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What's News

This newsletter's article completes this discussion about biological indicators (BIs) and is to be read along with the first article in this series about BIs published in this newsletter's February-March, 2008, issue. This article's recommendations, which are provided on p. 16S, are not unique to one processing device or system, but rather apply to any device that uses a liquid chemical sterilant (LCS) and similarly claims to "sterilize" instruments and to rinse them with "sterile" water.

Editor-in-Chief

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What is 'Q-Net'?

Q-Net is a technology-assessment, Internet-based network of questions and answers. Its newsletter is *The Q-Net™ Monthly*.

The main goal of **Q-Net** is to encourage the infection control, endoscopy, and OR communities not only to ask good questions but also to demand well referenced responses.

Q-Net addresses the needs of both the health care provider whose goal is to provide the best care possible and the patient who deserves affordable quality health care.

BIs for monitoring H₂O?

QUESTION: "Can a biological indicator (BI) be used to monitor a water filtration system and verify that the automated processor's 0.2 micron bacterial membrane is producing 'sterile' filtered rinse water"?

■ This article, the second in a series of two, provides recommendations for the proper use of biological indicators (BIs). The first article in this series about BIs was published in this newsletter's February-March, 2008, issue.

■ A position statement on the proper use of BIs is presented.

■ Recommendations are provided to the Food and Drug Administration.

INTRODUCTION: THE FIRST ARTICLE in this series, which was published in the February-March, 2008, issue of this newsletter, discusses the characteristics of biological indicators (BIs) labeled to challenge and monitor the effectiveness of sterilization processes in the healthcare setting. This article, which is the second and final in this series, completes this

Keywords: Biological indicators, "sterile" filtered rinse water, position statements, quality assurance

discussion about BIs.

Specifically, this article responds to a question about whether a BI can be used to monitor the effectiveness of a water filtration system used by an automated processor to "sterilize" a healthcare facility's tap water. Unpublished anecdotal discussions suggest that this question is based on a hitherto unreported practice that may not be uncommon, especially in the operating room setting. (Please review the April, 2008, issue of this newsletter.)

POSITION STATEMENT: THE PROPER USE of a BI to monitor a sterilization process is crucial for the BI to yield valid results and, to be sure, to prevent healthcare-acquired infections (**Box A, B**). Failure by a healthcare facility to monitor (routinely and microbiologically) processes labeled to sterilize surgical instruments or labeled to produce on-site "sterile" water: (a) violates both infection-control and quality-assurance requirements and standards; (b) precludes the requisite assurance that the processes were effective and performing as labeled; and (c) may prevent the prompt identification of a malfunctioning process, the consequences of which may be the reuse of contaminated surgical instruments and an increased risk of disease transmission.

BACKGROUND: BIS CONTAIN A standardized and known number of resistant biological markers, or indicators, known as

(Continued on page 14)

bacterial endospores. Certain species of bacterial endospores are resistant to adverse environmental conditions including extreme heat and, therefore, are ideal for monitoring sterilization processes. Demonstration that a traditional sterilization process destroyed all of a BI's bacterial endospores—indicated by a *negative* (or “no growth”) result—provides a level of assurance (but never a guarantee) that the requisite conditions for the sterilization of instruments, potentially contaminated with a high number of organisms, were achieved.

Biological indicators used to monitor rinse water?

◆ **BACKGROUND:** BIs are routinely used to monitor the effectiveness of sterilization processes in the healthcare setting.

◆ **QUESTION:** “Can a standard type of biological indicator (BI) be used to monitor the effectiveness of a water filtration system that features a 0.2 micron bacterial membrane and is labeled to produce “sterile” filtered rinse water?”

◆ **ANSWER:** No. The principles, design, and labeling of a BI preclude its use for monitoring “sterile” rinse water (Box A).

■ *The destruction of all of a BI's endospores implies successful sterilization. But, using a BI to monitor the effectiveness of a liquid chemical sterilant (LCS)—or the microbial quality of “sterile” rinse water presumably by incubating the BI's spore strip after its immersion in the rinse water, followed by an evaluation of the spore strip for growth—is a dubious practice that is inconsistent with aseptic technique and the well-established principles of BIs (Box A, B).¹*

Because the resistance of different species of bacterial endospores varies and depends on the mode of sterilization, one species of bacterial endospores is not universally used to monitor every type of sterilization process. Rather, each mode of sterilization is monitored using the most resistant species of endospores, to provide the greatest margin of assurance that the conditions for sterilization were achieved. For instance, endospores of *Geobacillus stearothermophilus* are used to monitor steam sterilizers, whereas endospores of *Bacillus atrophaeus* are used to monitor sterilization processes that use ethylene oxide (EtO) gas. (Please review the revised Table 1 on p. 4 of the updated February-March, 2008, issue of this newsletter available on-line at: www.myendosite.com.)

