For: *Infection Control, Operating Room, Endoscopy and Reprocessing Staff, Risk Managers, et al.*

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What's News

In February (2008) at least 6 cases of patient-to-patient transmission of the hepatitis C virus (HCV) were linked to a GI endoscopy center. Officials determined that the reuse of both syringes and single-dose vials of medicine was to blame. *Implementation of the recommendations provided in the following 4 issues of this newsletter will prevent the transmission of HCV during the administration of IV medications*: ● **March 2002**; ● **May-June 2003**; ● **July 2003**; and ● **December 2007**.

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.

Biological Indicators (BIs)

Question: ***"Can you explain the different types of biological indicators (BIs) used to monitor sterilization processes?"***

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Introduction: A BIOLOGICAL STERILIZATION process indicator, or "BI," may be defined as a microbiological test system that provides a defined resistance to a specified sterilization process.[1] More simply, BIs are (class 2) medical devices used by healthcare staff members to challenge and monitor sterilization processes. The periodic use of a BI to monitor a sterilization process and verify its effectiveness is crucial to prevent surgical instruments from transmitting disease.

BIs are also ordinarily used by manufacturers: to understand and characterize a sterilization process; to establish the duration of its exposure phase; and to validate the performance and effectiveness of its complete cycle prior to its clearance and marketing in the U.S.

BIs contain a standardized number of viable (alive), if dormant, structures known as bacterial endospores. Primarily formed by bacteria in the *Bacillus* and *Clostridium* genera, some species of bacterial endospores are ideally suited for monitoring sterilization processes. First, these durable structures are more resistant—that is, they can survive exposure to adverse conditions, such as extreme heat, for a longer period of time—than any other type of microorganism encountered in the clinical setting, including viruses, fungi, and the vegetative cells of bacteria (but not necessarily prions). Not all species of endospores are resistant to sterilization processes, however. The endospores of *Clostridium difficile,* for example, are readily destroyed by high-level disinfection. (Refer to the *November 2007* issue of this newsletter.)

Second, the number of resistant bacterial endospores that a BI contains is reported to be higher than the number of less resistant microorganisms that may remain on a pre-cleaned surgical instrument prior to sterilization.[2] As a result, a sterilization process that has been validated to destroy all of a BI's resistant bacterial endospores (within a specified period of time) provides a level of assurance, with some restrictions, that any microorganisms remaining on a pre-cleaned surgical instrument will also be destroyed, rendering the instrument safe for patient use.

A single species of bacterial endospores is not universally used to monitor all modes of sterilization, in part because the resistance of different species of endospores varies and depends on the mode of sterilization. To ensure that the BI poses the most formidable biologi-

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Keywords: Biological indicators, aseptic technique, spore-strip and self-contained designs, chemical indicators, sterility assurance level

cal challenge possible and reliably indicates whether the conditions for sterilization were achieved, guidelines specify that the BI contain the species of endospores most resistant to the mode of sterilization. (Some BIs contain two species of endospores and may be used to monitor two modes of sterilization.) Two species of bacterial endospores—*Geobacillus stearothermophilus* and *Bacillus atrophaeus*—used to monitor four different modes of sterilization are listed in Table 1.

A *negative*, *positive* BI result: DEMONSTRATION THAT AT least one of the endospores contained in a BI exposed to a sterilization process survived and grew, or germinated, is known as a "*positive*" result and, if reproducible, indicates sterilization failure. And, demonstration that none of a BI's endospores survived exposure to a sterilization process is known as a "*negative"* result and generally verifies that the conditions within the sterilizer were sufficiently lethal to achieve sterilization at the site where the BI was placed. (Whenever a BI yields a *positive* result, guidelines recommend that the sterilization process be inspected for improper operation, its cycle parameters checked, and the process monitored again using a BI.[3] In general, if the result of the second BI is *negative*, the sterilization process can be returned to service.[4])

