

**Bactericidal activity of GAMA Healthcare Ltd.
biocide determined using the European
Standard Test method BS EN 1276:1997
against: *Klebsiella pneumoniae* NCTC 13368
(ATCC 700603)**

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Tests Carried Out By:

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Microbiological Tests

Test Method

British/European Standard BS EN 1276:1997.
Dilution-neutralisation

Test Procedures

Full details of all the test and control procedures used are given in the Test Method

Disinfectant

GAMA Healthcare Ltd biocide as used in Clinell Universal Sanitising Wipes
Batch number: N/A
Date of delivery: June 2006
Storage conditions: 20°C – 25°C
Active substances: not specified
Appearance product dilutions: colourless, clear product solution.

Interfering Substance (Organic Challenge)

1. Simulated clean conditions:
0.3 g l⁻¹ bovine albumin (final concentration)
2. Simulated dirty conditions:
3.0 g l⁻¹ bovine albumin (final concentration)

Temperature

Ambient (23 - 25°C)

Contact Time Tested

5 (± 10 s) minute.

Test Organisms

Klebsiella pneumoniae NCTC 13368

Culture Medium

Tryptone Soya Agar, Lab M

Incubation

Plates were incubated at 37 °C for 24-48h

Diluent

MRD, Lab M

Neutraliser

Neutraliser, containing 60g/l Tween 80, 60g/l Saponin, 2g/l L-histidine, 2g/l L-cysteine in MRD.

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General Method

A standard suspension of test organisms containing $1.5 - 5.0 \times 10^8$ cells ml^{-1} of bacteria was prepared. 1 ml of interfering substance was pipetted into a Universal bottle, followed by 1 ml of test organism suspension. The mixture was mixed and left for 2 minutes. After 2 minutes 8 ml of the Gama Health Care Ltd biocide was added. After a contact time of 5 minutes, a 1 ml sample of the reaction mixture was pipetted into 9 ml of neutraliser and left for 5 minutes. A 1 ml sample was then pipetted into 2 Petri dishes and mixed with 15 ml of culture medium tempered at 47 °C. After setting, the Petri dishes were incubated at 37 °C. Colony forming units were counted after 2-3 days incubation and the fraction of surviving organisms calculated.

Test Organism

The test organism *Klebsiella pneumoniae* NCTC 13368 (ATCC 700603) is a multi-drug resistant strain which:

- has intermediate resistance to ceftriaxone and gentamicin,
- is used as a control organism for extended-spectrum beta-lactamase production and produces beta-lactamase SHV-18,
- is resistant to ampicillin, aztreonam, ceftazidime, ceftazidime, chloramphenicol, piperacillin and tetracycline.

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Requirements of this standard

The product, when tested as stipulated under simulated clean conditions (0.3 g l⁻¹ bovine albumin) or dirty conditions (3 g l⁻¹ bovine albumin) under the test conditions of ambient temperature (23 to 25 °C), 5 minute contact, for *Klebsiella pneumoniae* NCTC 13368 shall demonstrate at least a 5 log₁₀ reduction in viable counts.

Results¹

Results from the test are summarised in Tables 1 and 2, a full set of results can be found in Table 3.

| Test Conditions | Contact Time (minutes) | Log ₁₀ Reduction Achieved |
|-------------------------------|------------------------|--------------------------------------|
| 0.3 g l ⁻¹ (clean) | 5 | >5 ¹ |
| 3.0 g l ⁻¹ (dirty) | 5 | >5 ¹ |

Table 1. Log₁₀ reductions in *Klebsiella pneumoniae* NCTC 13368 viable counts following a 5 minute exposure to the test material.

| Referenced Organism | Starting concentration CFU ml ⁻¹ | Final concentration CFU ml ⁻¹ clean 0.3 g l ⁻¹ Bovine Albumin | Final concentration CFU ml ⁻¹ dirty 3.0 g l ⁻¹ Bovine Albumin |
|--|---|---|---|
| <i>Klebsiella pneumoniae</i> NCTC 13368 | 1.6 x10 ⁸ (150,164 ¹ , 15, 16 ²) | Plate count 0, 0. (Actual 6 log ₁₀ reduction) | Plate count 0, 4. (Actual 6 log ₁₀ reduction) |
| CFU = colony forming units ¹ viable count of bacterial colonies, 1 ml sample of 10 ⁻⁶ bacterial suspension ² viable count of bacterial colonies, 1 ml sample of 10 ⁻⁷ bacterial suspension | | | |

Table 2. Reductions in *Klebsiella pneumoniae* NCTC 13368 viable counts following a 5 minute exposure to the test material.