FREQUENCY OF MONITORING: HEALTHCARE STAFF MEMBERS use BIs to monitor sterilization processes daily, weekly, or with each load of instruments, depending on the mode of sterilization and the type of surgical instrument. Guidelines recommend that a BI be used to monitor: (a) a traditional steam sterilization process (not a flash sterilizer) at least *once a week*; (b) *each cycle* of an EtO gas sterilization process; and (c) *each load* of implantable or intravascular devices.²⁻⁴ (Guidelines also recommend, for example, that a sterilization process be monitored after its installation or a major repair.) Review of a medical facility's policies and procedures is

recommended to ensure they provide the proper frequency for monitoring each mode of sterilization. (For the reader's convenience, reference No. 2 provides guidance in the event that a BI yields a *positive*, or “growth,” result.²)

■ *Proper monitoring of a sterilization process using a (legally marketed) BI is necessary to ensure that the process was effective and is performing as labeled. Failure to monitor the process would invalidate its “sterilization” claim and may require healthcare staff members to conclude that the exposed surgical instruments are potentially contaminated.*

STERILITY ASSURANCE LEVEL: ALTHOUGH THE POLICIES and procedures of most healthcare facilities recommend that several additional types of indicators including mechanical and chemical indicators (CIs) be used routinely to monitor a sterilization process, BIs are irreplaceable and a linchpin of every infection-control and quality-assurance program. Unlike CIs—the results of which are limited and may merely indicate

(Continued on page 15)

Box A: The reasons why using a BI to monitor “sterile” filtered rinse water is contraindicated.

- ✓ Removing the spore strip or carrier from the BI's sealed packaging for immersion in rinse water is inconsistent with the well-established principles of BIs, is without precedent, and violates the BI's integrity.
- ✓ No legally marketed BI in the U.S. is labeled to monitor the effectiveness of a water filtration system or the microbial quality of an automated processor's rinse water.
- ✓ Validation and verification data have not been published demonstrating:
 - that a water filtration system can reliably and consistently produce “sterile” filtered rinse water from a healthcare facility's tap water supply (during *worst-case* testing conditions as the FDA requires),^{5,10,11}
 - the proper sampling techniques for on-site microbiological monitoring of “sterile” filtered rinse water;
 - the location within the automated processor (or water filtration system) where the production of “sterile” rinse water is most difficult to achieve and where monitoring the rinse water would be necessary; and
 - the quality-assurance and sterility-testing parameters associated with the “sterile” filtered rinse water produced at this most challenging location.
- ✓ The incubation temperature of 55-60°C—which is used to promote the growth of surviving endospores of *G. stearothermophilus*¹²—inhibits, if not prevents, the growth of waterborne microorganisms for which an incubation temperature of 37°C is recommended, if not necessary.

whether a set of instruments (if only for a few seconds) was exposed to a sterilizing agent—BIs uniquely confirm, by way of a *negative* result, that conditions sufficiently lethal to achieve sterilization were produced, at least at the location within the sterilizer where the BI was placed.

■ *Proper placement of the BI to ensure it is located (and remains) at the specific site(s) within the sterilizer validated by the sterilizer's manufacturer to be the most difficult to achieve the conditions for sterilization is necessary for the BI to yield valid and reproducible results (Box A, B).¹*

Most important, sterilization is an inferred state that is associated with a probability, or sterility assurance level (SAL), which for invasive surgical instruments generally equals 10^{-6} . This very small SAL describes the probability that no more than one resistant endospore on one instrument (or a BI spore strip) would survive exposure of one million (10^{+6}) instruments, each contaminated with one million endospores, to a validated sterilization process. The lower the SAL, the less likely the instrument will be contaminated after sterilization. (Please review the July-August, 1997, issue of this newsletter, which discusses the three “levels” of sterilization.)

