

**DELIVER WITH PRODUCT
TO END USER**

MagnaAmp®

Geobacillus stearothermophilus

TECHNICAL REPORT

Complies with:
USP
ISO 11138
and all appropriate subsections.

Technical Data and Use of MagnaAmp

SGM Part #7704
Rev. 4
11/2/06

INTRODUCTION

MagnaAmp® is a biological indicator (BI) produced for the manufacturers of sterile solutions. The bacterial spores in this unit respond predictably to specific F₀ exposures measured inside the product container by certified thermocouples. It is a totally self-contained unit. MagnaAmp 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 1.2 mL of the spore/medium suspension is sealed inside a 1 mL scored snap-top 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 wire or string, in the location of the liquid that is most difficult to sterilize. This is especially useful for large volumes of liquids. The flat bottom of the ampoule allows the ampoule to be set next to a container of liquid if the container is the same size as the MagnaAmp ampoule.

STORAGE

MagnaAmp 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, ampoules that have turned positive should be autoclaved and discarded.

USE

MagnaAmp 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. If the container is the same size as the MagnaAmp ampoule then the ampoule itself can be placed next to the container of liquid being sterilized.

The product containers should be filled to the same level or fill volume used for the product. Each MagnaAmp displaces approximately 2.7 mL of liquid and weighs approximately 2.7 grams. The liquid may be the product or simulated product. If a simulated product is used, it should

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

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 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 MagnaAmp may remain inside the product container if the color change can be easily observed. Growth inside the MagnaAmp will turn the growth medium from purple to 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 the 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. If the control is negative because of one of the latter two causes, do not use any of the biological indicators from the same box. Discard the box and its contents 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 MagnaAmp 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 MagnaAmp with spores. The distinguishing characteristic of the negative control is the absence of the MagnaAmp label on the glass vial.

INCUBATION CONDITIONS

The recommended incubation temperature is 55° to 60°C. Since MagnaAmp is a totally self-contained system, it can be incubated in either a water bath or standard bacteriological incubator. Bench top incubators are available from SGM Biotech for incubation of MagnaAmp. If the MagnaAmp 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.

INCUBATION READ OUT TIME

The recommended incubation time for MagnaAmp 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 MagnaAmp 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 MagnaAmp 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 ampoules 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⁾
1	8.25	53	54	98.1%
2	8.5	35	35	100%
3	9.75	66	67	98.5%

⁽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 48 hours as the numerator.

This data shows that the 48 hour incubation time claim was valid (ratio of positives at hours vs. seven 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. MagnaAmp 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 20										Population/Unit	D-value ⁽²⁾ (Minutes)
	Exposure Times (in minutes)											
	9	10	11	12	13	14	15	16	17	18		
1	20	13	10	4	1	2	2	0	0	N/A	2.1 x 10 ⁵	2.0
2	N/A	20	20	20	16	11	10	8	0	0	2.0 x 10 ⁶	2.3
3	N/A	20	19	11	8	4	1	0	0	0	2.0 x 10 ⁶	1.9

⁽²⁾Calculated according to USP methods.

