BIOLOGICAL INDICATORS IN MODERN STERILIZATION PRACTICE

Rapid Readout Technology, Sterility Assurance,
and Practical Applications of Terragene Self-Contained Systems

Product Service Team

Terragene S.A.

2026

For academic purposes and professional development

ABSTRACT

Biological indicators (BIs) are indispensable tools for verifying the efficacy of sterilization processes in healthcare and industrial settings. The evolution of self-contained biological indicator (SCBI) systems has enabled faster, more reliable, and more standardized monitoring of sterilization cycles. This work examines the scientific principles underpinning biological indicator technology, with particular emphasis on rapid readout systems, the demonstration of a Sterility Assurance Level (SAL) of 10^{-6}, and practical applications in routine sterilization monitoring. Throughout, Terragene Bionova® SCBI product lines, including models with readout times as short as 7 seconds for steam and 5 minutes for H_2O_2 sterilization, are presented as state-of-the-art examples of rapid readout technology. The work draws upon FDA guidance, ISO 11138 series standards, USP pharmacopeial chapters, and peer-reviewed literature to contextualize the advantages of validated reduced incubation time (RIT) systems for modern sterilization assurance.

1. INTRODUCTION

Sterilization is a validated process used to render a product free of all forms of viable microorganisms, including bacterial endospores (AAMI/ISO TIR 11139:2006). The effective performance of sterilizers used in healthcare facilities and manufacturing environments is critical for preventing nosocomial infections and ensuring patient safety. Within this framework, biological indicators serve as the most direct and definitive method for evaluating the lethality delivered by a sterilization process.

A biological indicator is defined as a microbiological test system providing a defined resistance to a specified sterilization process (AAMI, 2006). Unlike chemical indicators, which respond to one or more physical or chemical parameters of the process, BIs measure the actual microbiocidal effect by challenging the sterilization cycle with a known population of highly resistant bacterial spores. The survival or inactivation of these organisms provides direct evidence of whether the sterilization conditions were sufficient to achieve the desired level of microbial kill.

The evolution from simple spore strips to self-contained biological indicator (SCBI) systems has represented a major advancement in sterilization monitoring technology. Modern SCBIs such as those manufactured by Terragene S.A. integrate the spore-laden carrier and a specially formulated growth medium within a single sealed unit, providing well-defined test limits, controlled recovery conditions, easy result interpretation, and validated reduced incubation times (RIT). Terragene has further advanced this technology with rapid fluorescence-based indicators capable of delivering confirmed biological results in as little as 7 seconds.

This paper provides a comprehensive review of BI technology with emphasis on three areas of particular relevance to sterilization practitioners: (a) rapid readout capability enabled by Terragene SCBI design and fluorescence detection technology; (b) the scientific basis for demonstrating a Sterility Assurance Level (SAL) of 10^{-6}; and (c) practical examples of how Terragene biological indicators support and confirm sterility assurance across diverse sterilization modalities.

2. SCIENTIFIC FOUNDATION OF BIOLOGICAL INDICATORS

2.1 Spore Biology and Selection of Indicator Organisms

Bacterial endospores are the cornerstone of biological indicator technology. Sporulation is a survival strategy triggered by nutrient deprivation, resulting in a dormant cell form that exhibits extraordinary resistance to environmental stresses including heat, chemical agents, and radiation. This resistance, combined with the ability to remain stable during prolonged storage and to germinate reliably when favorable conditions are restored, makes bacterial spores ideally suited for sterilization process monitoring (Ridgeway, 2013).

The species most widely employed in BI manufacture belong to the genera Bacillus, Geobacillus, and Clostridium, all Gram-positive, rod-shaped bacteria. For established sterilization processes, international standards (ISO 11138 series) and pharmacopeial references (USP) recommend the following organism–process pairings:

Table 1. Recommended indicator organisms by sterilization process

Sterilization Process	Indicator Organism	Terragene Bionova® Products
Steam (moist heat)	*Geobacillus stearothermophilus*	BT225, BT224, BT222
Ethylene Oxide (EO)	*Bacillus atrophaeus*	BT110
Dry Heat	*Bacillus atrophaeus*	BT30
Hydrogen Peroxide (H_2O_2)	*Geobacillus stearothermophilus*	BT98, BT96
Formaldehyde (LTSF)	*Geobacillus stearothermophilus*	BT102

Source: Adapted from FDA CDRH Guidance (2007), ISO 11138 series, and USP <1035>.

