



Organisms and Their Uses in Biological Indicators

by Beth Ridgeway

Bacterial spores are the superstars in the biological indicator (BI) world. Their structure and characteristics make them the best candidates for manufacture of biological indicators used in moist heat, dry heat, radiation and chemical sterilization processes. Sporulation is basically a self-preservation strategy by the bacterium. When nutrients become deficient to the bacteria, sporulation is triggered and the formation of the spore occurs. The spore is in a dormant stage and can remain in that state for long periods of time.

Some of their characteristics that make them ideal for use in validation and monitoring cycles (compared to vegetative cells) are their stability for long periods of time, the fact that they are fairly easy to suspend in homogenous solutions with the exception of a few hydrophobic species, and that they are highly resistant to environmental stresses including sterilization processes. Once conditions become favorable, they can reactivate to the vegetative state. This makes them a perfect fit for BIs because they can be manufactured in a stable configuration and then exposed in their most resistant stage to monitor sterilization cycles and then activated by giving them the nutrients (media) and temperature they need to germinate.

Not all bacteria can form spores and the species most widely used in the BI industry are of the genus *Bacillus*, *Geobacillus* and *Clostridium*. All are gram positive bacteria with rod shaped cells. *Bacillus* and *Geobacillus* are aerobic species while *Clostridium* is an anaerobic species. Within the *Bacillus* genus are many species and each species has different tolerances to sterilization processes. There are widely known and accepted species in the BI industry that exhibit better resistance to particular sterilization processes. These species are recommended in the USP and ISO 11138. The most widely used species are *Geobacillus stearothermophilus*, *Bacillus atrophaeus* and *Bacillus subtilis* 5230. These are the most commonly recognized species for steam, ethylene oxide, dry heat and hydrogen peroxide sterilization processes. The ISO standard does state however that other species can be used that demonstrate equivalent performance as required by ISO. In some cases it may be feasible to use other organisms, for example when it has been determined that the bioburden organism may have a stronger resistance than the BI organism or when a heat sensitive product is being sterilized. In these instances, it may be necessary to perform bioburden validation studies.

The following paragraphs outline the typical species used at Mesa Labs for BI manufacturing. In addition to the species used in the BIs, quite a number of organisms are sold in suspension form for use in direct inoculation studies and/or use in custom biological indicator development.

Geobacillus stearothermophilus

This is the heavy hitter in the BI world. It is used in Mesa Labs BIs intended for steam, hydrogen peroxide, formaldehyde, propylene oxide and ozone sterilization processes. *Geobacillus stearothermophilus* is a thermophilic organism with an optimum growth temperature range of 55°C – 60°C. Resistance characteristics vary depending on BI configuration and sterilization process. To comply with ISO 11138-3, steam D₁₂₁-values for a standard BI must be ≥ 1.5 minutes and a Z-value ≥ 6°C determined from three temperatures ranging from 110°C – 130°C. USP guidelines suggest D₁₂₁-values of 1.5 minutes – 3.0 minutes. Currently, the only requirements for chemical sterilization are in ISO 11138-5 for formaldehyde which states the BIs shall have a D₆₀ value of ≥ 6.0 minutes. Mesa Labs has two BI manufacturing sites, Bozeman, Montana and Omaha, Nebraska. Table 1 displays BIs available from these sites using spores of *G. stearothermophilus*:

Table 1: Biological Indicators containing *G. stearothermophilus*.

	Bozeman Facility	Omaha Facility
Self-Contained	EZTest steam EZTest H ₂ O ₂ EZTest Propylene oxide SterilAmp MagnaAmp SterilFlex	ProTest and ProSpore2 ProTest H ₂ O ₂ * ProAMP ProSpore Ampoule SterilFlex
Strips in Glassine	MESAStrip MESAStrip-Duals MESAStrip-LTS Formaldehyde	Spore Strips Combined Spore Strips Spore Strip for LTSF (formaldehyde)
Custom/Micro Strips	1 mm x 25 mm 3 mm x 25 mm 2 mm x 10 mm	* * 2 mm x 10 mm
Custom Paper Discs	6 mm round	6 mm and 9 mm round
Borosilicate Discs	*	7 mm round
Apex Products	stainless steel discs and ribbons for H ₂ O ₂	stainless steel discs and ribbons for H ₂ O ₂
Industrial Use BIs	*	Steel discs/coupons/wires threads/polyester sutures
Process Challenge Device	*	ProLine
Culturing Sets	Releasat for Steam	Culture Test Kits (RCT Kits)
Spore Suspensions	aqueous or ethanol	aqueous or ethanol

* not a standard product from this facility

Bacillus atrophaeus

Bacillus atrophaeus is used in Mesa Labs BIs intended for ethylene oxide, dry heat, chlorine dioxide and ozone sterilization processes. *B. atrophaeus* is a mesophilic organism with an optimum growth temperature range of 30°C – 39°C. Resistance characteristics vary depending on BI configuration and sterilization process. ISO 11138-2 requires an EO D₅₄-value of not less than 2.5 minutes for a standard BI. ISO 11138-4 requires a dry heat D₁₆₀-value of not less than 2.5 minutes and a Z-value ≥ 20°C determined from three temperatures ranging from 150°C - 180°C. USP only lists typical resistance characteristics with these being: 2.5 minutes – 5.8 minutes for EO D₅₄-value and 1.0 minutes – 3.0 min for dry heat D₁₆₀-values. Currently, there are no resistance requirements listed for chlorine dioxide. Table 2 displays BIs from Mesa Labs using spores of *B. atrophaeus*:

