ETIGAM

TECHNICAL DATASHEET

ETIGAM NSC self-contained biological indicator for Steam

Geobacillus stearothermophilus (Bacillus stearothermophilus)

This technical datasheet provides relevant data and instructions for use of the NSC biological indicator for Steam.

in compliance with: USP, ISO 11138 and all appropriate subsections

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PRODUCT

The NSC biological indicator consists of a self-contained unit that includes bacterial spores of *Geobacillus stearothermophilus* ATCC #7953 inoculated onto a paper filter carrier and a small glass ampoule containing a nutrient broth culture medium containing Bromocresol purple as a pH indicator encased in a plastic vial that serves as the culture tube. Biochemical activity of the *Geobacillus stearothermophilus* organism produces acid by-products that cause the media to change color from purple to yellow. A visual pH color change and/or turbidity indicates a steam sterilization process failure. NSC BI's are conventional spore growth readout biological indicators specifically designed for reliable monitoring of Steam sterilization processes without the use of enzyme based technology or specific and specialized incubators or monitoring devices.

NSC BI's comply with the performance requirements of ANSI/AAMI/ISO 11138-1 and the USP requirements for self-contained biological indicators.

STORAGE

Store at controlled room temperature as defined by USP. USP-controlled room temperature is thermostatically controlled to 20-25°C (68-77°F) while allowing for excursions between 15-30°C (56-86°F). Reference the USP for the complete definition. Protect from light, chemicals and sterilants, excessive heat and moisture. Optimal humidity range for long term storage is 20 to 70%. Do not desiccate.
NSC BI's for steam sterilization processes have a shelf life of 24 months from the date of manufacture.

INSTRUCTIONS FOR USE

Exposure:

1. Record the sterilizer number, load number and processing date on the BI label.
2. Place the BI inside a test pack or area within the package deemed as the most difficult area to achieve sterilization.
3. Test the most challenging area in the sterilizer as indicated in the sterilizer's instruction manual (e.g., the middle of the sterilizer chamber) using an appropriate number of BI's.
4. Process the load according to the sterilizer manufacturer's instructions.
5. Remove the BI and confirm the chemical indicator printed on the label has turned brown/black.

Activation and Incubation:

1. Activate the processed BI following exposure by gently crushing the inner glass media tube using a vial crusher (or with the crushing well in the incubator). Assure that the growth medium has saturated the spore carrier. Prevent over crushing to ensure the culture medium does not come into contact with the filter in the cap. Place the activated biological indicator in the incubator rack or well and incubate within 24 hours.
2. Incubate at 55-60°C for 24 hours checking for spore growth (visual color change from purple to yellow and/or turbidity) at regular intervals (e.g., 6, 12 and 18 hours). Results should be read after 24 hours but no later than 48 hours after incubation.

Test Results:

1. Record negative (no growth) results after full incubation according to your standard operating procedures. No color change in the purple media indicates proper sterilization conditions were achieved.
2. Any positive (growth indicated by purple to yellow color change) result, should be reported immediately to a supervisor and the sterilizer taken out of service until resolved.

Use of Controls:

As a control, an unprocessed BI (from the same lot) should be gently crushed using a vial crusher and incubated each day the sterilizer is tested and in each incubator used. The positive control shall turn yellow within 24 hours of activation and incubation. Once the control turns yellow, it should be recorded and then autoclaved and discarded according to the instructions for use. The control is intended to ensure that viable spores are present on the BI and the incubator performs properly, they are not intended to be used for comparing test results. Incubation of positive controls should be read after 24 hours but no later than 48 hours.

INCUBATION CONDITIONS

A laboratory microbiological incubator that is adjusted to 55-60ºC will satisfy the incubation conditions for the NSC indicator for steam sterilization. To culture the disc in the NSC BI, compress the plastic vial within the crushing well of the incubator, or vial crusher to crush the glass ampoule. Assure that the growth medium has saturated the spore disc. Prevent over crushing to ensure the culture medium does not come into contact with the filter in the cap. Place the activated biological indicator in the incubator rack or well and incubate for 24 hours. Check for spore growth (visual color change from purple to yellow and/or turbidity) at regular intervals. Results should be read after 24 hours but no later than 48 hours.

READ OUT INTERPRETATION

No color change indicates the spores were inactivated and the sterilization process was lethal. The appearance of a yellow color indicates bacterial growth. Any positive (growth indicated by purple to yellow color change and/or turbidity) result, should be reported immediately to a supervisor and the sterilizer taken out of service until resolved. Always retest the sterilizer with additional NSC BI's within the test load. NSC BI's can be sub-cultured to verify organism when desired.

