recommended incubation for SterilAmp II 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 SterilAmp II is a totally self-contained system, it can be incubated in either a water bath or standard bacteriological incubator. If the SterilAmp II 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. SterilAmp II ampoules can be placed in zip lock bags for convenience during incubation.

The incubation time of SterilAmp II 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.

MesaLabs ph: 877.746.8883 • 625 Zoot Way, Bozeman, MT 59718 • mesalabs.com

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 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.

Negative Controls: The negative control (without spores) was developed for those users who run a longer sterilization cycle. The longer sterilization cycles break down certain growth media components and make it difficult to distinguish whether a SterilAmp II is turning positive.

The negative control is placed in the sterilizer load along with units that contain spores. Color changes due to thermal degradation can be observed and compared. This demonstrates the normal shift in color from the process. The negative control is used as a comparison to show what a negative result should look like even if the media experiences color change due to the thermal insult of the cycle. After incubation of both processed ampoules, the ampoule that contained spores is compared to the negative control ampoule. If there is a significant change in the color of the ampoule that contained spores as compared to the negative control ampoule, the result is recorded as positive. If there is not a significant change in the color of the ampoule that contained spores as compared to the negative control ampoule, the result is recorded as negative.

The negative control is manufactured with the same media formulation as the SterilAmp II with spores. The distinguishing characteristic of the negative control is a 2-mm stainless steel bead that is placed in the glass tube before it is sealed.

VIII. Resistance Performance Characteristics

Steam resistance assessment testing is performed by exposing SterilAmp II 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.

Survival and Kill times at 121°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.

F° Survival and Kill times are empirically derived data

IX. Population Determination

Detailed population assay instructions, TS-404 SterilAmp II, SterilAmp II "5230" and MagnaAmp, are available on Mesa's website: https://biologicalindicators.mesalabs.com/documents-manuals/

X. Compliance

SterilAmp II 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 population of the 10^4 SterilAmp II.

MesaLabs ph: 877.746.8883 • 625 Zoot Way, Bozeman, MT 59718 • mesalabs.com