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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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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/l |

d) OPERATING PROCEDURE

Methods used

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

Deviations from methods

None

Neutralisation method

Dilution Neutralisation

Neutraliser/ rinse media

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: 92, 90 A: 9.1 x 10 ¹ | Vc: 95, 101 B: 9.8 x 10 ¹ | Vc: 98, 98 C: 9.8 x 10 ¹ | | cfu/ml in test pot | Neat (~250 ppm) | 1:10 (~25 ppm) | |
| <i>Escherichia coli</i> FH 64/g Clean | Vc: 94, 100 -2: 9, 8 Nv: 9.7 x 10 ² | Vc: 92, 90 A: 9.1 x 10 ¹ | Vc: 95, 101 B: 9.8 x 10 ¹ | Vc: 98, 98 C: 9.8 x 10 ¹ | Vc: 161, 182 -7: 18, 12 N: 1.7 x 10 ⁸ | 1.7 x 10 ⁷ | Vc: >300, >300 Na: > 3.0 x 10 ³ R: <10 ⁵ | Pass Neat Valid |
| <i>Escherichia coli</i> FH 64/g Dirty | Vc: 94, 100 -2: 9, 8 Nv: 9.7 x 10 ² | Vc: 123, 106 A: 1.2 x 10 ² | Vc: 95, 101 B: 9.8 x 10 ¹ | Vc: 99, 119 C: 1.1 x 10 ² | Vc: 161, 182 -7: 18, 12 N: 1.7 x 10 ⁸ | 1.7 x 10 ⁷ | Vc: >300, >300 Na: > 3.0 x 10 ³ R: <10 ⁵ | Pass Neat Valid |
| <i>Staphylococcus aureus</i> FH 73/e Clean | Vc: 183, 168 -2: 18, 16 Nv: 1.8 x 10 ³ | Vc: 140, 132 A: 1.4 x 10 ² | Vc: 135, 148 B: 1.4 x 10 ² | Vc: 130, 111 C: 1.2 x 10 ² | Vc: >300, >300 -7: 30, 31 N: 3.0 x 10 ⁸ | 3.0 x 10 ⁷ | Vc: >300, >300 Na: > 3.0 x 10 ³ R: <10 ⁵ | Pass Neat Valid |
| <i>Staphylococcus aureus</i> FH 73/e Dirty | Vc: 183, 168 -2: 18, 16 Nv: 1.8 x 10 ³ | Vc: 133, 131 A: 1.3 x 10 ² | Vc: 135, 148 B: 1.4 x 10 ² | Vc: 125, 135 C: 1.3 x 10 ² | Vc: >300, >300 -7: 30, 31 N: 3.0 x 10 ⁸ | 3.0 x 10 ⁷ | Vc: >300, >300 Na: > 3.0 x 10 ³ R: <10 ⁵ | Fail Valid |
| <i>Enterococcus hirae</i> FH 65/f Clean | Vc: 101, 109 -2: 17, 18 Nv: 1.0 x 10 ³ | Vc: 81, 79 B: 8.0 x 10 ¹ | Vc: 71, 86 B: 7.8 x 10 ¹ | Vc: 73, 80 C: 7.6 x 10 ¹ | Vc: 151, 190 -7: 18, 16 N: 1.7 x 10 ⁸ | 1.7 x 10 ⁷ | Vc: >300, >300 Na: > 3.0 x 10 ³ R: <10 ⁵ | Pass Neat Valid |
| <i>Enterococcus hirae</i> FH 65/f Dirty | Vc: 101, 109 -2: 17, 18 Nv: 1.0 x 10 ³ | Vc: 88, 75 A: 8.2 x 10 ¹ | Vc: 71, 86 B: 7.8 x 10 ¹ | Vc: 67, 84 C: 7.6 x 10 ¹ | Vc: 151, 190 -7: 18, 16 N: 1.7 x 10 ⁸ | 1.7 x 10 ⁷ | Vc: >300, >300 Na: > 3.0 x 10 ³ R: <10 ⁵ | Fail Valid |
| <i>Pseudomonas aeruginosa</i> FH72/h Clean | Vc: 137, 130 -2: 12, 11 Nv: 1.3 x 10 ³ | Vc: 131, 132 A: 1.3 x 10 ² | Vc: 142, 136 B: 1.4 x 10 ² | Vc: 129, 120 C: 1.2 x 10 ² | Vc: 214, 211 -7: 18, 22 N: 2.1 x 10 ⁸ | 2.1 x 10 ⁷ | Vc: >300, >300 Na: > 3.0 x 10 ³ R: <10 ⁵ | Pass Neat Valid |
