

Spore News

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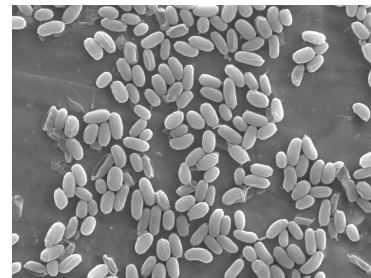
Consistent Biological Indicators Require Clean Spores



Inter and intra lot reproducibility of Biological Indicators (BIs) is enhanced by processing techniques to remove debris from spore suspensions prior to the production of BIs.

INTRODUCTION:

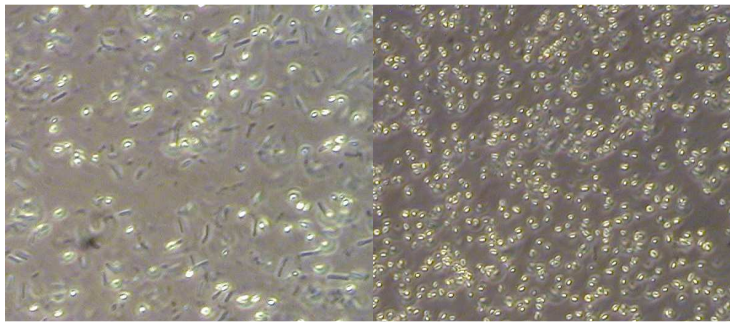
For more than 50 years, bacterial endospores have been used as biological indicators for monitoring sterilization processes. The microorganisms used are referenced in USP, ISO, and European Norms from well characterized type cultures such as ATCC (American Type Culture Collection). The reality is that some sources of biological indicators prepared from identical type cultures demonstrate a wide variation of sensitivity to sterilizing conditions as measured by D-values in BIER vessels. This variability can be attributed to how the spores are grown and prepared.



Cleaned *G. stearothermophilus* spores

PREPARING BIs:

It is important that the harvested spores from the bulk culture are free from nutrients and debris that can act as insulation or protection around individual spores. Cellular debris and residual media should be thoroughly removed from spore suspensions. When the spores are dried onto carriers this debris will dry around the spore and protect it from the sterilizing agent. The exact procedures used by BI manufacturers are proprietary and will vary. From the user's perspective, the importance is not in the method used but its consistency. Consistency in BI performance should be the most important quality characteristic used by customers in selecting a BI supplier.



Before Processing

After Processing

D-values from 21 lots of BIs from SGM and another manufacturer appear in Table 1. Figure 1 illustrates a frequency distribution of SGM spore crop resistance over five years compared to a competitor’s product variation over two years. The carriers for both products are paper and the spores are *G. stearotherophilus* ATCC 7953. The 21 lots of SGM product are sequential spore crops over the past five years. The competitor’s data progresses over approximately two years and the spore crops are identified by a sequential number system.

SGM Biotech, Inc.		Company 2	
Lot ⁽¹⁾	D-value	Lot ⁽²⁾	D-value
BST-203P	2.0	1	1.1 ⁽³⁾
BST-208P	2.0	2	1.3 ⁽³⁾
BST-216P	2.0	3	1.2 ⁽³⁾
BST-221P	2.0	4	1.5
BST-222P	2.0	5	2.3
BST-232P	2.2	6	1.8
BST-235P	2.3	7	1.5
BST-239P	2.0	8	1.9
BST-246P	2.3	9	1.9
BST-251P	2.4	10	1.5
BST-255P	1.9	11	1.9
BST-256P	1.9	12	2.2
BST-262P	2.0	13	1.5
BST-273P	1.7	14	1.8
BST-275P	2.0	15	2.4
BST-279P	2.0	16	1.6
BST-281P	2.0	17	1.5
BST-284P	1.8	18	1.3 ⁽³⁾
BST-286P	1.7	19	1.0 ⁽³⁾
BST-288P	1.8	20	1.2 ⁽³⁾
BST-289P	2.1	21	0.7
Average D-value	2.0	Average D-value	1.5
Highest	2.4	Highest	2.4
Lowest	1.7	Lowest	0.7

