Comparison Study of the Growth Promotion Capabilities of Self Contained Biological Indicator Culture Medium in Extended Steam Sterilization Cycles

By Charles Hughes, Gary Socola, Donald Tumminelli, and Mike Nolan

Abstract

Self Contained Biological Indicators (SCBIs) from four different manufacturers were subjected to extended steam sterilization cycles and tested per United States Pharmacopeia (USP) for growth promotion capabilities. Testing consisted of inoculating the processed SCBIs with less than 100 G. stearothermophilus bacterial endospores in order to determine if the culture medium could support growth of a low number of spores after exposure to extended cycle times. All SCBI test samples were processed, activated and incubated per their manufacturers' instructions. This study demonstrates the validity of commercially available SCBIs for use in commonly used extended steam sterilization cycles. Testing was conducted independently by three separate laboratories utilizing different lots of SCBIs in order to statistically verify results.

Introduction

Proper sterilization of instruments and materials is a critical aspect of infection control. For hospital steam sterilizers, biological indicators containing *Geobacillus stearothermophilus* spores offer the highest level of sterility assurance and are recommended to be tested at least weekly, preferably daily and every load with a medical implant.[1-3]

Steam biological indicators are commercially available in paper strip biological indicator (PSBI) and self-contained biological indicator (SCBI) formats. PSBIs are packaged in glassine envelopes and commonly used by device manufacturers and/or independent testing laboratories when validating sterilization cycles. After processing, the PSBI is aseptically transferred into a tube of sterile culture medium and incubated at 55-60 degrees C for one to seven days to observe for turbidity (spore growth). The quality of the culture medium is well documented as being critical to support growth of *G. stearothermophilus* spores that are injured, but not killed during a steam sterilization cycle.[4-9] SCBIs that contain a BI strip or disc and culture medium contained inside a crushable glass ampoule are commonly used by healthcare facilities when verifying steam sterilization cycles. The BI strip or disc and culture medium ampoule are packaged together in a plastic transparent vial with a cap and filter placed on top to seal the SCBI. Openings in the plastic cap allow steam to penetrate and inactivate the spores inoculated on the strip or disc. After processing, the SCBI is activated by breaking the glass ampoule allowing the culture medium to come into contact with the spores. The SCBI is then incubated for 24 or 48 hours at 55-60 degrees C to observe visible spore growth (a yellow color change in the culture medium).

SCBIs with an enzyme-based early readout capability are also available and widely used by healthcare facilities. This type of SCBI utilizes a culture medium that includes a fluorescent agent

Table 1 Extended Cycle Test Results 270º F Pre-vacuum for 6 minutes

Manufacturer	A			B			C			D					
	24 hours			24 hours			24 hours			3 hour			48 hours		
Laboratory	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
SCBI #1	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #2	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #3	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #4	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #5	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #6	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #7	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #8	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #9	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #10	P	P	P	P	P	P	P	P	P	N	P	N	P	P	P
Negative Control	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Positive Control	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P

