



## APPENDIX VII

Total number of pages = 20

**Test for Adequacy of Neutralizer**

## TEST FOR ADEQUACY OF THE NEUTRALIZER

### 1.0 OBJECTIVE

To determine an appropriate antimicrobial neutralizer system for use in a Health Care Personnel Handwash study, HTR Study No. 03-122085-106.

### 2.0 TEST ARTICLES

The test articles, HTR Code A and HTR Code B were received by Hill Top Research, Inc. on July 9, 2003, for use in this study. Test article, HTR Code C, was purchased by Hill Top Research, Inc. on March 6, 2003.

<u>HTR CODE</u>	<u>SPONSOR CODE</u>	<u>DESCRIPTION</u>
A	Test Formulation: 3554-194	clear to slightly cloudy liquid
B	Test Formulation: 3554-196	clear to slightly cloudy liquid
C	Hibiclens®, Lot 4652F, Exp.: 01/2004	Aqua plastic bottle with white cap, containing liquid

### 3.0 PROCEDURE

The procedure used to determine the efficacy of the neutralizer system was Standard Test Method for Evaluation of Inactivators of Antimicrobial Agents ASTM Standard E1054-2, "Neutralization Assay with Recovery on Solid Medium".

Prior to washing with the test articles, subjects performed a conditioning wash using Johnson and Johnson *head to toe* according to the protocol directions. The subjects then washed their hands eleven times, three using HTR Code A and three using HTR Code B, and three using HTR Code C according to the protocol directions. The subjects' hands were sampled after treatment 1, using stripping fluid with neutralizer<sup>1</sup> according to the protocol directions and samples were discarded. Following the sampling after treatment 1, the subjects' hands were rinsed with tap water and dried. The subjects also dried after treatments 2 through 10 with at least five minutes elapsing between treatments. One hand of each subject was sampled after the 11<sup>th</sup> treatment using stripping fluid with neutralizer and the other hand was sampled using stripping fluid without neutralizer<sup>2</sup>.

### 3.0 PROCEDURE (CONT.)

#### TEST A Test for Neutralizer Effectiveness

Aliquots from the subjects' stripping fluid containing neutralizer were removed as follows and used to test the adequacy of the neutralizer: a 10.0 mL aliquot of the stripping fluid with neutralizer was removed and placed in a sterile tube. An additional 1.0 mL aliquot of the stripping fluid with neutralizer was added to a tube containing 9.0 mL of dilution fluid with neutralizer<sup>3</sup>.

A 0.1 mL aliquot of diluted broth culture of *Staphylococcus aureus* ATCC 6538 was added to each of the prepared tubes. The diluted culture was a  $24 \pm 4$  hour Tryptic Soy Broth<sup>4</sup> culture of *S. aureus* ATCC 6538 serially diluted to  $10^{-5}$  in 0.9% saline<sup>5</sup>.

In addition a replicate set of test article HTR Code C tubes was challenged with *Serratia marcescens* ATCC 14756. A 0.1 mL aliquot of diluted *S. marcescens* ATCC 14756 was added to each of the prepared tubes of test article HTR Code C. The diluted culture was a  $24 \pm 4$  hour Tryptic Soy Broth<sup>4</sup> culture of *S. marcescens* ATCC 14756 serially diluted to  $10^{-5}$  in 0.9% saline<sup>5</sup>.

After mixing, a 1.0 mL aliquot from each inoculated tube was surface plated immediately and again at 30 minutes by distributing the 1.0 mL across three Tryptic Soy Agar<sup>6</sup> plates in duplicate. The plates were incubated at  $25 \pm 2^\circ\text{C}$  for  $48 \pm 4$  hours. After incubation, the numbers of colony forming units resembling the challenge organisms were enumerated.

#### TEST B Neutralizer Toxicity Control

A neutralizer toxicity control tube was prepared containing 10.0 mL stripping fluid with neutralizer.

#### TEST C Test Organism Viability

A test organism viability control (numbers control) tube was prepared containing 10.0 mL 0.9% saline.

#### TEST D Test Material Control

This control is required to demonstrate that the neutralizer actually did neutralize the activity of an antimicrobial agent.

A 10.0 mL aliquot of stripping fluid without neutralizer was removed from each subject's glove and placed in a tube.

### 3.0 PROCEDURE (CONT.)

The control tubes for B. Neutralizer Toxicity Control, C. Test Organism Viability and D. Test Material Control were inoculated, plated, incubated, and enumerated in the same manner as the tubes prepared for A. Test for Neutralizer Effectiveness.

### 4.0 RESULTS

Table 1A shows the plate counts obtained for the challenge with *S. aureus* ATCC 6538 and Table 1B for the challenge with *S. marcescens* ATCC 14756.

### 5.0 CONCLUSIONS

The neutralizer system is considered effective if Test A recovery population is statistically different from the test organisms viability population (Test C) and if the Test D recovery population is statistically less than the test organism viability population (Test C).

