

Spore News

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Biological Indicator for Monitoring Low Temperature Steam Sterilization



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The use of low temperature steam sterilization for special solutions or special packaging is becoming very common in industry today. Automated liquid packaging machines using form, fill, and seal operations commonly require temperatures of 106°C for sterilization of solutions because this is the maximum temperatures that many of these (LDPE) packages can take.

The biological indicator of choice for this process is *Bacillus subtilis* “5230” (ATCC #35021). A spore forming *Bacillus* which is much more sensitive to these steam sterilization temperatures than the most commonly used spores of *Geobacillus stearothermophilus*. *B. subtilis* “5230” was first used by Baxter (Travanol Laboratories) more than 20 years ago¹. Properly validated steam sterilization processes using this organism have been accepted by FDA. Baxter submitted this specific strain of *B. subtilis* “5230” to the American Type Culture Collection (ATCC) so it would be available for use by the industry.

SGM Biotech, Inc. is now offering this organism as a standard product in our inventory. The packaging configuration is in SterilAmp. SterilAmp is a small glass ampoule 6.25mm in diameter and 26mm long. The glass ampoule contains 0.3ml of liquid culture media and 10⁶ spores of *B. subtilis* “5230”. SGM provides D-values at 110, 115, and 121°C as well as a Z-value. These spores are very stable when refrigerated. Refrigeration is required since these spores are suspended in nutrient growth medium which allows the spores to germinate and grow at room temperature. Units should not be held at room temperature for longer than two (2) hours.



D-values from lot to lot may vary by $\pm 20\%$ or by ± 0.1 minute at the higher temperatures where the assessed resistance is typically less than 0.5 minutes.

The following data is typical of the performance of the SterilAmp *B. subtilis* “5230”.

¹ Caputo, R.A., Odlaug, T.E., Wilkinson, R.L., and Mascoli, C.C., “Biological Validation of a Sterilization Process for a Parenteral Product-Fractional Exposure Method”, *Journal of the Parenteral Drug Association*, July/Aug, Vol. 33 #4 (1979).

The Z-value for the following D-values is 8.5°C and has a correlation coefficient of 0.9991 (Figure 1). In the process of collecting these data, SGM tests over 600 individual BI's for the release of each lot of product.

Fractional Negative Data Set from AAMI Steam BIER Exposures at 121°C	
Time	# killed/total
0.75	0/20
1	0/20
1.25	8/20
1.5	19/20
1.75	20/20
2	20/20
2.25	20/20

Resistance Performance Data	
<u>D₁₂₁</u>	<u>0.2 minutes</u>
D upper 95% confidence limit	0.203 min
D lower 95% confidence limit	0.184 min
Survival time*	0.87 min
Kill time*	2.08 min
Z = 8.5°C	8.5°C
F _{T121} (Z = 8.5°C) kill	1.75 min
*Calculated using formula in USP 27, ISO 11138, and EN 866	

F_{T121} = equivalent of 1 minute at 121°C with a Z = 8.5°C.

Fractional Negative Data Set from AAMI Steam BIER Exposures at 115°C	
Time	# killed/total
4	0/20
5	0/20
6	0/20
7	3/20
8	18/20
9	20/20
10	20/20
11	20/20
12	20/20
13	20/20

Resistance Performance Data	
<u>D₁₁₅</u>	<u>1.1 minutes</u>
D upper 95% confidence limit	1.15 min
D lower 95% confidence limit	1.09 min
Survival time	4.83 min
Kill time	11.44 min
Z = 8.5°C	8.5°C
F _{T115} (Z = 8.5°C) kill	9.0 min

F_{T115} = equivalent of 1 minute at 115°C with a Z = 8.5°C

**Fractional Negative Data Set
from Controlled Glycol Bath
Exposures at 110°C**

Time	# killed/total
16	0/20
20	0/20
24	1/20
28	10/20
32	20/20
36	20/20
40	18/20
44	20/20
48	20/20
52	20/20

