

MesaStrip for Chlorine Dioxide

Bacillus atrophaeus
Geobacillus stearothermophilus

TECHNICAL REPORT

Complies with:
ISO 11138
and all appropriate subsections.

Technical Data and Use of MesaStrip for Chlorine Dioxide

Rev.1
TR-011

INTRODUCTION

MesaStrip is a biological indicator used in monitoring the efficacy of chlorine dioxide sterilization. MesaStrip contains spores of *Bacillus atrophaeus* 9372¹ or *Geobacillus stearothermophilus* 7953¹ and meets ISO 11138-1 requirements.

STORAGE

MesaStrip should be stored at room temperature. The strips should not be stored near sterilants or other chemicals and have a 24-month shelf life. Do not desiccate.

MEDIUM

MesaStrip can be cultured into Mesa Labs Releasat medium consisting of a proprietary formulated soybean casein digest base, providing 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 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.

Soybean casein digest broth will also provide the spores with a nutrient medium for growth.

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 Tyvek/Mylar package into a tube of medium.
6. If using Releasat medium: Any microbiological incubator that is adjusted to $37^{\circ} \pm 1^{\circ}\text{C}$ will satisfy the incubation conditions using this medium. **NOTE: It is critical that this temperature be maintained to achieve accurate results.** If using TSB: Any microbiological incubator that is adjusted for 30-35°C will satisfy the incubation conditions using this medium. **NOTE: It is important that this temperature be maintained to achieve accurate results.**
7. 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. If using TSB the medium should be observed for growth for no less than seven days. If using Releasat, the medium should be observed for color change at 24 hours and 48 hours.

INTERPRETATION

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

For TSB: The appearance of a cloudy medium or the formation of sediment indicates bacterial growth. Clear medium indicates no growth and that the spores were killed in the sterilization process.

Act on a positive test (color change to yellow or turbidity) as soon as the color change or turbidity is noted. These results are to be interpreted as “inadequate sterilization”. Carefully review sterilizer process records to assure 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. The media 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 or turbid within 24 to 48 hours of incubation. As soon as the control turns yellow or turbid, 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 media will support the growth of the test organism prior to testing the sterilizer. Mesa recommends that positive controls be incubated for no more than 48 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.

A negative control (a tube incubated without a spore strip) tests the medium for contamination. It should show no signs of growth.

INCUBATION READ-OUT TIME

The recommended incubation time for MesaStrip in TSB is no less than seven days.

The recommended incubation time for Releasat medium with MesaStrips in Chlorine Dioxide is 48 hours. Mesa Labs, Inc. has performed the FDA protocol for determining the incubation read-out time and the data meets the FDA criteria after 48 hours of incubation.

The incubation time of Mesa’s Releasat product with MesaStrips in Chlorine Dioxide 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’s Standard Operating Procedures. For each lot 100 biological indicator strips were exposed to a chlorine dioxide BIER cycle for the times indicated in Table 1. Chlorine dioxide exposure conditions were 5.0 mg/L chlorine dioxide gas at ambient temperature, 65-70% relative humidity. The exposed strips were transferred to Releasat medium and incubated at $37^{\circ} \pm 1^{\circ}\text{C}$ for seven days. The tubes that had microbial growth were counted at one, two, 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 below.

Table 1: Results of the Reduced Incubation Time Study (Chlorine Dioxide)

Releasat Lot Number	Exposure Time (Minutes)	# Positive 24 Hours	# Positive 48 Hours	# Positive 7 Days	Percent Positive ⁽¹⁾
1	35.0	68/100	69/100	69/100	100%
2	35.0	59/100	67/100	67/100	100%
3	31.0	25/100	32/100	32/100	100%

⁽¹⁾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 the number of positive biological indicators at 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%). Forty-eight 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. Chlorine dioxide exposure conditions were 5.0 mg/L chlorine dioxide gas at ambient temperature, 65 - 70% relative humidity. Twenty units per exposure were used unless specified. Following exposure, samples were cultured in Releasat media and incubated at 37° ± 1°C for seven days. Chlorine dioxide exposure performance data is presented in Table 3.

Table 3: Chlorine Dioxide Resistance Performance Data

BI Lot Number	Number of Positive Units / Number of Total Units Tested							Population/Unit	D-value ⁽¹⁾ (Minutes)
	Exposure Times (in minutes)								
	20	25	30	35	40	45	50		
1	8/20	3/20	7/20	3/20	1/20	0/20	2/20	2.0 x 10 ⁶	4.7
2	-	-	15/20	14/20	12/20	16/20	9/20	4.4 x 10 ⁶	6.0
3	-	-	1/10	0/10	1/10	2/10	0/10	2.9 x 10 ⁶	5.2

⁽¹⁾Calculated according to USP methods.

*Testing performed in Releasat media

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).

CERTIFICATION

Units are manufactured in compliance with Mesa Laboratories, Bozeman Manufacturing Facility's quality standards, ISO 11138 guidelines and all appropriate subsections.

MESASTRIP

BIOLOGICAL INDICATOR
For Industrial Use Only
CERTIFICATE OF ANALYSIS

Reorder No.:	
Species	Culture ⁽¹⁾
For:	Chlorine dioxide sterilization
Culture:	Soybean casein digest broth.
Purity:	No evidence of contaminants using standard plate count techniques.
Lot No.:	XXX-000
Manufacture Date:	YEAR MONTH DAY
Expiration:	YEAR MONTH DAY
Heat Shocked Population:	0.0 x 10 ⁰ Spores/Unit
Carrier Size:	1/4" x 3/4" (6 x 19 mm)

Units are manufactured in compliance with Mesa Laboratories, Bozeman Manufacturing Facility's quality standards, USP, and ISO 11138 guidelines and all appropriate subsections with the exception of the population.

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

Certified By: _____
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