SterilAmp® II

Bacillus subtilis '5230'

TECHNICAL REPORT

Complies with:
USP
ISO 11138
and all appropriate subsections.

Technical Data and Use of SterilAmp® II

Rev.3
TR-013

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INTRODUCTION

SterilAmp® II is a biological indicator produced for the manufacturers of sterile solutions. The product is manufactured for low temperature steam sterilization of solutions. 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 *Bacillus subtilis* "5230", 35021[(1)], suspended in a specially formulated culture medium.

The 0.3 mL of the spore/medium suspension is sealed inside a small thin walled pharmaceutical grade glass ampoule. These ampoules are approximately 6.75 mm in diameter and 27 mm long. This size allows them to be placed inside even the smallest product vials or ampoules. It also allows them to be packaged inside the small medical device plastic trays containing liquid such as those used for packaging contact lenses. These units can also be placed inside thermowells to effectively monitor Sterilization-in-Place (SIP) of product transport lines and filling machines.

STORAGE

SterilAmp II should be refrigerated upon receipt. *Bacillus subtilis* "5230" spores contained in the SterilAmp II has a recommended growth temperature of 35 – 39 °C. Refrigeration is necessary to assure stable indicators. In our laboratory, we have determined refrigerated stability for at least 15 months.

MEDIUM

The culture medium has phenol red, a pH indicator to aid in the early detection of growth. If the spores grow, the medium changes to yellow indicating viable spores were present and acid is being produced. If the medium remains red/orange, the spores did not grow, indicating they were killed in the sterilization process. Therefore, if the sterilization process was not effective, the spores will grow and turn the medium cloudy and yellow. If any ampoules show signs of a visual color change or turbidity prior to use, they should be autoclaved and discarded.

USE

The SterilAmp II biological indicators should be removed from the refrigerator and allowed to warm to room temperature. NOTE: Once the ampoules have reached room temperature, they must be used or refrigerated within a half-hour. The spores will germinate at room temperature. 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 displaces approximately 0.8 mL of liquid and weighs approximately 0.7 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.

It is recommended that a minimum of 10 BIs be distributed throughout each load. The positions in the load should be based on thermocouple profiling of the loaded chamber to assure that the "most difficult to

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

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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 35 - 39 °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 red/orange 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. It should not be held any longer than necessary because of the possibility of contaminating your work area with organisms resistant to sterilization. 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 72 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 users to differentiate the color between a positive ampoule compared to an ampoule that experienced color change from thermal degradation. Some 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. 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 35 – 39 °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. The

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recommended incubation time for SterilAmp II is 72 hours.

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 115 °C ± 0.5 °C and 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 35 – 39 °C for 72 hours. Performance data is presented in Tables 1 and 2.

Table 1: Resistance Performance Data at 115°

BI Lot Number	Number Positive Out of 20 Exposure Times (in minutes)					Population/Unit	D-value[1] (Minutes)
	6	8	10	12	14		
SASU-223	20	20	2	0	0	2.1×10^6	1.4
SASU-224	20	13	4	0	0	3.8×10^6	1.3
SASU-225	20	19	4	0	0	1.3×10^6	1.5

[1] Calculated according to USP methods.

Table 2: Resistance Performance Data at 121°C

BI Lot Number	Number Positive Out of 20 Exposure Times (in minutes)				Population/Unit	D-value[1] (Minutes)
	1	1.5	2	2.5		
SASU-223	20	2	0	0	2.1×10^6	0.2
SASU-224	20	1	0	0	3.8×10^6	0.2
SASU-225	20	7	1	0	1.3×10^6	0.2

[1] Calculated according to USP methods.

POPULATION DETERMINATION

Detailed population assay instructions are available in PDF format on the Mesa Labs – Bozeman Manufacturing Facility website. Log onto the mesalabs.com home page. Under documents & Downloads, select Documents; then select Biological Indicators. Under Population Assays/Protocol/Procedures, select Population Assay Procedures (Bozeman Products).

CERTIFICATE

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

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SteriAmp® II
BIOLOGICAL INDICATOR

FOR INDUSTRIAL USE ONLY

CERTIFICATE OF ANALYSIS

Reorder No: SASU/6

Bacillus subtilis '5230' 35021[1]

Biological Indicator for: Low Temperature Steam Sterilization of solutions

Culture: 35 – 39 °C. The supplied bacteriological medium will meet requirements for growth promoting ability.

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

Lot No: **SASU-000** Manufacture Date: YEAR MONTH DAY

Expiration: YEAR MONTH DAY

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

Assayed Resistance:

	D-Value[2]	Survival	Kill	
Steam 110 °C	0.0	00.0[3]	00.0[3]	min
Steam 115 °C	0.0	00.0[4]	00.0[4]	min
Steam 121 °C	0.0	00.0[4]	00.0[4]	min

Z-value: 00.0 °C

D-value reproducible only when exposed in an AAMI BIER vessel and cultured under the exact conditions used to obtain results reported here. MPN method used.

Units are manufactured in compliance with Mesa Laboratories, Bozeman Manufacturing Facility's quality standards.

[1] Culture is traceable to a recognized culture collection identified in USP and ISO 11138.
[2] D-value calculated using the Limited-Holcomb-Spearman-Karber method.
[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

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