BI DESIGNS: BIS FEATURE ONE of a few different designs. The “spore-strip” design includes a strip of paper that is inoculated with viable (alive) bacterial endospores. This type of BI does not contain accompanying growth medium, and, for the BI to yield valid and reproducible results, its spore strip remains sealed in and is not removed from the BI's packaging during exposure to the sterilizing agent. A second type of BI design is “self-contained” and features an inoculated spore strip (or other type of carrier) and growth medium, which is enclosed in a sealed glass ampoule to prevent its premature contact with and germination of the BI's endospores. Another type of “self-contained” BI is enzyme-based and provides results in as few as 3 to 4 hours. (Please review the February-March, 2008, issue of this newsletter for more details about the different types and designs of BIs.)

■ *BIs are uniquely designed so that the sterilizing agent can penetrate their sealed packaging and contact the enclosed viable endospores. This design is necessary for the BI to yield valid and reproducible results. Removing a spore strip (or carrier) from the BI's sealed packaging to monitor the effectiveness of a liquid chemical sterilant (LCS)—or, as suggested by this article's question on page 13, to monitor the microbial quality of rinse water labeled as “sterile”—is inconsistent with the well-established principles of BIs and can cause the BI to yield erroneous results (Box A, B).^{1,5}*

Among other concerns, manually removing a spore strip from the BI's packaging prior to the strip's immersion in a LCS may result in the inadvertent inactivation (or removal) of some of the BI's viable endospores—due to the handling of the BI's spore strip, not effective sterilization—yielding a

Box B: *The reasons why using a BI to monitor a liquid chemical sterilant (LCS) is improper:*

- ✓ Removing the spore strip or carrier from the BI's sealed packaging for immersion in a swirling LCS:
 - is inconsistent with the well-established principles and traditional labeling of BIs, is without precedent, and violates the BI's integrity, which may prevent the BI from yielding valid and reproducible results;^{1,2,5,13}
 - is inconsistent with the methodology of standardized sporicidal tests (i.e., the AOAC sporicidal test);¹
 - may result in the inactivation of some of the spore strip's viable endospores during handling (or, environmental contamination of the spore strip);^{1,6} and
 - can result in the washing- or rinsing-off of unrecoverable viable endospores from the BI's spore strip, yielding a *false-negative* result.¹
- ✓ According to the CDC, the effectiveness of a process that uses a LCS “cannot be verified” with BIs.²
- ✓ According to the FDA, LCSs “cannot be routinely monitored biologically.”¹³
- ✓ Validation and verification data demonstrating where within a processor the conditions for sterilization are most difficult to achieve and, therefore, where specifically to place the BI to monitor the LCS have not been published (monitoring a swirling LCS is especially problematic).¹
- ✓ Data demonstrating that *G. stearothermophilus* is the species of bacterial endospores most resistant to a LCS have not been published.¹

false-negative result and the potential for the release of improperly sterilized surgical instruments for patient use.¹ Similarly, removing a spore strip from the BI's packaging for immersion in a swirling LCS may result in the washing-off (or otherwise dislodging) of some of the BI's viable endospores—a scenario that would prevent the recovery and incubation of surviving endospores, possibly also yielding a false-negative BI result.¹

Further, handling and manually removing a spore strip from the BI's packaging may result in environmental contamination of the spore strip,⁶ yielding a false-positive result, the consequence of which may be to quarantine surgical instruments despite effective sterilization.

INCUBATION TEMPERATURE, TIME: AFTER ITS EXPOSURE to a completed sterilization process, the BI's spore strip is cultured and incubated in a growth medium at a temperature that promotes the germination of any endospores that might have survived due to ineffective sterilization. Displayed in the revised Table 1 on page 4 of the updated February-March, 2008, issue of this newsletter (accessible on-line), BIs that

(Continued on page 16)

contain *G. stearothermophilus* are incubated at 55–60° C (for as long as 7 days). For comparison, the recommended incubation temperature to promote the growth of waterborne bacteria—namely, gram-negative bacteria (e.g., *Pseudomonas* spp.) and atypical mycobacteria—is 37° C (for 1 to 2 days).

DISCUSSION: INFECTION CONTROL GUIDELINES require that processes labeled to achieve sterilization in the healthcare setting be microbiologically monitored as part of a complete quality assurance program.²⁻⁴ Failure to comply with these guidelines calls into doubt the effectiveness of the sterilization process and raises the specter that surgical instruments used on patients might not have been properly sterilized, posing an increased risk of healthcare-acquired infections.