READ OUT TIME

The validated incubation time for the NSC BI Steam is 24 hours. The U.S. FDA biological guidance document was utilized to determine the incubation read out time and the data has demonstrated that it meets the criteria for 24 hours incubation. The procedure followed for reduced incubation time determination is the same as that described in Attachment II of the FDA document entitled "Guidance for Industry and FDA staff, Biological Indicator (BI) Premarket Notification [510(k)] Submissions", issued October 4, 2007. This procedure allows for the reduction in incubation time, to the time which 97% of growth occurs relative to the growth at 7 days, provided 100 BI's are exposed and the 7 day result in the range of 30 to 80 out of 100 are positive for growth.

Three (3) separate lots of NSC biological indicators manufactured with *Geobacillus stearothermophilus* spores of ATCC strain #7953 with differing spore crops and culture media were exposed to steam sterilization 121-132°C and 135°C. Following exposure, the indicators were activated and incubated. The BI's were observed for growth at 24, 48, 72, 96, 120, 144 and 168 hours of incubation. A BI was considered positive for growth upon observation of yellow color change or turbidity within the plastic vial. The results of testing are displayed in Tables 1 and 2.

TABLE 1- REDUCED INCUBATION TIME (RIT) RESULTS 121°C

Lot #	24 hr.	48 hr.	72 hr.	96 hr.	120 hr.	144 hr.	168 hr.	RIT Results[1]
020	51/100	51/100	51/100	52/100	52/100	52/100	52/100	98.1%
044	71/100	71/100	71/100	72/100	72/100	72/100	72/100	98.6%
063	38/100	38/100	38/100	38/100	38/100	38/100	38/100	100%
Totals	160/300	160/300	160/300	162/300	162/300	162/300	162/300	

TABLE 2 - REDUCED INCUBATION TIME (RIT) RESULTS 135°C

Lot #	24 hr.	48 hr.	72 hr.	96 hr.	120 hr.	144 hr.	168 hr.	RIT Results[1]
237	30/100	30/100	30/100	30/100	30/100	30/100	30/100	100%
252	74/100	74/100	74/100	74/100	74/100	74/100	74/100	100%
377	60/100	60/100	60/100	60/100	60/100	60/100	60/100	100%
Totals	164/300	164/300	164/300	164/300	164/300	164/300	164/300	

[1]Each of the lots tested must have 30% to 80% of the indicators surviving (i.e., test positive) after 7 days incubation. Validation is accomplished by demonstrating that the NSC biological indicator when run at partial cycle times will show a 97% or greater level of growth in each partial cycle for the proposed incubation time to be acceptable.

NOTE: *The test is reproducible only under the exact conditions as in which it was determined. The user may not obtain the same result, and therefore the user is responsible for determining the suitability for their particular use.*

RESISTANCE PERFORMANCE TESTING

The D-values were determined using the fraction negative method as described in ANSI/AAMI/ISO 11138-1 biological indicator standard. The results of the D-value are illustrated in Table 3.

TABLE 3 - RESULTS OF D-VALUE RESISTANCE TESTING

Lot #	Labeled			Temp. °C	Labeled		Verified	
	Population	D-value	Z-value		Survival	Kill	Survive	Kill
089	$2.3x10^5$	1.6	15.1	121	5.4	15.0	20/20	0/20
		0.52		132	1.7	4.9	20/20	0/20
		0.15*		134	0.51*	1.4*	20/20	20/20
		0.12*		135	0.40*	1.1*	20/20	20/20
091	$2.1x10^5$	1.6	15.1	121	5.3	14.9	20/20	0/20
		0.52		132	1.7	4.8	20/20	0/20
		0.15*		134	0.50*	1.4*	20/20	20/20
		0.12*		135	0.40*	1.1*	20/20	20/20
096	$2.6x10^5$	1.9	14.2	121	6.5	17.9	20/20	0/20
		0.68		132	2.3	6.4	20/20	0/20
		0.17*		134	0.58*	1.6*	20/20	20/20
		0.12*		135	0.41*	1.1*	20/20	20/20
6580	$1.4x10^6$	2.2	26.0	121	9.2	22.3	20/20	0/20
		0.78		132	3.3	7.9	20/20	0/20
		0.70*		134	3.0*	7.1*	20/20	20/20
		0.64*		135	2.7*	6.4*	20/20	20/20

*Extrapolated or derived from extrapolated data

Each lot tested in evaluating the performance of the response NSC biological indicator to sub-lethal conditions in an AAMI/ISO compliant steam resistometer met their survival requirements by visually indicating growth of the organism.

