

**DELIVER WITH PRODUCT
TO END USER**

SterilAmp[®] II

Geobacillus stearothermophilus

TECHNICAL REPORT

Complies to:
USP
ISO 11138
and all appropriate subsections.

Technical Data and Use of SterilAmp[®] II

SGM Part #7709
Rev. 3
11/2/06

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.

The 0.3 mL of the spore/medium suspension is sealed inside a small, thin-walled, pharmaceutical-grade glass ampoule. These ampoules are approximately 1/4" in diameter and 1" 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. *Geobacillus stearothermophilus* is a thermophile and has a recommended growth temperature of 131° to 140°F (55° to 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 displaces approximately 0.8 mL of liquid and weighs approximately 0.7 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.

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

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

INCUBATION CONDITIONS

The recommended incubation temperature is 55° to 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.

INCUBATION READ-OUT TIME

The recommended incubation time for SterilAmp II is 48 hours. SGM Biotech has performed the FDA protocol at 121°C for determining the incubation read-out time and the data meets the FDA criteria after 48 hours of incubation.

The incubation time of SGM’s 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 SGM’s 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 Lot Number	Exposure Time (Minutes)	# Positive 48 Hours	# Positive 7 Days	Percent Positive ⁽¹⁾
SA-311	12	68	69	98.6%
SA-315	12.2	47	48	97.9%
SA-320	14.4	72	72	100%

⁽¹⁾Acceptable protocol results require greater than 97% of the base number of biological indicators to test positive. This % 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° to 60°C for 48 hours. Performance data is presented in Table 2:

Table 2: Resistance Performance Data

BI Lot Number	Number Positive Out of Twenty (20)										Population/Unit	D-value ⁽¹⁾ (Minutes)
	Exposure Times (in minutes)											
	9	10	11	12	13	14	15	16	17	18		
SA-314	N/A	20	13	11	2	1	0	0	0	0	1.3 x 10 ⁶	1.9
SA-318	N/A	20	19	10	5	5	2	1	0	0	1.9 x 10 ⁶	1.9
SA-321	20	19	19	14	8	4	0	0	0	0	1.7 x 10 ⁵	2.3

⁽¹⁾Calculated according to USP methods.

