REPORT FOR THE VALIDATION OF RAVEN'S SPORE STRIPS FOR USE WITH ALTAPURE'S PERACETIC ACID DECONTAMINATION

RAVEN BIOLOGICAL LABORATORIES
8607 Park Drive, Omaha, Nebraska

Report080731 Rev A

APPROVAL
OF
FINAL TESTING RESULTS

The information contained in this report has been reviewed and found acceptable.

Prepared By:

Zach Born – R&D Specialist
Raven Biological Laboratories

12-9-10
Date

Approved By:

Wendy Royalty – Director of Quality Affairs
Raven Biological Laboratories

12/9/10
Date

Deborah Dwyer – Director of Operations
Raven Biological Laboratories

12/9/10
Date

Robert Bradley – Director of Laboratory Production
Raven Biological Laboratories

12/09/10
Date

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1.0 PURPOSE

The purpose of this testing was to validate the use of Raven's *Geobacillus stearothermophilus* spore strips for use with Altapure's peracetic acid decontamination system.

2.0 SYSTEM DESCRIPTION

Three separate lots of Raven spore strips inoculated with *G. stearothermophilus* ATCC #7953 at a nominal population of 1.0×10^6 CFU's per strip were tested for population, resistance and growth inhibition in conjunction with Altapure's peracetic acid decontamination system. Testing was also performed to validate a reduced incubation time.

3.0 RESPONSIBILITIES

All testing was performed in accordance US Pharmacopeia as well as the FDA guidance for validation of reduced incubation for Biological Indicators.

4.0 EQUIPMENT AND MATERIALS USED

1. Altapure's peracetic acid decontamination system, Model HJ600.
2. Raven *G. stearothermophilus* ATCC #7953 bacterial spore strips at a nominal population of 1.0×10^6 packaged in Tyvek/Tyvek envelopes, Lot# 908 Test #2, Lot # 976 Test and Lot # 1021 Test.
3. *G. stearothermophilus* ATCC #7953 bacterial spore suspension at a known population to permit dispension of aliquots with a population of 10-100 viable organisms, Lot# 3029951.
4. Uninoculated spore strips and Tyvek/Tyvek envelopes
5. Raven Modified Tryptic Soy Broth with Bromocresol Purple culture media, Lot #: 000201, 000691, 002361, 001691, 002581, 002981.
6. Laminar Flow Hood #1, SN#5860.
7. Incubator at 60°C $\pm$2°C #3, SN#0700900.
8. Standard laboratory equipment.
9. Tryptic Soy Agar, Lot # 102.904
10. Cold Sterilant, Lot #: 654619, 638846, 654875, 658892, 657289.

5.0 TEST METHOD

5.1 Population Determination

5.1.1 For each lot of strips manufactured, population assays were performed per Raven's Inprocess Inspection Population Assay per LWI-1503.

5.2 Resistance Testing

5.2.1 Performed D-value testing on the 3 lots of *G. stearothermophilus* 10^6 spore strips using the Stumbo-Murphy Cochran Fraction Negative D-Value method following SOP LWI-TestingSMC.

5.2.2 For the purposes of this testing the strips were exposed with no primary packaging.

5.2.3 Recorded Results on LF-1502.

5.3 Reduced Incubation Testing

5.3.1 Reduced incubation times were validated for each lot of *G. stearothermophilus* 10^6 spore strips in accordance with FDA guidance on Validation of Reduced Incubation for Biological Indicators.

5.3.2 Exposed 100 strips at an exposure that produced dichotomous results with between 30-80 percent survivors.

5.3.3 After each exposure aseptically transferred each spore strip into an individual tube of sterilized TSB-BP and incubated at 60°C for 7 days.

5.3.4 Recorded Growth / No Growth daily on LF-1509.

5.4 Determination of Growth Inhibition Testing

5.4.1 Exposed 5 spore strips with no primary packaging to Altapure's Peracetic Acid decontamination system.

5.4.2 After exposure to the process and within 120 minutes of the end of the decontamination the following steps were completed:

5.4.2.1 Aseptically placed each strip into individual tubes of Tryptic Soy Broth with Bromocresol Purple pH indicator (TSB-BP).

5.4.3 Incubated at 60 ± 2°C for 2 hours to allow any inhibitory substances to desorb from the materials.

5.4.4 Removed the tubes from the incubator and aseptically removed the caps. The bacterial suspension was vortexed to ensure even distribution of the spores. Aseptically inoculated each tube with 0.1mL of the test organism suspension and replaced the cap.

5.4.5 Returned the inoculated vials to the incubator and incubated at 60 ± 2°C for 24 hours.

5.4.6 Negative Control: Aseptically placed 5 unexposed strips into tubes of TSB-BP and incubated at 60 ± 2°C for 2 hours.