Geobacillus stearothermophilus is the predominant organism for steam and hydrogen peroxide sterilization monitoring. It is a thermophilic organism with an optimal growth temperature range of 55–60°C. For steam BIs conforming to ISO 11138-3, the minimum D-value must be ≥ 1.5 minutes with a Z-value ≥ 6°C. *Bacillus atrophaeus*, a mesophilic organism (optimal 30–39°C), is the reference species for EO and dry heat sterilization, with minimum D-values specified by ISO 11138-2 and ISO 11138-4 respectively.

2.2 D-value, Z-value, and the Survivor Curve

The resistance characteristics of a biological indicator are quantified through several interrelated parameters. The D-value (decimal reduction value) is the time or dose required to achieve a 90% reduction (1-log) in the viable spore population under stated exposure conditions. The Z-value, applicable to thermal processes, represents the temperature change corresponding to a 10-fold change in D-value. Together, these parameters define the survivor curve, a semi-logarithmic plot of viable spore count versus exposure time that, under ideal conditions, follows first-order inactivation kinetics.

These parameters are not merely academic; they are the mathematical foundation for calculating the Sterility Assurance Level and for designing sterilization cycles that provide the required margin

of safety. The FDA recommends that D-values be determined on final finished product using a resistometer conforming to ANSI/AAMI standards, and that testing be conducted on at least three different spore lots prepared from different spore crops (FDA CDRH, 2007).

3. RAPID READOUT TECHNOLOGY IN TERRAGENE SCBIs

3.1 The Self-Contained Biological Indicator Advantage

Self-contained biological indicators represent a fundamental design advancement over traditional spore strip systems. In an SCBI, the spore-laden carrier and a proprietary growth medium are enclosed within a single hermetic unit. After sterilization exposure, the user activates the unit (typically by crushing an internal ampoule to release the medium), and incubates it at the validated temperature. This closed-system design eliminates the risk of post-exposure environmental contamination, standardizes recovery conditions, and ensures that every user evaluates the BI under the same controlled parameters that the manufacturer validated.

Terragene Bionova® SCBIs incorporate pH-sensitive chromogenic indicators in the growth medium for conventional readout models, and advanced enzyme-based fluorescence detection in the rapid florescence product lines. During incubation, metabolic activity of surviving spores produces either acid byproducts triggering a visible color change, or fluorescent enzyme substrates detectable by the Bionova® auto-reader. The fluorescence-based approach provides an unambiguous positive/negative result with dramatically faster turnaround, making the system ideally suited for high-throughput central sterile supply departments (CSSDs) and pharmaceutical manufacturing environments.

The new 7-seconds SCBI (Bionova® BT225) from Terragene is different. This BI operates by exploiting the structural properties of spore proteins. The spores used in the indicator contain highly stable proteins with specific conformations that are resistant to sterilization processes. When exposed to effective sterilization conditions, these protein structures undergo denaturation and irreversible conformational changes. This loss of native structure directly correlates with spore inactivation, and the BT225 detects this transition as a proxy for lethality, providing a rapid and reliable indication that the sterilization process has achieved the required microbial kill level.

3.2 Reduced Incubation Time: Principles and Validation

One of the most clinically and operationally significant attributes of modern SCBIs is their validated reduced incubation time (RIT). While the conventional incubation period for biological indicators is 7 days (as referenced in ISO 11138-1, Section 7.3), SCBI manufacturers may validate shorter incubation periods provided that rigorous criteria are met.

