

**DELIVER WITH PRODUCT
TO END USER**

DriAmp™
Dry Heat Biological Indicator
Culturing Set With Releasat® Medium

TECHNICAL REPORT

Complies with:
USP
ISO 11138
and all appropriate subsections.

Technical Data and Use of DriAmp™
Dry Heat Biological Indicator
Culturing Set With Releasat® Medium

SGM Part #7712
Rev.2
10/27/06

INTRODUCTION

DriAmp™ biological indicator (BI) with Releasat® culture medium is used in monitoring the efficacy of dry heat sterilization cycles. The DriAmp BI with Releasat culture medium consists of 1.0 mL scored snap-top glass ampoules containing silica sand inoculated with 10^6 spores of *Bacillus atrophaeus* # 9372⁽¹⁾, and culture tubes (16 x 100 mm) containing 3.6 to 4.0 mL of sterile proprietary Releasat culture medium. The Releasat medium is specially formulated for rapid outgrowth of *B. atrophaeus* spores that may have survived the dry heat process. Resistance performance of the DriAmp biological indicator has been determined in combination with Releasat culture media. The DriAmp BI meets the USP and ISO 11138 requirements.

STORAGE

The DriAmp BI with Releasat culture medium should be stored at room temperature. Do not dessicate. The DriAmp BI with Releasat culture medium has a 12 month shelf life.

MEDIUM

The Releasat culture medium, consisting of a proprietary formulated soybean casein digest base, provides the spores with a nutrient medium for growth. The culture medium has a pH indicator added to it, which appears as a red-orange color. If viable spores are added, the medium changes to yellow as the acidic metabolic products of the growing bacteria accumulate. If the medium remains red-orange and clear after the inoculated sand is added, no microbial growth occurred, indicating that the spores were killed in the sterilization process. Therefore, if the sterilization process was not effective, the spores will grow and the medium will turn yellow and cloudy. If a media tube shows signs of a visual color change or turbidity prior to use, it should be autoclaved and discarded.

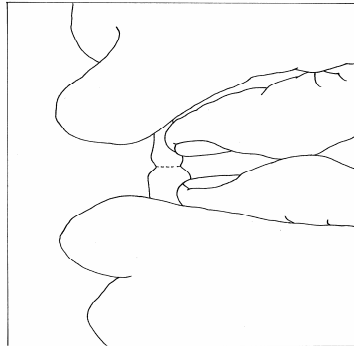
USE

1. Identify the ampoule(s) by labeling pertinent process or load location information.
2. Position the ampoule(s) inside the product or product package and place in the most difficult location to sterilize. Refer to the manufacturer's operating manual for guidelines.
3. Expose the load to the validated sterilization cycle.
4. Following exposure, remove the ampoule(s) and transfer them to the laboratory for culturing.
5. In the laboratory, ampoule(s) should be handled using strict aseptic technique and working in a Class 100 certified workstation.
6. Open each ampoule to culture the sand by holding the top of the ampoule with one hand and the body of the ampoule with the other hand and positioning thumb tips spread away from scored line of the ampoule (see Figure 1).
 - a. Safety goggles should be worn as a precaution.
 - b. The first knuckle of each hand should touch to form a fulcrum with the knuckles acting as a hinge.

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

- c. Apply pressure at the scored glass line.

Figure 1



NOTE: Thumbs placed tip-to-tip can result in laceration.

7. The spore sand from each ampoule should then be poured into individual Releasat medium tubes.
8. Dispose of empty glass. Identify tubes.
9. Any microbiological incubator that is adjusted to $37\pm 1^{\circ}\text{C}$ will satisfy the incubation conditions for the Releasat medium.

NOTE: It is critical that this temperature be maintained to achieve accurate results. The tubes should be placed in the incubator immediately after the sand is cultured. Their placement in an optimized growth environment is necessary to gain accurate results. The medium should be observed for color change at 24 hours, 48 hours, and 72 hours.

INTERPRETATION

The appearance of a yellow color read-out indicates bacterial growth. No color change indicates that the spores were killed in the sterilization process.

Act on a positive test (color change to yellow) as soon as the color change is noted. Color change is to be interpreted as “inadequate sterilization”. Carefully review sterilizer process records to assure that all physical process parameters are within specifications. Always assure that loading configuration and product and package specifications are in agreement with the sterilization validation process. Releasat culture medium may be subcultured if identification of positive growth is desired.

