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**Investigation into the effectiveness of Sagewash Sanitiser 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'**  
**(MA-FH-004)**

CONFIDENTIAL TO:

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10<sup>th</sup> December 2009

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## SUMMARY

When tested in accordance with the method of BS EN 1276:1997, Sagewash Sanitiser (FH/118555/1b and FH/118555/1c) tested neat (~250 ppm), possesses bactericidal activity in 5 minutes at 20°C under clean (0.03% bovine albumen final concentration in the test) and dirty (0.3% bovine albumen final concentration in the test) conditions against: *Escherichia coli* and the additional test strains: *Escherichia coli* O157:H7 and *Salmonella* Typhimurium.

When tested in accordance with the method of BS EN 1276:1997, Sagewash Sanitiser (FH/118555/1b and FH/118555/1c) tested neat (~250 ppm), possesses bactericidal activity in 5 minutes at 20°C under clean (0.03% bovine albumen final concentration in the test) conditions against: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus hirae* and the additional test strains: *Listeria monocytogenes* and Methicillin Resistant *Staphylococcus aureus*.

When tested in accordance with the method of BS EN 1276:1997, Sagewash Sanitiser (FH/118555/1b and FH/118555/1c) tested neat (~250 ppm), does not possess bactericidal activity in 5 minutes at 20°C under dirty (0.3% bovine albumen final concentration in the test) conditions against: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus hirae* and the additional test strains: *Listeria monocytogenes* and Methicillin Resistant *Staphylococcus aureus*.

## INTRODUCTION

Sage Systems requested the Food Hygiene Department of Campden Technology Limited (Campden BRI) to assess the efficacy of Sagewash Sanitiser 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' (MA-FH-004).

## 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' (MA-FH-004) was followed.

## NOTES

The test was carried out at only 2 concentrations: neat (~250 ppm) and 1:10 (~25 ppm), under clean and dirty conditions.

## Bactericidal activity in general use conditions

a) IDENTIFICATION OF THE TEST LABORATORY	Food Hygiene Department, Campden BRI
b) IDENTIFICATION OF THE SAMPLE	FH/118555/1b, FH/118555/1c
Name of the product	Sagewash Sanitiser
Batch number	Not supplied
Manufacturer	Sage Systems
Condition on receipt	OK
Date of delivery	22.09.09
Storage conditions	Food Hygiene Sample Store, Dark, Ambient
Product diluent recommended by the manufacturer for use	N/A
Active substance(s) and its (their) concentrations(optional)	N/A
c) EXPERIMENTAL CONDITIONS	
Period of analysis	December 2009
Product diluent used during the test	Water of Standard Hardness (WSH)
Product test concentrations	Neat (~250 ppm) , 1:10 (~25 ppm)
Appearance of diluted product	Clear
Contact time	Bactericidal Tests – 5 minutes
Test temperature	Bactericidal Tests - 20°C
Interfering substance	Bovine Serum Albumin @ 0.03% (“CLEAN”) and 0.30% (“DIRTY”) in Sterile Distilled Water (SDW)
Stability of the mixture (interfering substance and product diluted in hard water)	Stable
Temperature of incubation	Bactericidal Tests - 37°C ± 1°C
Identification of bacterial strains used	Standard bactericidal test strains: <i>Escherichia coli</i> Ec FH 64/g <i>Enterococcus hirae</i> Eh FH 65/f <i>Staphylococcus aureus</i> Sa FH 73/f <i>Pseudomonas aeruginosa</i> Pa FH 72/h  Additional test strains: <i>Listeria monocytogenes</i> Lm FH 66/d <i>Salmonella</i> Typhimurium St FH 68/d <i>Escherichia coli</i> O157:H7 FH 45/a (non toxigenic) Methicillin Resistant <i>Staphylococcus aureus</i> MRSA FH 80/I

d) OPERATING PROCEDURE  
Methods used  
  
Deviations from methods  
  
Neutralisation method  
  
Neutraliser/ rinse media

**Bactericidal Test – BS EN 1276:1997  
(MA-FH-004)**

**None**

**Dilution Neutralisation**

**Sodium thiosulphate neutraliser - sodium thiosulphate 5 g/l, deionised water 1000ml**

e) TEST RESULTS

**See attached tables**

f) CONCLUSION

**When tested according to the method of BS EN 1276:1997, the supplied Sagewash Sanitiser (FH/118555/1b and 118555/1c), tested neat (~250 ppm) possesses bactericidal activity in 5 minutes at 20°C under clean and dirty conditions for the bactericidal test strains:**

*Escherichia coli*

**And additional test strains:**

*Escherichia coli* O157:H7

*Salmonella* Typhimurium.