The FDA CDRH guidance document “Biological Indicator (BI) Premarket Notification [510(k)] Submissions” (2007) establishes the methodology for validating reduced incubation times. The protocol requires exposure of a minimum of 300 BIs (100 from each of three different manufacturing lots) to partial sterilization cycles that produce between 30% and 80% spore survival. All BIs are incubated for the full 7-day reference period, with daily scoring. The validated

RIT is the greatest number of incubation days required to achieve ≥ 97% of the total 7-day positive count in any single lot. Critically, results are not averaged across lots, the most conservative lot defines the minimum incubation time.

This methodology ensures that even under worst-case conditions, when only a single spore survives a near-lethal process exposure, the validated incubation time is sufficient for that last survivor to germinate, multiply, and produce a detectable positive signal within the SCBI system.

3.3 Terragene Bionova® Rapid Readout Portfolio

Terragene has developed a comprehensive portfolio of Bionova® self-contained biological indicators with validated readout times that span a remarkably wide range, from a leading 7 seconds for steam sterilization to 4 hours for ethylene oxide. This spectrum of readout speeds allows sterilization professionals to select the product best suited to their operational tempo, process type, and regulatory requirements.

The Terragene Bionova® rapid product line leverages advanced enzyme-based fluorescence detection technology. Unlike conventional colorimetric SCBIs that rely on pH-driven color change from spore metabolism, which inherently requires hours of incubation for sufficient acid production, fluorescence-based systems detect the enzymatic activity or protein structure within seconds to minutes of incubation. This approach indirectly identifies metabolic viability at a much earlier stage, before visible turbidity or pH change occurs, enabling dramatically faster results without compromising sensitivity or specificity.

Table 2 presents the complete Terragene Bionova® rapid readout portfolio across all major sterilization modalities:

Table 2. Terragene Bionova® SCBI portfolio: validated readout times by product and sterilization process

Product Code	Sterilization Process	Validated Readout	Detection	Conv. Time
BT225	Steam (moist heat 132-135 ºC)	**7 seconds**	Fluorescence	7 days
BT224	Steam (moist heat 132-135 ºC)	**20 minutes**	Fluorescence	7 days
BT222	Steam (moist heat 121-135 ºC)	**1 hour**	Fluorescence	7 days
BT98	Hydrogen Peroxide (H_2O_2)	**5 minutes**	Fluorescence	7 days
BT96	Hydrogen Peroxide (H_2O_2)	**30 minutes**	Fluorescence	7 days
BT110	Ethylene Oxide (EO)	**4 hours**	Fluorescence	7 days
BT102	Formaldehyde (LTSF)	**2 hours**	Fluorescence	7 days

Note: All readout times validated per FDA CDRH 510(k) guidance methodology. Fluorescence detection requires use of the corresponding Bionova® auto-reader.

3.4 Clinical and Operational Significance of Rapid Readout

The BT225, with its 7-second readout for steam sterilization, represents the fastest biological indicator result available in the industry. This near-instantaneous feedback fundamentally transforms the workflow in high-throughput central sterile supply departments: rather than holding processed loads for hours or days awaiting BI results, facilities can obtain definitive biological confirmation of sterilization efficacy within seconds of incubation initiation. The BT224 (20-minute readout) and BT222 (1-hour readout) offer intermediate options that balance speed with cost considerations for facilities with varying operational demands.

For hydrogen peroxide sterilization, an increasingly critical modality for heat-sensitive devices, the BT98 provides a confirmed biological result in just 5 minutes. The BT96, with a 30-minute readout, offers an alternative for applications where a slightly longer window is acceptable.

The EO sterilization indicator BT110 delivers results in 4 hours, a dramatic reduction from the conventional 48-hour or 7-day incubation, enabling faster release of EO-sterilized loads. The formaldehyde indicator BT102 provides a 2-hour readout for LTSF processes, supporting rapid turnaround in facilities using low-temperature steam and formaldehyde sterilization.

As described by McCauley (2009), the speed of positive signal detection in a conventional SCBI is directly related to the number of surviving spores, an unexposed control BI with a full spore population typically shows positive growth within 3–4 hours using colorimetric methods. Terragene's fluorescence-based detection technology compresses this timeline by orders of magnitude, identifying enzymatic markers of spore viability at concentrations far below the threshold required for visible color change. This is the technological basis enabling the rapid readout times shown in Table 2.

