SCM BIOTECH, INC.

REPORT #000802
March 20, 2002
CROSS TESTING DIFFERENT LOTS OF RELEASAT SPORE STRIPS WITH DIFFERENT LOTS OF RELEASAT GROWTH MEDIA

I. Purpose:

The purpose of this study was to determine if using different lots of Releasat spore strips with different lots of Releasat growth media resulted in D-values similar to those obtained during initial qualification testing.

II. Scope:

Three different lots of Releasat spore strips, each produced from a different *Bacillus subtilis* spore crop, were tested in three different lots of Releasat growth media in order to determine variation due to cross testing. Three different lots of Releasat spore strips, each produced from the same crop dilution, were tested in the same lot of Releasat growth medium. One lot of Releasat spore strips was tested in its corresponding lot of growth medium three times in order to determine normal experimental variation. Testing consisted of obtaining D-values in ethylene oxide for each spore strip lot-growth medium lot combination.

III. Equipment and Supplies Used:

- Releasat spore strip lot numbers RS000101 (Bsub020497/S13-10), RS000102 (Bsub071999/S2-2), RS000103 (Bsub071999/S2-3), RS000104 (Bsub071999/S2-4) and RS000106 (Bsub020398/S9-2)
- Releasat growth media lot numbers RM000102, RM000104, RM000108 and RM000109
- Joslyn Sterilizer Corp. ethylene oxide B.I.E.R. (54º ± 1ºC, 600 ± 30mg/L ethylene oxide, 60 ± 10% RH)
- Incubator set at 37ºC ± 1ºC

IV. Procedures:

Part A. Cross Testing Different Strips with Different Media

Three different lots of Releasat spore strips, each produced from a different spore crop, were tested with three different lots of Releasat growth media. Spore strip lot number RS000101 was tested with media lot numbers RM000102, RM000108 and RM000109. Spore strip lot number RS000104 was tested with media lot numbers RM000102, RM000108 and RM000109. Spore strip lot number RS000106 was tested with media lot numbers RM000102, RM000108 and RM000109. These combinations of spore strip lots and growth media are presented graphically in Figure 1.

Testing consisted of D-value determination in ethylene oxide. Each exposure consisted of 10 spore strips, exposed in 2-minute increments. Exposed spore

strips were cultured into growth media within four hours after exposure. Tubes were incubated at 37ºC ± 1ºC. Results were recorded after 72 hours incubation and D-values were calculated using the Stumbo, Murphy, Cochran method. Data were analyzed to determine if any of the combinations resulted in significantly different D-values.

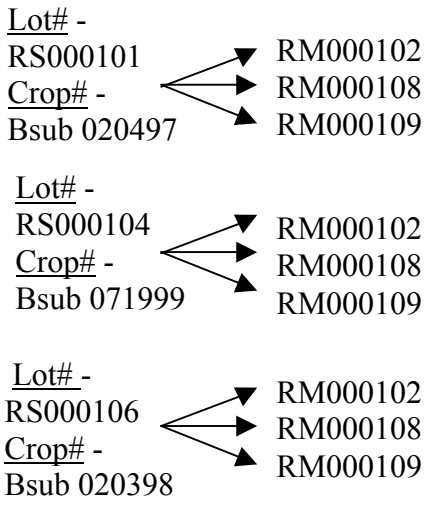


Figure 1. Combinations of Spore Strips and Growth Media Tested in Part A.

Part B. Cross Testing Different Strips with the Same Media

Three different lots of spore strips (produced from the same crop dilution) were tested in one lot of growth medium. Spore strip lot numbers RS000102, RS000103 and RS000104 were each tested with growth medium lot number RM000109. These combinations of spore strip lots and the growth medium lot are presented graphically in Figure 2. Testing was conducted and data were analyzed as described in Part A.

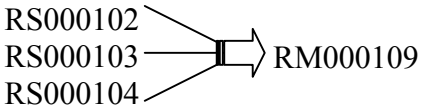


Figure 2. Combinations of Spore Strips and Growth Medium Tested in Part B.

