GIBRALTAR LABORATORIES, INC.
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LABORATORY REPORT

FINAL REPORT

Sponsor: (1124)
Enviro Tech Chemical Services, Inc.
213 Primo Way
Modesto, CA 95358
Attn: Mike Harvey
Purchase Order #: N/A

GBL Ref. #: 132-527-1070
GBL Sample #: 30808/1.114
Lot #1: 13347
Lot #2: None
Date Received: 01/14/02
Date Tested: 04/22/02, 06/27/02
Date Completed: 07/10/02

Killing Time on BioSide HS 5%

Description of Test Material
One plastic bottle containing BioSide HS 5%; Lot # 13347; Mfg. Date: 11-03-01

Conclusion: Bioside HS 5% when tested at its lowest recommended rate (2 ppm) shows very low to modest efficacy, depending upon organisms and contact time. However at 9 ppm it was completely effective against *Pseudomonas aeruginosa, Candida albicans* and *Chlorella vulgaris* after a 3 hour contact time inactivating greater than five logs.

Respectfully submitted,
GIBRALTAR LABORATORIES, INC.

Date Written: 7/11/02
Analyst: 114

Approved by: _____
Daniel L. Prince, Ph.D.

1 **Purpose:** To determine in vitro killing times of *Pseudomonas aeruginosa, Candida albicans* and *Chlorella vulgaris* in contact with the test materials.

2 **Test Systems**

2.1 *Pseudomonas aeruginosa*, ATCC #: 15442, GBL # 26892/8

2.2 *Candida albicans*, ATCC # 10231, GBL # 26892/4

2.3 *Chlorella vulgaris*, ATCC # 30821, GBL # 115302

3 **Test Material**

3.1 BioSide HS 5% (Lot #: 13347) at a 2ppm test concentration in 400ppm Hard Water, against *Pseudomonas aeruginosa, Candida albicans* and *Chlorella vulgaris* at temp: 95^{0}F (35C).

3.2 BioSide HS 5% (Lot #: 13347) at a 9ppm test concentration in 400ppm Hard Water, against *Pseudomonas aeruginosa, Candida albicans* and *Chlorella vulgaris* at temp: 95^{0}F (35C).

4 **Time Intervals:** 3 hours and 6 hours

5 **Media and Equipment:**

GBL Stat Broth (Trypticase Soy Broth containing 0.5% Lecithin and 4% Tween 20)
Lot # D-127
Trypticase Soy Agar (TSA) Lot # D-125
Sabouraud Dextrose Agar (SDA) Lot # D-180
ATCC Medium 5 Lot # F-34
Saline Lot # D-162
20 to 25C Incubator GBL # 29480
33 to 35C Incubator GBL # 78280

6 **Method**

6.1 **Cultivation of the Test Organisms:**

6.1.1 *Pseudomonas aeruginosa* was grown into 15 mL Trypticase Soy Broth (TSB) at 30 to 35C for 24 hours

6.1.2 *Candida albicans* was grown into 15 mL Trypticase Soy Broth (TSB) at 20 to 25C for 48 hours.

6.1.3 *Chlorella vulgaris* was grown into ATCC Medium 5 for up to 14 days at room temperatures (approximately 25^{0}C).

6.2 Preparation of the Test Systems

6.2.1 BioSide HS 5% (2ppm)

0.1mL of the BioSide HS 5% was aseptically added into a sterile 2499.9mL of sterile 400ppm Hard Water and mixed (dilution 1:25000).

6.2.2 BioSide HS 5% (9ppm)

0.1mL of the BioSide HS 5% was aseptically added into a sterile 555.5mL of sterile 400ppm Hard Water and mixed (dilution 1:5555.6).

6.3.1 Inoculation of the Test System Solution

10.0 mL aliquots of the test materials were transferred to sterile 25 x 150mm glass test tube and the tubes were placed in a 35^{0}C water bath. Approximately 10^6 per mL aliquots of the test organism were added into the test material. The tube was mixed immediately and was placed in the water bath. 1.0 mL aliquots was removed at the specified time intervals and transferred into 9.0 mL sterile GBL STAT broth and mixed. Ten fold serial dilutions were made thereafter into 9.0 mL sterile GBL STAT broth. 2 - 1.0 mL from the each dilution tube were plated into sterile petri dishes. The *Candida albicans* plates were poured with SDA and the plates were incubated at 20 to 25C for 5 to 7 days. At the end of the incubation periods, the plates were counted using a Quebec colony counter and the number of viable organisms was determined.