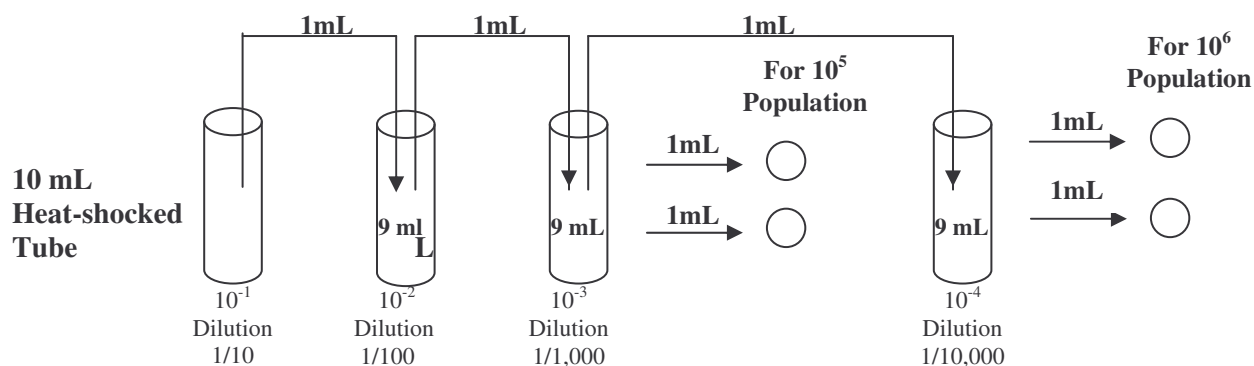
POPULATION DETERMINATION

METHOD I: One Ampoule/Volume Population Assay

1. Randomly select four ampoules from the lot to be assayed.
2. Place each ampoule into a sterile, screw cap, 19.5 x 145 mm flat-bottomed tube. Crush the ampoule using either a sterile stainless steel rod or sterile forceps.
3. Measure an appropriate volume of sterile purified water that will allow a total of 10 mL when the ampoule is added (e.g., if the ampoule has a fill of 0.3 mL, then measure 9.7 mL of sterile purified water for a total volume 10 mL.)
4. Rinse the crushing device with sterile purified water as it is added to the test tube.
5. Vortex the sample for no less than one minute.
6. Sonicate the tube for no less than three minutes using 47 kHz.
7. Heat shock procedure:
 - 7.1. Place the dilution tube in which the ampoule was crushed in a preheated bath at 95°-100°C for 15 minutes.
 - 7.2. Remove tubes and cool rapidly in ice bath (0° to 4°C).
8. Dilution Series:

For a 10^5 and 10^6 population:

A dilution series will be made from each tube. Vortex each heat-shocked tube for at least 10 seconds. From each tube, transfer a 1 mL aliquot to a dilution tube containing 9 mL of sterile purified water. Vortex the dilution tube for at least 10 seconds. Transfer 1 mL to a second dilution tube containing 9 mL of sterile purified water (**repeat this step one more time for a 10^6 population**). Vortex this tube for at least 10 seconds. Pipette 1 mL each from this dilution tube into two 15 x 100 mm Petri dishes. Pour approximately 20 ml of melted TSA Difco agar cooled to 45° to 50°C into the Petri dishes. Swirl to assure adequate mixing and allow the agar to solidify. Do not use agar that has been melted and held longer than eight hours.



NOTE: It is extremely important to make each serial transfer immediately after vortexing.

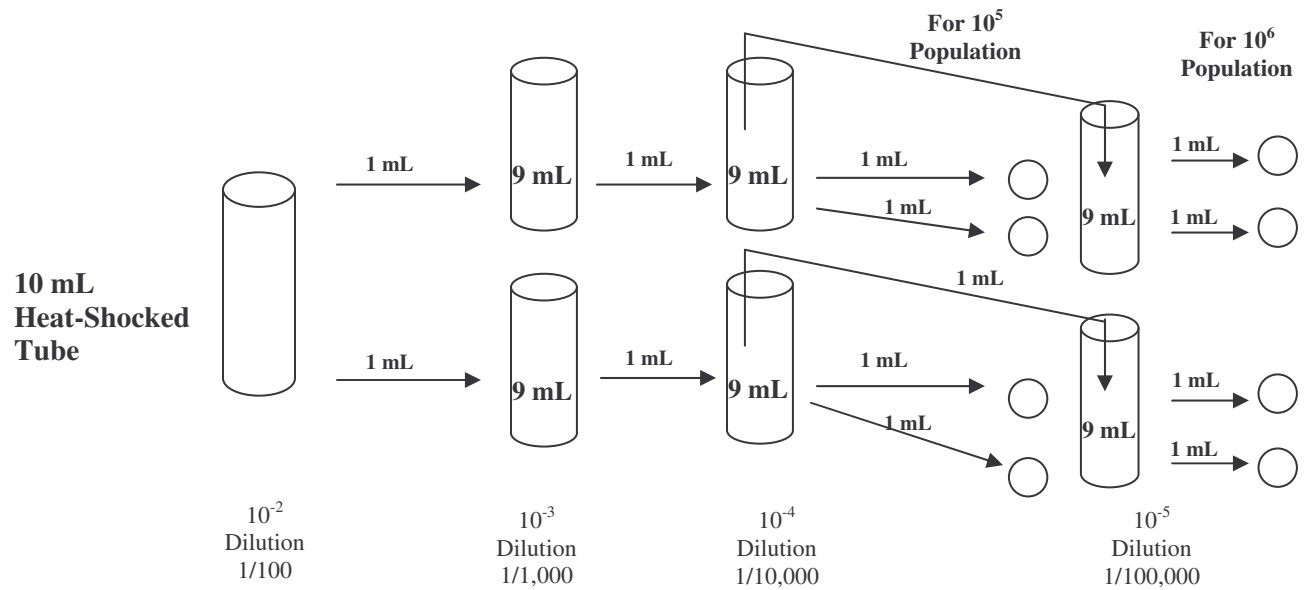
9. Invert and incubate plates at 55°-60°C.
10. After 48 hours of incubation count the plates. Preferably plates with counts between 30 and 300 CFUs should be used, but not less than 6 per USP.
11. Average counts and then multiply by the dilution factor to calculate population per original unit.
12. Document all information.

