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REPORT No.

G-38290

06/22/01

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EDUCATION *Ph.D. Supervised*  
EXPERIENCE *Serving since 1970*  
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LABORATORY REPORT

EXCELLENCE = GIBALTAR

FINAL REPORT

Purchase Order #: 65614  
Sponsor #: 1124  
Enviro Tech Chemical Services, Inc.  
213 Primo Way  
Modesto, CA 95358  
Attn: Mike Harvey

GBL Reference #: 452-33-3185  
GBL-NJ Sample No.: 28441/1.266  
GBL-NC Sample No.: C4688  
Lot #: 01206  
Date Received: 03/07/01  
Date Tested: 06/08/01  
Date Completed: 06/15/01

EPA Food Contact Sanitizer Screening Test on BioSide HS 5%

Description of Test Material

One white plastic bottle with a white plastic screw cap approximately 6" x 2" containing approximately 500 mL of a clear liquid.  
Dilute: 1 ounce/5 gallons (1:640) in 400 ppm hard water  
Screen test against *Campylobacter jejuni*

Conclusion

BioSide HS 5%, killed  $\geq 99.999\%$  of *Campylobacter jejuni* within 30 seconds when diluted 1 ounce/5 gallons (1:640) in 400 ppm AOAC hard water.

Respectfully submitted,  
GIBALTAR LABORATORIES, INC.



Date Written: 6/22/01  
Analyst: 17

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



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1 **Purpose**

To determine whether or not the test materials kills 99.999% of *Campylobacter jejuni* within 30 seconds in a suspension test.

2 **Test System**

*Campylobacter jejuni*; GBL-NC Stock # 223; ATCC # 29428

3 **Disinfectant Tested:** BioSide HS 5%

4 **Test Conditions**

- 4.1 **Contact Time:** 30 seconds and 60 seconds
- 4.2 **Organic Soil:** none
- 4.3 **Test Concentration:** 1 ounce/5 gallons (1:640)
- 4.4 **Test Dilution:** 2 mL Test Material + 1278 mL Diluent
- 4.5 **Diluent:** sterile 400 ppm AOAC hard water
- 4.6 **Test Temperature:** 20 ± 0.2C.

5 **Preparation of Culture:** The organism was grown on Chocolate Agar (CA) under microaerophilic conditions.

6 **Method**

6.1 99 mL of the diluted test material prepared as in section 4.4 was aliquoted into a wide-mouth erlenmeyer flask. The flask was allowed to equilibrate for ≥ 20 minutes in a 20C water bath. In parallel, a flask was prepared as a numbers controls wherein sterile phosphate buffer dilution water was substituted for the 99 mL of test material.

6.2 The efficacy assay was performed by adding 1 mL of the appropriate test system organism to the 99 mL flask as per AOAC 960.09(H). The number of bacteria present in the erlenmeyer flasks was determined after the 30 seconds and 60 seconds contact time. One mL from the erlenmeyer flasks was pipetted into 9 mL of Fluid Thioglycollate Medium (FTM) containing 5% bovine calf serum in 20 x 150 mm glass test tubes. Four – 1 mL and four - 0.1 mL aliquots were individually plated onto CA plates. 1.0 mL from the FTM tube was inoculated into 20 mL FTM tubes. The tubes were incubated for 7 days at 37 ± 1C and the plates were incubated at 37 ± 1C under microaerophilic conditions for 7 days. The typical turbidity in the tubes was observed after incubation and the plates counted for typical colonies.

6.3 The numbers control was performed by adding 1 mL of the test system organism to a wide-mouth erlenmeyer flask containing 99 mL of the sterile phosphate buffer dilution water as per AOAC 960.09(H). The number of bacteria present in the erlenmeyer flask was determined after ≤ 30 seconds. Ten-fold serial dilutions up to 10<sup>-7</sup> were made into 9 mL FTM containing 5% bovine calf serum in 20 x 150 mm glass test tubes. Four – 1 mL and four - 0.1 mL aliquots were individually plated onto CA plates. 1.0 mL from the



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FTM tube was inoculated into 20 mL FTM tubes. The tubes were incubated for 7 days at  $37 \pm 1C$  and the plates were incubated at  $37 \pm 1C$  under microaerophilic conditions for 7 days. The typical turbidity in the tubes was observed after incubation and the plates counted for typical colonies.

**7. Sterility Controls (Neutralizer Broth, AOAC Hard Water and Phosphate Buffer Dilution Water)**

0.1 mL of each was individually pipetted onto Campylobacter agar medium (BBL) containing 10% sheep blood and five antimicrobials and were surface streaked. The plates were then incubated under microaerophilic conditions for 72 hours. The plates were counted using a Quebec colony counter.

**8. Media and Reagents**

- 8.1 Chocolate Agar, Remel Lot # 173442
- 8.2 Phosphate Buffer Stock Solution, Lot # C-517
- 8.3 Phosphate Buffer Dilution Water, Lot # C-496
- 8.4 Fluid Thioglycollate Medium, Lot # E-43
- 8.5 Sterile 400 ppm AOAC Hard Water, Lot # C-543

**Table 1: Results for *Campylobacter jejuni***

	<b>BioSide HS 5%</b>	
	<b>Cfu/mL</b>	<b>Log<sub>10</sub></b>
<b>Numbers Control</b>	$3.7 \times 10^7$	7.57
<b>30 Seconds</b>	<100	<2.0
<b>60 Seconds</b>	<100	<2.0
<b>Log Reduction in 30 Seconds</b>	>5.57 (complete kill)	