**When tested according to the method of BS EN 1276:1997, the supplied Sagewash Sanitiser (FH/118555/1b and FH/118555/1c), tested neat (~250 ppm) possesses bactericidal activity in 5 minutes at 20°C under clean conditions for the bactericidal test strains:**

*Staphylococcus aureus*

*Pseudomonas aeruginosa*

*Enterococcus hirae*

**And additional test strains:**

*Listeria monocytogenes*

Methicillin Resistant *Staphylococcus aureus*

**When tested according to the method of BS EN 1276:1997, the supplied Sagewash Sanitiser (FH/118555/1b and FH/118555/1c), tested neat (~250 ppm) does not possess bactericidal activity in 5 minutes at 20°C under dirty conditions for the bactericidal test strains:**

*Enterococcus hirae*  
*Staphylococcus aureus*  
*Pseudomonas aeruginosa*

**And additional test strains:**

*Listeria monocytogenes*  
Methicillin Resistant *Staphylococcus aureus*

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

Vc:	viable count
R:	reduction in viability
Na:	the number of cfu/ml in the test mixture
Nv:	the number of cfu/ml of the bacterial suspension for validation controls
N:	the number of cfu/ml of the bacterial test suspension
A:	the number of cfu/ml of the experimental conditions control
B:	the number of cfu/ml of the neutraliser toxicity validation or of the filtration validation
C:	the number of cfu/ml of the dilution neutralisation test validation, or of the membrane filtration test validation