Part C. Testing the Same Lot of Strips and Media

Releasat spore strip lot RS000104 was tested with its corresponding growth medium lot, RM000104, three separate times. Testing was conducted and data were analyzed as described in Part A.

V. Results and Discussion:

In the Biological Indicator for Ethylene Oxide Sterilization, Paper Carrier section, the USP24 states that "the requirements of the test are met if the determined D-value is within 20% of the labeled D-value for the selected sterilizing

temperature". The D-values obtained during initial qualification testing for each of the Releasat lots used in this study are summarized in Table 1.

Table 1. D-values Obtained During Initial Qualification Testing.

Releasat Lot	Initial D-value (min)	± 20% of Initial D-value
RM000101/RS000101	3.0	2.4 – 3.6
RM000102/RS000102	3.2	2.6 – 3.8
RM000103/RS000103	3.3	2.6 – 4.0
RM000104/RS000104	3.0	2.4 – 3.6
RM000106/RS000106	3.1	2.5 – 3.7
RM000108/RS000108	3.2	2.6 – 3.8
RM000109/RS000109	3.0	2.4 – 3.6

Part A. Cross Testing Different Strips with Different Media

The D-values obtained during cross testing with different strips and different media are summarized in Table 2. The D-values ranged from 3.0 to 3.6 minutes. However, all of the D-values were within 20% of the original D-values for each of the lots tested.

Table 2. D-values from Cross Testing Different Strips with Different Media.

Spore Strip Lot	**Strip Lot Initial D-value**	Growth Medium Lot				
		RM000102	RM000108	RM000109		
		D-value (min)	D-value (min)	D-value (min)	Max % Difference	Min % Difference
RS000101	**3.0**	3.4	3.6	3.4	+ 20%	+ 13%
RS000104	**3.0**	3.3	3.3	3.4	+ 13%	+ 10%
RS000106	**3.1**	3.0	3.2	3.3	+ 7%	- 3%

Part B. Cross Testing Different Strips with the Same Growth Medium

The D-values obtained during cross testing with different strips and the same growth medium are summarized in Table 3. The D-values ranged from 3.2 to 3.4 and were also within 20% of the original D-values obtained for each lot tested.

Table 3. D-values from Cross Testing Different Strips with the Same Medium.

Spore Strip Lot	**Strip Lot Initial D-value**	Growth Medium Lot	
		RM000109	
		D-value (min)	% Difference
RS000102	**3.2**	3.2	0%
RS000103	**3.3**	3.3	0%
RS000104	**3.0**	3.4	+ 13%

Graphpad Prism statistics software was used to analyze the cross testing data. All of the data points collected are summarized in Table 4. The D-values obtained during initial qualification testing are highlighted with boxes. All of the other D-values were obtained by cross testing.

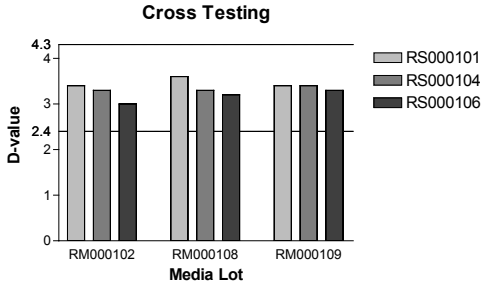
Table 4. All Data Collected During Cross Testing.

Releasat Lot	RS000101	RS000102	RS000103	RS000104	RS000106	RS000108	RS000109
RM000101	[3.0]						
RM000102	3.4	[3.2]		3.3	3.0		
RM000103			[3.3]				
RM000104				[3.0]			
RM000106					[3.1]		
RM000108	3.6			3.3	3.2	[3.2]	
RM000109	3.4	3.2	3.3	3.4	3.3		[3.0]

A complete data table was necessary in order to perform statistical analyses, so data points were eliminated until a complete table was formed (Table 5). The condensed data are also presented graphically. The dotted lines represent + 20% of the highest D-value and – 20% of the lowest D-value.