The ASTM Method indicates that if the difference between two comparative means is  $\leq 0.2 \log_{10}$  then the variability of the data is smaller than what is required to detect a minimal difference detectable by the prescribed statistical test.

As the  $\log_{10}$  differences between comparatives means A vs. C, B vs. C, and D vs. C were in all cases  $< 0.2 \log_{10}$  it can be concluded that there was no significant difference between the comparative means, because the variability was small and the difference between the comparative means was less than the minimal difference to be detected.

In this study the neutralizer system adequately neutralized the test products and the neutralizer system was found to be non-toxic.

The data analysis is presented in Table 2A for the challenge with *S. aureus* ATCC 6538 and Table 2B for the challenge with *S. marcescens* ATCC 14756.

**REFERENCES**

1. **Stripping Fluid with Neutralizer**  
The stripping fluid with neutralizer used for sampling contained 0.4 g  $\text{KH}_2\text{PO}_4$ , 10.1 g  $\text{Na}_2\text{HPO}_4$ , 3.0 g Lecithin, 10.0g Tween 80, and 1.0 g Triton X-100 in one liter purified water. The pH was adjusted to  $7.8 \pm 0.1$  prior to dispensing into water dilution bottles, or other suitable containers, to yield a final volume of  $75 \pm 1.0$  mL after autoclaving at  $121^\circ\text{C}$ .
2. **Stripping Fluid without Neutralizer**  
The stripping fluid with neutralizer used for sampling contained 0.4 g  $\text{KH}_2\text{PO}_4$ , 10.1 g  $\text{Na}_2\text{HPO}_4$ , and 1.0 g Triton X-100 in one liter purified water. The pH was adjusted to  $7.8 \pm 0.1$  prior to dispensing into water dilution bottles, or other suitable containers, to yield a final volume of  $75 \pm 1.0$  mL after autoclaving at  $121^\circ\text{C}$ .
3. **Dilution Fluid: Butterfield's Phosphate Buffer Water with Neutralizer**  
The dilution fluid contained 1.25 mL AOAC Phosphate Buffer Stock\*, 10.0 g Tween 80, and 3.0 g Lecithin in one liter purified water. The pH was adjusted to  $7.2 \pm 0.2$  prior to dispensing into tubes, to yield a final volume of  $9.0 \pm 0.1$  mL after autoclaving at  $121^\circ\text{C}$ .
4. **Tryptic Soy Broth**  
The broth consisted of 30.0 g Tryptic Soy Broth powder in one liter purified water. The pH was  $7.3 \pm 0.2$ . The media was dispensed into tubes and sterilized by autoclaving at  $121^\circ\text{C}$ .
5. **0.9% Saline**  
The saline contained 9.0 g NaCl in one liter purified water. The material was dispensed into tubes to yield a final volume of  $9.0 \pm 0.1$  mL after autoclaving at  $121^\circ\text{C}$ .
6. **Tryptic Soy Agar**  
The plating medium contained 40.0 g Tryptic Soy Agar powder in one liter purified water. The pH was  $7.3 \pm 0.2$ . The media was autoclaved at  $121^\circ\text{C}$ . After autoclaving and tempering, the media was aseptically dispensed into sterile Petri dishes, approximately 18 - 20 mL per plate.

\*The AOAC Phosphate Buffer Stock contained 34.0 g  $\text{KH}_2\text{PO}_4$  in one liter purified water. The pH was adjusted to  $7.2 \pm 0.1$  prior to dispensing into bottles, to yield a final volume of approximately 100 mL after autoclaving at  $121^\circ\text{C}$ .