**TABLE 1 - RESULTS AND VALIDATIONS (Dilution Neutralisation)**

Test criteria	Validation Test						Test suspension	Test procedure at concentration % (v/v)			Result
	Vc: 94, 100 -2: 9, 8 Nv: 9.7 x 10 <sup>2</sup>	Vc: 92, 90 A: 9.1 x 10 <sup>1</sup>	Vc: 95, 101 B: 9.8 x 10 <sup>1</sup>	Vc: 98, 98 C: 9.8 x 10 <sup>1</sup>	Vc: 161, 182 -7: 18, 12 N: 1.7 x 10 <sup>8</sup>	Neat (~250 ppm)		1:10 (~25 ppm)	Pass/Fail/Invalid		
<i>Escherichia coli</i> FH 64/g Clean	Vc: 94, 100 -2: 9, 8 Nv: 9.7 x 10 <sup>2</sup>	Vc: 92, 90 A: 9.1 x 10 <sup>1</sup>	Vc: 95, 101 B: 9.8 x 10 <sup>1</sup>	Vc: 98, 98 C: 9.8 x 10 <sup>1</sup>	Vc: 161, 182 -7: 18, 12 N: 1.7 x 10 <sup>8</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: >300, >300 Na: > 3.0 x 10 <sup>3</sup> R: <10 <sup>5</sup>	Pass Neat Valid			
<i>Escherichia coli</i> FH 64/g Dirty	Vc: 94, 100 -2: 9, 8 Nv: 9.7 x 10 <sup>2</sup>	Vc: 123, 108 A: 1.2 x 10 <sup>2</sup>	Vc: 95, 101 B: 9.8 x 10 <sup>1</sup>	Vc: 99, 119 C: 1.1 x 10 <sup>2</sup>	Vc: 161, 182 -7: 18, 12 N: 1.7 x 10 <sup>8</sup>	Vc: 8, 4 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: >300, >300 Na: > 3.0 x 10 <sup>3</sup> R: <10 <sup>5</sup>	Pass Neat Valid			
<i>Staphylococcus aureus</i> FH 73/e Clean	Vc: 183, 168 -2: 18, 16 Nv: 1.8 x 10 <sup>3</sup>	Vc: 140, 132 A: 1.4 x 10 <sup>2</sup>	Vc: 135, 148 B: 1.4 x 10 <sup>2</sup>	Vc: 130, 111 C: 1.2 x 10 <sup>2</sup>	Vc: >300, >300 -7: 30, 31 N: 3.0 x 10 <sup>8</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: >300, >300 Na: > 3.0 x 10 <sup>3</sup> R: <10 <sup>5</sup>	Pass Neat Valid			
<i>Staphylococcus aureus</i> FH 73/e Dirty	Vc: 183, 168 -2: 18, 16 Nv: 1.8 x 10 <sup>3</sup>	Vc: 133, 131 A: 1.3 x 10 <sup>2</sup>	Vc: 135, 148 B: 1.4 x 10 <sup>2</sup>	Vc: 126, 136 C: 1.3 x 10 <sup>2</sup>	Vc: >300, >300 -7: 30, 31 N: 3.0 x 10 <sup>8</sup>	Vc: >300, >300 Na: > 3.0 x 10 <sup>3</sup> R: <10 <sup>5</sup>	Vc: >300, >300 Na: > 3.0 x 10 <sup>3</sup> R: <10 <sup>5</sup>	Fail Valid			
<i>Enterococcus hirae</i> FH 65/f Clean	Vc: 101, 109 -2: 17, 18 Nv: 1.0 x 10 <sup>3</sup>	Vc: 81, 79 B: 8.0 x 10 <sup>1</sup>	Vc: 71, 86 B: 7.8 x 10 <sup>1</sup>	Vc: 73, 80 C: 7.6 x 10 <sup>1</sup>	Vc: 151, 190 -7: 18, 16 N: 1.7 x 10 <sup>8</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: >300, >300 Na: > 3.0 x 10 <sup>3</sup> R: <10 <sup>5</sup>	Pass Neat Valid			
<i>Enterococcus hirae</i> FH 65/f Dirty	Vc: 101, 109 -2: 17, 18 Nv: 1.0 x 10 <sup>3</sup>	Vc: 88, 75 A: 8.2 x 10 <sup>1</sup>	Vc: 71, 86 B: 7.8 x 10 <sup>1</sup>	Vc: 67, 84 C: 7.6 x 10 <sup>1</sup>	Vc: 151, 190 -7: 18, 16 N: 1.7 x 10 <sup>8</sup>	Vc: >300, >300 Na: > 3.0 x 10 <sup>3</sup> R: <10 <sup>5</sup>	Vc: >300, >300 Na: > 3.0 x 10 <sup>3</sup> R: <10 <sup>5</sup>	Fail Valid			
<i>Pseudomonas aeruginosa</i> FH 72/h Clean	Vc: 137, 130 -2: 12, 11 Nv: 1.3 x 10 <sup>3</sup>	Vc: 131, 132 A: 1.3 x 10 <sup>2</sup>	Vc: 142, 136 B: 1.4 x 10 <sup>2</sup>	Vc: 129, 120 C: 1.2 x 10 <sup>2</sup>	Vc: 214, 211 -7: 18, 22 N: 2.1 x 10 <sup>8</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: >300, >300 Na: > 3.0 x 10 <sup>3</sup> R: <10 <sup>5</sup>	Pass Neat Valid			
<i>Pseudomonas aeruginosa</i> FH 72/h Dirty	Vc: 137, 130 -2: 12, 11 Nv: 1.3 x 10 <sup>3</sup>	Vc: 134, 148 A: 1.4 x 10 <sup>2</sup>	Vc: 142, 136 B: 1.4 x 10 <sup>2</sup>	Vc: 137, 110 C: 1.2 x 10 <sup>2</sup>	Vc: 214, 211 -7: 18, 22 N: 2.1 x 10 <sup>8</sup>	Vc: 34, 30 Na: 3.2 x 10 <sup>2</sup> R: <10 <sup>5</sup>	Vc: >300, >300 Na: > 3.0 x 10 <sup>3</sup> R: <10 <sup>5</sup>	Fail Valid			

