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**REPORT No.**  
G-38559.1  
8/9/2001

**EDUCATION** *Ph.D. Supervised*  
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**LABORATORY REPORT**

**EXCELLENCE = GIBRALTAR**

**Study Title**

Food Contact Sanitizer Test on BioSide HS 5%  
Against *Listeria monocytogenes*

**Product Identity**

BioSide HS 5%

**Data Requirement**

EPA Pesticide Assessment Guidelines Subdivision G, 1982, 91-2.

**Author**

Daniel L. Prince, Ph.D.  
President

**Study Completion Date**

July 15, 2001

**Testing Facility**

Gibraltar Laboratories, Inc.  
122 Fairfield Road  
Fairfield, NJ 07004

**Laboratory Project Number (Study File)**

GBL Study # GR 1722

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**1 Purpose**

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

**2 Test System**

*Listeria monocytogenes*; GBL-NJ Login # 106771/11; ATCC # 984

**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: 1 mL Test Material + 639 mL Diluent
- 4.5 Diluent: sterile 400 ppm AOAC hard water
- 4.6 Test Temperature: 20 ± 0.2C.

**5 Preparation of Culture: Organisms were prepared using Brain Heart Infusion Agar (BHIA).**

**6 Method**

99 mL of the diluted test material prepared as in section 4.4 was aliquoted into each of two wide-mouth erlenmeyer flasks. The flasks were allowed to equilibrate for ≥ 20 minutes in a 20± 0.2C water bath. In parallel, two flasks were prepared as numbers controls wherein sterile phosphate buffer dilution water was substituted for the 99 mL of test material. The efficacy assay was performed by adding 1 mL of the appropriate test system organism to the 99 mL flask as per AOAC. The number of bacteria present in the erlenmeyer flasks was determined after the 30 seconds and 60 seconds contact time. Ten-fold serial dilutions were made into 9 mL GBL STAT Broth (GBL STAT Broth = Trypticase Soy Broth containing 4% Tween 20 and 0.5% Azolectin) and pour plates were performed in quadruplicate using BHIA containing neutralizer. Incubation was for 48 hours at 37 ± 1C. The colony forming units were counted using a Quebec colony counter.

The numbers control was performed by adding 1 mL of the appropriate test system organism to duplicate wide-mouth erlenmeyer flasks containing 99 mL of the sterile phosphate buffer dilution water as per AOAC. The number of bacteria present in the erlenmeyer flasks was determined after ≤ 30 seconds. Ten-fold serial dilutions were made into 9 mL GBL STAT Broth. Pour plates were performed in quadruplicate were performed using BHIA. Incubation was for 48 hours at 37 ± 1C. The colony forming units were counted using a Quebec colony counter.



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**7. Sterility Controls**

For neutralizer broth, AOAC hard water and Phosphate buffer dilution water, 1.0 mL of each was individually plated into sterile petri dishes and the plates were poured with BHIA. The plates were incubated at  $37 \pm 1C$  for  $48 \pm 8$  hours. The colony forming units were counted using Quebec colony counter.

**8. Confirmation of Surviving Organisms**

After counting the plates, colonies of the surviving organisms, if any, were selected and subcultured onto respective agar plates and incubated at  $37 \pm 1C$  for 24 to 48 hours to confirm the typical growth.

**9 Media and Reagents**

- 9.1 Brain Heart Infusion Agar, Lot # F-251
- 9.2 Phosphate Buffer Stock Solution, Lot # F-211
- 9.3 Phosphate Buffer Dilution Water, Lot # F-226
- 9.4 GBL STAT Broth, (Trypticase Soy Broth containing 4.0% Tween 20 and 0.5% Azolectin), Lot # F-196
- 9.5 AOAC Hard Water, Lot # C-801

**10 Additional Information**

- 10.1 **Purchase Order #:** NA  
**Sponsor #:** (1124)  
Enviro Tech Chemical Services, Inc.  
213 Primo Way  
Modesto, CA 95358  
**Attn:** Mike Harvey  
**Tel #:** 209/581-9578  
**Fax #:** 209/581-9653  
**GBL Reference #:** 30-527-142  
**GBL-NJ Sample #:** 28545/1-2.262  
**Study Initiation:** 06/29/01  
**Date Received:** 06/19/01  
**Date Tested:** 07/13/01  
**Date Completed:** 07/15/01  
**Protocol No.:** 2290



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**10.2 Description of Test Material**

Two plastic bottles with white plastic screw caps each containing BioSide HS 5%.  
28545/1 = Lot # 01206                      Manufacturing Date: Dec. 12, 2000  
28545/2 = Lot # 10102                      Manufacturing Date: Jan. 2, 2001

**10.3 Statistical Method:** None. Basic arithmetic was used.

**10.4 Records to be Maintained:** The test findings reflected in this experiment will be kept on file for a period of at least five years in the Gibraltar Laboratories archives. Specific records to be maintained include a copy of this report, all raw data, sample information as provided by sponsor, and the findings of the QAU.