**Note:** Recipes which are given as liter volumes may be prepared in greater or lesser volumes.

TABLE 1A

NEUTRALIZATION RESULTS

*Staphylococcus aureus* ATCC 6538

Neutralization of Product

ASTM TEST*	ARTICLE	TIME	PLATE COUNTS				Avg CFU/mL
			Duplicate	1.0 mL sample over 3 plates			
				Plate 1	Plate 2	Plate 3	
A Neutralizer Efficacy	HTR Code A-1 10mL Stripping Fluid w/ Neut.	immediate	1	8	10	15	3.6x10 <sup>1</sup>
			2	12	18	10	
		30 min	1	17	14	16	4.4x10 <sup>1</sup>
			2	17	12	13	
	HTR Code A-2 10mL Stripping Fluid w/ Neut.	immediate	1	2	4	3	9.0x10 <sup>0</sup>
			2	2	3	4	
		30 min	1	5	4	2	1.2x10 <sup>1</sup>
			2	6	4	4	
	HTR Code A-3 10mL Stripping Fluid w/ Neut.	immediate	1	3	2	0	7.0x10 <sup>0</sup>
			2	4	2	3	
		30 min	1	2	3	5	1.0x10 <sup>1</sup>
			2	3	3	4	
A Neut Efficacy	HTR Code A-1 1mL Stripping Fluid w/ Neut into 9mL Dil. Fluid w/ Neut.	immediate	1	8	8	4	1.6x10 <sup>1</sup>
			2	1	4	6	
		30 min	1	6	7	4	1.4x10 <sup>1</sup>
			2	3	3	4	
	HTR Code A-2 1mL Stripping Fluid w/ Neut into 9mL Dil. Fluid w/ Neut.	immediate	1	2	3	2	1.0x10 <sup>1</sup>
			2	3	5	5	
		30 min	1	6	5	2	1.2x10 <sup>1</sup>
			2	1	5	4	
	HTR Code A-3 1mL Stripping Fluid w/ Neut. into 9mL Dil. Fluid w/ Neut.	immediate	1	2	3	2	6.5x10 <sup>0</sup>
			2	2	3	1	
		30 min	1	4	0	2	1.0x10 <sup>1</sup>
			2	2	8	4	
A Neut Efficacy	HTR Code B-1 10mL Stripping Fluid w/ Neut.	immediate	1	2	2	1	8.0x10 <sup>0</sup>
			2	4	6	1	
		30 min	1	5	7	10	1.5x10 <sup>1</sup>
			2	3	3	2	
	HTR Code B-2 10mL Stripping Fluid w/ Neut.	immediate	1	2	6	5	1.1x10 <sup>1</sup>
			2	5	2	2	
		30 min	1	4	8	6	1.4x10 <sup>1</sup>
			2	0	4	5	
	HTR Code B-3 10mL Stripping Fluid w/ Neut.	immediate	1	2	4	4	1.0x10 <sup>1</sup>
			2	4	4	3	
		30 min	1	2	3	3	9.0x10 <sup>0</sup>
			2	3	5	2	

NA= Not applicable

Counted by: JNB/08-07-03

Raw Data Reviewed BY/Date: SA/8-8-03

Calculated By/Date:

JNB/08-08-03

Calculations  
Checked By/Date:

SA/8-8-03

TABLE 1A

NEUTRALIZATION RESULTS

*Staphylococcus aureus* ATCC 6538

Test Material Control

TM ICST*	ARTICLE	TIME	PLATE COUNTS				Avg CFU/mL
			1.0mL sample over 3 plates				
			Duplicate	Plate1	Plate 2	Plate 3	
D Test Material Control	HTR Code A-1 10.0mL Stripping Fluid w/o Neut.	immediate	1	2	0	3	6.0x10 <sup>0</sup>
			2	4	2	1	
		30 min	1	0	1	0	3.0x10 <sup>0</sup>
			2	0	3	2	
	HTR Code A-2 10.0mL Stripping Fluid w/o Neut.	immediate	1	2	0	1	4.5x10 <sup>0</sup>
			2	2	4	0	
		30 min	1	2	6	3	9.5x10 <sup>0</sup>
			2	5	2	1	
	HTR Code A-3 10.0mL Stripping Fluid w/o Neut.	immediate	1	3	4	3	8.5x10 <sup>0</sup>
			2	2	2	3	
		30 min	1	4	1	2	7.5x10 <sup>0</sup>
			2	4	2	2	
D Test Material Control	HTR Code B-1 10.0mL Stripping Fluid w/o Neut.	immediate	1	0	0	0	<1.0x10 <sup>0</sup>
			2	0	0	0	
		30 min.	1	0	0	0	<1.0x10 <sup>0</sup>
			2	0	0	0	
	HTR Code B-2 10.0mL Stripping Fluid w/o Neut.	immediate	1	0	0	0	<1.0x10 <sup>0</sup>
			2	0	0	0	
		30 min	1	0	0	1	<1.0x10 <sup>0</sup>
			2	0	0	0	
	HTR Code B-3 10.0mL Stripping Fluid w/o Neut.	immediate	1	1	0	1	1.5x10 <sup>0</sup>
			2	1	0	0	
		30 min	1	0	0	1	2.0x10 <sup>0</sup>
			2	0	0	3	
D Test Material Control	HTR Code C-1 10.0mL Stripping Fluid w/o Neut.	immediate	1	0	0	0	<1.0x10 <sup>0</sup>
			2	0	0	1	
		30 min	1	0	1	0	<1.0x10 <sup>0</sup>
			2	0	0	0	
	HTR Code C-2 10.0mL Stripping Fluid w/o Neut.	immediate	1	0	1	0	<1.0x10 <sup>0</sup>
			2	0	0	0	
		30 min	1	0	0	0	<1.0x10 <sup>0</sup>
			2	0	0	0	
	HTR Code C-3 10.0mL Stripping Fluid w/o Neut.	immediate	1	0	0	0	<1.0x10 <sup>0</sup>
			2	0	0	0	
		30 min	1	0	0	0	<1.0x10 <sup>0</sup>
			2	0	0	0	

NA= Not applicable

Counted by: JNB | 08-07-03

Raw Data Reviewed BY/Date: AVR 8-14

Calculated By/Date:

JNB | 08-08-03

Calculations

Checked By/Date:

SRH | 8-8-03

TABLE 1A

NEUTRALIZATION RESULTS

*Staphylococcus aureus* ATCC 1538 Neutralizer Toxicity & Numbers Control

ASTM TEST	ARTICLE	TIME	PLATE COUNTS				Avg CFU/mL		
			Duplicate	1.0 mL sample over 3 plates					
				Plate 1	Plate 2	Plate 3			
B Neutralizer Toxicity	Toxicity - 1 1.0mL Stripping Fluid w/Neut. into 9.0mL Dilution Fluid w/Neut.	immediate	1	3	5	3	8.5x10 <sup>0</sup>		
			2	1	4	1			
		30 min	1	3	10	2		1.2x10 <sup>1</sup>	
			2	2	6	2			
		Toxicity - 2 1.0mL Stripping Fluid w/ Neut. into 9.0mL Dilution Fluid w/Neut.	immediate	1	1	5		5	1.1x10 <sup>1</sup>
				2	5	4		2	
	30 min		1	3	4	7	1.4x10 <sup>1</sup>		
			2	3	3	7			
	Toxicity - 3 1.0mL Stripping Fluid w/Neut. into 9.0mL Dilution Fluid w/Neut.		immediate	1	7	5	3	1.5x10 <sup>1</sup>	
				2	8	4	3		
		30 min	1	0	4	1			
			2	3	6	9			

B Neutralizer Toxicity		immediate	1						
			2						
		30 min	1						
			2						
			immediate	1					
				2					
	30 min		1						
			2						
		immediate	1						
			2						
		30 min	1						
			2						

C Numbers Control	# - 1 10.0mL Saline	immediate	1	5	4	9	1.8x10 <sup>1</sup>		
			2	3	7	7			
		30 min	1	2	6	5		1.3x10 <sup>1</sup>	
			2	5	6	2			
		# - 2 10.0mL Saline	immediate	1	1	1		3	7.0x10 <sup>0</sup>
				2	3	3		3	
	30 min		1	4	3	6	1.2x10 <sup>1</sup>		
			2	5	2	4			
	# - 3 10.0mL Saline		immediate	1	9	6	6	1.7x10 <sup>1</sup>	
				2	7	0	6		
		30 min	1	5	6	2			
			2	4	11	2			

Counted by: JNB 08.07.03

NA= Not applicable

Raw Data Reviewed BY/Date: SLH 8-14

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JNB / 08.08.03

Calculations

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SLH / 8.8.03

TABLE 1A

NEUTRALIZATION RESULTS

*Staphylococcus aureus* 6538

Neutralization of Product

ASTM TEST*	ARTICLE	TIME	PLATE COUNTS				Avg CFI	
			Duplicate	1.0 mL sample over 3 plates				
				Plate 1	Plate 2	Plate 3		
A Neutralizer Efficacy	HTR Code B-1 1.0mL Stripping Fluid w/ Neut. into 9.0mL Dil. Fluid w/ Neut.	immediate	1	2	3	3	8.0x10 <sup>6</sup>	
			2	4	3	1		
		30 min	1	3	2	2	1.2x10 <sup>1</sup>	
			2	7	7	4		
	HTR Code B-2 1.0mL Stripping Fluid w/ Neut. into 9.0mL Dilution Fluid w/ Neut.	immediate	1	5	5	4	1.3x10 <sup>1</sup>	
			2	1	3	8		
		30 min	1	3	7	6	1.6x10 <sup>1</sup>	
			2	4	2	10		
	HTR Code B-3 1.0mL Stripping Fluid w/ Neut. into 9.0mL Dilution Fluid w/ Neut.	immediate	1	2	3	3	8.0x10 <sup>0</sup>	
			2	1	6	1		
		30 min	1	7	6	19	2.0x10 <sup>0</sup>	
			2	2	3	3		
* @ 4 errors JNB 08-07-03								
A Neut Efficacy	HTR Code C-1 10.0mL Stripping Fluid w/ Neut.	immediate	1	2	4	* + 5	9.5x10 <sup>0</sup>	
			2	2	3	3		
		30 min	1	* 2	5	3	* 2	1.2x10 <sup>1</sup>
			2	1	* + 7	4		
	HTR Code C-2 10.0mL Stripping Fluid w/ Neut.	immediate	1	13	15	12	3.8x10 <sup>1</sup>	
			2	10	12	14		
		30 min	1	9	5	7	1.6x10 <sup>1</sup>	
			2	3	5	4		
	HTR Code C-3 10.0mL Stripping Fluid w/ Neut.	immediate	1	2	4	1	6.5x10 <sup>0</sup>	
			2	2	3	1		
		30 min	1	2	3	2	9.0x10 <sup>0</sup>	
			2	1	6	4		
A Neut Efficacy	HTR Code C-1 1.0mL Stripping Fluid w/ Neut. into 9.0mL Dilution Fluid w/ Neut.	immediate	1	7	5	5	1.0x10 <sup>0</sup>	
			2	1	3	0		
		30 min	1	11	6	6	1.6x10 <sup>0</sup>	
			2	3	5	2		
	HTR Code C-2 1.0mL Stripping Fluid w/ Neut. into 9.0mL Dilution Fluid w/ Neut.	immediate	1	15	17	9	4.0x10 <sup>1</sup>	
			2	19	11	10		
		30 min	1	24	18	15	5.8x10 <sup>1</sup>	
			2	19	14	25		
	HTR Code C-3 1.0mL Stripping Fluid w/ Neut. into 9.0mL Dilution Fluid w/ Neut.	immediate	1	7	10	6	1.9x10 <sup>1</sup>	
			2	6	7	2		
		30 min	1	3	6	9	1.4x10 <sup>1</sup>	
			2	2	5	4		

