DriAmp™

Dry Heat Biological Indicator Culturing
Set with Releasat® Medium

TECHNICAL REPORT

Complies to
USP, ISO 11138,
and all appropriate subsections

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

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 desiccate. 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 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

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

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.

c. Apply pressure at the scored glass line.
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° ± 1°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.

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 ensure that all physical process parameters are within specifications. Always ensure 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 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. Mesa Labs 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; inadvertent sterilization of the positive control BI; or inadvertent sterilization of the box of indicators - due to improper storage.

INCUBATION READ-OUT TIME

The recommended incubation time for the DriAmp BI with Releasat Culture Medium is 72 hours. Mesa Labs 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 Mesa Labs 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

Biological Indicator Lot Number	Exposure Time (Minutes)	# Positive 72 Hours	# Positive 7 Days	Percent Positive[(1)]
DA-1RD BATR-073002A RM-000174	34	49	49	100.0%
DA-2RD BSUB-010602 PD-0705/1	34	50	51	98.0%
DA-3RD BATR-031103 PD-0705/2	34	59	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%). 72 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 D_{150}, D_{160} and D_{170}-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. 20 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 (Minutes)	**72 hour read (# negatives per 20)**	**Exposure Time (Minutes)**	**72 hour read (# negatives per 20)**	**Exposure Time (Minutes)**	**72 hour read (# negatives per 20)**
42	0	16	0	11	0
50	0	18	0	12	0
48	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 minutes**	32	14	19	20
		34	15	**D_{170}-value:**	**2.2 minutes**
		36	18		
		38	16		
		40	20		
		42	20		
		D_{160}-value:	**4.6 minutes**		

Table III
Resistance Testing for Lot DA-2RD

Testing at 150°C		Testing at 160°C		Testing at 170°C	
Exposure Time (Minutes)	**72 hour read (# negatives per 20)**	**Exposure Time (Minutes)**	**72 hour read (# negatives per 20)**	**Exposure Time (Minutes)**	**72 hour 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 minutes**	17	19
90	17			18	17
96	19			19	20
102	20			20	19
108	20			21	20
D_{150}-value:	**11.2 minutes**			22	20
				D_{170}-value:	**2.2 minutes**

Table IV
Resistance Testing for Lot DA-3RD

Testing at 150°C		Testing at 160°C		Testing at 170°C	
Exposure Time (Minutes)	**72 hour read (# negatives per 20)**	**Exposure Time (Minutes)**	**72 hour read (# negatives per 20)**	**Exposure Time (Minutes)**	**72 hour 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 minutes	19	17
D_{150}-value:	11.2 minutes			20	20
				21	20
				22	20
				D_{170}-value:	2.4 minutes

Z-values for each lot of BIs were calculated using log linear regression with the D_{150}, D_{160} and D_{170}-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 minutes	11.2 minutes	11.2 minutes
D_{160}-value	4.6 minutes	4.4 minutes	4.6 minutes
D_{170}-value	2.2 minutes	2.2 minutes	2.4 minutes
Z-value	29.9°C	28.3°C	29.9°C
Spore Population	2.3×10^6 (spores per unit)	2.3×10^6 (spores per unit)	2.4×10^6 (spores per 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

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

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

	Sets per Box	Catalog Number
DriAmp™ Biological Indicator with Releasat® Culture Medium *Bacillus atrophaeus* 10^6 spores/unit	50	DH/50

DriAmp™

Dry Heat Biological Indicator Culturing Set with Releasat® Culture Medium

For Industrial Use Only

CERTIFICATE OF ANALYSIS

Reorder No.: DH/50

Bacillus atrophaeus 9372[1]

Biological Indicator for: Dry Heat Sterilization.

Culture: 36 to 38°C. The supplied bacteriological medium will meet requirements for growth promoting ability.

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

DriAmp Lot No.: **DH-000**

Ampoule Lot No. : DA-000

Media Lot No.: DM-000

Manufacture Date: YEAR MONTH DAY

Expiration Date: YEAR MONTH DAY

Heat Shocked Population: 0.0×10^6 Spores / Unit

Assayed Resistance:	D-Value[2]	Survival	Kill	
Dry Heat 150°C:	0.0	00.0[4]	00.0[4]	
Dry Heat 160°C:	0.0	00.0[3]	00.0[3]	
Dry Heat 170°C:	0.0	00.0[4]	00.0[4]	minutes

Z-value: 00.0°C

D-value reproducible only when exposed in a 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, USP, and ISO 11138 guidelines and all appropriate subsections.

(1) Culture is traceable to a recognized culture collection identified in USP and ISO11138.
(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. Mesa Labs uses a D-value rounded four decimal places in this calculation.
(4) Empirically derived data.

Certified By: _____
Quality Representative

Complete Quality Control testing results available upon request.

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