The *Pseudomonas aeruginosa* plates were poured with TSA and the plates were incubated at 30 to 35C for 2 days. At the end of the incubation periods, the plates were counted using a Quebec colony counter and the number of viable organisms was determined.

6.3.2 Inoculation of the Test System Solution

10.0 mL aliquots of the test materials were transferred to sterile 25 x 150mm glass test tube and the tubes were placed in a 35^{0}C water bath. Approximately 1.0mL aliquots of the test organism were added into the test material. The tube was mixed immediately and was placed in the water bath. 1.0 mL aliquots was removed at the specified time intervals and transferred into 9.0 mL sterile ATCC Medium 5 and mixed. The test tubes were incubated at room temperatures (approximately 25^{o}C) for up to 14 days. At the end of the incubation periods, the algae were determined by hemocytometer.

6.3.3 Inoculation of the Control Solution (Numbers Control)

10.0 mL aliquots of sterile deionized water were transferred to sterile 25 x 150mm glass test tube. Approximately 10^6 per mL aliquots of the test organism were added into the sterile deionized water. Each tube was mixed and 1.0 mL aliquots were removed and transferred into 9.0 mL sterile GBL STAT broth and was mixed. Ten fold serial dilutions were made thereafter into 9.0 mL sterile GBL STAT broth. 2 - 1.0 mL from the each dilution tube were plated into sterile petri dishes.

The *Candida albicans* plates were poured with SDA and the plates were incubated at 20 to 25C for 5 to 7 days. At the end of the incubation periods, the plates were counted using a Quebec colony counter and the number of viable organisms was determined.

The *Pseudomonas aeruginosa* plates were poured with TSA and the plates were incubated at 30 to 35C for 2 days. At the end of the incubation periods, the plates were counted using a Quebec colony counter and the number of viable organisms was determined.

6.3.4 Inoculation of the ATCC Medium 5 (Numbers Control)

10.0mL aliquots of sterile ATCC Medium 5 were transferred to sterile 25 x 150mm glass test tube. Approximately 10^6 per mL aliquots of the test organism were added into the test tube containing sterile deionized water. The test tube was mixed and 1.0mL aliquot were removed and transferred into 9.0mL sterile ATCC Medium 5 and was mixed. The test tubes were incubated at room temperatures (approximately 25°C) for up to 14 days. The algae were determined by hemocytometer.

7 Results: Table # 1 to 6

Table 1: Enumeration of the *Pseudomonas aeruginosa* after exposure to BioSide HS 5%; 2 ppm test concentration; Temp: 35°C; GBL Sample #: 30808/1; Lot #: 13347

Dilution	Contact Time (Minutes)					
	3 hours		6 hours		Control	
	A	B	A	B	A	B
10^{-1}	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
10^{-2}	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
10^{-3}	>300	>300	>300	>300	TNTC	TNTC
10^{-4}	244	284	178	187	>300	>300
10^{-5}	25	27	19	20	73	78
Cfu / mL	2.4×10^6	2.8×10^6	1.8×10^6	1.9×10^6	7.3×10^6	7.8×10^6
Ave. cfu/mL	2.6×10^6		1.9×10^6		7.6×10^6	
Log $_{10}$	6.41		6.28			
Log$_{10}$ Control	6.88		6.88		6.88	
Log Reduction	0.47		0.6			

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Table 2: Enumeration of the *Pseudomonas aeruginosa* after exposure to BioSide HS 5% 9ppm test concentration; Temp: 35°C; GBL Sample #: 30808/1; Lot #: 13347

Dilution	Contact Time (Minutes)					
	3 hours		6 hours		Control	
	A	B	A	B	A	B
10^{-1}	0	0	0	0	TNTC	TNTC
10^{-2}	0	0	0	0	TNTC	TNTC
10^{-3}	0	0	0	0	TNTC	TNTC
10^{-4}	0	0	0	0	>300	>300
10^{-5}	0	0	0	0	73	78
Cfu / mL	<10	<10	<10	<10	7.3×10^6	7.8×10^6
Ave. cfu/mL	<10		<10		7.6×10^6	
Log_{10}	<1		<1			
Log_{10} Control	6.88		6.88		6.88	
Log Reduction	5.88		5.88			

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MICROBIOLOGY
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Table 3: Enumeration of the *Candida albicans* after exposure to BioSide HS 5%; 2 ppm test concentration; Temp: 35°C; GBL Sample #: 30808/1; Lot #: 13347