| <i>Pseudomonas aeruginosa</i> FH 72/h Dirty | Vc: 137, 130 -2: 12, 11 Nv: 1.3 x 10 ³ | Vc: 134, 148 A: 1.4 x 10 ² | Vc: 142, 136 B: 1.4 x 10 ² | Vc: 137, 110 C: 1.2 x 10 ² | Vc: 214, 211 -7: 18, 22 N: 2.1 x 10 ⁸ | 2.1 x 10 ⁷ | Vc: >300, >300 Na: > 3.0 x 10 ³ R: <10 ⁵ | 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 ³ | Vc: 163, 158 A: 1.6 x 10 ² | Vc: 170, 169 B: 1.7 x 10 ² | Vc: 161, 171 C: 1.7 x 10 ² | | 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 ³ | Vc: 163, 158 A: 1.6 x 10 ² | Vc: 170, 169 B: 1.7 x 10 ² | Vc: 161, 171 C: 1.7 x 10 ² | Vc: >300, >300 -7: 30, 37 N: 3.4 x 10 ⁸ | Vc: 0, 0 Na: <1.5 x 10 ² R: >10 ⁵ | Vc: >300, >300 Na: > 3.0 x 10 ³ R: <10 ⁵ | Pass Neat Valid | |
| <i>Listeria monocytogenes</i> FH 66/d Dirty | Vc: 163, 179 -2: 16, 17 Nv: 1.7 x 10 ³ | Vc: 142, 178 A: 1.6 x 10 ² | Vc: 170, 169 B: 1.7 x 10 ² | Vc: 174, 170 C: 1.7 x 10 ² | Vc: >300, >300 -7: 30, 37 N: 3.4 x 10 ⁸ | Vc: >300, >300 Na: > 3.0 x 10 ³ R: <10 ⁵ | Vc: >300, >300 Na: > 3.0 x 10 ³ R: <10 ⁵ | Fail Valid | |
| <i>Escherichia coli</i> O157: H7 FH 45/a Clean | Vc: 95, 96 -2: 9, 8 Nv: 9.6 x 10 ² | Vc: 71, 78 A: 7.4 x 10 ¹ | Vc: 78, 91 B: 8.4 x 10 ¹ | Vc: 85, 89 C: 8.7 x 10 ¹ | Vc: 171, 163 -7: 16, 16 N: 1.6 x 10 ⁸ | Vc: 0, 0 Na: <1.5 x 10 ² R: >10 ⁵ | Vc: >300, >300 Na: > 3.0 x 10 ³ R: <10 ⁵ | Pass Neat Valid | |
| <i>Escherichia coli</i> O157: H7 FH 45/a Dirty | Vc: 95, 96 -2: 9, 8 Nv: 9.6 x 10 ² | Vc: 94, 102 A: 9.8 x 10 ¹ | Vc: 78, 91 B: 8.4 x 10 ¹ | Vc: 85, 89 C: 8.7 x 10 ¹ | Vc: 171, 163 -7: 16, 16 N: 1.6 x 10 ⁸ | Vc: 0, 0 Na: <1.5 x 10 ² R: >10 ⁵ | Vc: >300, >300 Na: > 3.0 x 10 ³ R: <10 ⁵ | Pass Neat Valid | |
| MRSA FH 80/b Clean | Vc: 96, 97 -2: 8, 10 Nv: 9.6 x 10 ² | Vc: 96, 97 B: 9.6 x 10 ¹ | Vc: 85, 85 B: 8.5 x 10 ¹ | Vc: 89, 80 C: 8.4 x 10 ¹ | Vc: 172, 168 -7: 17, 19 N: 1.7 x 10 ⁸ | Vc: 0, 0 Na: <1.5 x 10 ² R: >10 ⁵ | Vc: >300, >300 Na: > 3.0 x 10 ³ R: <10 ⁵ | Pass Neat Valid | |
| MRSA FH 80/b Dirty | Vc: 96, 97 -2: 8, 10 Nv: 1.2 x 10 ³ | Vc: 98, 118 A: 1.0 x 10 ² | Vc: 85, 85 B: 8.5 x 10 ¹ | Vc: 106, 95 C: 1.0 x 10 ² | Vc: 172, 168 -7: 17, 19 N: 1.7 x 10 ⁸ | Vc: >300, >300 Na: > 3.0 x 10 ³ R: <10 ⁵ | Vc: >300, >300 Na: > 3.0 x 10 ³ R: <10 ⁵ | Fail Valid | |
| <i>Salmonella</i> Typhimurium FH 68/d Clean | Vc: 138, 102 -2: 10, 9 Nv: 1.2 x 10 ³ | Vc: 114, 115 A: 1.1 x 10 ² | Vc: 110, 112 B: 1.1 x 10 ² | Vc: 117, 106 C: 1.1 x 10 ² | Vc: 203, 200 -7: 24, 24 N: 2.0 x 10 ⁸ | Vc: 0, 0 Na: <1.5 x 10 ² R: >10 ⁵ | Vc: >300, >300 Na: > 3.0 x 10 ³ R: <10 ⁵ | Pass Neat Valid | |
| <i>Salmonella</i> Typhimurium FH 68/d Dirty | Vc: 138, 102 -2: 10, 9 Nv: 1.2 x 10 ³ | Vc: 158, 138 A: 1.4 x 10 ² | Vc: 110, 112 B: 1.1 x 10 ² | Vc: 134, 135 C: 1.3 x 10 ² | Vc: 203, 200 -7: 24, 24 N: 2.0 x 10 ⁸ | Vc: 0, 0 Na: <1.5 x 10 ² R: >10 ⁵ | Vc: >300, >300 Na: > 3.0 x 10 ³ R: <10 ⁵ | Pass Neat Valid | |