

# REDUCED INCUBATION TIME (RIT) ETHYLENE OXIDE (EO) SCBI, SCE-06

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## REDUCED INCUBATION TIME STUDY PER THE CENTER FOR DEVICES AND RADIOLOGICAL HEALTH, FDA GUIDANCE FOR VALIDATION OF BIOLOGICAL INDICATOR INCUBATION TIME FOR NAMSA SELF-CONTAINED BIOLOGICAL INDICATOR (SCBI) FOR MONITORING ETHYLENE OXIDE (EO) STERILIZATION PROCESSES

### SCOPE

The test was based on the methodology outlined in The Center for Devices and Radiological Health, FDA Guidance for Validation of Biological Indicator Incubation Time. The incubation period for biological indicators may be reduced from the standard seven days, if the reduced incubation period is validated adequately. FDA recommends manufacturers validate reduced incubation time using this methodology.

### PURPOSE

The RIT study is performed to determine the minimum incubation period at 30-35°C required to obtain  $\geq 97\%$  correlation to seven (7) day outgrowth of sub-lethally exposed NAMSA SCBIs.

### INTRODUCTION

NAMSA has developed a SCBI for monitoring EO sterilization processes. As a standard seven day incubation time was not desirable for end-users, a Reduced Incubation Time (RIT) study was conducted to provide data to support a label claim with an incubation time less than seven days.

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### MATERIALS

Self-Contained Biological Indicators (SCBIs) with  $10^6$  spores per SCBI (NAMSA Code SCE-06), minimum of 100 from each of three lots (300 total)

Incubator, 30-35°C

### METHODS

Individual groups of 100 SCE-06 NAMSA SCBIs, from each of three different lots, were exposed to partial sterilization cycles using an EO resistometer. The resistometer exposure parameters were  $54^\circ\text{C} \pm 1^\circ\text{C}$ ,  $60 \pm 10\%$  Relative Humidity (RH) and EO concentration of  $600 \pm 30$  mg/L. These exposure times are based on the exposure parameters outlined in ANSI/AAMI/ISO 11138-1:2006 "Sterilization of health care products – Biological indicators – Part 1: General". The resistometer exposure times were selected and adjusted as needed to yield between 30% and 80% viable spore growth for each of the 100 SCBIs exposed. The number of viable SCBIs following each resistometer exposure was determined by crushing the media ampule (allowing the media to contact the spore carrier) and incubating at 30-35°C. The SCBIs were examined for signs of growth (media exhibiting turbidity or yellow color) daily (i.e.,  $24 \pm 2$  hours), for seven days.

Only resistometer cycles that yielded between 30% to 80% SCBI survival were considered valid. The greatest length of incubation time required to obtain  $\geq 97\%$  growth (number of positive SCBIs divided by number of positive SCBIs on the seventh day of incubation) in any one of the valid resistometer cycles will be the minimum length of time required for a reduced incubation time claim.

### INTERPRETATION

The greatest number of hours or days of incubation required to obtain 97% or more positive SCBIs (based on the seven (7) day incubation time) in any one of the partial cycles is the minimum incubation time that will be outlined as a claim on NAMSA SCBIs.

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## RESULTS

The number of positive SCBIs / number of SCBIs tested for each day of incubation for product code SCE-06 is reported in Tables 1-3.

**TABLE 1**

20.0 Minute EO Resistometer Exposure, Cycle 06S-1204-3, SCBI Lot N25616, SCE-06

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
Number of viable SCBIs (SCBIs yielding spore growth)	0	74	75	76	76	76	76
Number Tested	100	100	100	100	100	100	100
Percent Growth (# Positive / # Positive at Day 7)	0	97	99	100	100	100	100

**TABLE 2**

20.0 Minute EO Resistometer Exposure, Cycle 06S-1206-1, SCBI Lot N26002, SCE-06

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
Number of viable SCBIs (SCBIs yielding spore growth)	0	30	30	31	31	31	31
Number Tested	100	100	100	100	100	100	100
Percent Growth (# Positive / # Positive at Day 7)	0	97	99	100	100	100	100

**TABLE 3**

34.0 Minute Steam Resistometer Exposure, Cycle 06S-1206-3, SCBI Lot N25410, SCE-06

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
Number of viable SCBIs (SCBIs yielding spore growth)	0	62	64	64	64	64	64
Number Tested	100	100	100	100	100	100	100
Percent Growth (# Positive / # Positive at Day 7)	0	97	100	100	100	100	100

For additional information:

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## DISCUSSION

This reduced incubation time study was designed to determine the incubation period of sub-lethally injured NAMSA manufactured *Bacillus atrophaeus* SCBIs. The media (modified Tryptic Soy Broth) has been formulated to enhance growth of sub-lethally injured spores and is an integral component of the SCBI. The parameters under which this study was conducted ( $54 \pm 1^\circ\text{C}$ ,  $60 \pm 10\% \text{RH}$ ,  $600 \pm 30 \text{ mg/L}$ ) represent EO exposure conditions required by ANSI/AAMI/ISO 11138-1:2006. Because the mechanism by which spores are sub-lethally injured is the same regardless of the temperature of the EO chamber, the EO concentration of the humidity within the chamber, this reduced incubation time study characterizing the response of NAMSA SCBIs allows for the use of the NAMSA SCBIs in a wide variety of EO sterilization cycles. This study documents how sub-lethally injured spores in NAMSA SCBIs (NAMSA code SCE-06), manufactured using concise standard operating procedures, react when activated and incubated at 30-35°C.

## CONCLUSION

This study was conducted in compliance with Appendix H entitled "The Center for Devices and Radiological Health, FDA Guidance for Validation of Biological Indicator Incubation Time" from the FDA guidance document entitled "Pre-market Notifications [510(k)] for Biological Indicators Intended to Monitor Sterilizers Used in Health Care Facilities; Draft Guidance for Industry and FDA Reviewers (March 1999)". Three lots of SCBIs, each exhibiting between 30% and 80% growth following EO resistometer exposure, resulted in a  $\geq 97\%$  correlation at 48 hours (two days) to results observed at seven days. The results of this study support a minimum reduced incubation period of 48 hours for NAMSA *Bacillus atrophaeus* SCBIs, NAMSA code SCE-06.

## REFERENCES

Appendix H of FDA Guidance Document on Pre-market Notifications [510(k)] for Biological Indicators Intended to Monitor Sterilizers Used in Health Care Facilities; Draft Guidance for Industry and FDA Reviewers, May 21, 2001.

All raw data pertaining to this study and a copy of the final report will be stored in the designated archive files at NAMSA, 6750 Wales Road, Northwood, Ohio 43619-1397 under Lab No. 06G\_53166\_04.

For additional information:

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