But, a BI's *negative* result does *not* prove or guarantee the sterility of the processed load (as some guidelines may suggest). A BI can yield a *negative* result despite processed instruments remaining contaminated due to, among other factors, inadequate pre-cleaning of the instruments, overloading the sterilizer with too many instruments, and improper placement of the BI inside the sterilizer. Confirmation that a processed instrument was successfully sterilized would require aseptically sampling all of its internal and external surfaces for all types of microorganisms (and viruses and prions)—a procedure that is impractical, time-consuming, expensive, and would result in re-contamination of the instrument. In lieu of sampling all of its surfaces, the sterility of the instrument is instead inferred and described by the probability that the instrument remained contaminated after exposure to a validated sterilization process. ➡ That the sterility of an instrument (or of a sterile field) can never be guaranteed, but rather is inferred and described by a probability, is one of the most important principles of sterilization and aseptic technique.

What is a "sterility assurance level"? THE EFFECTIVENESS OF a sterilization process is commonly described by a *sterility assurance level* (SAL). The lower the SAL, the lower the risk of infection and the more statistically improbable the instrument will remain contaminated after exposure to a sterilization process. To minimize the risk of infection, surgical

> A BI's *negative* result does not guarantee that the processed instrument is sterile.

instruments, such as biopsy forceps, are associated with a SAL of 10^{-6}, whereas a higher SAL of 10^{-3} might be permissible for some other types of items, such as those that only contact the skin and, therefore, pose a lower risk of infection if contaminated at the time of use. A SAL of 10^{-6} establishes the likelihood that no more than one resistant endospore, from an initial population of one million, may survive exposure to a validated sterilization process's *half-cycle* (i.e., a period of time equal to one-half of the process's exposure phase).

A sterilization process validated to achieve a SAL of 10^{-6} in 10 minutes, for example, would be required to destroy within 5 minutes (a half-cycle) all but one endospore on an item, such as a BI, inoculated with one million endospores. Similarly, no more than one endospore on one item would be permitted to survive the exposure of one million items, each contaminated with one million endospores, to this sterilization process's 10-minute exposure phase.

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Species of Bacterial Endospores	Sterilization Mode	Growth Temperature	Incubation Time	Gram Stain
Geobacillus stearothermophilus	Pressurized steam (moist heat)	55° – 60° C	Up to 7 days	Gram positive
Bacillus atrophaeus	Dry heat, ethylene oxide gas (EtO)	30° – 35° C	Up to 7 days	Gram positive
Geobacillus stearothermophilus	Hydrogen peroxide gas plasma	55° – 60° C	Up to 7 days	Gram positive
(Pseudomonas aeruginosa) *	Not applicable	37° C	1 – 2 days	Gram negative
(Mycobacterium chelonae) *	Not applicable	37° C	10 or more days	Gram negative

Table 1. ***The species of bacterial endospores contained in a biological indicator (BI) to monitor different modes of sterilization, and its growth temperature, incubation time, and gram stain***. Incubation times for BIs whose results are based on enzyme activity are not listed. (* *Pseudomonas aeruginosa* and *M. chelonae* are not spore-forming bacteria; both are waterborne microorganisms listed in this table for the purpose of reference.)

A *test* BI, *positive-control* BI: THE BI USED to monitor a sterilization process is referred to as the *test* BI. This BI's result may be evaluated for germination along with a second BI known as a *positive-control*. These two BIs are treated identically, subjected to the same conditions and handling techniques, and taken from the same lot (with the same manufacturing date). But, while the *test* BI *is* exposed to the sterilization process, the *positive-control* is *not*.

The purpose of the *positive-control* is to confirm, by observing the germination of its endospores, that failure of any of the *test* BI's endospores to germinate is due to the effectiveness of the sterilization process (a valid *negative* result). If the *positive-control*'s endospores fail to germinate, however, the *test* BI's result is invalid. Factors that may cause a *positive-control*'s endospores not to germinate include: expiration of the lot of BIs from which the *positive-control* (and *test* BI) was taken; an incubation temperature that does not support germination of the BI's endospores; or inactive endospores.

