SterilAmp® II

Geobacillus stearothermophilus

TECHNICAL REPORT

Complies to
USP, ISO 11138,
and all appropriate subsections

Technical Data and Use of SterilAmp® II

Rev.4
TR-012

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INTRODUCTION

SterilAmp® II is a biological indicator 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. It is a totally self-contained unit. SterilAmp II is easy to use, no sophisticated laboratory testing or analysis is required. These specially engineered ampoules contain spores of *Geobacillus stearothermophilus* 7953[(1)], 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. 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.

STORAGE

SterilAmp II should be refrigerated upon receipt. *Geobacillus stearothermophilus* is a thermophile and has a recommended growth temperature of 131° to 140°F (55° – 60 °C). The spores are dormant at room temperature (65° to 75°F/18° to 24 °C). Since some areas of the world can reach ambient temperatures above 100°F (38°C), refrigeration is recommended to assure stable indicators. In our laboratory, we have determined refrigerated stability for at least 18 months.

MEDIUM

The growth media 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. Following incubation, the ampoules should be autoclaved and discarded.

USE

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

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

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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° - 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. Growth inside the SterilAmp II will turn the purple growth medium yellow. This indicates a positive test (non-sterile).

A positive control should be run for each cycle tested or at least once per week. The positive control typically turns yellow within 24 hours of incubation. As soon as a control turns yellow, it should be appropriately recorded and then autoclaved and discarded. The positive control is intended to confirm that viable spores are present in the biological indicators. 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 bag of indicators - due to improper storage. If the control is negative because of one of the latter two causes, do not use any of the biological indicators from the same bag. Discard the entire bag of units after confirmation of test results.

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 documents 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.

INCUBATION CONDITIONS

The recommended incubation temperature is 55° - 60°C. 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.

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INCUBATION READ-OUT TIME

The 48-hour incubation time was validated according to the CDRH Guidance for Industry and FDA Staff: Biological Indicator (BI) Premarket Notification [510(k)] Submissions, issued October 4, 2007. The CDRH RIT protocol for validation of reduced incubation time (RIT) may or may not meet each user's requirements for regulatory compliance. Users should therefore confirm regulatory requirements for RIT, or incubate for 7 days.

The incubation time of Mesa Labs SterilAmp II product was validated according to the Center for Devices and Radiological Health, FDA protocol entitled "Guide for Validation of Biological Indicator Incubation Time". Three lots of SterilAmp II were prepared according to Mesa Labs Standard Operating Procedures. For each lot, 100 biological indicators were exposed to a steam BIER cycle for the times indicated in Table 1. Exposure conditions were 121°C ± 0.5 °C. The exposed biological indicators were incubated at 55° – 60 °C for seven days. The results of the test that were valid according to the FDA protocol (30 % - 80 % of the tubes positive for microbial growth) are shown in Table 1.

Table 1: Results of the Reduced Incubation Time Study at 121° C

Biological Indicator Crop/Dilution Number	Exposure Time (Minutes)	# Positive 48 Hours	# Positive 7 Days	Percent Positive[(1)]
GST-093014/SA5	10.5	34	35	97.1%
GST-101613/SA4	10.8	39	40	97.5%
GST-102912/SA4	11.3	48	49	98%

[(1)] Acceptable protocol results require greater than 97 % of the base number of biological indicators to test positive. This percentage is calculated by using the number of positive biological indicators on day 7 as the base number (denominator data) and using the number of positive biological indicators at forty-eight (48) hours as the numerator.

This data shows that the 48-hour incubation time claim was valid (ratio of positives at 48 hours vs. 7 days greater than 97 %). A 48-hour incubation time provides users with a rapid release of sterilized product. It should be emphasized that incubator performance is critical to achieve these incubation times.

RESISTANCE PERFORMANCE TESTING

D-value determination was performed by fraction negative analysis and a population assay was performed on the biological indicators. SterilAmp II biological indicators were exposed in a steam BIER vessel that meets the AAMI BIER standard. Exposure conditions were at 121°C ± 0.5 °C in saturated steam using a pre-vacuum cycle. Twenty units per exposure were used. Following exposure, samples were incubated at 55° – 60 °C for 48 hours. Performance data is presented in Table 2:

Table 2: Resistance Performance Data

BI Crop / Dilution Number	Number Negative Out of Twenty-Five (25) Exposure Times (in minutes)											Population/Unit	D-value[(1)] (Minutes)
	8	9	10	11	12	13	14	15	16	17	18		
GST-110116/SA2	NA	N/A	NA	0	0	2	5	16	24	25	25	3.1×10^6	2.2
GST-112116/SA3	NA	N/A	NA	0	0	4	13	22	23	25	25	3.6×10^6	2.1
GST-012117/SA4	0	0	5	19	21	23	25	25	NA	NA	NA	2.5×10^6	1.6

[(1)] Calculated according to USP methods

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POPULATION DETERMINATION

Detailed population assay instructions are available in PDF format on the Mesa Labs – Bozeman Manufacturing Facility website. Log onto the http://biologicalindicators.mesalabs.com . Under Documents & Resources, select Documents & Manuals; then select Procedures. Scroll down to Population Assay—SterilAmp, MagnaAmp link.

CERTIFICATE

Units are manufactured in compliance with Mesa Labs quality standards, USP, and ISO 11138 guidelines and all appropriate subsections. Sample Certificate of Analysis can be found in Figure 1.

Figure 1: Sample Certificate of Analysis

SterilAmp® II

BIOLOGICAL INDICATOR

FOR INDUSTRIAL USE ONLY

CERTIFICATE OF ANALYSIS

Reorder No: SA/

Geobacillus stearothermophilus 7953 (1)

Biological Indicator for: Steam Sterilization of solutions at 121°C.

Culture: 55 – 60°C. The supplied bacteriological medium will meet requirements for growth promoting ability.

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

Lot No: SA-000 Manufacture Date: YEAR MONTH DAY

Expiration: YEAR MONTH DAY

Heat Shocked Population: 0.0 x 10^0 Spores / Unit

Assayed Resistance:

	D-Value	Survival	Kill	
Steam 121°C	0.0 (2)	00.00 (3)	00.00 (3)	min
F_0		00.0 (4)	00.0 (4)	min

Z-value: 00.0°C

Units are manufactured in compliance with Mesa Laboratories, Bozeman Manufacturing Facility's quality standards, USP, and ISO 11138 guidelines and all appropriate subsections.

(1) Culture is traceable to a recognized culture collection identified in USP and ISO 11138.
(2) Resistance was determined in an AAMI BIER vessel and calculated using the Fraction Negative method. The D-value is reproducible only when exposed and cultured under the exact conditions used to obtain results reported here.
(3) Survival/Kill values are calculated according to a formula in USP and ISO 11138. A D-value rounded to four decimal places is used in this calculation.
(4) Empirically derived data.

Certified by: _____ _____
Quality Representative Date

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