Which may cause a quandary for healthcare facilities, some of which use a liquid-based processor that is labeled to achieve “sterilization” and produce “sterile” filtered rinse water. Although quality assurance standards require that (in addition to the LCS) the microbial (and endotoxin) level of this (or any) processor’s rinse water labeled as “sterile” be monitored—*microbiologic* (not pressure) monitoring of the rinse water is necessary to verify that the 0.2 micron bacterial membrane is producing “sterile” rinse water as labeled and is not failing, allowing bacteria to pass, and requiring replacement—no BIs marketed in the U.S. are labeled for this intended use (**Box A**). So, understanding that failure to monitor the filtered water to assure its “sterility” raises the possibility that the rinsed instruments are contaminated and pose an infection risk, what’s a healthcare facility to do?

The answer to this question is unclear (although the manufacturer may provide guidance.⁷) Resolving this quandary, however, is important to public health and to the reduction of the risk of healthcare-acquired infections. Only if this automated processor’s filtered rinse water were monitored and found to be contaminated with microorganisms might some healthcare facilities dry the wet instruments after reprocessing (using 70% alcohol and forced air). Clinical use of wet instruments poses an increased risk of healthcare-acquired infections and patient morbidity and mortality.⁸

Ostensibly to resolve this quandary, unpublished anecdotal reports and this newsletter’s posed question suggest that some healthcare facilities are confused and may be misusing BIs to monitor the microbial quality of filtered rinse water, to ensure it is “sterile” as labeled. This misuse of a BI may be advanced, in part, by confusing language used to describe the “intended use” of BIs labeled for monitoring a LCS-based processor (for example, refer to 510[k] clearances: K960570, K060568, K062269). As if also (erroneously) to suggest, possibly, that using a BI to monitor filtered rinse water may be a valid practice, a manufacturer report states: “if the tap water filtration had not been effective, there would have been organisms other than the ones on the test devices detected.”⁹

Using a BI’s spore strip (or another type of carrier or “test device”) to monitor the effectiveness of a water filtration process, however, is an unsound and unprecedented practice

(**Box A**). And, any claim or 510(k) clearance intimating that a BI’s *negative* result indicates (in addition to the LCS being effective) that the processor’s filtered rinse water is “sterile” is in error, if for no other reason than that the specified incubation temperature of the BI’s spore strip (inoculated with endospores of *G. stearothermophilus*) is 55–60° C (**Box A**)—a temperature range that *inhibits*, if not prevents, the growth of waterborne bacteria (for which a much lower incubation temperature of 37° C is recommended).

■ *Incubation at an improper temperature can prevent the growth of surviving microorganisms and yield a false-negative result. For example, incubation at 55–60° C of a sample of contaminated rinse water (or a BI’s spore strip) would inhibit the growth of waterborne bacteria.*

CONCLUSION: BIS ARE INTEGRAL components of every infection-control and quality-assurance program. Their proper use is crucial to prevent contaminated surgical instruments from transmitting disease. Using a BI’s spore strip to monitor the microbial (and endotoxin) level of a processor’s rinse water labeled as “sterile,” therefore, is contraindicated (**Box A**). Similarly, the use of a BI’s spore strip to monitor the effectiveness of a LCS is not recommended (**Box B**).^{1,2,13} (the FDA-clearance of a BI for monitoring a LCS notwithstanding). ♦ LFM ➔ *Important—Article continued, see box, below.*

➔ **Several RECOMMENDATIONS for the proper use of BIs and this article’s REFERENCES are provided on the enclosed PAGE 16S, which is also available at:**

www.myendosite.com/htmlsite/2008/p16s.pdf

Thank you for your interest in this newsletter. *I have addressed each issue and topic to the best of my ability.* Respectfully, *Lawrence F. Muscarella, Ph.D.* Please direct all correspondence to:

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(Continued from page 16)

RECOMMENDATIONS: CONTAMINATED RINSE WATER used to reprocess flexible endoscopes has been causally linked to patient morbidity and mortality. Assurance that the rinse water used during endoscope reprocessing is not contaminated with microorganisms, therefore, is important to prevent healthcare-acquired infections. In response to a question about whether a BI can be used to monitor the “sterility” of rinse water and provide this assurance, the following recommendations are provided, to clarify the proper use of BIs. Review of a medical facility’s policies and procedures to ensure compliance with these recommendations is advised.

Recommendations #1, #2: Do not use a traditional BI’s spore strip to monitor the effectiveness of a water filtration system or the microbial quality (and endotoxin level) of rinse water labeled as “sterile.” Similarly, the use of a BI’s spore strip to monitor the effectiveness of a LCS, whether the LCS is stagnant or circulating in an automated processor’s basin, is not recommended.^{1,2,13} Review **Box A** (p. 14) and **Box B** (p. 15) (as well as this newsletter’s February-March, 2008, issue).