N = negative for growth
P = positive for growth

Figure 1 Extended Cycle Test Results 270º F Pre-vacuum for 8 minutes

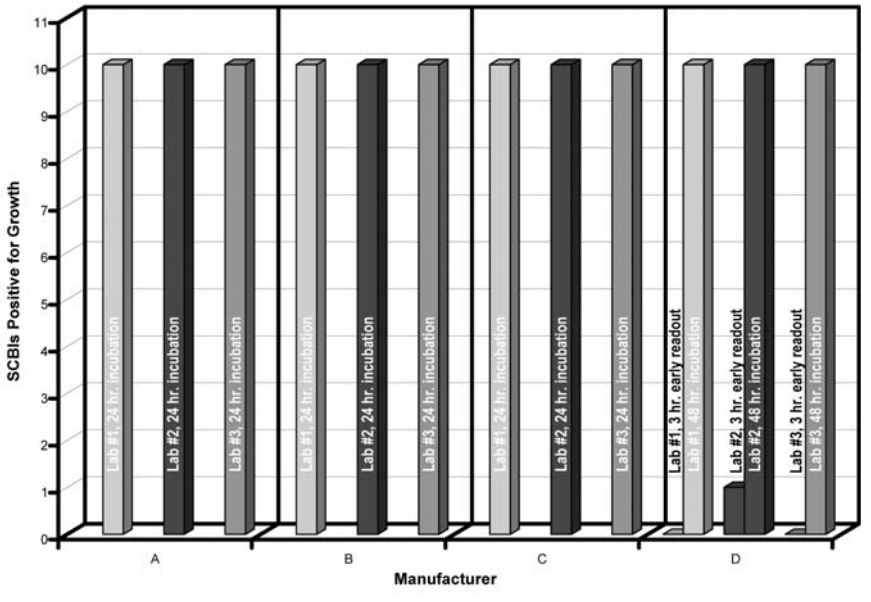
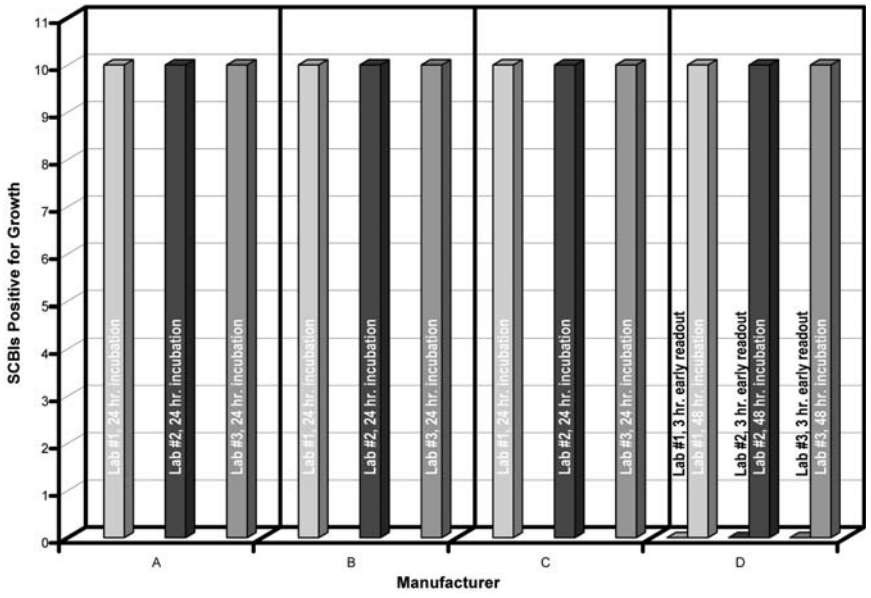


Table 2 Extended Cycle Test Results 270º F Pre-vacuum for 8 minutes

Manufacturer	A			B			C			D					
	24 hours			24 hours			24 hours			3 hour			48 hours		
Laboratory	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
SCBI #1	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #2	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #3	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #4	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #5	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #6	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #7	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #8	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #9	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #10	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
Negative Control	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Positive Control	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P

N = negative for growth
P = positive for growth

Figure 2 Extended Cycle Test Results 270º F Pre-vacuum for 8 minutes



detectable within three hours of incubation when used in conjunction with the manufacturer's electronic reader. The electronic reader is also designed to allow for continued incubation for 48 hours at 60 degrees C in order to observe visible spore growth (a yellow color change in the culture medium). This study details the methods used in evaluating the growth promotion capabilities of the culture medium contained within four commercially available SCBIs processed in extended steam sterilization cycles.

In recent years, steam sterilization cycle times have begun to increase beyond the standard pre-vacuum cycle of 270 degrees F for four minutes exposure time.[10-14] Extended cycles are due primarily to an increase in the weight and/or complexity of instruments, particularly orthopedic sets. Culture medium in SCBIs is typically validated for use at only standard sterilization cycle times. This study was designed to evaluate if commonly used extended cycle times would adversely affect the growth promotion performance of the culture medium contained within the SCBI vials. All SCBI test samples were processed, activated and incubated per their manufacturers' instructions.

Three separate testing laboratories were used in this study, processing four different SCBIs. Three of the four SCBI test samples were incubated for 24 hours in a conventional incubator. The fourth SCBI was incubated in the manufacturer's electronic reader designed to detect a three-hour early fluorescent readout, then allowed to continue incubation for 48 hours at 60 degrees C in order to observe visible spore growth. The SCBI manufacturers are identified as A, B, C and D. The testing laboratories are disclosed herein and identified as 1, 2, and 3 in the accompanying tables and figures.