JA= Not applicable

Counted by: JNB 08-07-03

Raw Data Reviewed BY/Date: SRH

Calculated By/Date:

JNB | 08-08-03

Calculations

Checked By/Date:

SRH /

TABLE 1B

NEUTRALIZATION RESULTS

*Serratia marcescens* ATCC 14756

Neutralization of Product

ASTM TEST*	ARTICLE	TIME	PLATE COUNTS				Avg CFI
			Duplicate	1.0mL sample over 3 plates			
				Plate1	Plate 2	Plate 3	
9A Neutralizer Efficacy	HTR Code C-1 10mL Stripping Fluid w/ Neut.	immediate	1	70	63	68	2.1x10 <sup>2</sup>
			2	66	84	64	
		30 min	1	59	59	60	1.8x10 <sup>2</sup>
			2	64	74	54	
	HTR Code C-2 10 mL Stripping Fluid w/ Neut.	immediate	1	72	57	34	1.8x10 <sup>2</sup>
			2	56	62	66	
		30 min	1	68	125	76	2.4x10 <sup>2</sup>
			2	72	73	67	
	HTR Code C-3 10mL Stripping Fluid w/ Neut.	immediate	1	37	53	60	1.7x10 <sup>2</sup>
			2	69	76	56	
		30 min	1	71	66	64	2.1x10 <sup>2</sup>
			2	89	64	66	
A Neut Efficacy	HTR Code C-1 1.0mL Stripping Fluid w/ Neut. into 9.0mL Dilution Fluid w/ Neut.	immediate	1	65	63	62	1.8x10 <sup>2</sup>
			2	49	59	66	
		30 min	1	58	75	78	1.9x10 <sup>2</sup>
			2	72	53	47	
	HTR Code C-2 1.0mL Stripping Fluid w/ Neut. into 9.0mL Dilution Fluid w/ Neut.	immediate	1	68	64	48	1.7x10 <sup>2</sup>
			2	64	55	44	
		30 min	1	43	58	64	2.0x10 <sup>2</sup>
			2	124	62	47	
	HTR Code C-3 1.0mL Stripping Fluid w/ Neut. into 9.0mL Dilution Fluid w/ Neut.	immediate	1	193	61	54	2.5x10 <sup>2</sup>
			2	76	55	59	
		30 min	1	65	78	59	2.0x10 <sup>2</sup>
			2	67	55	75	
A Neut Efficacy	NA	immediate	1				
			2				
		30 min	1				
			2				
		immediate	1				
			2				
		30 min	1				
			2				
		immediate	1				
			2				
		30 min	1				
			2				

A = Not applicable

Counted by: JNB

08-06-03

Raw Data Reviewed BY/Date: JM

Calculations

TABLE 1B

NEUTRALIZATION RESULTS

*Serratia marcescens* ATCC 14756

Neutralizer Toxicity & Numbers Control

ASTM TEST	ARTICLE	TIME	PLATE COUNTS				Avg CFU/r		
			Duplicate	mL sample over 3 plates					
				Plate 1	Plate 2	Plate 3			
B Neutralizer Toxicity	Toxicity -1 1.0mL Stripping Fluid w/ Neut. into 9.0mL Dilution Fluid w/ Neut.	immediate	1	55	82	298 <sup>A</sup>	3.2x10 <sup>2</sup>		
			2	68	71	67			
		30 min	1	61	70	71		1.8x10 <sup>2</sup>	
			2	46	62	55			
		Toxicity -2 1.0mL Stripping Fluid w/ Neut. into 9.0mL Dilution Fluid w/ Neut.	immediate	1	54	48		56	2.0x10 <sup>2</sup>
				2	63	75		109	
	30 min		1	46	54	58	1.6x10 <sup>2</sup>		
			2	53	50	63			
	Toxicity -3 1.0mL Stripping Fluid w/ Neut. into 9.0mL Dilution Fluid w/ Neut.		immediate	1	63	55	64	1.9x10 <sup>2</sup>	
				2	64	63	70		
		30 min	1	62	65	67			
			2	56	63	53			

B Neutralizer Toxicity	NA	immediate	1					
			2					
		30 min	1					
			2					
		NA	immediate	1				
				2				
	30 min		1					
			2					
	NA	immediate	1					
			2					
		30 min	1					
			2					