Dilution	Contact Time (Minutes)					
	3 hours		6 hours		Control	
	A	B	A	B	A	B
10^{-1}	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
10^{-2}	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
10^{-3}	>300	>300	>300	>300	TNTC	TNTC
10^{-4}	136	148	56	41	156	141
10^{-5}	12	10	4	4	10	11
Cfu / mL	1.4×10^6	1.5×10^6	5.6×10^5	4.1×10^5	1.6×10^6	1.4×10^6
Ave. cfu/mL	1.5×10^6		4.9×10^5		1.5×10^6	
Log $_{10}$	6.18		5.69			
Log$_{10}$ Control	6.18		6.18		6.18	
Log Reduction	0		0.49			

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Table 4: Enumeration of the *Candida albicans* after exposure to BioSide HS 5% 9 ppm test concentration; Temp: 35°C; GBL Sample #:30808/1; Lot #: 13447

Dilution	Contact Time (Minutes)					
	3 hours		6 hours		Control	
	A	B	A	B	A	B
10^{-1}	0	0	0	0	TNTC	TNTC
10^{-2}	0	0	0	0	TNTC	TNTC
10^{-3}	0	0	0	0	>300	>300
10^{-4}	0	0	0	0	156	141
10^{-5}	0	0	0	0	10	11
Cfu / mL	<10	<10	<10	<10	1.6×10^6	1.4×10^6
Ave. cfu/mL	<10		<10		1.5×10^6	
Log 10	<1		<1			
Log10 Control	6.18		6.18		6.18	
Log Reduction	5.18		5.18			

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Table 5: Enumeration of the *Chlorella vulgaris* after exposure to BioSide HS 5% 2 ppm test concentration; Temp: 35°C; GBL Sample #: 30808/1; Lot #: 13447

	Contact Time (Minutes) Number of Algae / mm^3											
	3 hours				6 hours				Control			
	A		B		A		B		A		B	
Hemocytometer count representing 10^{-2} dilution	202	211	198	206	110	115	106	93	252	251	244	239
	241	236	196	199	121	111	96	112	245	240	248	251
Ave. Algae count	223		200		114		102		247		246	
Algae/mL	2.2×10^8		2.0×10^8		1.2×10^8		1.0×10^8		2.5×10^8		2.5×10^8	
Ave. Algae/mL	2.1×10^8				1.1×10^8				2.5×10^8			
Log $_{10}$	8.32				8.04							
Log_{10} Control	8.40				8.40				8.40			
Log Reduction	0.08				0.36							

Algae/mL = 247 avg. X 10^2 dilution X $10^4 = 2.5 \times 10^8$ **Control A**
Algae/mL = 246 avg. X 10^2 dilution X $10^4 = 2.5 \times 10^8$ **Control B**
Algae/mL = 223 avg. X 10^2 dilution X $10^4 = 2.2 \times 10^8$ **3 hours A**
Algae/mL = 200 avg. X 10^2 dilution X $10^4 = 2.0 \times 10^8$ **3 hours B**
Algae/mL = 114 avg. X 10^2 dilution X $10^4 = 1.1 \times 10^8$ **6 hours A**
Algae/mL = 102 avg. X 10^2 dilution X $10^4 = 1.0 \times 10^8$ **6 hours B**

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Table 6: Enumeration of the *Chlorella vulgaris* after exposure to BioSide HS 5% 9 ppm test concentration; Temp: 35°C; GBL Sample #: 30808/1; Lot #: 13447

	Contact Time (Minutes) Number of Algae / mm^3											
	3 hours				6 hours				Control			
	A		B		A		B		A		B	
Hemocytometer count representing 10^{-2} dilution	0	0	0	0	0	0	0	0	252	251	244	239
	0	0	0	0	0	0	0	0	245	240	248	251
Ave. Algae count	<10		<10		<10		<10		247		246	
Algae/mL	<10		<10		<10		<10		$2.5x\ 10^8$		$2.5\ x\ 10^8$	
Ave. Algae/mL	<10				<10				$2.5\ x\ 10^8$			
Log $_{10}$	<1				<1							
Log_{10} Control	8.40				8.40				8.40			
Log Reduction	7.40				7.40							

Algae/mL = 247 avg. X 10^2 dilution X $10^4 = 2.5 \times 10^8$ **Control A**
Algae/mL = 246 avg. X 10^2 dilution X $10^4 = 2.5 \times 10^8$ **Control B**