

Releasat[®]
Biological Indicator
Culturing Set for Steam Sterilization

TECHNICAL REPORT

Complies to
USP, ISO 11138,
and all appropriate subsections

Technical Data and Use of Releasat[®] for Steam

Rev.1
TR-006

INTRODUCTION

Releasat[®] Biological Indicator Culturing Set for Steam is used in monitoring the efficacy of steam sterilization cycles. The Releasat Biological Indicator (BI) Culturing Set for Steam consists of MesaStrips containing spores of *Geobacillus stearothermophilus*, 7953¹, 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 *G. stearothermophilus* spores that may have survived the steam 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 for Steam should be stored at room temperature. The strips should not be stored near sterilants or other chemicals. Do not desiccate. The Releasat Biological Indicator Culturing Set 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 purple 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 purple and clear after the exposed 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 strip(s) 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 55-60°C will satisfy the incubation conditions for Releasat medium. **NOTE: It is critical that this temperature be maintained to achieve**

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

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 results. The medium should be observed for color change at 24 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 control for each cycle tested. The positive control typically turns yellow within 24 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 strip and the culture medium will support the growth of the test organism prior to testing the sterilizer. Positive controls are not intended to be “color standard” for comparing test results. Mesa Labs recommends that positive controls be incubated for no more than 24 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 strip.

INCUBATION READ-OUT TIME

The recommended incubation time for the Releasat Steam medium is 24 hours. Mesa Labs has performed the FDA protocol for determining the incubation read-out time and the data meets the FDA criteria after 24 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 according to Mesa Labs’ standard operating procedures for steam exposure. For each lot, 100 biological indicator strips were exposed to a steam BIER cycle for the times indicated in Table 1. Steam exposure conditions were as specified in ISO 18472. The exposed strips were transferred to Releasat medium and incubated at 55-60°C for seven days. The tubes that had microbial growth were counted at 24 hours 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 1.

**Table I
Results of the Reduced Incubation Time Study (Steam)**

Releasat Lot Number	Exposure Time (Minutes)	Number Positive 24 Hours	Number Positive 7 days	Percent Positive ⁽¹⁾
1	14.0	46	47	98
2	13.5	79	80	99
3	14.5	43	43	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), the number of positive biological indicators at 24 hours as the numerator, and multiplying by 100.

This data shows that the 24 hour incubation time claim was valid (ratio of positives at 24 hours vs. seven days greater than 97%). 24 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

The D-value calculation was performed using fraction negative data and the Limited-Holcomb-Spearman-Karber calculation. A population assay was performed on the biological indicators. Steam exposure conditions were controlled as specified in ISO 18472. 20 units per exposure were used. Following exposure, samples were incubated at 55-60°C for 24 hours. Steam exposure performance data is presented in Tables II. Z-value data is presented in Table III.

Table II
Steam Resistance Performance Data at 121°C, 124°C, 129°C, and 134°C

121°C									
Lot	Number Positive Out of 20							Population/Unit	D-value⁽¹⁾ (Minutes)
	Exposure Time (in minutes)								
	9.5	10.75	12	13.25	14.5	15.75	17		
1	20	20	19	13	6	1	0	2.3 x 10 ⁶	2.1
2	20	16	11	0	0	--	--	2.2 x 10 ⁵	2.1
3	--	--	20	20	11	1	0	1.2 x 10 ⁶	2.3
124°C									
Lot	Number Positive Out of 20				Population/Unit	D-value⁽¹⁾ (Minutes)			
	Exposure Time (in minutes)								
	4	5	6	7					
1	20	20	3	0	2.3 x 10 ⁶	0.9			
2	20	14	20	0	2.2 x 10 ⁵	1.1			
3	20	20	10	0	1.2 x 10 ⁶	0.9			
129°C									
Lot	Number Positive Out of 20				Population/Unit	D-value⁽¹⁾ (Minutes)			
	Exposure Time (in minutes)								
	1	1.33	1.66	1.99					
1	20	20	1	0	2.3 x 10 ⁶	0.2			
2	20	14	2	0	2.2 x 10 ⁵	0.3			
3	20	20	1	0	1.2 x 10 ⁶	0.2			
134°C									
Lot	Number Positive Out of 20				Population/Unit	D-value⁽¹⁾ (Minutes)			
	Exposure Time (in minutes)								
	0.17	0.34	0.51	0.68					
1	20	20	1	0	2.3 x 10 ⁶	0.1			
2	20	20	4	0	2.2 x 10 ⁵	0.1			
3	20	15	0	0	1.2 x 10 ⁶	0.1			

⁽¹⁾ Calculated according to USP methods.

**Table III
Z-value Data**

Lot	Population	D₁₂₁	D₁₂₄	D₁₂₉	Z-value
1	2.3 x 10 ⁶	2.1	0.9	0.2	7.8°C
2	2.2 x 10 ⁵	2.1	1.1	0.3	9.4°C
3	1.2 x 10 ⁶	2.3	0.9	0.2	7.6°C

POPULATION DETERMINATION

Detailed population assay instructions are available on the company website (www.mesalabs.com)

CERTIFICATE

MesaStrip biological indicators are certified for population, D-value and Survival/Kill confirmation times are determined using Releasat medium

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

	<u>Sets per Box</u>	<u>Reorder Number</u>
<i>G. stearothermophilus</i> (10 ⁵ spores/strip)	100	RS5/100
<i>G. stearothermophilus</i> (10 ⁶ spores/strip)	100	RS6/100

Releasat[®]

BIOLOGICAL INDICATOR CULTURING SET
For Industrial Use Only

CERTIFICATE OF ANALYSIS

Reorder No: RS0/100

Geobacillus stearothermophilus 7953⁽¹⁾

Biological Indicator for: Steam Sterilization

Culture: **Releasat[®]** Media, 55 – 60°C. The supplied bacteriological medium will meet requirements for growth promoting ability.

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

Releasat Steam Lot No.: RS-000

Spore Strip Lot No.: RSS0-000

Media Lot No.: PM-000

Manufacture Date: YEAR MONTH DAY

Expiration Date: 12 months from Manufacture Date

Heat Shocked Population: 0.0 x 10⁰ Spores / Unit

Carrier Size: 1" x ¼" (25 mm x 6 mm)

Assayed Resistance: D-Value⁽²⁾ Survival⁽³⁾ Kill⁽³⁾

Steam 121°C 0.0 00.0⁽³⁾ 00.0⁽³⁾ min

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 Mesa Labs' 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. Mesa Labs uses a D-value rounded to four decimal places in this calculation.

Certified By: _____
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