

MagnaAmp®  
*Geobacillus stearothermophilus*

TECHNICAL REPORT

Complies to  
USP, ISO 11138,  
and all appropriate subsections

Technical Data and Use of MagnaAmp®

Rev.2  
TR-004

## **INTRODUCTION**

MagnaAmp® 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. MagnaAmp is easy to use, no sophisticated laboratory testing or analysis is required. These specially engineered ampoules contain spores of *Geobacillus stearothermophilus* 7953<sup>(1)</sup>, suspended in a specially formulated culture medium.

The 1.2 mL of the spore/medium suspension is sealed inside a 1 mL 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 fine gauge wire or string, in the location of the liquid that is most difficult to sterilize, which is typically 1/3 to 1/2 the way up from the bottom of the container or the geometric center of the liquid. This is especially useful for large volumes of liquids greater than 150 mL. 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, the ampoules should be autoclaved and discarded.

## **USE**

The 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 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

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

based on thermocouple profiling of the loaded chamber to ensure 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. 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 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 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 Mesa Labs, Bozeman Manufacturing Facility 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 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' 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 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  $121^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ . The exposed biological indicators were incubated at  $55^{\circ}\text{-}60^{\circ}\text{C}$  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  $121^{\circ}\text{C}$**

Biological Indicator Crop/Dilution Number	Exposure Time (Minutes)	# Positive 48 Hours	# Positive 7 Days	Percent Positive <sup>(1)</sup>
GST-031915/MA2	12.5	80	81	98.8%
GST-020816/MA2	10.0	53	53	100%
GST-062816/MA2	12.5	71	72	98.6%

<sup>(1)</sup> 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. MagnaAmp biological indicators were exposed in a steam BIER vessel that meets the AAMI BIER standard. Exposure conditions were at  $121^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  in saturated steam using a pre-vacuum cycle. 20 units per exposure were used. Following exposure, samples were incubated at  $55^{\circ}$  to  $60^{\circ}\text{C}$  for 48 hours. Performance data is presented in Table 2:

**Table 2: Resistance Performance Data**

BI Crop/Dilution Number	Number Negative Out of 25											Population/Unit	D-value <sup>(1)</sup> (Minutes)
	Exposure Times (in minutes)												
	8	9	10	11	12	13	14	15	16	17	18		
GST-031915/MA2	NA	NA	0	0	1	4	15	24	24	25	25	$3.3 \times 10^6$	2.0
GST-020816/MA2	0	1	3	20	22	25	25	NA	NA	NA	NA	$2.5 \times 10^6$	1.6
GST-062816/MA2	NA	NA	NA	0	0	8	15	24	25	25	NA	$1.5 \times 10^6$	2.1

<sup>(1)</sup> Calculated according to USP methods