C Numbers Control	#-1 10.0mL Saline	immediate	1	72	67	63	1.9x10 <sup>2</sup>		
			2	71	56	50			
		30 min	1	72	67	93		2.0x10 <sup>2</sup>	
			2	61	56	60			
		#-2 10.0mL Saline	immediate	1	68	71		68	1.9x10 <sup>2</sup>
				2	56	64		62	
	30 min		1	64	71	71	1.9x10 <sup>2</sup>		
			2	60	53	59			
	#-3 10.0mL Saline		immediate	1	65	55	63	1.8x10 <sup>2</sup>	
				2	71	64	52		
		30 min	1	72	58	66			
			2	57	64	58			

©SAS 8-6-03  
 NA= Not applicable  
 Counted By/Date: SAS/8-6-03  
 Calculated by/Date:

JNB/080803

Raw Data Reviewed BY/Date: ALZ  
 Calculations  
 Checked By/Date: CEL/B.

TABLE 1B

NEUTRALIZATION RESULTS

*Serratia marcescens* ATCC 14756

Test Material Control

TM TEST*	ARTICLE	TIME	PLATE COUNTS				Avg CFU/r
			Duplicate	mL sample over 3 plates			
				Plate 1	Plate 2	Plate 3	
D Test Material Control	HTR Code C-1 10.0mL Stripping Fluid w/o Neut.	immediate	1	61	73	54	
			2	67	64	75	
		30 min	1	25	31	25	8.4x10 <sup>1</sup>
			2	31	32	23	
	HTR Code C-2 10.0mL Stripping Fluid w/o Neut.	immediate	1	72	69	63	1.9x10 <sup>2</sup>
			2	53	59	62	
		30 min	1	25	29	25	8.4x10 <sup>1</sup>
			2	20	40	29	
	HTR Code C-3 10.0mL Stripping Fluid w/o Neut.	immediate	1	35	23	34	8.8x10 <sup>1</sup>
			2	25	34	24	
		30 min	1	8	5	7	2.0x10 <sup>1</sup>
			2	5	8	7	

D Test Material Control		immediate	1				
			2				
		30 min	1				
			2				
	N/A	immediate	1				
			2				
		30 min	1				
			2				
		immediate	1				
			2				
		30 min	1				
			2				

D Test Material Control		immediate	1				
			2				
		30 min	1				
			2				
	N/A	immediate	1				
			2				
		30 min	1				
			2				
		immediate	1				
			2				
		30 min	1				
			2				

NA= Not applicable

Counted By/Date: EAS 8-6-03

Raw Data Reviewed BY/Date: AKZ

Calculations  
Checked By/Date:

TRR/08.08.02

CLL/R-S

TABLE 2A

ANALYSIS OF NEUTRALIZATION STUDY DATA

Test organism: *Staphylococcus aureus* ATCC 6538

ASTM Test	Product Code	Replicate	Sample Time Min	Number of Survivors	
				CFU/mL	Log Cfu/ML
A	HTR Code A in 10 ml Stripping fluid w/ neutralizer	1	0	3.60E+01	1.5563
		2	0	9.00E+00	0.9542
		3	0	7.00E+00	0.8451
		1	30	4.40E+01	1.6435
		2	30	1.20E+01	1.0792
		3	30	1.00E+01	1.0000
B	Toxicity	1	0	8.50E+00	0.9294
		2	0	1.10E+01	1.0414
		3	0	1.50E+01	1.1761
		1	30	1.20E+01	1.0792
		2	30	1.40E+01	1.1461
		3	30	1.20E+01	1.0792
C	Numbers Control	1	0	1.80E+01	1.2553
		2	0	7.00E+00	0.8451
		3	0	1.70E+01	1.2304
		1	30	1.30E+01	1.1139
		2	30	1.20E+01	1.0792
		3	30	1.50E+01	1.1761
D	HTR Code A in 10 ml Stripping fluid w/o neutralizer	1	0	6.00E+00	0.7782
		2	0	4.50E+00	0.6532
		3	0	8.50E+00	0.9294
		1	30	3.00E+00	0.4771
		2	30	9.50E+00	0.9777
		3	30	7.50E+00	0.8751

Mean  
A 1.1797 6  
C 1.1167 6  
Diff 0.0630

Mean  
B 1.0752 6  
C 1.1167 6  
Diff -0.0414

Mean  
D 0.7818 6  
C 1.1167 6  
Diff -0.3349

TABLE 2A

ANALYSIS OF NEUTRALIZATION STUDY DATA

Test organism: *Staphylococcus aureus* ATCC 6538

ASTM Test	Product Code	Replicate	Sample Time Min	Number of Survivors	
				CFU/mL	Log Cfu/ML
A	HTR Code A in 1.0 ml Stripping Fluid w/ neutralizer +9.0mL of Diln. Fluidw/ neutraliz	1	0	1.60E+01	1.2041
		2	0	1.00E+01	1.0000
		3	0	6.50E+00	0.8129
		1	30	1.40E+01	1.1461
		2	30	1.20E+01	1.0792
		3	30	1.00E+01	1.0000
B	Toxicity	1	0	8.50E+00	0.9294
		2	0	1.10E+01	1.0414
		3	0	1.50E+01	1.1761
		1	30	1.20E+01	1.0792
		2	30	1.40E+01	1.1461
		3	30	1.20E+01	1.0792
C	Numbers Control	1	0	1.80E+01	1.2553
		2	0	7.00E+00	0.8451
		3	0	1.70E+01	1.2304
		1	30	1.30E+01	1.1139
		2	30	1.20E+01	1.0792
		3	30	1.50E+01	1.1761