Resistance Performance Data

<u>D₁₁₀</u>	<u>4.2 minutes</u>
D upper 95% confidence limit	4.33 min
D lower 95% confidence limit	4.03 min
Survival time	18.47 min
Kill time	43.68 min
Z = 8.5°C	8.5°C
F _{T110} (Z = 8.5°C) kill	44.0 min

F_{T110} = equivalent of 1 minute at 110°C with a Z = 8.5°C

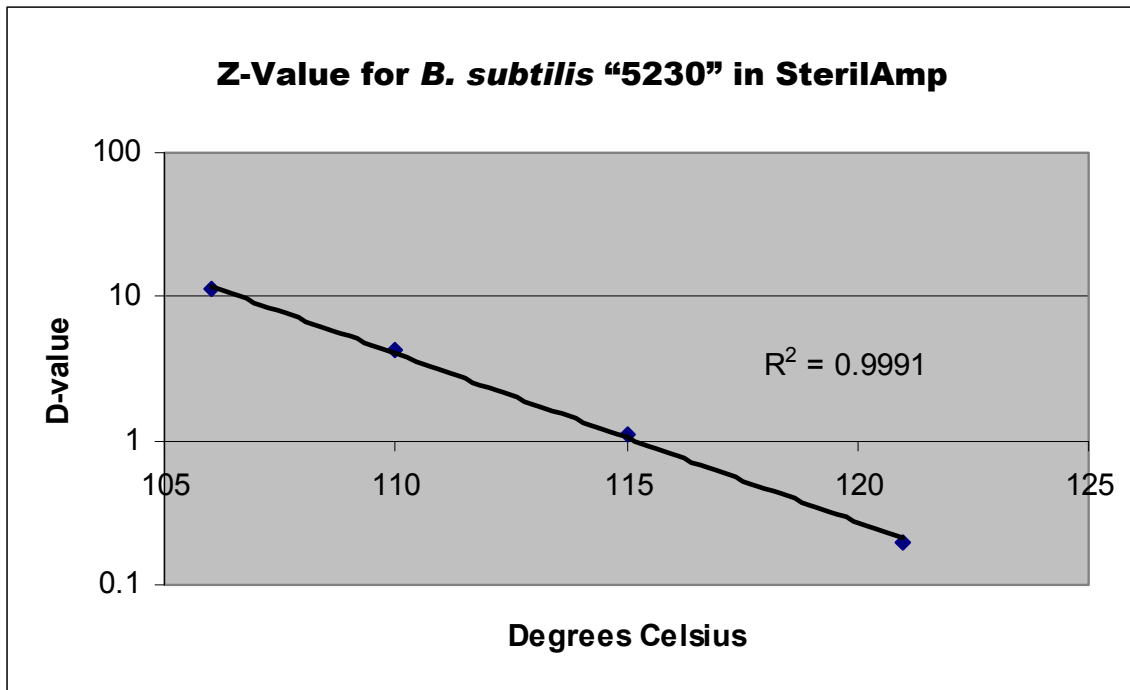
**Fractional Negative Data Set from
Controlled Glycol Bath
Exposures at 106°C**

Time	# killed/total
32	0/20
48	0/20
64	0/20
80	17/20
96	19/20
112	20/20

Resistance Performance Data

<u>D₁₀₆</u>	<u>11.3 minutes</u>
D upper 95% confidence limit	11.37 min
D lower 95% confidence limit	11.25 min
Survival time	49.69 min
Kill time	117.50 min
Z = 8.5°C	8.5°C
F _{T106} (Z = 8.5°C) kill	112.0 min

F_{T106} = equivalent of 1 minute at 106°C with a Z = 8.5°C



SGM's SterilAmp *B. subtilis* "5230" is a very reliable and predictable BI for use in routine monitoring of these low temperature sterilization cycles. Controlled manufacturing methods can provide you with consistent resistance performance from batch to batch.

SterilAmp is easy to use. There is no post processing manipulations to do. Simply remove the BI from the container and place into a $37 \pm 2^\circ\text{C}$ incubator for 48 hours. Positive samples (surviving spores) turn a yellow color. Negative samples (sterile) remain a pale red-orange color.

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