**TABLE 1 (cont'd) - RESULTS AND VALIDATIONS (Dilution Neutralisation)**

Test criteria	Validation Test			Test suspension	Test procedure at concentration % (v/v)			Result
	Vc: 163, 179 -2: 16, 17 Nv: 1.7 x 10 <sup>3</sup>	Vc: 163, 158 A: 1.6 x 10 <sup>2</sup>	Vc: 170, 169 B: 1.7 x 10 <sup>2</sup>		cfu/ml in test pot	Neat (~250 ppm)	1:10 (~25 ppm)	
<i>Listeria monocytogenes</i> FH 66/d Clean	Vc: 163, 179 -2: 16, 17 Nv: 1.7 x 10 <sup>3</sup>	Vc: 163, 158 A: 1.6 x 10 <sup>2</sup>	Vc: 170, 169 B: 1.7 x 10 <sup>2</sup>	Vc: >300, >300 -7: 30, 37 N: 3.4 x 10 <sup>8</sup>	3.4 x 10 <sup>7</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: >300, >300 Na: > 3.0 x 10 <sup>3</sup> R: <10 <sup>5</sup>	Pass Neat Valid
<i>Listeria monocytogenes</i> FH 66/d Dirty	Vc: 163, 179 -2: 16, 17 Nv: 1.7 x 10 <sup>3</sup>	Vc: 142, 178 A: 1.6 x 10 <sup>2</sup>	Vc: 170, 169 B: 1.7 x 10 <sup>2</sup>	Vc: >300, >300 -7: 30, 37 N: 3.4 x 10 <sup>8</sup>	3.4 x 10 <sup>7</sup>	Vc: >300, >300 Na: > 3.0 x 10 <sup>3</sup> R: <10 <sup>5</sup>	Vc: >300, >300 Na: > 3.0 x 10 <sup>3</sup> R: <10 <sup>5</sup>	Fail Valid
<i>Escherichia coli</i> O157: H7 FH 45/a Clean	Vc: 95, 96 -2: 9, 8 Nv: 9.6 x 10 <sup>2</sup>	Vc: 71, 78 A: 7.4 x 10 <sup>1</sup>	Vc: 78, 91 B: 8.4 x 10 <sup>1</sup>	Vc: 171, 163 -7: 16, 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: >300, >300 Na: > 3.0 x 10 <sup>3</sup> R: <10 <sup>5</sup>	Pass Neat Valid
<i>Escherichia coli</i> O157: H7 FH 45/a Dirty	Vc: 95, 96 -2: 9, 8 Nv: 9.6 x 10 <sup>2</sup>	Vc: 94, 102 A: 9.8 x 10 <sup>1</sup>	Vc: 78, 91 B: 8.4 x 10 <sup>1</sup>	Vc: 171, 163 -7: 16, 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: >300, >300 Na: > 3.0 x 10 <sup>3</sup> R: <10 <sup>5</sup>	Pass Neat Valid
MRSA FH 80/b Clean	Vc: 96, 97 -2: 8, 10 Nv: 9.6 x 10 <sup>2</sup>	Vc: 96, 97 B: 9.6 x 10 <sup>1</sup>	Vc: 85, 85 B: 8.5 x 10 <sup>1</sup>	Vc: 172, 168 -7: 17, 19 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: >300, >300 Na: > 3.0 x 10 <sup>3</sup> R: <10 <sup>5</sup>	Pass Neat Valid
MRSA FH 80/b Dirty	Vc: 96, 97 -2: 8, 10 Nv: 1.2 x 10 <sup>3</sup>	Vc: 98, 118 A: 1.0 x 10 <sup>2</sup>	Vc: 85, 85 B: 8.5 x 10 <sup>1</sup>	Vc: 172, 168 -7: 17, 19 N: 1.7 x 10 <sup>8</sup>	1.7 x 10 <sup>7</sup>	Vc: >300, >300 Na: > 3.0 x 10 <sup>3</sup> R: <10 <sup>5</sup>	Vc: >300, >300 Na: > 3.0 x 10 <sup>3</sup> R: <10 <sup>5</sup>	Fail Valid
<i>Salmonella</i> Typhimurium FH 68/d Clean	Vc: 138, 102 -2: 10, 9 Nv: 1.2 x 10 <sup>3</sup>	Vc: 114, 115 A: 1.1 x 10 <sup>2</sup>	Vc: 110, 112 B: 1.1 x 10 <sup>2</sup>	Vc: 203, 200 -7: 24, 24 N: 2.0 x 10 <sup>8</sup>	2.0 x 10 <sup>7</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: >300, >300 Na: > 3.0 x 10 <sup>3</sup> R: <10 <sup>5</sup>	Pass Neat Valid
<i>Salmonella</i> Typhimurium FH 68/d Dirty	Vc: 138, 102 -2: 10, 9 Nv: 1.2 x 10 <sup>3</sup>	Vc: 158, 138 A: 1.4 x 10 <sup>2</sup>	Vc: 110, 112 B: 1.1 x 10 <sup>2</sup>	Vc: 203, 200 -7: 24, 24 N: 2.0 x 10 <sup>8</sup>	2.0 x 10 <sup>7</sup>	Vc: 0, 0 Na: <1.5 x 10 <sup>2</sup> R: >10 <sup>5</sup>	Vc: >300, >300 Na: > 3.0 x 10 <sup>3</sup> R: <10 <sup>5</sup>	Pass Neat Valid