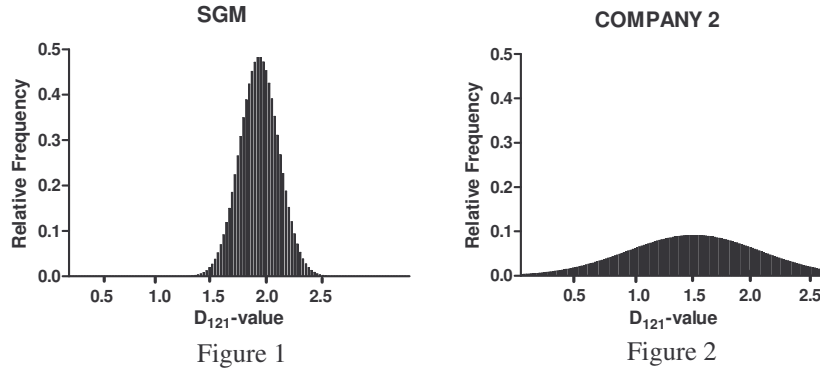
¹Different lot numbers relate to different spore crops. Gaps in the lot number sequence indicate that the same spore crop was used.

²Actual lot numbers are unknown. Therefore, a sequential numbering system is used.

³Values below the ISO 11138-3 minimum acceptable value of 1.5 minutes.

Figure 1 illustrates a frequency distribution plot of the 21 SGM BI lots. Figure 2 illustrates a frequency distribution plot for Company 2 D-value data.

D-value Distribution Comparison



Statistics	SGM	Co. 2
Minimum	1.70	0.700
Maximum	2.40	2.40
Mean	2.00	1.58
Std. Deviation	0.183	0.436
Std. Error	0.0399	0.0951
Lower 95% CI of mean	1.92	1.38
Upper 95% CI of mean	2.09	1.77
Coefficient of variation	9.13%	27.65%

D-values:

In this specific case, the BIs consist of *G. stearothermophilus* for steam sterilization; the ISO 11138 and EN 866 require a minimum D-value at 121°C of 1.5 minutes.

Company 1 - SGM Biotech, Inc.

The SGM data provided indicates an average D-value of 2.0 minutes. This represents a normal thermal resistance of many different spore crops grown under similar conditions. SGM D-values ranged from 1.7 to 2.4 minutes, which is equal to +20% above the average and -15% below the average.

Company 2

The data accumulated indicates an average D-value of 1.5 minutes. The competing product D-values ranged from 0.7 to 2.4 minutes, which is equal to +60% above the average and -46% below the average. There are seven D-values actually falling below the minimum 1.5 minutes required by USP and EN.

SPORES ARE PREDICTABLE:

Biological indicators for validation and monitoring sterilization processes constitute a test method which to be useful needs to demonstrate a measure of reproducibility. Because of the biological nature of spores, there will be some inherent variation in their survival characteristics, depending on individual differences. But normal response variation of spores should not be sufficient to cause any concern or interlot unacceptability for sterilization monitoring purposes.

EXPLAINING UNACCEPTABLE D-VALUES:

Manufacturers use different media and techniques for culturing spore forming bacteria as well as different techniques for harvesting and handling spore suspensions. It is in these techniques that factors are introduced which can either raise or reduce D-values. Over a period of five years the SGM lots consistently gave D-value results close to 2.0 minutes. The lowest D-value was 1.7 minutes and still within requirements of the standards. The second manufacturer's data as listed in Table 1, show seven results are below the ISO and EN requirements for use as a BI and the range of D-values is much wider.

Variable cultural conditions and extraneous cellular debris would tend to influence thermal resistance of spores. Well controlled laboratory SOPs and materials aid in maintaining the highest quality standards and reduce inter and intralot variability.

CONCLUSIONS:

Biological indicators based on bacterial spores provide a proven, reliable validation tool for sterilization processes. BIs are unique as a single test that challenges **all of the critical parameters of sterilization processes**.

But from a user's perspective, major differences between lots as expressed by D-values are frustrating and can be avoided. SGM will make available, upon request, all of the release data of purchased product.

It would be unreasonable to expect every lot to have a D-value of exactly 2.0 minutes because of the limitations of the test procedure and the nature of biologicals. But a range of 1.7 to 2.4 minutes is a reasonable expectation.

Please email us with topics you would like to see addressed in "Spore News".