Table 2: Biological indicators containing *B. atrophaeus*

	Bozeman Facility	Omaha Facility
Self-Contained	EZTest EO	ProTest and ProSpore2
Strips in Glassine	MESAStrip MESAStrip-Duals MESAStrip-ClO ₂	Spore Strips Combined Spore Strips Spore Strip for ClO ₂
Custom/Micro Strips	1 mm x 25 mm 3 mm x 25 mm 2 mm x 10 mm 2 mm x 19 mm	* * 2 mm x 10 mm *
Custom Paper Discs	6 mm round	6 mm and 9 mm round
Borosilicate Discs	*	7 mm round
Apex Products	Stainless steel ribbon and discs	Stainless steel ribbon and discs
Industrial Use BIs	Stainless steel discs	Steel discs, coupons, threads, wires, poly sutures
Process Challenge Device	*	ProLine
Culturing Sets	Releasat for Gas Releasat for ClO ₂ DriAmp	TSB w/Phenol Red and spore strips Releasat for ClO ₂ DriAmp
Spore Suspensions	aqueous or ethanol	aqueous or ethanol

* not a standard product from this facility

Bacillus subtilis 5230

Bacillus subtilis 5230 is employed in low temperature steam sterilization processes and is a good choice for customers who may have heat sensitive products that may be compromised by the time and temperature of the standard 121°C steam sterilization cycles. A properly validated cycle is necessary for use of this organism for steam processes but is an excellent and reliable choice for low temperature sterilization processes. *B. subtilis* 5230 is a mesophile with an optimum growth temperature range of 30°C – 39°C. It can grow up to 56°C. Table 3 displays BIs from Mesa Labs using spores of *B. subtilis* 5230:

Table 3: Biological indicators using *B. subtilis* 5230

	Bozeman Facility	Omaha Facility
Self Contained	SterilAmp 5230	ProAMP 5230
Strips in Glassine	MESAStrip	Spore Strip
Spore Suspensions	aqueous or ethanol	aqueous or ethanol

Bacillus pumilus

Bacillus pumilus is used in Mesa Labs BIs intended for ionizing radiation and ultraviolet sterilization processes. *B. pumilus* is a mesophilic organism with an optimum growth temperature range of 30°C – 35°C. No standards in ISO or USP exist regarding resistance requirements for this species. Table 4 displays BIs from Mesa Labs sites using spores of *B. pumilus*:

Table 4: Biological indicators using *B. pumilus*

	Bozeman Facility	Omaha Facility
Strips in Glassine	MESAStrip	Spore Strip
Spore Suspensions	aqueous or ethanol	aqueous or ethanol
Custom Paper Discs	6 mm round	*
Industrial Use BIs	stainless steel discs	*

*not a standard product from this facility

In addition to the commonly used organisms described above, Mesa Labs manufactures a variety of spores that can be intended for direct inoculation in customer product for validation purposes or for BI manufacture by the customer. The following organisms are manufactured in aqueous or ethanol solutions:

***Bacillus subtilis* 6633** – mesophilic organism, optimum growth temperature 30°C – 35°C. Commonly used as an organism for media growth promotion testing.

Bacillus megaterium – mesophilic organism, optimum growth temperature 30°C – 35°C. A commonly used organism in environmental and industrial research applications.

Bacillus cereus - mesophilic organism, optimum growth temperature 30°C – 35°C. Commonly used in food industry research as this organism is known to cause food-borne illnesses. Very closely related to *Bacillus anthracis* and used as a surrogate organism.

Clostridium sporogenes – mesophilic anaerobic organism, optimum growth temperature 30°C – 35°C. Commonly used as a surrogate to *Clostridium botulinum* in food industry sterilization processes and is currently being researched as potential bacteria to fight cancerous tumors.

***Bacillus smithii* (formerly *Bacillus coagulans*)** – Mesa Labs has found optimum growth temperature of 48°C – 52°C and has observed growth up to 65°C for this organism. It is most commonly used for probiotic purposes.

Bacillus thuringiensis – mesophilic organism, optimum growth temperature 30°C – 35°C. Most commonly known for its use in pesticides and as a surrogate for *Bacillus anthracis*.

This overview of organisms and their applications in BIs illustrates what is typically used for standard industry practices. There may be times during validation process development where an altogether different BI and/or organism combination may be needed. In addition to BIs, Mesa Labs offers contract studies to help with development of sterilization studies in product and/or bioburden resistance determinations. Mesa Labs has an exceptional team of customer service and technical support that is happy to accommodate any questions or concerns that may arise in your sterilization/validation processes.

Beth graduated from University of Montana with a B.S. in Microbiology and Medical Technology. She has worked for Mesa Labs (previously SGM Biotech) for nine years holding positions in the BI production laboratory, Research and Development, and Contract Studies. Beth's previous position was Spore Lab Supervisor and currently she is Director of Laboratory Production.