Recommendations #3, #4: Review the BI’s labeling. Use the BI containing the most resistant species of endospores to monitor each mode of sterilization as frequently as guidelines require (using a BI to monitor a LCS, however, is not recommended;^{1,2,13} see above).²⁻⁴ For instance, use *G. stearothermophilus* to monitor traditional steam sterilizers at least once a week. Similarly, use chemical indicators (CIs) as frequently as guidelines recommend (e.g., on the outside of every load).

Recommendation #5: Because infections associated with implants can be insidious and result in significant morbidity, use a BI to monitor *each* load of implantable or intravascular devices.²⁻⁴ Flash sterilization of implants is not recommended (refer to this newsletter’s September-October, 2007, issue).

Recommendations #6, #7: Use a *positive-control* as often as described in the BI’s labeling (e.g., with every *test* BI). Incubate both the *test* BI and the *positive-control* at the recommended temperature (e.g., 55-60° C) and for the required number of days (e.g., 7 days). Verify that the *positive-control*’s endospores have germinated. (Refer to the revised Table 1 of the updated February-March, 2008, issue of this newsletter, which is available on-line.) References No. 2 and No. 14 provide guidance in the event that a *test* BI yields a *positive* result.^{2,14} Also, confirm that the shelf-life of the both the *test* BI and the *positive-control* (including the growth medium) has not expired invalidating their results.

Recommendation #8: In accordance with infection-control guidelines and the principles of BIs (as well as the principles of process validation, hazard analysis, and *worst-case* testing conditions), place the BI at the location inside the sterilizer that is *most* difficult to sterilize—for example, the sterilizer’s

“cold spot” or the center of the load.⁵ Refer to the manufacturer of the sterilizer for this specific location.

Recommendation #9: As part of a complete infection-control and quality-assurance program, microbiological monitoring of “sterile” (or “bacteria-free”) filtered rinse water used by automated processors is recommended, to evaluate whether the water filtration system’s 0.2 (or 0.1) micron bacterial membrane is failing, allowing bacteria to pass, and warranting replacement.¹⁴ (Do not, however, use a BI for this purpose; see above. Nor are pressure measurements a validated method for monitoring the microbiological effectiveness of a processor’s bacterial filter.¹⁵) Contact the manufacturer of the processor for the proper technique to sample the rinse water and verify its “sterility.” Assume to be potentially contaminated any instrument that is exposed to a mode of sterilization—or, rinsed terminally by an processor’s water labeled as “sterile” (or “bacteria-free”)—that is not monitored microbiologically. Apply to monitoring the rinse water used by a processor the same techniques and principles employed to monitor the microbial quality (and endotoxin levels) of rinse water used to reprocess hemodialyzers.¹⁵ In addition, a review of microbiological textbooks or handbooks for proper techniques to sample rinse water is suggested.

Recommendation #10: Thoroughly dry instruments wet with rinse water after each completed reprocessing cycle. Perform this step using 70% alcohol followed by forced air (and aseptically wiping the instruments with a dry towel, if necessary). (Please review the April, 2008, issue of this newsletter—which discusses “sterile” filtered rinse water.)

Final recommendations: The FDA is requested to: (a) re-evaluate for scientific validity the 510(k) clearances of all currently marketed BIs labeled to monitor the effectiveness of a LCS;^{1,2,13} (b) review the intended use of these clearances to ensure it is clear and cannot be misinterpreted to suggest that a BI (or kit) can be used to monitor the microbial quality of “sterile” filtered rinse water; and (c) publish guidance on the proper sampling techniques for monitoring the rinse water used by automated processors. Also, because of their inherent limitations,^{1,2,13,16} the FDA is requested to consider re-labeling LCSs (i.e., high-level disinfectants/sterilants) to specify the immersion temperature and time necessary to be “100% sporicidal”—*not* to achieve “sterilization,” which is a misleading claim that the labeling of almost all LCSs, including 2% glutaraldehyde, currently display. Review the November-December, 2001, issue of this newsletter for more details about this proposed label change.¹⁷ Using a LCS to achieve “sterilization” is controversial and has been frequently questioned by this newsletter’s editor. ♦ LFM *The End*

➔ This article’s REFERENCES are provided at:
www.myendosite.com/htmlsite/2008/refs070808.pdf