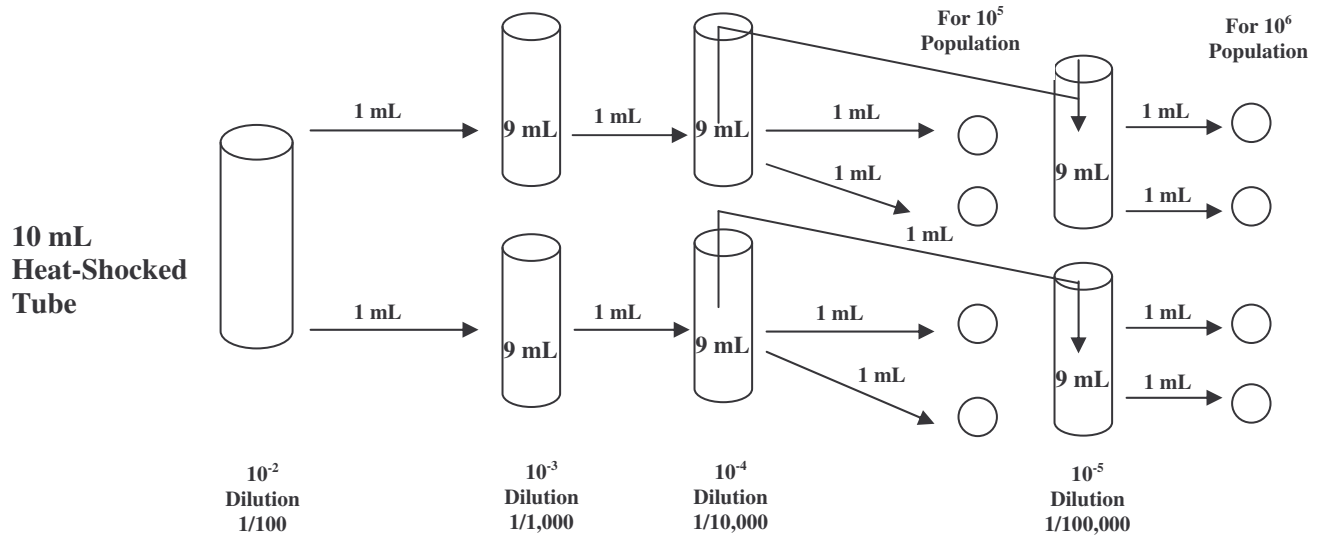
POPULATION DETERMINATION

The following procedure has been provided to evaluate the spore population of MagnaAmp.

1. Randomly select four ampoules from the lot to be assayed.
2. Place all four ampoules in a 250mL screw cap bottle. 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 1.2 mL, then place in the bottle 95.2 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 by shaking vigorously.
4. Rinse the crushing device (if used) when adding the dilution fluid.
5. Vortex the sample for no less than one minute.
6. Prior to sonication, allow the sample to sit for approximately five minutes to allow air bubbles to dissipate.
7. Sonicate the sample for no less than three minutes using 45-60 kHz and vortex again.
8. Heat shock procedure:
 - 8.1 Place a 10 mL aliquot of the dilution fluid into a sterile, screw cap, 19.5 x 145 mm tube.
 - 8.2 Place the dilution tube in a preheated bath at 95°C for 15 minutes.
9. Remove tube and cool rapidly in ice bath (0° to 4°C).
10. Dilution Series:

For a 10^5 and 10^6 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 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.

NOTE: For each transfer of liquid it is important to rinse the pipette. After each transfer into a 9mL water blank, re-pipette 1 mL of liquid into the pipette and again dispense into the same tube. Rinsing will ensure any spores remaining in the pipette will be recovered; failure to do this may result in a low recovery.

11. Invert and incubate plates at 55°-60°C for 48 hours.
12. 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.
13. Average counts and then multiply by the dilution factor. Divide by four to calculate population per original unit.
14. Document all information.

CERTIFICATE

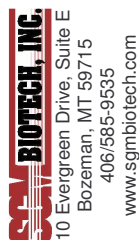
Each lot of MagnaAmp is manufactured in compliance with SGM Biotech's quality standards, USP, and ISO 11138 guidelines and all appropriate subsections. Each lot of MagnaAmp is supplied with the following certificate:



BIOLOGICAL INDICATOR

FOR INDUSTRIAL USE ONLY

CERTIFICATE OF ANALYSIS



Reorder No.: MA/

Geobacillus stearothermophilus 7953⁽¹⁾

For: Steam Sterilization of Solution.

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

Storage: Under refrigeration (2-8°C).

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

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

Lot No.: MA-

Manufacture Date: YEAR MONTH DAY

Expiration: 18 months from Manufacture Date.

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

Assayed Resistance:

	D-value ⁽²⁾	Survival	Kill	
Steam (121°C)	0.0	00.0 ⁽³⁾	00.0 ⁽³⁾	minutes
F ₀		00.0 ⁽⁴⁾	00.0 ⁽⁴⁾	minutes
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 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.

