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**Investigation into the Effectiveness of VIRKON S (FH/73540/1) when Tested in Accordance with BS EN 1276:1997 - 'Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas' (TES-FH-004)**

CONFIDENTIAL TO: Mr. Mark Squire,  
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Karen Hall

7th August 2003

Report Approved By : Report Checked By :

Name :

**Approved  
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## SUMMARY

When tested in accordance with BS EN 1276:1997 the Virkon S tested at 1% v/v possesses bactericidal activity in 5 minutes at 10°C under clean (0.03% bovine albumen) and dirty (0.3% bovine albumen) conditions. Against the four standard organisms - *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Enterococcus hirae* plus *Listeria monocytogenes*, *Salmonella* Typhimurium, *Yersinia enterocolitica* and *Escherichia coli* O157:H7. Thus meeting the bacterial requirements only of the M&S protocol TES-FH-013; part 1.

## INTRODUCTION

Antec International requested the Food Hygiene Department of CCFRA Technology Limited (CCFRA) to assess the efficacy of Virkon S, for bactericidal activity in accordance with BS EN 1276:1997 - 'Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas' (TES-FH-004). With the addition of *Listeria monocytogenes*, *Salmonella* Typhimurium, *Yersinia enterocolitica* and *Escherichia coli* O157:H7.

## METHOD

For the bactericidal tests, BS EN 1276:1997 - 'Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas' (TES-FH-004) was followed.

### **Bactericidal activity in general use conditions**

a) IDENTIFICATION OF THE TEST LABORATORY	<b>Food Hygiene Department, CCFRA</b>
b) IDENTIFICATION OF THE SAMPLE	
Name of the product	<b>VIRKON S</b>
Batch number	<b>PJW 1/87/B 500g</b>
Manufacturer	<b>Antec International</b>
Condition on receipt	<b>OK</b>
Date of delivery	<b>13th June, 2003</b>
Storage conditions	<b>Food Hygiene Sample Store, Dark, Ambient</b>
Product diluent recommended by the manufacturer for use	<b>Water of Standard Hardness (WSH)</b>
Active substance(s) and its (their) concentrations(optional)	<b>N/A</b>
c) EXPERIMENTAL CONDITIONS	
Period of analysis	<b>16th June, 2003 - 15th July, 2003</b>
Product diluent used during the test	<b>Water of Standard Hardness (WSH)</b>
Product test concentrations	<b>0.5%, 1.0% and 2.0%</b>
Appearance product dilutions	<b>Clear</b>
Contact time	<b>Bactericidal Tests – 5 mins ±10 secs</b>
Test temperature	<b>Bactericidal Tests - 10°C ±1°C</b>
Interfering substance	<b>Bovine Serum Albumin @ 0.03% (“CLEAN”) and 0.30% (“DIRTY”) in Sterile Distilled Water (SDW)</b>
Stability of the mixture (interfering substance and product diluted in hard water)	<b>Some precipitation formation at 2% in Dirty conditions. Breaks down on agitation</b>
Temperature of incubation	<b>Bactericidal Tests - 37°C ± 1°C</b>

c) EXPERIMENTAL CONDITIONS (CONTINUED)

Identification of bacterial strains used	<b>Standard bactericidal test strains -</b> <i>Escherichia coli</i> <i>Enterococcus hirae</i> <i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i> plus <i>Listeria monocytogenes</i> <i>Salmonella Typhimurium</i> <i>Yersinia enterocolitica</i>	Ec FH 64/a Eh FH 65/a Pa FH 72/a Sa FH 73/a Lm FH 66/a St FH 68/a Ye FH 67/a
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d) OPERATING PROCEDURE

Methods used	<b>Bactericidal Test – BS EN 1276:1997</b>
Deviations from methods	<b>NONE</b>
Neutraliser method	<b>Dilution Neutralisation</b>
Neutraliser/ rinse media	<b>BS Inactivator</b>

e) TEST RESULTS

**PASS @ 1.0%**

f) CONCLUSION

**According to BS EN 1276:1997 the supplied 'Virkon S' (FH/73540/1), at the in use concentration (1%) possess bactericidal activity in 5 minutes at 10°C under clean and dirty conditions for the referenced strains...**  
*Escherichia coli*  
*Enterococcus hirae*  
*Pseudomonas aeruginosa*  
*Staphylococcus aureus*  
 plus  
*Listeria monocytogenes*  
*Salmonella Typhimurium*  
*Yersinia enterocolitica*

N.B. KEY TO CODES ON RESULT SHEETS...

