Releasat®

Biological Indicator
Culturing Set

TECHNICAL REPORT

Complies to
USP, ISO 11138,
and all appropriate subsections

Technical Data and Use of Releasat® Biological Indicator Culturing Set with Medium

Rev.1
TR-005

INTRODUCTION

Releasat® Biological Indicator Culturing Set is used in monitoring the efficacy of ethylene oxide gas and dry heat sterilization cycles. The Releasat Biological Indicator (BI) Culturing Set consists of MesaStrips™ containing spores of *Bacillus atrophaeus* 9372[1], and culture tubes (16 x 100 mm) containing 3.8 ± 0.2 mL of sterile proprietary culture media. The Releasat medium is specially formulated for rapid outgrowth of *B. atrophaeus* spores that may have survived the ethylene oxide gas and dry heat process. Performance of the BI has been determined for the combination of culture medium and spore strips. The MesaStrip used in the culture set meets the USP and ISO 11138 requirements.

STORAGE

The Releasat Biological Indicator Culturing Set should be stored at room temperature. The strips should not be stored near sterilants or other chemicals and have a 12-month shelf life. Do not desiccate.

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 spore strip 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 spore strips by labeling pertinent process or load location information. Position the strip inside the product or product package and place in the most difficult location to sterilize. Refer to the manufacturer's operating manual for guidelines.

2. Place a sufficient number of spore strips throughout the load to be sterilized.
   NOTE: Generally, a minimum of 10 strips is recommended.

3. Expose the load to the validated sterilization cycle.

4. Following exposure and appropriate aeration remove the spore strips and transfer them to the laboratory for culturing.

5. In the laboratory, using strict aseptic technique and working in a Class 100 certified workstation, transfer each spore strip from the glassine package into a tube of Releasat medium.

6. Any microbiological incubator that is adjusted to 37 ± 1 °C will satisfy the incubation conditions for 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 strips are cultured. Their placement in an optimized growth environment is necessary to gain accurate

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

results. The medium should be observed for color change at 24, 48, 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 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 and negative 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 spore strip and the culture medium will support the growth of the test organism prior to testing the sterilizer. Mesa Labs, Bozeman Manufacturing Facility 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 strip; or inadvertent "sterilization" of the entire box of indicators due to improper storage.

INCUBATION READ-OUT TIME

The recommended incubation time for the Releasat medium is 72 hours. Mesa Labs, Bozeman Manufacturing Facility 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 Mesa Labs' Releasat product 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 Releasat medium were prepared accordingto Mesa Labs' Standard Operating Procedures for ethylene oxide and dry heat exposure. For each lot 100 biological indicator strips were exposed to an ethylene oxide BIER cycle or dry heat cycle for the times indicated in Tables 1 and2.. Ethylene oxide exposure conditions were 600 ± 30 mg/L ethylene oxide gas, 54 ± 1 °C, 60 ± 10 % relative humidity. Dry heat exposure conditions were 160 ± 2 °C. The exposed strips were 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 accordingto the FDA protocol (between 30 and 80 % of the tubes positive for microbial growth) are shown in Table 1 and 2 below.

Table 1
Results of the Reduced Incubation Time Study (Ethylene Oxide)

Releasat Lot Number	Exposure Time (Minutes)	Number Positive 72 Hours	Number Positive 7 days	Percent Positive[1]
1	19.5	39	40	97.5 %
2	21.0	50	51	98.0 %
3	18.75	79	80	98.8 %

[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), and the number of positive biological indicators at 72 hours as the numerator.

Table 2
Results of the Reduced Incubation Time Study (Dry Heat)

Releasat Lot Number	Exposure Time (Minutes)	Number Positive 72 Hours	Number Positive 7 days	Percent Positive[1]
4	19.0	73	73	100 %
5	20.0	37	37	100 %
6	18.0	59	59	100 %

[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), and the number of positive biological indicators at 72 hours as the numerator.

This data shows that the 72 hour incubation time claim was valid (ratio of positives at 72 hours vs. 7 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

D-value determination was performed by fraction negative analysis and a population assay was performed on the biological indicators. Ethylene oxide exposure conditions were 600 ± 30 mg/L ethylene oxide gas, 54 ± 1 °C, 60 ± 10 % relative humidity. Dry heat exposure conditions were 160 ± 2 °C. Twenty-five units per exposure were used. Following exposure, samples were cultured in Releasat medium and incubated at 37 ± 1 °C for 72 hours. Ethylene oxide exposure performance data is presented in Table 3. Dry heat exposure performance data is presented in Table 4.

Table 3:
Ethylene Oxide Resistance Performance Data

BI Lot Number	Number Positive Out of Twenty (25) Exposure Times (in minutes)								Population/Unit	D-value[1] (Minutes)
	14	16	18	20	22	24	26	28		
RG-289	25	25	5	6	1	1	0	0	2.7×10^6	2.7
RG-290	25	22	13	3	2	1	1		2.9×10^6	2.7
RG-296	25	25	23	6	4	1	0	0	2.1×10^6	3.0

[1]Calculated according to USP methods.

Table 4:
Dry Heat Resistance Performance Data

BI Lot	Number Positive Out of Twenty (25)	Population/Unit	D-value[1]

Number	Exposure Times (in minutes)											(Minutes)
	5	**6**	**7**	**8**	**9**	**10**	**11**	12	13	14		
RG-288	-	-	25	25	15	16	4	9	0	0	2.4×10^6	1.5
RG-289	25	24	25	23	23	14	3	0	0	0	2.7×10^6	1.5
RG-290	25	25	23	20	18	18	13	5	3	0	2.9×10^6	1.6

(1)Calculated according to USP methods.

POPULATION DETERMINATION

Detailed population assay instructions are available in PDF format on the Mesa Labs website; http://biologicalindicators.mesalabs.com/documents-manuals/

CERTIFICATION

MesaStrip biological indicators are certified for population, D-value and survival/kill. Times are determined using Releasat medium.

Releasat Biological Indicator Culturing Sets (includes MesaStrips and tubes of culture medium) are available as follows:

	Sets per box	Cat. No.
Releasat Biological Indicator Culturing Set *B. atrophaeus* 10^6 spores/strip	100	RG-100

CERTIFICATE OF ANALYSIS

Reorder No: RG/100

Bacillus atrophaeus 9372[1]

Biological Indicator for: Ethylene Oxide/Dry Heat Sterilization

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

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

Releasat Kit Lot No.: **RG-000**

Manufacture Date: YEAR MONTH DAY

Expiration Date: YEAR MONTH DAY

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

Carrier Size: 1" x ¼" (25 mm x 6 mm

Media fill volume: 3.6 ± 0.2 mL

Assayed Resistance:	D-Value[2]	Survival	Kill	
Ethylene Oxide (600 ± 30 mg/l, 60 ± 10 % RH, 54 ± 1 °C) **Oxyfume® 2000**[5]		(3)	(3)	min
Ethylene Oxide (600 ± 30 mg/l, 60 ± 10 % RH, 54 ± 1 °C) **100 % EtO**		(3)	(3)	min
Dry Heat (160 °C) [6]		(4)	(4)	min

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 Mesa Laboratories' 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 a formula in USP and ISO 11138. A D-value rounded to four decimal places is used in this calculation.
[4] Empirically derived data.
[5] Oxyfume 2000 is a registered trademark of Honeywell.
[6] With exception to D_{160}-value specified in ISO 11138-4.

Certified By: _____
Quality Representative

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