For healthcare facilities, the practical impact is transformative: with the BT225, sterilization loads can be biologically confirmed within seconds of processing. Even the longer-readout products in the portfolio enable same-day release. This dramatically improves instrument availability, reduces inventory requirements, accelerates surgical scheduling, and eliminates the operational burden of managing recall protocols for loads released before conventional BI results are available.

4. STERILITY ASSURANCE LEVEL (SAL) OF 10^{-6}

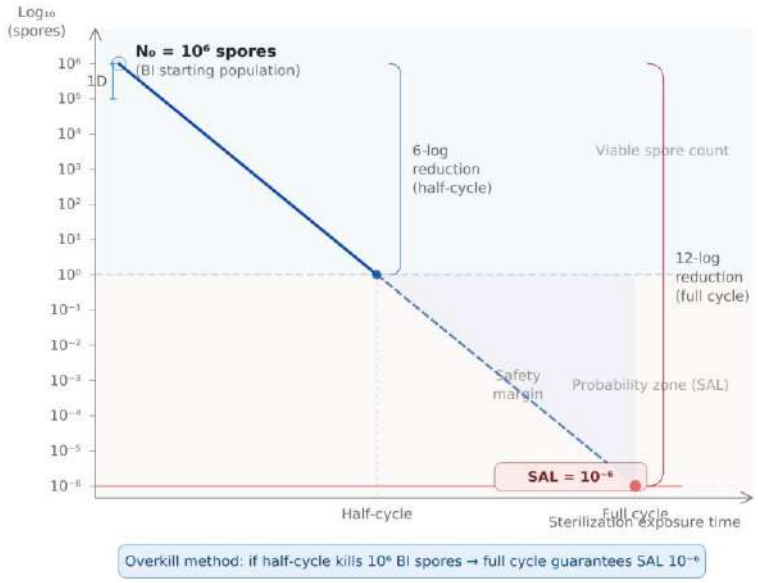
4.1 Definition and Regulatory Basis

The Sterility Assurance Level (SAL) is defined as the probability of a single viable microorganism occurring on a product after sterilization (AAMI/ISO TIR 11139:2006). For terminally sterilized medical devices and pharmaceutical products, the internationally accepted target is an SAL of 10^{-6}, meaning a probability of no more than one non-sterile unit per million units processed.

The SAL is calculated from the initial spore population (N_0) and the spore log reduction (SLR) delivered by the process. For a BI with a population of 10^6 spores exposed to a sterilization cycle designed to deliver a 12-log reduction (the classic overkill approach), the resulting SAL is 10^{-6}. This calculation depends critically on two assumptions: (1) that the D-value and Z-value of the

challenge organism are accurately characterized, and (2) that the BI is placed at the location of minimum lethality within the sterilizer load.

Figure 1. The Sterility Assurance Level and the overkill approach



4.2 The Overkill Method and BI Population Requirements

The overkill approach to sterilization validation is the most widely used strategy in healthcare and manufacturing. It employs a BI with a known spore population—typically 10^5 to 10^6 CFU of an organism far more resistant than any expected bioburden, and demonstrates that the half-cycle (50% of the full exposure time) is sufficient to achieve complete kill of all BIs. The full cycle then delivers at least double the lethality, providing a substantial safety margin.

The FDA recommends minimum spore populations and D-values for cleared BIs. For steam at 121°C, the recommended minimum is 10^5 spores with a D-value of ≥ 1.5 minutes and survival time of ≥ 5 minutes. For EO (600 mg/L, 54°C, 60% RH), the minimum is 10^6 spores with a D-value of ≥ 2.5 minutes. These specifications ensure that the BI provides a meaningful and conservative challenge to the sterilization process.

Terragene Bionova® SCBIs, including the rapid models BT225, BT224, BT98, BT110, and BT102 are manufactured with spore populations and resistance characteristics that meet or exceed these regulatory benchmarks. Each production lot undergoes ISO11138-based characterization on final finished product, with D-value, Z-value, survival/kill window, and spore population verified across multiple spore crops before lot release. The rapid readout time does not compromise the resistance challenge: the same spore population and resistance profile that ensures a valid SAL demonstration is present in both conventional and rapid Bionova® models.

