



122 FAIRFIELD RD., FAIRFIELD, NJ 07004-2405 • PHONE: [973] 227-6882 • FAX: [973] 227-0812  
e-mail: info@gibraltarlabsinc.com • internet: www.gibraltarlabsinc.com

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Project No.: GR2556  
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**Study Title**

EPA Food Contact Sanitizer Test for Previously Cleaned Food-Contact Surfaces (AOAC Germicidal and Detergent Sanitizing Action of Disinfectants)

**Product Identity**

"Perasan C-5"

mRID

**Data Requirement**

EPA DIS/TSS-4 of January 30, 1979

**Author**

Daniel L. Prince, Ph.D.  
President

**Study Completion Date**

01/23/2009

**Testing Facility**

Gibraltar Laboratories, Inc.  
16 Montesano Road  
Fairfield, NJ 07004

**Laboratory Project Number (Study File)**

GBL Study # GR 2556



## **STUDY METHOD**

### **PREPARATION OF TEST SUBSTANCE AND METHOD**

One mL of the test substance was added to 639 mL of 200 ppm AOAC hard water (volume to volume). 99 mL of water to be used in the test, containing bactericide at the concentration to be tested, was measured into sterile, 250 mL wide-mouth Erlenmeyer flasks and placed in a constant temperature bath until it reached  $25 \pm 0.2C$ , for  $\geq 20$  min. Duplicate flasks were prepared for each germicide to be tested. A similar flask was also prepared containing 99 mL sterile phosphate buffer dilution  $H_2O$ , as "initial numbers" control. One mL of culture suspension was added to each test flask as follows: The flask was whirled, stopping just before suspension was added, creating enough residual motion of liquid to prevent pooling of suspension at the point of contact with the test water. The suspension was added midway between center and edge of surface with tip of pipet slightly immersed in the test solution. Care was taken to avoid touching the pipet to the neck or side of flask during the addition. One mL portions of this exposed culture were added to neutralizer blank exactly 30 and 60 seconds after the addition of the suspension and mixed well immediately after transfer. For test samples, the following dilution procedure was followed: 1 mL-exposed culture was transferred into 9 mL neutralizer broth and vortexes to dislodge adhering organisms. 1 mL and 0.1 mL were plated in **quadruplicate** and poured with Tryptone Glucose Extract Agar (TGEA). Plates were incubated for 48 hours at  $37 \pm 1C$ . The colony forming units were counted using Quebec colony counter.

### **PREPARATION OF TEST SYSTEM/STRAINS**

*Staphylococcus aureus* and *Escherichia coli* were prepared according to the AOAC 18<sup>th</sup> Edition, section 960.09D.

### **EXPOSURE CONDITIONS**

Contact Time: 30 and 60 seconds  
Organic Soil: none  
Test Concentration: 1ounce/ 5gallons (1:640)  
Test Dilution: 1 mL test substance + 639 mL diluent  
Diluent: sterile 200 ppm AOAC hard water  
Test Temperature:  $25 \pm 0.2C$

### **TEST SYSTEM RECOVERY**

For incubation, the organisms will be incubated at  $37 \pm 1C$  and not  $35C$ , as this is an acceptable temperature range for the mesophilic test system organisms for  $48 \pm 8$  hours or longer according to the best judgment of the bacteriologist.

### **PROTOCOL CHANGES**

#### **PROTOCOL AMENDMENTS**

None

#### **PROTOCOL DEVIATIONS**

None

**Table 1: Raw Data Results for *Staphylococcus aureus***

Test Substance	Test Dilution (v/v)	Exposure Time	Flask	Plate Counts (CFU/plate)			
				Number Surviving		Number Controls (10 <sup>6</sup> ) (Microbes Initially Present)	
				10 <sup>-1</sup>	10 <sup>-2</sup>	Flask A	Flask B
"Perasan C-5" Lot # 843-8-1003-1	1 ounce/5gallons (1:640)	30 seconds	Flask A	0,0,0,0	0,0,0,0	8.9 x 10 <sup>7</sup> 8.6 x 10 <sup>7</sup> 9.2 x 10 <sup>7</sup> 9.1 x 10 <sup>7</sup>	8.8 x 10 <sup>7</sup> 9.3 x 10 <sup>7</sup> 9.3 x 10 <sup>7</sup> 8.6 x 10 <sup>7</sup>
			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		
"Perasan C-5" Lot # 843-8-1203-1	1 ounce/5gallons (1:640)	30 seconds	Flask A	0,0,0,0	0,0,0,0		
			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		
"Perasan C-5" Lot # 843-8-1203-2	1 ounce/5gallons (1:640)	30 seconds	Flask A	0,0,0,0	0,0,0,0		
			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		
						Avg. of Flask A and B 9.0 x 10 <sup>7</sup> = 7.95 Log	

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

Test Substance / Lot #	Exposure Time	Test Dilution (v/v)	Average Number Surviving (cfu/mL)	Microbes Initially Present (cfu/mL)	Microbes Initially Present (Log <sub>10</sub> )	Log <sub>10</sub> Reduction	Percent Reduction
"Perasan C-5" Lot # 843-8-1003-1	30 seconds	1 ounce/5gallons (1:640)	<10	9.0 x 10 <sup>7</sup>	7.95	≥6.95	>99.999%
"Perasan C-5" Lot # 843-8-1203-1	30 seconds	1 ounce/5gallons (1:640)	<10	9.0 x 10 <sup>7</sup>	7.95	≥6.95	>99.999%
"Perasan C-5" Lot # 843-8-1203-2	30 seconds	1 ounce/5gallons (1:640)	<10	9.0 x 10 <sup>7</sup>	7.95	≥6.95	>99.999%



**Table 3: Raw Data Results for *Escherichia coli***

Test Substance	Test Dilution (v/v)	Exposure Time	Flask	Plate Counts (CFU/plate)			
				Number Surviving		Number Controls (10 <sup>6</sup> ) (Microbes Initially Present)	
				10 <sup>-1</sup>	10 <sup>-2</sup>	Flask A	Flask B
"Perasan C-5" Lot # 843-8-1003-1	1 ounce/5gallons (1:640)	30 seconds	Flask A	0,0,0,0	0,0,0,0	1.1 x 10 <sup>8</sup>	1.1 x 10 <sup>8</sup>
			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		
"Perasan C-5" Lot # 843-8-1203-1	1 ounce/5gallons (1:640)	30 seconds	Flask A	0,0,0,0	0,0,0,0		
			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		
"Perasan C-5" Lot # 843-8-1203-2	1 ounce/5gallons (1:640)	30 seconds	Flask A	0,0,0,0	0,0,0,0		
			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		
						Avg. of Flask A and B 1.1 x 10 <sup>8</sup> = 8.04 Log	

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

Test Substance / Lot #	Exposure Time	Test Dilution (v/v)	Average Number Surviving (cfu/mL)	Microbes Initially Present (cfu/mL)	Microbes Initially Present (Log <sub>10</sub> )	Log <sub>10</sub> Reduction	Percent Reduction
"Perasan C-5" Lot # 843-8-1003-1	30 seconds	1 ounce/5gallons (1:640)	<10	1.1 x 10 <sup>7</sup>	8.04	≥7.04	>99.999%
"Perasan C-5" Lot # 843-8-1203-1	30 seconds	1 ounce/5gallons (1:640)	<10	1.1 x 10 <sup>7</sup>	8.04	≥7.04	>99.999%
"Perasan C-5" Lot # 843-8-1203-2	30 seconds	1 ounce/5gallons (1:640)	<10	1.1 x 10 <sup>7</sup>	8.04	≥7.04	>99.999%