METHOD II: Four Ampoule/Volume Population Assay

1. Randomly select four ampoules from the lot to be assayed.
2. Place all four ampoules in a bottle large enough to hold a volume of 100 mL of sterile purified water. The total volume of sterile purified water after the ampoules have been added will need to be 100 mL (e.g., if the ampoules have a fill of 0.3 mL, then place in the bottle 98.8 mL of sterile purified water for a total of 100 mL).
3. Crush the ampoules before adding the dilution fluid using either a sterile stainless steel rod or sterile forceps or shaking vigorously.
4. Rinse the crushing device (if used) when adding the dilution fluid and swirling.
5. Vortex the sample for no less than one minute.
6. Sonicate the sample for no less than three minutes using 47 kHz, and vortex again.
7. Heat shock procedure:
 - 7.1. Place a 10 mL aliquot of the dilution fluid into a sterile, screw cap, 19.5 x 145 mm tube.
 - 7.2. Place the dilution tube in a preheated bath at 95°-100°C for 15 minutes.
8. Remove tube and cool rapidly in ice bath (0° to 4°C).
9. Dilution Series:

For a 10⁵ and 10⁶ population:

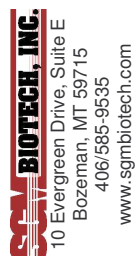
Vortex the heat-shocked tube for at least 10 seconds. Transfer two (1 mL) aliquots to dilution tubes containing 9 mL of sterile purified water. **A dilution series will be made from each tube.** Vortex the dilution tube for at least 10 seconds. Transfer 1 mL to a second dilution tube containing 9 mL of sterile purified water (**repeat this step one more time for a 10⁶ population**). Vortex this tube for at least 10 seconds. Pipette 1 mL each from this dilution tube into two 15 x 100 mm Petri dishes. Pour approximately 20 mL of melted TSA Difco agar cooled to 45° to 50°C into the Petri dishes. Swirl to assure adequate mixing and allow the agar to solidify. Do not use agar that has been melted and held longer than eight hours.



10. Invert and incubate plates at 55°-60°C for 48 hours.
11. After 48 hours of incubation count plates. Preferably plates with counts between 30 and 300 CFUs should be used, but not less than six per USP.
12. Average counts and then multiply by the dilution factor. Divide by four to calculate population per original unit.
13. Document all information.

CERTIFICATE

Each lot of SterilAmp II is manufactured in compliance with SGM Biotech's quality standards, USP, and ISO 11138 guidelines and all appropriate subsections.



Reorder No. SA/

Geobacillus stearothermophilus 7953⁽¹⁾

For: Steam Sterilization of Solutions.

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

Storage: Under refrigeration (2-8°C). Protect from light.

Disposal: Autoclave at 121°C for not less than 30 minutes.

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

Lot No.: SA-

Manufacture Date: YEAR MONTH DAY

Expiration: 18 months from Manufacture Date.

Heat Shocked Population: x 10 Spores/Unit

Assayed Resistance:

D-value ⁽²⁾	Survival	Kill	
Steam (121°C)	(3)	(3)	minutes
F ₀	(4)	(4)	minutes
Z-value			°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 SGM Biotech'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⁾ D-value calculated using the Limited-Holcomb-Spearman-Karber method.

⁽³⁾ Survival/Kill values are calculated according to USP and ISO 11138.

⁽⁴⁾ Empirically derived data.

Certified By: _____

Complete Quality Control testing results available upon request.