A positive control should be prepared periodically or at least weekly. Many users perform a positive control for each cycle tested. The positive control typically turns yellow within 24 to 48 hours of incubation. As soon as the control turns yellow, it should be appropriately recorded, autoclaved, and discarded. The positive control should not be held longer than necessary because of the possibility of contaminating the work area with organisms that are resistant to sterilization. The control is intended to confirm that viable spores are present on the inoculated sand and the culture medium will support the growth of the test organism prior to testing the sterilizer. Positive controls are not intended to be a “color standard” for comparing test results. SGM recommends that positive controls be incubated for no more than 72 hours.

A positive control that has not grown is a serious problem. Fortunately, the causes are few: a grossly malfunctioning incubator; or inadvertent sterilization of the positive control ampoule.

INCUBATION READ-OUT TIME

The recommended incubation time for the DriAmp BI with Releasat culture medium is 72 hours. SGM Biotech has performed the FDA protocol for determining the incubation read-out time and the data meets the FDA criteria after 72 hours of incubation.

The incubation time of DriAmp BI with Releasat culture medium was validated according to the Center of Devices and Radiological Health, FDA protocol entitled “Guide for Validation of Biological Indicator Incubation Time”. Three lots of DriAmp BIs with three lots of Releasat medium were tested according to SGM’s Standard Operating Procedures for dry heat exposure. For each lot, 100 biological indicator ampoules were exposed to a dry heat cycle that would result in a fractional cycle of 30%-80% positive results. Dry heat exposure conditions were 158° to 162°C. After exposure, sand was removed from the ampoules and transferred to Releasat medium and incubated at 37±1°C for seven days. The tubes that had microbial growth were counted at three and seven days. The results of the tests that were valid according to the FDA protocol (between 30% and 80% of the tubes positive for microbial growth) are shown in Table I.

Table I: Results of DriAmp BI with Releasat Culture Medium Reduced Incubation Time Study

| BI lot Number / Crop Number/ Media lot Number | 72 hours | | 7 days | |
|---|----------------------------------|---------------------------|----------------------------------|---------------------------|
| | Number Positive Units per 100 | Percentage ⁽¹⁾ | Number Positive Units per 100 | Percentage ⁽¹⁾ |
| DA-1RD Batr 073002A RM 000174 | 49 | 100.0 % | 49 | 100.0 % |
| DA-2RD Bsub 010602 PD 0705/1 | 50 | 98.0 % | 51 | 100.0 % |
| DA-3RD Batr 031103 PD 0705/2 | 59 | 100.0 % | 59 | 100.0 % |

⁽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), the number of positive biological indicators at 72 hours as the numerator, and multiplying by 100.

This data shows that the 72 hour incubation time claim was valid (ratio of positives at 72 hours vs. seven days greater than 97%). Seventy-two hour incubation times provide 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

Dry heat D₁₅₀, D₁₆₀ and D₁₇₀-values were performed by fraction negative analysis on three lots of DriAmp BIs, each manufactured with a different *Bacillus atrophaeus* spore crop. A population assay was also performed on each BI lot. Twenty units per exposure were used for the testing at each temperature. Following exposure, sand was removed from the ampoules and cultured into the Releasat medium. Tubes of medium were incubated at 37±1°C for seven days and D-values

were calculated with the 72 hour data using the Limited-Holcomb-Spearman-Karber calculation. The BI resistance data are presented in Tables II-IV.

Table II: Resistance Testing for Lot DA-1RD

| Testing at 150°C | | Testing at 160°C | | Testing at 170°C | |
|-------------------------|---------------------------------|-------------------------|---------------------------------|-------------------------|---------------------------------|
| Exposure Time (min.) | 72 hr read (# negatives per 20) | Exposure Time (min.) | 72 hr read (# negatives per 20) | Exposure time (min.) | 72 hr read (# negatives per 20) |
| 42 | 0 | 16 | 0 | 11 | 0 |
| 50 | 0 | 18 | 0 | 12 | 0 |
| 58 | 1 | 20 | 2 | 13 | 2 |
| 66 | 9 | 22 | 5 | 14 | 9 |
| 74 | 16 | 24 | 2 | 15 | 11 |
| 82 | 18 | 26 | 0 | 16 | 14 |
| 90 | 20 | 28 | 7 | 17 | 17 |
| 98 | 20 | 30 | 7 | 18 | 20 |
| D ₁₅₀ -value | 10.3 min. | 32 | 14 | 19 | 20 |
| | | 34 | 15 | D ₁₇₀ -value | 2.2 min. |
| | | 36 | 18 | | |
| | | 38 | 16 | | |
| | | 40 | 20 | | |
| | | 42 | 20 | | |
| | | D ₁₆₀ -value | 4.6 min. | | |