TABLE 4 - BI SURVIVAL RESPONSE

BI SURVIVAL RESULTS				
Lot	**Temperature Tested**	**Survive Cycle**	**Result[1]**	**Positive Growth Observed**
376	134°C	0.9 min	30/30	3.0 – 4.0 hr
603	135°C	1.1 min	30/30	2.5 – 3.5 hr
604	132°C	1.2 min	30/30	3.0 – 3.5 hr
605	121°C	6.9 min	30/30	4.5 – 5.5 hr

[1]Expressed as the number of samples positive for growth compared with the number of samples exposed.

Survival and kill formulas are:

- **Survival Time** (in minutes) = not less than (labeled D-value) x (log (population) – 2)
- **Kill Time** (in minutes) = not greater than (labeled D-value) x (log (population) + 4)

POPULATION DETERMINATION

Materials:

- Trypticase soy agar
- Sterile petri dishes 15 x 100 mm
- Blender with sterile (sterilized as per J.5.1) stainless steel blender cups VWR 58983-004 or equivalent
- Powerstat Variable Autotransformer, Type 3PN116C
- 13 x 100 mm screw top tubes
- Dilution bottles filled with 100 ml sterile water
- Sterile pipettes
- Water bath
- Sterile tweezers
- Timer
- Incubator
- Colony counter
- Hand tally counter
- Calibrated thermometer

Procedure:

1. To assess the sterility of the dilution water, remove a sample (generally 1.0 ml) from each final dilution bottle and place in a petri dish labeled "water check" (used in step 8). The total volume in the "water check" petri dish should not exceed 5 mls.
2. QS all of the 100 ml dilution bottles using the QS bottle. Note: Dilution bottles for Low Count Strips require 50 ml of sterile water.
3. Gather four biological indicators. Using sterile tweezers, place one biological indicator into a stainless steel blender cup. For low count strips, gather eight biological indicators. Using sterile tweezers, place one biological indicator into a stainless steel blender cup.
4. Refer to the Dilution Table for specific instructions depending on the population of the strip being assayed.
5. When all the tubes have been prepared, heat shock the tubes at the following time and temperature, assuring that the water bath is above the level with the heat shock tube contents. Geobacillus stearothermophilus 95-100°C 15.0 minutes.

Begin timing when the heat shock tube is secured in the water bath. There is a limit of 8 heat shock tubes placed in the bath at one time.

6. Upon removal from the water bath, immediately cool to room temperature in a metal block test tube holder.
7. Label petri dishes with lot number, strip number 1,2,3,4 etc., dilution factor, volume plated and date. Also label a petri dish with "agar check". This petri dish receives no sample and is used to assess the sterility of the agar.
8. Plate 0.5 ml to 1.0 ml in duplicate from each heat shock tube. Add 30-35 ml of trypticase soy agar cooled to 45-50°C to all petri dishes and swirl gently. The petri dishes should include the samples assayed, the "water check" dish, and the "agar check" dish. Note: For strips with a 10^3 population plate 2.5 ml in duplicate.
9. For Low Count Strips, plate 5.0 ml from each heat shock tube to separate petri dishes labeled HS. Thus, at the conclusion of the procedure, 40 petri dishes should be prepared (20 HS, 20 NHS).
10. Swirl the petri dishes to evenly mix the sample. Allow petri dishes to harden.
11. Invert the petri dishes and incubate them at the following temperatures: Geobacillus stearothermophilus 55-60°C.
12. Colonies are counted at 24 and 48 hours. If the 48 hour count is less than 30 or greater than 300 per plate, an adjustment in dilution volume is made and the procedure is repeated until each plate has 30-300 CFU. Contact management in the event more than three colonies are found in the "water check" or "agar check" dish.
13. To calculate a 1/100 population for a 0.5 ml population determination, add the colony counts together to represent 1.0 ml of a sample. Divide by 100 for the population.
14. To calculate a 1/100 population for 10^3 strips (2.5 ml sample), add the colony counts together and divide by 5 to represent 1.0 ml of a sample. Divide by 10 for the population.
15. To calculate the population for low count strips, add the colony counts for the five 5.0 ml samples together. Multiply by two for the number of CFUs/strip.

10 x 6 carrier:

Add 100 ml of sterile distilled water from a dilution bottle to the blender cup. Blend on low speed (Powerstat set at 30 for paper discs, 55 for paper strips) for 3 minutes. Return liquid to dilution bottle and from this, transfer 1.0 ml to a 100 ml final dilution bottle. Shake vigorously. Transfer 1-5 ml of this material to a heat shock tube. This is a 10^{-4} dilution. Wash the blender cup thoroughly with cold tap water. Repeat with the remaining three biological indicators, using one biological indicator per blend. Continue with step 5.