Chemical indicators (CIs): IN ADDITION TO BIs, healthcare personnel may use chemical indicators (CIs) and mechanical indicators to monitor sterilization processes. Examples of CIs include process indicators and chemical integrators, whereas thermometers, timers, and pressure gauges are examples of mechanical indicators.[5] CIs are used to indicate whether a load of instruments was exposed to one or more of a sterilization process's physical parameters. But, CIs do not indicate whether the conditions for sterilization were achieved. Moreover, the result of a CI is not always reliable. Especially if the conditions achieved by a sterilization process are marginally inadequate, a CI may undergo a chemical or physical change, such as changing color, despite ineffective sterilization (i.e., a *false-positive* result). For these reasons, CIs can be used to supplement—*but not to replace*—BIs. Together, the use of BIs and CIs is an important component of a comprehensive infection-control and sterilization quality assurance program.

Other characteristics of a BI: SEVERAL OF THE characteristics of a BI are listed in Box A. Whereas a CI may be used with each load of instruments, the frequency with which a BI is used to monitor a sterilization process depends on the clinical setting and the mode of sterilization (and type of surgical instrument). While a BI may be used only once a week to monitor a steam sterilizer in a dental setting, a BI may be used with each load of instruments to monitor an ethylene oxide (EtO) gas sterilizer in a hospital setting. BIs also may be used after installation (or relocation) of, or a major repair to, a sterilization process, to validate its proper operation; or to monitor each load of implantable or intravascular devices. Proper placement of the BI (and test pack) at the site(s) within the sterilizer validated by the sterilizer's manufacturer to be the most difficult to access and to achieve sterilization is required for the BI to yield reliable and meaningful results.[3]

The design of BIs—the *spore-strip* design: NOT ALL BIS are alike, and their designs vary, although each contains a carrier and a standardized (and known) number of one (or two) species of bacterial endospores. (A BI contains no other types of microorganisms other than its endospores.) BIs typically feature a *spore-strip* design or a *self-contained* design.

BIs of the *spore-strip* design include a strip of paper that is inoculated with a suspension of dried endospores and enclosed within a sealed container, such as a glassine envelope. (Although sealed, the BI's packaging permits the sterilant to enter the BI and contact the enclosed resistant endospores.) The BI remains within its sealed packaging during exposure to the sterilization process: to maintain the integrity of both the BI and its results, to maintain the viability of its endospores, and to prevent environmental contamination of the BI's inoculated carrier. If any of the BI's endospores are inactivated prior to sterilization, or become dislodged from the carrier during handling and are not recovered, then the BI may yield a *false-negative* result, possibly causing un-sterile instruments to be released for patient use. Similarly, depending on the incubation temperature (Table 1), the BI might yield a *false-positive* result if its carrier were to become contaminated with environmental bacteria, such as waterborne

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Box A: ***Characteristics of a biological indicator (BI)***

- ✓ Used by healthcare facilities to verify periodically the effectiveness of a sterilization process (Table 1).
- ✓ Used by manufacturers to validate a sterilization process and establish a sterility assurance level (SAL) of 10^{-6}.
- ✓ Contains the species of endospores most resistant to the mode of sterilization (Table 1).
- ✓ Usually gram-positive (Table 1).
- ✓ Provides a defined resistance to the sterilization process.
- ✓ Demonstrates whether the conditions produced by a process were sufficiently lethal to achieve sterilization.
- ✓ Contains a standardized and known number of endospores inoculated on to a carrier in a sealed package.
- ✓ May be used after installation of, or a major repair to, a sterilization process, to validate its proper operation.
- ✓ Features a spore-strip design or a self-contained design (and may also be based on enzyme activity).
- ✓ Located at the site within the sterilizer where the conditions for sterilization are most difficult to achieve.
- ✓ May require 7 days to yield results (Table 1).
- ✓ May be used weekly, daily, or with each load to monitor a sterilization process.
- ✓ Used to monitor each load of implantable devices.
- ✓ May contain the culture medium (and a pH indicator or dye) to promote germination of surviving endospores.
- ✓ May contain a fluorescent product to monitor the activity of an enzyme associated with endospore germination.
- ✗ (Does not prove or guarantee the sterility of the processed instruments.)

gram-negative bacilli,[6] possibly causing instruments to be quarantined despite their successful sterilization.