Mean  
A 1.0404 n 6  
C 1.1167 6  
Diff -0.0763

Mean  
B 1.0752 n 6  
C 1.1167 6  
Diff -0.0414

TABLE 2A

ANALYSIS OF NEUTRALIZATION STUDY DATA

Test organism: *Staphylococcus aureus* ATCC 6538

ASTM Test	Product Code	Replicate	Sample Time Min	Number of Survivors	
				CFU/mL*	Log Cfu/ML
A	HTR Code B in 10 ml Stripping fluid w/ neutralizer	1	0	8.00E+00	0.9031
		2	0	1.10E+01	1.0414
		3	0	1.00E+01	1.0000
		1	30	1.50E+01	1.1761
		2	30	1.40E+01	1.1461
		3	30	9.00E+00	0.9542
B	Toxicity Control	1	0	8.50E+00	0.9294
		2	0	1.10E+01	1.0414
		3	0	1.50E+01	1.1761
		1	30	1.20E+01	1.0792
		2	30	1.40E+01	1.1461
		3	30	1.20E+01	1.0792
C	Numbers Control	1	0	1.80E+01	1.2553
		2	0	7.00E+00	0.8451
		3	0	1.70E+01	1.2304
		1	30	1.30E+01	1.1139
		2	30	1.20E+01	1.0792
		3	30	1.50E+01	1.1761
D	HTR Code B in 10 ml Stripping fluid w/o neutralizer	1	0	1.00E+00	0.0000
		2	0	1.00E+00	0.0000
		3	0	1.50E+00	0.1761
		1	30	1.00E+00	0.0000
		2	30	1.00E+00	0.0000
		3	30	2.00E+00	0.3010

Mean  
A 1.0368 n 6  
C 1.1167 6  
Diff -0.0798

Mean  
B 1.0752 n 6  
C 1.1167 6  
Diff -0.0414

Mean  
D 0.0795 n 6  
C 1.1167 6  
Diff -1.0372

\*Values <1.0 are entered as 1.0

TABLE 2A

ANALYSIS OF NEUTRALIZATION STUDY DATA

Test organism: *Staphylococcus aureus* ATCC 6538

ASTM Test	Product Code	Replicate	Sample Time Min	Number of Survivors	
				CFU/mL	Log Cfu/ML
A	HTR Code B in 1.0 ml Stripping Fluid w/ neutralizer +9.0mL of Diln. Fluidw/ neutraliz	1	0	8.00E+00	0.9031
		2	0	1.30E+01	1.1139
		3	0	8.00E+00	0.9031
		1	30	1.20E+01	1.0792
		2	30	1.60E+01	1.2041
		3	30	2.00E+01	1.3010
B	Toxicity	1	0	8.50E+00	0.9294
		2	0	1.10E+01	1.0414
		3	0	1.50E+01	1.1761
		1	30	1.20E+01	1.0792
		2	30	1.40E+01	1.1461
		3	30	1.20E+01	1.0792
C	Numbers	1	0	1.80E+01	1.2553
		2	0	7.00E+00	0.8451
		3	0	1.70E+01	1.2304
		1	30	1.30E+01	1.1139
		2	30	1.20E+01	1.0792
		3	30	1.50E+01	1.1761

Mean		n
A	1.0841	6
C	1.1167	6
Diff	-0.0326	
Mean		
B	1.0752	6
C	1.1167	6
Diff	-0.0414	

TABLE 2A

Page 5 of 6

ANALYSIS OF NEUTRALIZATION STUDY DATA

Test organism: *Staphylococcus aureus* ATCC 6538

ASTM Test	Product Code	Replicate	Sample Time Min	Number of Survivors	
				CFU/mL	Log Cfu/ML
A	HTR Code C in 10 ml Stripping fluid w/ neutralizer	1	0	9.50E+00	0.9777
		2	0	3.80E+01	1.5798
		3	0	6.50E+00	0.8129
		1	30	1.20E+01	1.0792
		2	30	1.60E+01	1.2041
		3	30	9.00E+00	0.9542
B	Toxicity Control	1	0	8.50E+00	0.9294
		2	0	1.10E+01	1.0414
		3	0	1.50E+01	1.1761
		1	30	1.20E+01	1.0792
		2	30	1.40E+01	1.1461
		3	30	1.20E+01	1.0792
C	Numbers Control	1	0	1.80E+01	1.2553
		2	0	7.00E+00	0.8451
		3	0	1.70E+01	1.2304
		1	30	1.30E+01	1.1139
		2	30	1.20E+01	1.0792
		3	30	1.50E+01	1.1761

