ETIGAM

TECHNICAL DATASHEET

ETIGAM NSS biological indicator spore strips for steam

Geobacillus stearothermophilus (Bacillus stearothermophilus)

This technical report provides relevant data and instructions for use
of the NSS biological indicator for steam

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

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PRODUCT

The NSS biological indicator consists of bacterial spores of *Geobacillus stearothermophilus* ATCC #7953 inoculated onto a paper filter carrier contained within a glassine envelope for use in steam sterilizers. The spore strip is removed from the load and aseptically transferred into appropriate microbiological culture medium. Biochemical activity of the *Geobacillus stearothermophilus* organism produces acid by-products that, when transferred to standard broth, will demonstrate noticeable turbidity and in the case of using a culture media with PH indicator, the culture medium will have a noticeable color change. A visual pH color change and/or turbidity indicates a steam sterilization process failure. NSS 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.
NSS BI's comply with the performance requirements of ANSI/AAMI/ISO 11138-1 and the USP requirements for spore strip 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.
NSS BI's 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 in your record processing note book.
2. Place the NSS BI inside a test pack of similar design to items being sterilized or area within the package deemed as the most difficult area to achieve sterilization. Use a sufficient number of NSS BI's, a minimum of ten (10) is recommended.
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).
4. Process the load according to the sterilizer manufacturer's instructions.
5. Remove the NSS BI and aseptically transfer to appropriate microbiological culture medium.

Activation and Incubation:

1. Transfer each spore strip into a tube containing soybean casein digest broth. 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.
2. Incubate at 55-60°C for 7 days, regularly checking for spore growth (visual color change in medium and/or turbidity). Results should be read no later than 7 days after incubation.

Test Results:

1. Record negative (no growth) results after full incubation according to your standard operating procedures. No visual color change and/or turbidity in the media indicates proper sterilization conditions were achieved.
2. Any positive 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 NSS BI (from the same lot) should be prepared for incubation preferably for each cycle tested. The positive control shall become turbid and/or has a color change within the 7 days of incubation. When the control becomes positive, 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 NSS BI and the incubator performs properly, they are not intended to be used for comparing test results. Incubation of positive controls should be read no later than 7 days.

INCUBATION CONDITIONS

A laboratory microbiological incubator that is adjusted to 55-60°C will satisfy the incubation conditions for the NSS BI for steam sterilization. To culture the NSS BI, aseptically transfer to prepared or purchased medium. This will allow the growth medium to saturate the spore strip. Place the transferred biological indicator in the incubator rack or well and incubate for 7 days. Check for spore growth at regular intervals.

READ OUT INTERPRETATION
The medium should be observed for growth for no less than 7 days.
Clear medium indicates that the spores were killed in the sterilization process.
The appearance of a visual pH color change and/or turbidity indicates bacterial growth (positive).
Any positive result, should be reported immediately to a supervisor and the sterilizer taken out of service until resolved.
Always retest the sterilizer with additional NSS BI's within the test load. NSS BI's can be sub-cultured to verify the target organism when desired.

READ OUT TIME
The incubation time for the NSS BI for steam is 7 days.

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 and Z-values are illustrated in Table 1.

TABLE 1—RESULTS OF D-VALUE and Z-VALUE RESISTANCE TESTING STEAM

Lot	Labeled Population	Temp.	D-Value[1]	Survival/Kill[2]	Z-value (°C)
RU45	1.7×10^6	118.1°C	4.0 min	17.0/40.9 min	8.6
		121.1°C	1.6 min	6.8/16.3 min	
		126.1°C	28 sec	119/286 sec	
RU46	1.6×10^6	118.1°C	6.1 min	25.7/62.2 min	8.2
		121.1°C	2.5 min	10.6/25.5 min	
		126.1°C	38 sec	160/387 sec	
RU48	1.3×10^6	118.1°C	4.9 min	20.2/49.5 min	7.7
		121.1°C	1.7 min	7.0/17.1 min	
		126.1°C	26 sec	107/262 sec	

[1]Determined at the time of manufacture using fraction negative procedures in AAMI/ISO compliant test vessel.
[2]Calculated using USP, AAMI and ISO survival and kill time formulas.

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 ml.

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 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 100ml 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.