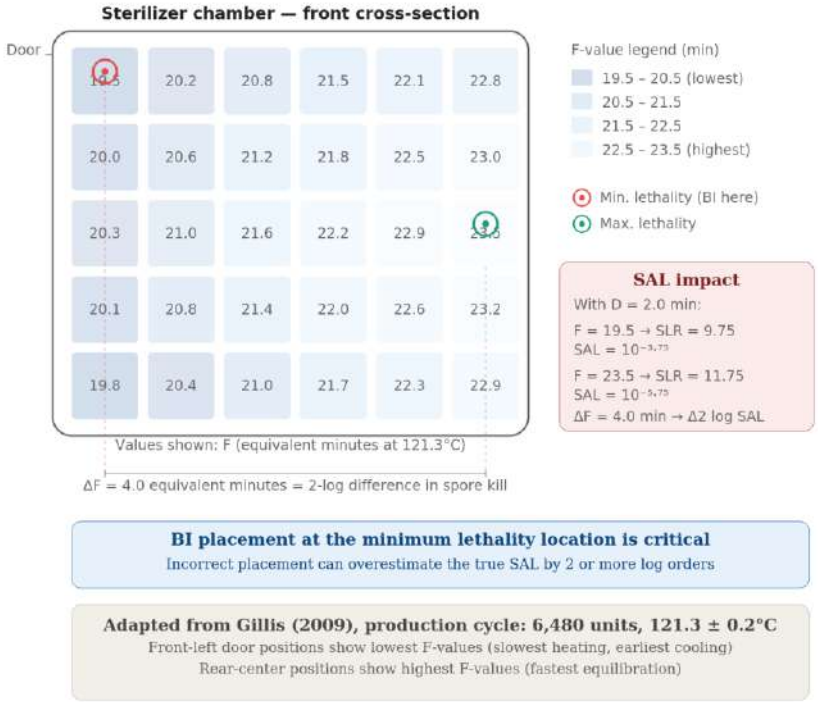
4.3 Importance of BI Placement and Lethality Mapping

A critical but frequently underappreciated factor in achieving a reliable SAL is the placement of the biological indicator at the location of minimum lethality within the sterilizer. As Gillis (2009) demonstrated using lethality mapping data from a production steam sterilization cycle containing 6,480 product units, the equivalent process time (F) across different locations within the chamber ranged from 19.5 to 23.5 minutes—a 4-minute differential that, for spores with a D-value of 2 minutes, translates to a 2-log difference in spore kill between the minimum and maximum lethality locations.

For large or thermally massive loads, this differential can be even greater: ranges of 10–12 equivalent minutes are not uncommon, corresponding to a potential SAL variation from 10^{-1} to 10^{-6} across the same load. This underscores why sterilization specialists must identify the worst-case location through systematic lethality mapping, and why guessing at BI placement based on limited information is, as Gillis aptly noted, a dangerous hypothesis.

In this context, the precision and consistency of Terragene biological indicators play a vital role in validating these challenging sterilization profiles. By providing highly standardized resistance characteristics, these indicators allow sterilization specialists to accurately correlate biological inactivation with the physical lethality mapped within the load. This synergy between precise biological monitoring and systematic mapping ensures that the demonstrated SAL reflects the true minimum lethality delivered to the product, even in the most complex configurations.

Figure 2. Example of the influence of biological indicator placement on the validation of sterilization process lethality



5. PRACTICAL APPLICATIONS AND CASE SCENARIOS

5.1 Routine Monitoring in Hospital CSSDs

In the healthcare central sterile supply department, biological indicators are used for routine process monitoring, qualification of new sterilizer installations, and periodic revalidation of existing equipment. Terragene Bionova® SCBIs are designed for seamless integration into these workflows. The typical routine monitoring protocol involves placing one SCBI per sterilization load, along with an unexposed control unit. After the sterilization cycle, both units are activated and incubated. The control must show positive growth (confirming viability of the spore population), while the exposed BI must remain negative at the conclusion of the validated incubation period.