After exposure of a BI of this *spore-strip* design to the sterilization process, aseptic technique is employed to open the sealed packaging and transfer the carrier into the culture medium, which provides nutrients that support the rapid germination of surviving endospores. (*Note*: *Aseptic technique* is not a perfunctory or causal practice; rather, it requires that specific, though oft-overlooked, procedures, such as opening the sealed BI and transferring its spore strip into the culture medium in a *Class 100* [ISO class 5] certified area, be performed under controlled conditions, to prevent environmental contamination of the BI, resulting in *false-positive* results.[7-12])

The carrier and culture medium are then promptly incubated for up to 7 days at the specific temperature indicated on the BI's labeling, to promote the growth of surviving endospores (Table 1). Germination of at least one of the BI's surviving endospores is displayed by the culture medium, which may contain a pH indicator, becoming cloudy or changing color. This *positive* result indicates that sterilization may not have been achieved (whereas a *negative* result implies that the sterilization process was effective).

The *self-contained* design: A SECOND TYPE of BI features the *self-contained* design and, like the *spore-strip* design, contains a carrier (e.g., a strip of paper, a disc) inoculated with endospores. Unlike spore-strip BIs, however, self-contained BIs include a sealed glass ampoule in which the culture medium is contained to prevent its contact with and the premature germination of the BI's endospores. The spore strip and ampoule (and the culture medium) are packaged together in a container, or tube, that is capped with a lid that permits penetration of the sterilant while preventing environmental contamination of the BI.

After exposure to the sterilization process, the BI is "activated" by crushing its glass ampoule, causing the BI's spore strip to contact the culture medium, which contains a pH indicator. Similar to a *spore-strip* BI, the carrier and the culture medium of a *self-contained* BI are promptly incubated for up to 7 days at the specific temperature indicated on the BI's labeling (Table 1). A visual change in the color of the culture medium indicates germination of surviving endospores (i.e., a *positive* result) due to ineffective sterilization. This self-contained design eliminates the potential for environmental contamination to which BIs of the spore-strip design are prone during the transfer of the spore strip from its envelope or packaging into the culture medium.

An enzyme-based "dual" BI: STERILIZATION PROCESSES may be monitored using yet another type of BI. This *self-contained* BI features a dry spore strip inoculated with endospores and a culture medium that, like other *self-contained* BIs, contains a pH indicator and is sealed in a crushable glass ampoule. Unlike other types of *self-contained* BIs, however, this BI's culture medium also contains a non-fluorescent substrate. After exposure to the sterilization process, this BI's ampoule is promptly crushed and incubated. A visual change in the color of this BI's culture medium after a few days typically indicates germination of surviving endospores due to failure of the sterilization process.[13]

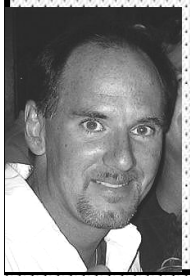
In addition to yielding results in a few days, this "dual" BI provides a more timely result for the rapid evaluation of sterilization effectiveness. The germination of endospores is associated with enzymes that mediate metabolic processes, and this BI employs a design that detects the activity of one of these enzymes. During germination of this BI's endospores due to sterilization ineffectiveness, this enzyme, α-D-glucosidase, is active and converts the non-fluorescent substrate contained in the BI's culture medium into a fluorescent substrate.[13,14] An electronic "reader," which is also used to incubate the BI, quickly evaluates the effectiveness of the sterilization process by detecting whether this fluorescent substrate has been produced—that is, whether this enzyme is active as a result of germination of this BI's endospores. (If the sterilization process is effective, this BI's endospores and this enzyme are inactive, and this fluorescent substrate is not produced.) This type of enzyme-based BI provides results in a few as 3 to 4 hours and may be used to monitor the effectiveness of steam and EtO gas sterilization processes. ● LFM

(Note: This discussion will continue in a future issue.)

> The sterility of an instrument can never be guaranteed, but rather is inferred.

References available on request.

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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