Table 5. Condensed Cross Testing Data.

Releasat Lot	RS000101	RS000104	RS000106
RM000102	3.4	3.3	3.0
RM000108	3.6	3.3	3.2
RM000109	3.4	3.4	3.3



The data in Table 5 were analyzed in a two-way ANOVA. The ANOVA results are presented in Table 6. Sixty-three percent of the total variation was due to the strips, while sixteen percent was due to the media. Although there was greater variation due to the strips than to the media, the differences between strips and media were not significant.

Table 6. Two-Way ANOVA of Condensed Cross Testing Data.

Parameter	Data Set-A	Data Set-B	Data Set-C	Data Set-D
Table Analyzed	Data Table-3			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Strips	62.89	0.0610		
Media	16.49	0.3086		
Source of Variation	P value summary	Significant?		
Strips	ns	No		
Media	ns	No		
Source of Variation	Df	Sum-of-squares	Mean square	F
Strips	2	0.14	0.068	6.1
Media	2	0.036	0.018	1.6
Residual	4	0.044	0.011	

Results from a column statistics analysis of the condensed cross testing data are summarized in Table 7. The coefficient of variation associated with testing media lot RM000102 with strip lot RS000101 was 3.33%. Likewise, the coefficient of variation associated with testing media lot RM000108 with strip lot RS000104 was 1.73% and the coefficient of variation associated with testing media lot RM000109 with strip lot RS000106 was 4.82%.

Table 7. Column Statistics of Condensed Cross Testing Data.

Releasat Lot	RS000101	RS000104	RS000106
Number of values	3	3	3
Minimum	3.400	3.300	3.000
Maximum	3.600	3.400	3.300
Mean	3.467	3.333	3.167
Std. Deviation	0.1155	0.05774	0.1528
Std. Error	0.06667	0.03333	0.08819
Lower 95% CI	3.180	3.190	2.787
Upper 95% CI	3.754	3.477	3.546
Coefficient of variation	3.33%	1.73%	4.82%

Part C. Testing the Same Lot of Strips and Media

The D-values obtained during testing the same lot of strips with its corresponding lot of media are summarized in Table 8. The D-values ranged from 3.2 to 3.4 and were also within 20% of the original D-values obtained for each lot tested.

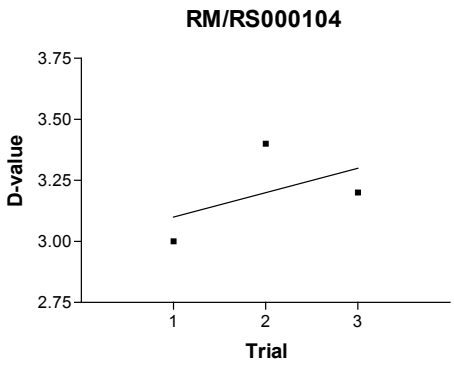
Table 8. D-values from Testing the Same Strips with the Same Medium.

Spore Strip Lot RS000104	**Strip Lot Initial D-value**	Growth Medium Lot RM000104 D-value (min)	% Difference
trial 1	**3.0**	3.4	+ 13%
trial 2	**3.0**	3.2	+ 6%

The data from Table 8 were also analyzed statistically. The linear regression results are summarized in Table 9. The differences between the D-values were not significant.

Table 9. Linear Regression of the Same Strips with the Same Medium Data.

	1
Variables	
Slope	0.10 ± 0.17
Y-intercept	3.0 ± 0.37
X-intercept	-30
1/slope	10
95% Confidence Intervals	
Slope	-2.1 to 2.3
Y-intercept	-1.8 to 7.8
Goodness of Fit	
r^2	0.25
Sy.x	0.24
Is slope significantly non-zero?	
F	0.33
DFn, DFd	1.0, 1.0
P value	0.6667
Deviation from zero?	Not Significant
Data	
Number of X values	3
Maximum number of Y replicates	1
Total number of values	3
Number of missing values	0



Results from a column statistics analysis of the RM/RS000104 data are summarized in Table 10. The coefficient of variation associated testing with same lot of media and strips together three times was 6.25%.