Table III: Resistance Testing for Lot DA-2RD

| Testing at 150°C | | Testing at 160°C | | Testing at 170°C | |
|-------------------------|---------------------------------|-------------------------|---------------------------------|-------------------------|---------------------------------|
| Exposure Time (min.) | 72 hr read (# negatives per 20) | Exposure Time (min.) | 72 hr read (# negatives per 20) | Exposure time (min.) | 72 hr read (# negatives per 20) |
| 36 | 0 | 16 | 0 | 9 | 0 |
| 42 | 0 | 19 | 0 | 10 | 0 |
| 48 | 1 | 22 | 1 | 11 | 0 |
| 54 | 0 | 25 | 2 | 12 | 1 |
| 60 | 1 | 28 | 8 | 13 | 3 |
| 66 | 3 | 31 | 10 | 14 | 9 |
| 72 | 11 | 34 | 20 | 15 | 13 |
| 78 | 13 | 37 | 20 | 16 | 12 |
| 84 | 18 | D ₁₆₀ -value | 4.4 min. | 17 | 19 |
| 90 | 17 | | | 18 | 17 |
| 96 | 19 | | | 19 | 20 |
| 102 | 20 | | | 20 | 19 |
| 108 | 20 | | | 21 | 20 |
| D ₁₅₀ -value | 11.2 min. | | | 22 | 20 |
| | | | | D ₁₇₀ -value | 2.2 min. |

Table IV: Resistance Testing for Lot DA-3RD

| Testing at 150°C | | Testing at 160°C | | Testing at 170°C | |
|-------------------------|---------------------------------|-------------------------|---------------------------------|-------------------------|---------------------------------|
| Exposure Time (min.) | 72 hr read (# negatives per 20) | Exposure Time (min.) | 72 hr read (# negatives per 20) | Exposure time (min.) | 72 hr read (# negatives per 20) |
| 18 | 0 | 16 | 0 | 7 | 0 |
| 26 | 0 | 19 | 0 | 8 | 0 |
| 34 | 1 | 22 | 2 | 9 | 1 |
| 42 | 0 | 25 | 4 | 10 | 1 |
| 50 | 2 | 28 | 7 | 11 | 1 |
| 58 | 4 | 31 | 10 | 12 | 0 |
| 66 | 4 | 34 | 17 | 13 | 2 |
| 74 | 11 | 37 | 16 | 14 | 4 |
| 82 | 16 | 40 | 18 | 15 | 9 |
| 90 | 15 | 43 | 19 | 16 | 12 |
| 98 | 16 | 46 | 20 | 17 | 15 |
| 106 | 20 | 49 | 20 | 18 | 16 |
| 114 | 20 | D ₁₆₀ -value | 4.6 min. | 19 | 17 |
| D ₁₅₀ -value | 11.2 min. | | | 20 | 20 |
| | | | | 21 | 20 |
| | | | | 22 | 20 |
| | | | | D ₁₇₀ -value | 2.4 min. |

Z-values for each lot of BIs were calculated using log linear regression with the D₁₅₀, D₁₆₀ and D₁₇₀-values. A summary of the performance data tested for each lot is presented in Table V.

Table V: Performance Data Summary for Three DriAmp BI Lots

| | Lot DA-1RD | Lot DA-2RD | Lot DA-3RD |
|-------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| D ₁₅₀ -value | 10.3 min. | 11.2 min. | 11.2 min. |
| D ₁₆₀ -value | 4.6 min. | 4.4 min. | 4.6 min. |
| D ₁₇₀ -value | 2.2 min. | 2.2 min. | 2.4 min. |
| Z-value | 29.9°C | 28.3°C | 29.9°C |
| Spore Population | 2.3 x 10 ⁶ spores/unit | 2.3 x 10 ⁶ spores/unit | 2.4 x 10 ⁶ spores/unit |