With the BT225 (7-second readout) or BT224 (20-minute readout), steam sterilization loads can be biologically confirmed before the instruments even reach the point-of-use. This eliminates the traditional paradigm of releasing loads based on chemical indicator results alone and then waiting days for biological confirmation, a practice that exposes facilities to the risk of costly and disruptive load recalls if a BI later tests positive. For H_2O_2 sterilization of heat-sensitive endoscopes and devices, the BT98 (5-minute readout) enables biological confirmation faster than the time typically required to transport the processed load to the operating theater.

5.2 Sterilization Validation with Overkill Approach

During initial sterilization validation, BIs play a central role in the overkill protocol. A typical validation study involves running three consecutive half-cycle exposures with BIs distributed at the identified worst-case locations within the load. All BIs must show no growth after incubation, demonstrating that the half-cycle alone delivers sufficient lethality to kill the challenge organism. The full cycle, being double the half-cycle exposure, then provides the required safety margin to guarantee an SAL of 10^{-6}.

Terragene Bionova® SCBIs are particularly well suited for validation studies because their controlled, closed-system design minimizes variability introduced by user technique. The resistance characteristics are tightly specified on each lot certificate, enabling precise calculation of expected survival and kill times. The availability of rapid readout products such as the BT224 and BT98 further accelerates validation timelines: where traditional protocols required 7-day holds between validation runs to confirm BI results, facilities using Terragene rapid indicators can confirm results within minutes and proceed to the next validation run the same day.

5.3 Addressing Discordant Results: When BIs Test Positive

One of the most challenging situations in sterilization practice is the occurrence of a positive BI in an otherwise apparently normal process cycle. Physical parameters (temperature, pressure, time) may all appear within specification, yet the BI indicates surviving spores. As Gillis (2009) emphasized, this discordance typically reveals conditions that physical instruments cannot detect, such as air pockets in porous loads during steam sterilization or inadequate humidification within product interstices during EO processing.

The appropriate response is never to dismiss the biological result. If spores survive the process and remain viable, the problem is real. The BI integrates all lethal and sub-lethal conditions experienced at its specific location, making it the most comprehensive single indicator of process efficacy. Terragene SCBIs, with their well-characterized resistance profiles, provide the technical foundation for root-cause investigations when discordant results occur. The rapid readout capability of models like the BT225 and BT98 adds an additional operational advantage: positive results are detected almost immediately, allowing corrective action to begin within minutes rather than days.

6. REGULATORY CONSIDERATIONS

6.1 FDA Classification and 510(k) Requirements

Biological indicators intended for monitoring sterilization in healthcare facilities are classified by the FDA as Class II medical devices (21 CFR 880.2800(a)) requiring premarket notification through the 510(k) pathway. The FDA CDRH guidance document (2007) specifies the performance testing, labeling, and documentation requirements for BI submissions, including viable spore population assays, resistometer-based D-value and Z-value determinations, survival/kill window verification, shelf-life studies, and incubation time validation.

Terragene maintains FDA 510(k) clearances for its Bionova® SCBI product lines and conducts all required performance characterization on final finished product in accordance with the guidance recommendations and applicable ANSI/AAMI/ISO 11138 standards.

6.2 Healthcare versus Manufacturing: The RIT Distinction

An important regulatory nuance concerns the applicability of reduced incubation times across different use settings. The FDA CDRH guidance and its RIT validation methodology were developed specifically for BIs used in healthcare facilities. ISO 14161, Section 12.3.3, acknowledges that a manufacturer-validated RIT need not be repeated by the end user provided the BI is used for its intended sterilization process. However, the FDA CDER (Center for Drug Evaluation and Research) has indicated that it may not recognize the CDRH RIT protocol for BIs used in pharmaceutical manufacturing settings, instead favoring the 7-day incubation period referenced in ISO 11138-1 (Bradley and Krushefski, 2014).