Table 10. Column Statistics of the Same Strips with the Same Medium Data.

X Labels	1
Number of values	3
Minimum	3.0
Maximum	3.4
Mean	3.2
Std. Deviation	0.20
Std. Error	0.12
Lower 95% CI	2.7
Upper 95% CI	3.7
Coefficient of variation	6.25%

In order to assess the amount of variation possible in any particular *B. subtilis* crop, the D-values obtained from four different Releasat lots each inoculated from the same crop, were obtained and compared statistically. The D-values are summarized in Table 11.

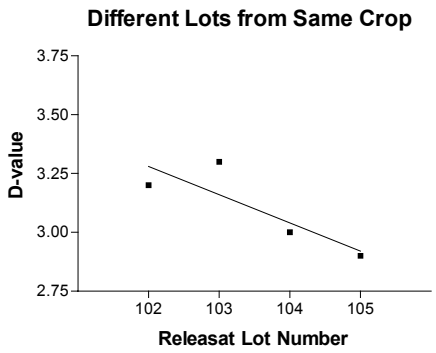
Table 11. D-values from Four Releasat Lots Inoculated from the Same Crop.

Releasat Lot	D-value
RM/RS000102	3.2
RM/RS000103	3.3
RM/RS000104	3.0
RM/RS000105	2.9

The data from Table 11 were analyzed statistically. The linear regression results are presented in Table 12. The differences between the D-values were not significant.

Table 12. Linear Regression of Same Crop Data.

	1
Variables	
Slope	-0.12 ± 0.053
Y-intercept	3.4 ± 0.14
X-intercept	28
1/slope	-8.3
95% Confidence Intervals	
Slope	-0.35 to 0.11
Y-intercept	2.8 to 4.0
Goodness of Fit	
r^2	0.72
Sy.x	0.12
Is slope significantly non-zero?	
F	5.1
DFn, DFd	1.0, 2.0
P value	0.1515
Deviation from zero?	Not Significant
Data	
Number of X values	4
Maximum number of Y replicates	1
Total number of values	4
Number of missing values	0



Results from a column statistics analysis of the same crop data are summarized in Table 13. The coefficient of variation associated with different lots of Releasat produced from the same crop, was 5.89%.

Table 13. Column Statistics of Same Crop Data.

Releasat Lot	D-value
Number of values	4
Minimum	2.9
Maximum	3.3
Mean	3.1
Std. Deviation	0.18
Std. Error	0.091
Lower 95% CI	2.8
Upper 95% CI	3.4
Coefficient of variation	5.89%

If there was an effect on D-value caused by cross testing different lots of Releasat media and strips, the greatest amount of variation would be expected in the cross testing part of this study. In fact, the coefficients of variation associated with the cross tested lots (1.73% - 4.82%) were lower than those associated with testing the same lot three times (6.25%) and with testing different lots from the same crop (5.89%). The highest coefficient of variation was associated with testing the same lot three times (6.25%), indicating that there is more variation in the procedure used to determine a D-value in ethylene oxide for Releasat than is inherent to the lot of media or strips used.

VI. Conclusions:

1. The coefficients of variation associated with the cross tested lots were lower than those associated with testing the same lot three times and with testing different lots from the same crop.
2. The highest coefficient of variation was associated with testing the same lot three times.
3. There is more variation in the procedure used to determine a D-value in ethylene oxide for Releasat than is inherent to the lot of media or strips used.
4. All of the D-values determined in this study met the USP requirements, regardless of the strip or medium lot used.
5. The differences between D-values were not statistically significant, regardless of whether they were determined with the same lot of media and strips or different lots of media and strips.
6. The amount of variation involved in determining a D-value in ethylene oxide for Releasat was not increased by cross testing different strip lots with different media lots.

This study has shown that it is acceptable to use different Releasat spore strip lots with different Releasat media lots.