Justification

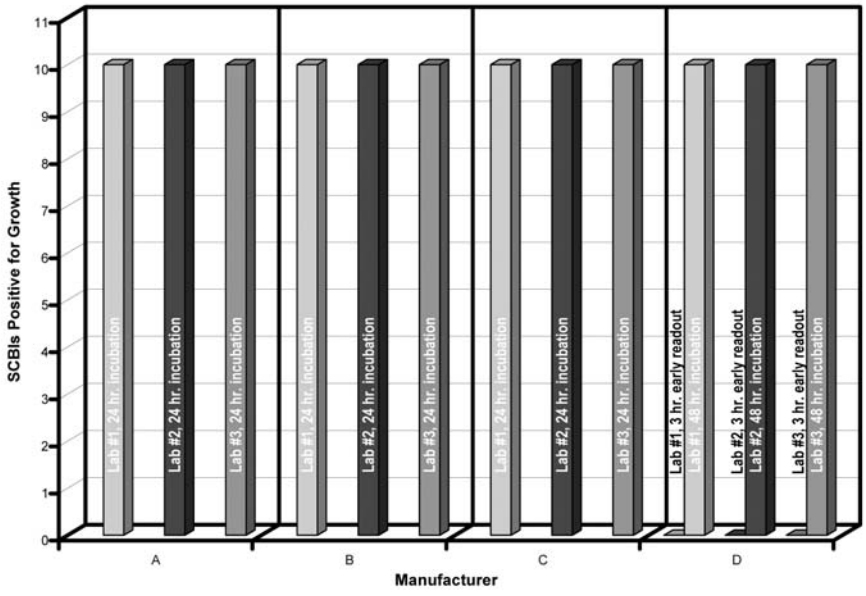
Section 12.4 of the ANSI/AAMI/ISO

Table 3 Extended Cycle Test Results 270º F Pre-vacuum for 10 minutes

Manufacturer	A			B			C			D					
	24 hours			24 hours			24 hours			3 hour			48 hours		
Laboratory	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
SCBI #1	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #2	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #3	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #4	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #5	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #6	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #7	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #8	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #9	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
SCBI #10	P	P	P	P	P	P	P	P	P	N	N	N	P	P	P
Negative Control	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Positive Control	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P

N = negative for growth
P = positive for growth

Figure 3 Extended Cycle Test Results 270º F Pre-vacuum for 10 minutes



14161:2000 standard for biological indicators states, "Selection of a suitable culturing medium requires consideration of many variables, such as pH of the culturing medium and the presence of inhibitory substances such as salts, pH indicators, or antibiotics. Other substances in the culturing medium can affect the recovery of sterilizing agent-stressed test organisms. Users should not overprocess the culture medium, as extended sterilization can induce changes that can affect its growth promoting properties. The ability of the culturing medium to promote the growth of low numbers of microorganisms should be demonstrated."

USP has an established procedure for testing culture medium. This testing requires inoculating the medium with less than 100 spores of a test organism, and then incubating at the appropriate incubation temperature for a specified time. Performing this test assures that the culture medium is capable of supporting growth of low numbers of microorganisms.[15]

Equipment and Materials

Pre-vacuum steam sterilizer
55-60 degrees C laboratory incubator
60 degrees C electronic readers
SCBI activators
Laminar flow hood
Micro-syringe
Perforated instrument tray
Manufacturer "A" SCBI
Manufacturer "B" SCBI
Manufacturer "C" SCBI
Manufacturer "D" SCBI
Disposable sterilization wrappers
Steam indicator tape
Disposable Bowie-Dick test packs
G. stearothermophilus ATCC 7953 spores

Methodology

A Bowie-Dick type test was performed each day in an empty chamber to document proper air removal prior to testing in the pre-vacuum steam sterilizer. Eleven test SCBIs from each lot of the four manufacturers were labeled 1-11 for traceability purposes. All 44 test SCBIs were placed inside a perforated instrument tray, wrapped with disposable sterilization wrap and secured with steam indicator tape. The sterilizer was set for an extended hospital pre-vacuum cycle of 270 degrees F for 6 minutes exposure time, with 20 minutes dry time. The tray was placed on the lowest shelf over the drain and processed. Upon cycle completion, the tray was removed from the sterilizer and allowed to cool for 30 minutes. Upon opening the tray, one processed SCBI from each of the four lots was removed, activated, and incubated as a negative control. Negative controls were expected to show no growth. The remaining 40 SCBIs were placed inside a laminar flow hood and each inoculated with approximately 50 spores from suspension. Following inoculation with viable spores, the SCBIs were activated

and then incubated along with one unprocessed SCBI from each lot. The unprocessed SCBI served as a positive control to verify the spores were indeed viable at time of use and the incubators were functioning properly. Positive controls were expected to show growth.

The inoculated SCBIs from manufacturers A, B, and C were placed inside a 55-60 degrees C laboratory incubator for 24 hours, per their instructions for use. The inoculated SCBIs from Manufacturer D were placed inside a 60 degrees C electronic reader for 48 hours, per their instructions for use. Manufacturer D's SCBI contained spores with an early-readout capability which was observed and recorded. The early readout is signaled by the electronic reader with a red light or (+) sign if fluorescence is detected or a green light or (–) sign if no fluorescence is detected within three hours of incubation. The electronic reader is designed to allow for continued incubation of 48 hours for visible spore growth (a yellow color change in the culture medium). The test procedure was repeated in pre-vacuum steam sterilization cycles of 270 degrees F for eight and 10 minutes of exposure time, with 20 minutes of drying time. All test results were observed and recorded.