For pharmaceutical manufacturers, a practical approach is to read BIs at both the validated RIT and again at 7 days. This dual-read strategy enables timely product release decisions while generating the 7-day data that satisfies conservative regulatory expectations. Terragene supports customers in both healthcare and manufacturing environments with product-specific guidance on incubation protocols appropriate to their regulatory context.

7. CONCLUSION

Biological indicators remain the gold standard for verifying sterilization process efficacy. The development of self-contained systems with rapid readout capabilities, exemplified by the

Terragene Bionova® portfolio, has transformed sterilization monitoring from a multi-day waiting period into a near-real-time reporting capability, without sacrificing the scientific rigor or safety margins that underpin sterility assurance.

Terragene Bionova® SCBIs represent the state of the art in biological indicator technology. By combining well-characterized spore populations of internationally recognized indicator organisms, proprietary growth media optimized for rapid and reliable recovery, fluorescence-based detection enabling readout times from 7 seconds (BT225 for steam) to 4 hours (BT110 for EO), and a closed-system design that eliminates user-introduced variability, these products enable healthcare facilities and sterilization professionals to confidently demonstrate SAL 10^{-6} compliance while maximizing operational efficiency.

The scientific principles reviewed in this work, from spore biology and D-value kinetics to lethality mapping and regulatory frameworks, provide the foundation for understanding why biological indicators are essential, how rapid readout technology works, and how Terragene products support sterility assurance in practice. As sterilization technologies continue to evolve, the role of well-designed, rigorously validated biological indicators will only grow in importance.

REFERENCES

[1] AAMI (2006a). Sterilization of health care products – Vocabulary. ANSI/AAMI/ISO TIR 11139:2006. Association for the Advancement of Medical Instrumentation, Arlington, VA.

[2] AAMI (2006b). Sterilization of health care products – Biological indicators – Part 1: General requirements. ANSI/AAMI/ISO 11138-1:2006.

[3] AAMI (2006c). Sterilization of health care products – Biological indicators – Part 3: Biological indicators for moist heat sterilization processes. ANSI/AAMI/ISO 11138-3:2006.

[4] FDA CDRH (2007). Guidance for Industry and FDA Staff: Biological Indicator (BI) Premarket Notification [510(k)] Submissions. U.S. Food and Drug Administration, Center for Devices and Radiological Health, October 4, 2007.

[5] Gillis, J.R. (2009). “It’s Time to Remove Guesswork from the Sterilization Process.” Spore News, Vol. 6, No. 1. SGM Biotech, Inc., Bozeman, MT.

[6] ISO (2017). ISO 11138-1:2017. Sterilization of health care products – Biological indicators – Part 1: General requirements. International Organization for Standardization, Geneva.

[7] ISO (2017). ISO 14161:2009. Sterilization of health care products – Biological indicators – Guidance for the selection, use and interpretation of results. International Organization for Standardization, Geneva.

[8] Krushefski, G. (2009). “Disinfection and Sterilization.” Spore News, Vol. 6, No. 5. SGM Biotech, Inc., Bozeman, MT.

[9] McCauley, K. (2009). “Defining Limits: The Beauty of a Self-Contained Biological Indicator (SCBI).” Spore News, Vol. 6, No. 6. SGM Biotech, Inc., Bozeman, MT.

[10] Pflug, I.J. (1990). Microbiology and Engineering of Sterilization Processes. Environmental Sterilization Laboratory, Minneapolis, MN.

[11] Bradley, R. and Krushefski, G. (2014). “Reduced Incubation Times for Biological Indicators.” Spore News, Vol. 11, No. 2. Mesa Laboratories, Inc.

[12] Ridgeway, B. (2013). “Organisms and Their Applications in Biological Indicators.” Spore News, Vol. 10, No. 1. Mesa Laboratories, Inc.

[13] United States Pharmacopeia (USP). USP <1035> Biological Indicators for Sterilization. Current edition.

[14] United States Pharmacopeia (USP). USP <135> Biological Indicators for Verification of Sterilization of Articles, Liquids, and Surfaces by Chemical or Physical Agents.