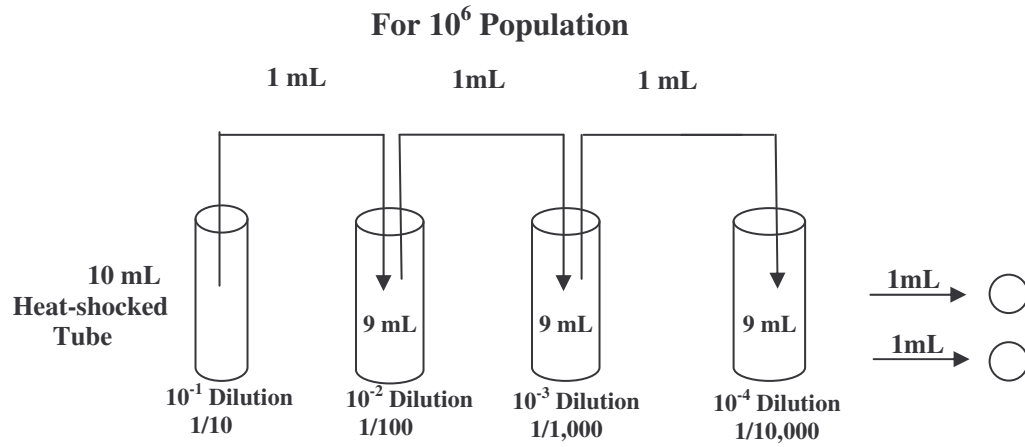
Spores contained in the DriAmp Biological Indicators are traceable to a recognized culture collection and are certified for identity and population. D-values and Z-values are determined using Releasat medium.

POPULATION DETERMINATION

The following procedure has been provided to evaluate the spore population of the sand contained inside the DriAmp Biological Indicator.

NOTE: To avoid inaccurate plate counts, it is important to perform the serial transfers using a 2.0 mL pipette or whichever pipet has the largest bore size. This will help avoid clogging of the pipet tip with sand.

1. Randomly select four ampoules from the lot to be assayed.
2. Remove spore sand from glass ampoule (reference USE Section, Figure 1, for proper removal from ampoule). Place contents of each ampoule in a sterile, screw cap 19.5 x 145 mm, flat-bottom tube with four, 6 mm glass beads, and 10 mL of sterile 0.1% Tween 80 solution.
3. Sonicate 5 minutes using 47 kHz.
4. Vortex 1 minute.
5. Heat shock procedure:
 - 5.1. Place the dilution tube in a preheated bath at 80°-85°C for ten minutes.
 - 5.2. Remove tubes and cool rapidly in ice bath (0° to 4°C).
6. Dilution Series:
 - 6.1 **A dilution series will be made from each tube.** Vortex each heat-shocked tube for at least ten seconds.
 - 6.2 From each tube, transfer a 1 mL aliquot to a dilution tube containing 9 mL of sterile purified water.
 - 6.3 Vortex the dilution tube for at least ten seconds. Transfer 1.0 mL to a second dilution tube containing 9 mL of sterile purified water (**repeat this step one more time for a 10⁶ population**).
 - 6.4 Vortex this tube for at least ten seconds. Pipette 1 mL from this dilution tube into two 15 x 100 mm petri dishes.
7. 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.

8. Invert and incubate plates at 30°-35°C.
9. After 48 hours of incubation, count plates. Preferably plates with counts between 30 and three 300 CFUs should be used, but not less than six per USP.
10. Average counts and then multiply by the dilution factor to calculate population per original unit.
11. Document all information.

CERTIFICATION

DriAmp Biological Indicator with Releasat[®] Culture Medium (includes ampoule containing spore sand and tubes of culture medium) is available as follows:

| | <u>Sets per box</u> | <u>Cat. No.</u> |
|---|---------------------|-----------------|
| DriAmp Biological Indicator with Releasat [®] Culture Medium <i>B. atrophaeus</i> 10 ⁶ spores/unit | 50 | DHR/50 |

DriAmp[™] Dry Heat Biological Indicator Culturing Set With Releasat[®] Medium

For Industrial Use Only

CERTIFICATE OF ANALYSIS

Reorder No. DHR/50

Bacillus atrophaeus 9372⁽¹⁾

For: Dry Heat Sterilization

Culture: 37±1°C. The supplied bacteriological medium will meet requirements for growth promoting ability.

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

Biological Indicator Lot No.: DA-000

Media Lot No.: DM-000

Manufacture Date:

Expiration: 12 months from Manufacture Date.

Minimum Heat Shocked Population: spores per unit

Assayed Resistance:

| | D-value ⁽²⁾ | Survival ⁽³⁾ | Kill ⁽³⁾ |
|----------------|------------------------|-------------------------|---------------------|
| Dry Heat 150°C | minutes | minutes | minutes |
| Dry Heat 160°C | minutes | minutes | minutes |
| Dry Heat 170°C | minutes | minutes | 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.

Units are manufactured in compliance with SGM Biotech's quality standards, and tested according to appropriate subsections of USP and ISO 11138.

⁽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.