ETIGAM

TECHNICAL DATA SHEET

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 BI is a self-contained biological indicator for use in monitoring ethylene oxide (EO) gas sterilization cycles. It consists of bacterial spores *Bacillus atrophaeus* ATCC #9372 inoculated onto a paper disc contained within a plastic vial that serves as the culture tube. The plastic vial also contains a small, breakable, glass ampoule with culture media containing Bromocresol purple as a pH indicator. 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.

- NSC-E6: 1.0 to 5.0 x 10^6 spores per indicator
- D values $\geq$ 2.5 minutes, meets current ISO 11138 minimum in 100% ethylene oxide
- meets USP/ISO/AAMI survival and kill specifications:

  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)

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.

INCUBATION:

A laboratory microbiological incubator that is adjusted to 37 +/- 2°C will satisfy the incubation conditions for the NSC BI's for EO Gas. Place the activated biological indicator in the incubator rack or well and incubate for 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 BI's are designed and validated for a read-out after 48 hours incubation time. Contact the supplier for additional instructions for incubation times in excess of 48 hours.

READ OUT INTERPRETATION

No color change (remains purple) and an absence of turbidity indicate the spores were inactivated and the sterilization process was lethal. The appearance of a yellow color and/or turbidity indicates bacterial growth and a sterilization failure. All sterilization failures (growth as indicated by purple to yellow color change or turbidity) 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.

INSTRUCTIONS FOR USE

A. 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 determined 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.

B. Activation and Incubation:

1. Activate the processed BI's following exposure by gently crushing the inner glass media tube using a vial crusher or crushing well within the incubator. Prevent over crushing to ensure the culture medium does not come into contact with the filter in the cap. Assure that the spore disc has been wetted with media prior to incubation.
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.

C. 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.

D. Use of Controls:

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 TIME DETERMINATION

The validated incubation time for the NSC BI is 48 hours. The FDA biological guidance document was utilized to determine the incubation 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 are displayed in Table 1.

TABLE 1: RESULTS OF INCUBATION TIME STUDY

Lot	Cycle#	24 hr	48 hr	72 hr	96 hr	120 hr	144 hr	168 hr	48 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.

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 2.

TABLE 2: D VALUE DETERMINATION

Lot Number	D-value
500	3.6 min
501	3.4 min
502	3.5 min
503	3.4 min
504	3.1 min

- D values for all lots meet the ISO 11138-2 minimum of 2.5 minutes.
- D values for all lots meet the FDA recommended minimum of 3.0 minutes.

The D-values were determined using the fraction negative method as described in ANSI/AAMI/ISO 11138-1 biological indicator standard.

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