5.4.7 Negative Control: Removed the tubes from the incubator and aseptically removed the caps. Vortexed the bacterial suspension to ensure even distribution of the spores. Aseptically inoculated each tube with 0.1mL of the test organism suspension and replaced the cap.

5.4.8 Positive Control: Incubated 5 tubes of TSB-BP containing no carriers or primary packaging at 60 ± 2°C for 2 hours.

5.4.9 Positive Control: Removed the tubes from the incubator and aseptically removed the caps. Vortexed the bacterial suspension to ensure even distribution of the spores. Aseptically inoculated each tube with 0.1mL of the test organism suspension and replaced the cap.

5.4.10 Recorded all results as growth or no growth of the test organism on LF-1509.

6.0 RESULTS

6.1 Population Verification

Lot #	Population
908 Test #2	2.9×10^6
976 Test	2.4×10^6
1021 Test	2.6×10^6

6.2 Resistance Testing

Lot #	Time	Run	Survive	Kill	SMC D-Value	Avg. D-Value
908 Test #2	5 min	-	50	0	NA	
	15 min	1	18	32	2.2	
		2	40	10	2.4	2.3 minutes
		3	30	20	2.3	
	25 min	-	0	50	NA	

Lot #	Time	Run	Survive	Kill	SMC D-Value	Avg. D-Value
976 Test	5 min	-	50	0	NA	
	15 min	1	6	44	2.8	
		2	2	48	2.6	2.6 minutes
		3	1	49	2.5	
	30 min	-	0	50	NA	

Lot #	Time	Run	Survive	Kill	SMC D-Value	Avg. D-Value
1021 Test	5 min	-	50	0	NA	
	15 min	1	49	1	2.6	
		2	49	1	2.6	2.6 minutes
		3	47	3	2.5	
	25 min	-	0	50	NA	

6.3 Reduced Incubation Testing

908 Test #2

Date	15 min Growth	No-Growth
8/17/2010	66	34
8/18/2010	69	31
8/19/2010	69	31
8/20/2010	69	31
8/21/2010	70	30
8/22/2010	70	30
8/23/2010	70	30

% Positive @ 48hrs: 98%

976 Test

Date	14 min Growth	No-Growth
9/9/2010	60	40
9/10/2010	62	38
9/11/2010	63	37
9/12/2010	63	37
9/13/2010	63	37
9/14/2010	63	37
9/15/2010	63	37

% Positive @ 48hrs: 98%

1021 Test

Date	12 min Growth	No-Growth
10/11/2010	61	39
10/12/2010	62	38
10/13/2010	63	37
10/14/2010	63	37
10/15/2010	63	37
10/16/2010	63	37
10/17/2010	63	37

% Positive @ 48hrs: 98%

6.4 Growth Inhibition Testing

BI #	Exposed Strip	Positive Control	Negative Control
1	+	+	+
2	+	+	+
3	+	+	+
4	+	+	+
5	+	+	+

+ = Positive for Growth
- = Negative for Growth

7.0 DATA RECORDING

7.1 Recorded population results on LWI-1503.

7.2 Recorded results of Stumbo-Murphy Cochran D-value determination on LF-1502.

7.3 Recorded results for Reduced Incubation Testing on LF-1509.

7.4 Recorded results of Growth Inhibition testing on LF-1509

8.0 ACCEPTANCE CRITERIA

Determination of Growth Inhibition

Viable microorganism population results were between 10 and 100 organisms used to inoculate the media. "Growth" occurred in all the tests.

Reduced Incubation

The results of the Reduced Incubation study exhibited between 30-80% survivors and all three lots showed positive growth greater than 97% of the total number of positive samples at 48 hours.

9.0 EXCURSIONS

No primary packaging was used in the growth inhibition testing because the product is intended to be used without any. Used .01mL/sample of lot #3029951 for growth inhibition testing.

10.0 CONCLUSIONS

The results from all three lots show that Raven's spore strips are suitable for use in conjunction with Altapure's Peracetic Acid decontamination system. D-values were obtained using the Stumbo-Murphy Cochran calculation and three runs of the same time were averaged to determine a D-value. The strips have a 48 hour reduced incubation time as determined by all 3 lots showing greater than 98% positive results after 48 hours. It has also been shown that neither the media nor the strips are inhibited by residual sterilant after exposure to Altapure's Decontamination process.

11.0 ATTACHMENTS

Attachment 1: 908 Test #2
- A: Production Record
- B: Population Assay
- C: Resistance Testing
- D: Reduced Incubation Testing

Attachment 2: 976 Test
- A: Production Record
- B: Population Assay
- C: Resistance Testing
- D: Reduced Incubation Testing

Attachment 3: 1021 Test
- A: Production Record
- B: Population Assay
- C: Resistance Testing
- D: Reduced Incubation Testing

Attachment 4: Growth Inhibition Data

Attachment 5: Original Protocol