Interpretation of the Results

When tested against *Klebsiella pneumoniae* NCTC 13368 with a 5 minute contact time a full strength GAMA Healthcare Ltd biocide met the requirements of the Standard under simulated clean and dirty conditions.

Conclusion

According to EN 1276:1997, the batch provided of GAMA Healthcare biocide possesses bactericidal activity in 5 minutes at ambient temperature (23-25°C) under clean conditions (0.3g/l bovine albumin) and dirty conditions (3g/l bovine albumin) for referenced strain *Klebsiella pneumoniae* NCTC 13368.

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¹ See Table of results in Appendix 1.

Appendix 1

| Test Organism | VALIDATIONS | | | | | | | | | | Bacterial Test Suspension | | | Test Procedure Results | | | | | | | | | | | | | | | | | | | | | | | |
|---|--------------------------------|------------------------------------|-------|---------|---------|------------------------------|---------|---------------------------------|---------|---------|---------------------------|-------|-----|------------------------|---------|-----|--------|----|--------|---|--------|---------------|--------------------------------|---|------------|---|------------|--|--|--|--|--|--|--|--|--|--|
| | Bacterial Suspension | Experimental Conditions Validation | | | | Neutraliser Toxicity Control | | Dilution Neutralisation Control | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | Clean | Dirty | | Clean | Dirty | Clean | Dirty | | | Clean | Dirty | | | | | | | | | | | | | | | | | | | | | | | | | |
| <i>Klebsiella pneumoniae</i> | Vc | 178 | 187 | 193 | 192 | Vc | 158 | 181 | Vc | 173 | 182 | 158 | 177 | 10-6 | 150 | 164 | Vc < | 15 | 15 | < | 15 | 15 | | | | | | | | | | | | | | | |
| | Nv | 1.6E+03 | A | 1.8E+02 | 1.9E+02 | B | 1.7E+02 | C | 1.8E+02 | 1.7E+02 | 10-7 | 15 | 16 | N | 1.6E+08 | R > | 2.E+05 | > | 2.E+05 | > | 2.E+05 | > | 2.E+05 | | | | | | | | | | | | | | |
| Verification of Methodology | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table border="0" style="width:100%; border-collapse: collapse;"> <tr> <td style="width:50%;">Passed</td> <td style="width:50%;">Log10 Reductions/cfu/ml</td> </tr> <tr> <td>N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N = 1.6E+08</td> <td>Clean 5.32</td> </tr> <tr> <td>Nv is between 6E+2 cfu/ml and 3E+3 cfu/ml, Nv = 1.6E+03</td> <td>Dirty 5.32</td> </tr> <tr> <td>CLEAN A ≥ 0.05 x Nv when 0.05 x Nv = 7.8E+01</td> <td></td> </tr> <tr> <td>DIRTY A ≥ 0.05 x Nv when 0.05 x Nv = 7.8E+01</td> <td></td> </tr> <tr> <td>B ≥ 0.05 x Nv when 0.05 x Nv = 7.8E+01</td> <td></td> </tr> <tr> <td>CLEAN C ≥ 0.5 x B when 0.5 x B = 8.5E+01</td> <td></td> </tr> <tr> <td>DIRTY C ≥ 0.5 x B when 0.5 x B = 8.5E+01</td> <td></td> </tr> </table> | | | | | | | | | | | | | | | | | | | | | | Passed | Log10 Reductions/cfu/ml | N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N = 1.6E+08 | Clean 5.32 | Nv is between 6E+2 cfu/ml and 3E+3 cfu/ml, Nv = 1.6E+03 | Dirty 5.32 | CLEAN A ≥ 0.05 x Nv when 0.05 x Nv = 7.8E+01 | | DIRTY A ≥ 0.05 x Nv when 0.05 x Nv = 7.8E+01 | | B ≥ 0.05 x Nv when 0.05 x Nv = 7.8E+01 | | CLEAN C ≥ 0.5 x B when 0.5 x B = 8.5E+01 | | DIRTY C ≥ 0.5 x B when 0.5 x B = 8.5E+01 | |
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Table 3. Testing of *Klebsiella pneumoniae* NCTC 13368 the GAMA Healthcare Ltd biocide using BS EN 1276:1997.