Mean		n
A	1.1013	6
C	1.1167	6
Diff	-0.0153	
t-test p-value	0.9049	
Mean		n
B	1.0752	6
C	1.1167	6
Diff	-0.0414	
t-test p-value	0.5689	

TABLE 2A

ANALYSIS OF NEUTRALIZATION STUDY DATA

Test organism: *Staphylococcus aureus* ATCC 6538

ASTM Test	Product Code	Replicate	Sample Time Min	Number of Survivors	
				CFU/mL	Log Cfu/ML
A	HTR Code C In 1.0 ml Stripping Fluid w/ neutralizer +9.0mL of Diln. Fluidw/ neutrali	1	0	1.00E+01	1.0000
		2	0	4.00E+01	1.6021
		3	0	1.90E+01	1.2788
		1	30	1.60E+01	1.2041
		2	30	5.80E+01	1.7634
		3	30	1.40E+01	1.1461
B	Toxicity	1	0	8.50E+00	0.9294
		2	0	1.10E+01	1.0414
		3	0	1.50E+01	1.1761
		1	30	1.20E+01	1.0792
		2	30	1.40E+01	1.1461
		3	30	1.20E+01	1.0792
C	Numbers	1	0	1.80E+01	1.2553
		2	0	7.00E+00	0.8451
		3	0	1.70E+01	1.2304
		1	30	1.30E+01	1.1139
		2	30	1.20E+01	1.0792
		3	30	1.50E+01	1.1761

Mean  
A 1.3324 n 6  
C 1.1167 6  
Diff 0.2157

Mean  
B 1.0752 n 6  
C 1.1167 6  
Diff -0.0414

TABLE 2B

ANALYSIS OF NEUTRALIZATION STUDY DATA

Test organism: *Serratia marcescens* ATCC 14756

ASTM Test	Product Code	Replicate	Sample Time Min	Number of Survivors	
				CFU/mL	Log Cfu/ML
A	HTR Code C in 10 ml Stripping fluid w/ neutralizer	1	0	2.10E+02	2.3222
		2	0	1.80E+02	2.2553
		3	0	1.70E+02	2.2304
		1	30	1.80E+02	2.2553
		2	30	2.40E+02	2.3802
		3	30	2.10E+02	2.3222
B	Toxicity	1	0	3.20E+02	2.5051
		2	0	2.00E+02	2.3010
		3	0	1.90E+02	2.2788
		1	30	1.80E+02	2.2553
		2	30	1.60E+02	2.2041
		3	30	1.80E+02	2.2553
C	Numbers Control	1	0	1.90E+02	2.2788
		2	0	1.90E+02	2.2788
		3	0	1.80E+02	2.2553
		1	30	2.00E+02	2.3010
		2	30	1.90E+02	2.2788
		3	30	1.90E+02	2.2788
D	HTR Code C in 10 ml Stripping fluid w/o neutralizer	1	0	2.00E+02	2.3010
		2	0	1.90E+02	2.2788
		3	0	8.80E+01	1.9445
		1	30	8.40E+01	1.9243
		2	30	8.40E+01	1.9243
		3	30	2.00E+01	1.3010

Mean  
A 2.2943 n 6  
C 2.2786 6  
Diff 0.0157

Mean  
B 2.2999 6  
C 2.2786 6  
Diff 0.0214

Mean  
D 1.9456 6  
C 2.2786 6  
Diff -0.3329

TABLE 2B

ANALYSIS OF NEUTRALIZATION STUDY DATA

Test organism: *Serratia marcescens* ATCC 14756

ASTM Test	Product Code	Replicate	Sample Time Min	Number of Survivors	
				CFU/mL	Log Cfu/ML
A	HTR Code C in 1.0 ml Stripping Fluid	1	0	1.80E+02	2.2553
		2	0	1.70E+02	2.2304
		3	0	2.50E+02	2.3979
	w/ neutralizer +9.0mL of Dihn. Fluidw/ neutralizer	1	30	1.90E+02	2.2788
		2	30	2.00E+02	2.3010
		3	30	2.00E+02	2.3010
B	Toxicity	1	0	3.20E+02	2.5051
		2	0	2.00E+02	2.3010
		3	0	1.90E+02	2.2788
		1	30	1.80E+02	2.2553
		2	30	1.60E+02	2.2041
		3	30	1.80E+02	2.2553
C	Numbers Control	1	0	1.90E+02	2.2788
		2	0	1.90E+02	2.2788
		3	0	1.80E+02	2.2553
		1	30	2.00E+02	2.3010
		2	30	1.90E+02	2.2788
		3	30	1.90E+02	2.2788

Mean		n
A	2.2941	6
C	2.2786	6
Diff	0.0155	
Mean		
B	2.2999	6
C	2.2786	6
Diff	0.0214	