Vc:	viable count
R:	reduction in viability
Na:	the number of cfu/ml in the test mixture (dilution factor of 10 <sup>-1</sup> )
C:	the number of cfu/ml in the dilution neutralisation test control, or of the membrane filtration test control
Nv:	the number of cfu/ml of the bacterial test suspension (dilution factor of 10 <sup>-1</sup> )
A:	the number of cfu/ml of the experimental conditions control
B:	the number of cfu/ml of the neutraliser toxicity control or of the filtration control
N:	the number of cfu/ml in the bacterial or fungal suspension (dilution factor of 10 <sup>-6</sup> for Bacterial Tests, 10 <sup>-5</sup> For Fungicidal Tests)

Test criteria	Validation Test				Test suspension	cfu/ml in test pot	Test procedure at concentration % (v/v / w/w)			Result
	Vc: 148, 166 -2 13, 21 Nv: 1.6 x 10 <sup>3</sup>	Vc: 135, 154 A: 1.4 x 10 <sup>2</sup>	Vc: 172, 167 B: 1.7 x 10 <sup>2</sup>	Vc: 269, 218 C: 2.4 x 10 <sup>2</sup>			0.5%	1.0%	2.0%	
<i>Escherichia coli</i> FH 64/a Clean	Vc: 148, 166 -2 13, 21 Nv: 1.6 x 10 <sup>3</sup>	Vc: 135, 154 A: 1.4 x 10 <sup>2</sup>	Vc: 172, 167 B: 1.7 x 10 <sup>2</sup>	Vc: 269, 218 C: 2.4 x 10 <sup>2</sup>	Vc: 171, 182 -7 22, 21 N: 1.8 x 10 <sup>8</sup>	1.8 x 10 <sup>7</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Pass @ 0.5%
<i>Escherichia coli</i> FH 64/a Dirty	Vc: 148, 166 -2 13, 21 Nv: 1.6 x 10 <sup>3</sup>	Vc: 165, 167 A: 1.6 x 10 <sup>2</sup>	Vc: 172, 167 B: 1.7 x 10 <sup>2</sup>	Vc: 187, 218 C: 2.0 x 10 <sup>2</sup>	Vc: 171, 182 -7 22, 21 N: 1.8 x 10 <sup>8</sup>	1.8 x 10 <sup>7</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 2, 3 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Pass @ 0.5%
<i>Staphylococcus aureus</i> FH 73/b Clean	Vc: 208, 252 -2 22, 23 Nv: 2.3 x 10 <sup>3</sup>	Vc: 195, 220 A: 2.1 x 10 <sup>2</sup>	Vc: 259, 255 B: 2.6 x 10 <sup>2</sup>	Vc: 229, 276 C: 2.5 x 10 <sup>2</sup>	Vc: 266, 271 -7 28, 26 N: 2.7 x 10 <sup>8</sup>	2.7 x 10 <sup>7</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Pass @ 0.5%
