ETIGAM

TECHNICAL DATASHEET

ETIGAM NSC self-contained biological indicator for EO Gas

Bacillus atrophaeus (Bacillus subtilis var. niger)

This technical report provides relevant data and instructions for use
of the NSC biological indicator for Ethylene oxide gas.

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 *Bacillus atrophaeus* ATCC #9372 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 *B. atrophaeus* organism produces acid by-products that cause the media to change color from purple to yellow. A visual pH color change and/or turbidity indicate an EO sterilization process failure.
NSC BI's are conventional spore growth read out biological indicators specifically designed for rapid and reliable monitoring of EO Gas sterilization processes without the use of enzyme based technology, 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 a 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 have a shelf life of 18 months after 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 to be 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's and confirm that the chemical indicator printed on the label has turned orange.

Activation and Incubation:

1. Activate the processed BI's 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 48 hours.
2. Incubate at 37 ± 2°C for 48 hours checking for spore growth (visual color change from purple to yellow and/or turbidity) at regular intervals (e.g. 12, 24 and 36 hours). Results should be read at 48 hours after incubation.

Test Results:

1. Record negative (no growth) results after full incubation according to your standard operating procedures. No color change and/or turbidity in the purple media indicates the spores were inactivated and the proper sterilization conditions were achieved.
2. 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.

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 48 hours of activation and incubation. Once the control turns yellow or shows turbidity, it should be recorded and then autoclaved and discarded according to the instructions for use. Not discarding the biological indicator when positives are identified, could potentially contaminate your work area. Positive controls are 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 at 48 hours.

INCUBATION CONDITIONS

A laboratory microbiological incubator that is adjusted to 37 ± 2ºC will satisfy the incubation conditions for the NSC BI's for EO Gas. To culture the strip in the NSC BI, compress the plastic vial by gently crushing the inner glass ampoule using a vial crusher (or with the crushing well in the incubator). Assure that the growth medium has saturated the spore strip. 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 48 hours.
Check for spore growth (visual color change from purple to yellow and/or turbidity) at regular intervals (e.g. 12, 24 and 36 hours). Results should be read at 48 hours after incubation.

Precaution:

The NSC-EO self-contained BI's are designed and validated for a read-out after 48 hours incubation time. In case a verification is required, the BI's may be incubated for 7 days. However, in this case it is necessary to avoid evaporation. Evaporation of more than 15% may give misleading results. It is recommended to incubate at min. 90% RH, and/or use caps to cover the tubes.

READ OUT INTERPRETATION

No color change and/or turbidity indicates the spores were inactivated and the sterilization process was lethal.
The appearance of a yellow color and/or turbidity 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 EO Gas is 48 hours. The FDA biological guidance document was utilized to determine the incubation read out time and the data has demonstrated that it meets the criteria for 48 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 at which 97% of growth occurs relative to the growth at seven (7) days, provided 100 NSC BI's are exposed and the 7 day result in the range of 30 to 80 out of 100 are positive for growth.
Five lots of NSC BI's were exposed to ethylene oxide in an ISO 18472 compliant 100% EO resistometer to times predicted to give 30 to 80 surviving indicators out of 100 exposed samples. The 5 NSC BI lots were comprised of 5 different lots of spore carriers and three different culture media lots. Exposures of ethylene oxide were conducted under the recommended conditions of 600 ± 30 mg/L (100%) ethylene oxide, 54.0 ± 1.0°C, and 60 ± 10% relative humidity. 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. An NSC BI was considered positive for growth upon observation of yellow color change and/or turbidity within the plastic vial. The results of testing are displayed in Table 1.

TABLE 1 - RESULTS OF REDUCED INCUBATION TIME STUDY

Lot	Cycle#	24 hr	48 hr	72 hr	96 hr	120 hr	144 hr	168 hr	%[1]
500	400	47/100	55/100	55/100	55/100	55/100	55/100	55/100	100.00
501	395	55/100	68/100	68/100	68/100	68/100	68/100	68/100	100.00
502	430	*RNT*	65/100	66/100	67/100	67/100	67/100	67/100	97.01
503	453	42/100	54/100	54/100	54/100	54/100	54/100	54/100	100.00
504	385	52/100	66/100	66/100	66/100	67/100	68/100	68/100	97.06

RNT=Reading Not Taken

[1]Acceptance criteria=All test results are in the range of 30 to 80 out of 100 as required by the FDA guidance document and greater than 97% growth is observed at 48 hours of incubation when compared to the 168 hour grow out result.

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 determination are illustrated in Table 2.

TABLE 2 - RESULTS OF D-VALUE RESISTANCE TESTING

Lot Number	D-value	≥ 3.0 minutes
500	3.6 min	yes
501	3.4 min	yes
502	3.5 min	yes
503	3.4 min	yes
504	3.1 min	yes

For each lot the D-value is ≥ 3.0 minutes, therefore this is an acceptable result.
The survival/kill window is typically calculated by the formula found in USP/ISO/AAMI standards. The FDA guidance document recommends a minimum survival time of 15 minutes for ethylene oxide indicators. Depending upon the indicator population and the D-value, the USP/ISO/AAMI formula may result in a calculated survival time that is less than 15 minutes. In this case, the FDA recommended that a minimum of 15 minutes was used in that this presents a greater challenge. 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 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. Bacillus atrophaeus 80-85°C 10.0 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 temperature range:
    Bacillus atrophaeus 35 ± 2°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 that 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.