Laboratory Identification

Lab No. 1: SPSmedical Supply Corp. (Rush, N.Y.)
Lab No. 2: Apptec Laboratories (Marietta, Ga.)
Lab No. 3: University of Ottawa (Ottawa, Canada)

Results

Results demonstrated that all SCBIs tested did support growth of a low number of spores per the USP recommended growth promotion procedure when incubated for their full incubation periods. Growth was documented by a yellow color change in the culture medium; however, Manufacturer D's SCBI consistently signaled negative -- in the electronic reader for its three-hour early-readout. The exception was a single positive + early readout observed by Lab No. 2 during the six-minute extended cycle test. (See Table 1 and Figure 1).

Discussion

The purpose of this study was to evaluate the affect of extended steam sterilization cycles on the growth promotion of culture medium contained in SCBIs. American and international standards caution users not to overprocess sterile culture medium, as extended sterilization can induce changes that may affect its growth promoting properties. Failure of the culture medium to detect low numbers of viable spores during incubation could lead to false negative results and subsequently, to the release of non-sterile instruments and materials.

In extended cycles, users would expect the *G. stearothermophilus* spores to be deactivated prior to the culture medium being adversely affected; however, this may not always be the case. Incomplete air removal due to tray design, e.g., multiple layers, can cause the items to be exposed to sub-optimum exposure conditions, e.g., dry heat and not saturated steam. Dry heat is considerably less effective as a sterilizing agent compared to saturated steam at the same temperature, and microorganisms could survive under these conditions. Other common causes of incomplete air removal and/or super heated steam, include: clogged or obstructed steam traps; poor steam quality that contains entrapped air; inadequate initial vacuum; radiant heat from the sterilizer jacket; presence of large packs containing absorbent material, rigid container systems or devices with lumens; and processing small loads which tend to concentrate all residual chamber air during evacuation.[16]

Conclusion

Testing conducted independently by three separate laboratories demonstrated that SCBIs are capable of supporting growth of a low number of G. stearothermophilus spores following extended steam sterilization cycles at 270 degrees F pre-vacuum for six, eight, and 10 minutes of exposure time when incubated for their full incubation period of 24 or 48 hours. **ICT**

Charles Hughes, Gary Socola, Donald Tumminelli, and Mike Nolan are with SPSmedical Supply Corp. in Rush, N.Y.

References

1. Association for the Advancement of Medical Instrumentation. Comprehensive Guide to Steam Sterilization in Healthcare Facilities. ANSI/AAMI ST79:2006. Arlington (VA): AAMI, 2006. American National Standard.
2. Association of periOperitive Registered Nurses. Standards, Recommended Practices and Guidelines. Denver: AORN. 2006 Ed.
3. Central Service Technician Manual. Chicago: IAHCSMM. Sixth Edition. 2005.
4. Biological Indicators - Guidance for Selection, use and interpretation of results. ANSI/AAMI/ISO 14161:2000. Arlington, Va.: AAMI, 2000. American National Standard.
5. Boris C and Graham GS. The effect of recovery medium and the methodology on biological indicators. Medical Device and Diagnostic Industry,1985,7,pp.43-48.
6. Busta FF, Foegeding PM , and Adams DM. Injury and resuscitation of germination and outgrowth of bacterial spores. In: Levinson HS, Sonenshein AL, and Tipper DJ, eds. Sporulation and Germination. Proc. Eighth Int. Spore Conference. Washington, D.C., 1981, ASM, pp.261-265.
7. Davis SB, Carls R A, and Gillis JR. Recovery of sublethal sterilization damaged Bacillus spores in various recovery medium, Dev. Ind. Micro.,1976. 18, pp. 427-438.
8. Roberts TA. Recovering spores damaged by heat, ionizing radiation, or ethylene oxide. J. Appl. Bact., 1970, 33, pp. 74-94.
9. Quesnel L B. Biological indicators and the sterilization process. In: Lea & Febiger, Philadelphia, 1991. The revival of injured microbes. London, Academic Press, 1984, pp. 257-284.
10. Canadian Journal of Infection Control. Problems with process monitors for extended steam sterilization cycles. Vol. 21 No. 3, Fall, 2006.
11. Recommendations for Care, Cleaning, Maintenance and Sterilization of Manual Orthopeadic Surgical Instruments. 97-5000-170-00 Rev. 3. Zimmer, Inc. 2006.
12. Sterilization Instructions - Large Bone Instruments. DePuy, A Johnson & Johnson Company. 0902-00-721. Rev. D, Page 2.
13. Sterilization Times: A gamble not worth taking. Healthcare Purchasing News. July 2006.
14. SYNTHES: Developing Sterilization Parameters. March 21-25, 2004. AORN Congress.
15. United States Pharmacopeia 28. Microbiological Test. Section 71.
16. Schneider, PM, Reich RR, Kirckof SS, and Foltz WG. Performance of Various Steam Sterilization Indicators Under Optimum and Sub-Optimum Exposure Conditions. APIC Sponsored Conference. San Antonio, June 12-13, 2003.