<i>Staphylococcus aureus</i> FH 73/b Dirty	Vc: 208, 252 -2 22, 23 Nv: 2.3 x 10 <sup>3</sup>	Vc: 250, 229 A: 2.4 x 10 <sup>2</sup>	Vc: 259, 255 B: 2.6 x 10 <sup>2</sup>	Vc: 252, 273 C: 2.6 x 10 <sup>2</sup>	Vc: 266, 271 -7 28, 26 N: 2.7 x 10 <sup>8</sup>	2.7 x 10 <sup>7</sup>	Vc: >300, >300 Na: >1.5 x 10 <sup>2</sup> R: <10 <sup>5</sup>	Vc: 2, 1 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Pass @ 1.0%
<i>Enterococcus faecalis</i> FH 65/a Clean	Vc: 217, 216 -2 13, 21 Nv: 2.1 x 10 <sup>3</sup>	Vc: 209, 219 A: 2.1 x 10 <sup>2</sup>	Vc: 224, 222 B: 2.2 x 10 <sup>2</sup>	Vc: 234, 200 C: 2.2 x 10 <sup>2</sup>	Vc: 230, 241 -7 21, 29 N: 2.4 x 10 <sup>8</sup>	2.4 x 10 <sup>7</sup>	Vc: 10, 10 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 0, 2 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Pass @ 0.5%
<i>Enterococcus faecalis</i> FH 65/a Dirty	Vc: 217, 216 -2 13, 21 Nv: 2.1 x 10 <sup>3</sup>	Vc: 178, 250 A: 2.1 x 10 <sup>2</sup>	Vc: 224, 222 B: 2.2 x 10 <sup>2</sup>	Vc: 240, 236 C: 2.4 x 10 <sup>2</sup>	Vc: 230, 241 -7 21, 29 N: 2.4 x 10 <sup>8</sup>	2.4 x 10 <sup>7</sup>	Vc: >300, >300 Na: >1.5 x 10 <sup>2</sup> R: <10 <sup>5</sup>	Vc: 20, 24 Na: 2.2 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Pass @ 1.0%
<i>Pseudomonas aeruginosa</i> FH 72/b Clean	Vc: 247, 209 -2 33, 33 Nv: 2.4 x 10 <sup>3</sup>	Vc: 272, 276 A: 2.7 x 10 <sup>2</sup>	Vc: 190, 208 B: 1.9 x 10 <sup>2</sup>	Vc: 212, 234 C: 2.2 x 10 <sup>2</sup>	Vc: 268, 324 -7 23, 29 N: 2.9 x 10 <sup>8</sup>	2.9 x 10 <sup>7</sup>	Vc: >300, >300 Na: >1.5 x 10 <sup>2</sup> R: <10 <sup>5</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 2, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Pass @ 1.0%
<i>Pseudomonas aeruginosa</i> FH 72/b Dirty	Vc: 247, 209 -2 33, 33 Nv: 2.4 x 10 <sup>3</sup>	Vc: 282, 263 A: 2.7 x 10 <sup>2</sup>	Vc: 190, 208 B: 1.9 x 10 <sup>2</sup>	Vc: 296, 275 C: 2.8 x 10 <sup>2</sup>	Vc: 268, 324 -7 23, 29 N: 2.9 x 10 <sup>8</sup>	2.9 x 10 <sup>7</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 1, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 1, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Pass @ 0.5%