**10.5 Other Remarks Pertaining To The Test Material**

**10.5.1 Chemical Nature and Concentration:** Hydrogen Peroxide/Peroxyacetic Acid; Concentration on file at Enviro Tech Chemical Services

**10.5.2 Expiration Date:** Not known by Gibraltar.

**10.5.3 Storage Conditions:** The test materials were stored at ambient room temperature at the testing facility.

**10.5.4 Stability under above Conditions:** Stability and purity are the responsibility of the sponsor.

**10.5.5 Sample Retention:** After all studies are complete the remaining test material, if any, will be discarded or destroyed in accordance with GBL policy and State and Federal regulations.

**10.6 Archive Location:** Gibraltar Laboratories, 122 Fairfield Road, Room 203A, Fairfield, New Jersey 07004-2405; or Guarantee Records Management, 215 Coles Street, Jersey City, New Jersey 07307.

**10.7 Changes from the Approved Protocol:** None.

**10.8 Circumstances**

No circumstances arose during the course of this study which compromised the quality or integrity of the data reported herein.



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**Table 1: Raw Data Results for *Listeria monocytogenes***

Test Substance	Concentration in ppm	Exposure Time	Flask	Plate Counts (CFU/plate)			
				Number Surviving		Microbes Initially Present (Control)	
				Test 0.1 mL (10 <sup>-2</sup> ) in neutralizer	Test 1.0 mL (10 <sup>-1</sup> ) in neutralizer	No. Control 1.0 mL of 10 <sup>-6</sup>	
						Flask A	Flask B
BioSide HS 5% Lot # 01206	78 ppm	30 seconds	Flask A	0,0,0,0	0,0,0,0	92,70, 73,72	83,67, 65,77
			Flask B	0,0,0,0	0,0,0,0		
		60 seconds	Flask A	0,0,0,0	0,0,0,0		
			Flask B	0,0,0,0	0,0,0,0		

**Table 2: Calculated Results for *Listeria monocytogenes* (cfu/mL) by Lot, Exposure, and corresponding Percent and Log<sub>10</sub> Reduction**

Test Substance	Exposure Time	Concentration in ppm	Average Number Surviving (cfu/mL)	Microbes Initially Present (cfu/mL)	Microbes Initially Present (Log <sub>10</sub> )	Log <sub>10</sub> Reduction	Percent Reduction
BioSide HS 5% Lot # 01206	30 seconds	78 ppm	<10	7.5 x 10 <sup>7</sup>	7.87	≥6.87	≥99.999%



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**Table 3: Raw Data Results for *Listeria monocytogenes***

Test Substance	Concentration in ppm	Exposure Time	Flask	Plate Counts (CFU/plate)				
				Number Surviving		Microbes Initially Present (Control)		
				Test 0.1 mL (10 <sup>-2</sup> ) in neutralizer	Test 2.0 mL (10 <sup>-1</sup> ) in neutralizer	No. Control 1.0 mL of 10 <sup>-6</sup>		
						Flask A	Flask B	
BioSide HS 5% Lot # 10102	78 ppm	30 seconds	Flask A	0,0,0,0	0,0,0,0	92,70, 73,72		83,67, 65,77
			Flask B	0,0,0,0	0,0,0,0			
		60 seconds	Flask A	0,0,0,0	0,0,0,0			
			Flask B	0,0,0,0	0,0,0,0			

**Table 4: Calculated Results for *Listeria monocytogenes* (cfu/mL) by Lot, Exposure, and corresponding Percent and Log<sub>10</sub> Reduction**

Test Substance	Exposure Time	Concentration in ppm	Average Number Surviving (cfu/mL)	Microbes Initially Present (cfu/mL)	Microbes Initially Present (Log <sub>10</sub> )	Log <sub>10</sub> Reduction	Percent Reduction
BioSide HS 5% Lot # 10102	30 seconds	78 ppm	<10	7.5 x 10 <sup>7</sup>	7.87	≥6.87	≥99.999%

**Conclusion**

BioSide HS 5%, Lot # 01206 and 10102, killed 99.999% of *Listeria monocytogenes* within 30 seconds when diluted 1ounce/5 gallons (1:640) in 400 ppm AOAC hard water.