Test criteria	Validation Test					Test suspension	cfu/ml in test pot	Test procedure at concentration % (v/v / w/w)			Result
	Vc: 261, 288 -2 21, 24 Nv: 2.7 x 10 <sup>3</sup>	Vc: 292, 260 A: 2.8 x 10 <sup>2</sup>	Vc: 271, 256 B: 2.6 x 10 <sup>2</sup>	Vc: 261, 314 C: 2.9 x 10 <sup>2</sup>	Vc: 261, 288 -7 21, 24 N: 2.7 x 10 <sup>8</sup>			0.5%	1.0%	2.0%	
<i>Listeria monocytogenes</i> FH66/a Clean	Vc: 261, 288 -2 21, 24 Nv: 2.7 x 10 <sup>3</sup>	Vc: 292, 260 A: 2.8 x 10 <sup>2</sup>	Vc: 271, 256 B: 2.6 x 10 <sup>2</sup>	Vc: 261, 314 C: 2.9 x 10 <sup>2</sup>	Vc: 261, 288 -7 21, 24 N: 2.7 x 10 <sup>8</sup>	2.7 x 10 <sup>7</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Pass @ 0.5%	
<i>Listeria monocytogenes</i> FH66/a Dirty	Vc: 261, 288 -2 21, 24 Nv: 2.7 x 10 <sup>3</sup>	Vc: 294, 256 A: 2.7 x 10 <sup>2</sup>	Vc: 271, 256 B: 2.6 x 10 <sup>2</sup>	Vc: 241, 313 C: 2.8 x 10 <sup>2</sup>	Vc: 261, 288 -7 21, 24 N: 2.7 x 10 <sup>8</sup>	2.7 x 10 <sup>7</sup>	Vc: >300, >300 Na: >1.5 x 10 <sup>2</sup> R: <10 <sup>5</sup>	Vc: 30, 24 Na: 2.7 x 10 <sup>2</sup> R: 10 <sup>5</sup>	Vc: 2, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Pass @ 1.0%	
<i>Salmonella</i> Typhimurium FH 68/a Clean	Vc: 300, 274 -2 33, 27 Nv: 2.9 x 10 <sup>3</sup>	Vc: 341 346 A: 3.4 x 10 <sup>2</sup>	Vc: 285, 302 B: 2.9 x 10 <sup>2</sup>	Vc: 248, 251 C: 2.5 x 10 <sup>2</sup>	Vc: 177, 179 -7 18, 27 N: 1.8 x 10 <sup>8</sup>	1.8 x 10 <sup>7</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Pass @ 0.5%	
<i>Salmonella</i> Typhimurium FH 68/a Dirty	Vc: 300, 274 -2 33, 27 Nv: 2.9 x 10 <sup>3</sup>	Vc: 256, 294 A: 2.7 x 10 <sup>2</sup>	Vc: 285, 302 B: 2.9 x 10 <sup>2</sup>	Vc: 258, 246 C: 2.5 x 10 <sup>2</sup>	Vc: 177, 179 -7 18, 27 N: 1.8 x 10 <sup>8</sup>	1.8 x 10 <sup>7</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Pass @ 0.5%	
<i>Yersinia enterocolitica</i> FH 67/a Clean	Vc: 178, 171 -2 14, 9 Nv: 1.7 x 10 <sup>3</sup>	Vc: 149, 122 A: 1.3 x 10 <sup>2</sup>	Vc: 154, 148 B: 1.5 x 10 <sup>2</sup>	Vc: 207, 185 C: 1.9 x 10 <sup>2</sup>	Vc: 176, 173 -7 15, 16 N: 1.7 x 10 <sup>8</sup>	1.7 x 10 <sup>7</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Pass @ 0.5%	
<i>Yersinia enterocolitica</i> FH 67/a Dirty	Vc: 178, 171 -2 14, 9 Nv: 1.7 x 10 <sup>3</sup>	Vc: 164, 145 A: 1.5 x 10 <sup>2</sup>	Vc: 154, 148 B: 1.5 x 10 <sup>2</sup>	Vc: 195, 163 C: 1.8 x 10 <sup>2</sup>	Vc: 176, 173 -7 15, 16 N: 1.7 x 10 <sup>8</sup>	1.7 x 10 <sup>7</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Pass @ 0.5%	
<i>Escherichia coli</i> O157:H7 FH Clean	Vc: 161, 157 -2 26, 12 Nv: 1.6 x 10 <sup>3</sup>	Vc: 110, 120 A: 1.1 x 10 <sup>2</sup>	Vc: 142, 163 B: 1.5 x 10 <sup>2</sup>	Vc: 142, 161 C: 1.5 x 10 <sup>2</sup>	Vc: 154, 165 -7 21, 16 N: 1.6 x 10 <sup>8</sup>	1.6 x 10 <sup>7</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Pass @ 0.5%	
<i>Escherichia coli</i> O157:H7 FH 72/a Clean	Vc: 161, 157 -2 26, 12 Nv: 1.6 x 10 <sup>3</sup>	Vc: 90, 112 A: 1.0 x 10 <sup>2</sup>	Vc: 142, 163 B: 1.5 x 10 <sup>2</sup>	Vc: 124, 116 C: 1.2 x 10 <sup>2</sup>	Vc: 154, 165 -7 21, 16 N: 1.6 x 10 <sup>8</sup>	1.6 x 10 <sup>7</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Pass @ 0.5%	