

**Bactericidal activity of Gamma Health Care
Ltd. biocide determined using the European
Standard Test method BS EN 1276:1997
against: *Vibrio cholerae* NCTC 11348**

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Tests Carried Out By:

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Microbiological Tests

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|--|---|
| 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 | Gamma Health Care Ltd biocide 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 (25°C) |
| Contact Time Tested | 5 (± 10 s) minute. |
| Test Organisms | <i>Vibrio cholerae</i> NCTC 11348 |
| Culture Medium | Tryptone Soya Agar, Lab M |
| Incubation | Plates were incubated at 37 °C for 48-60 h |
| Diluent | MRD, Lab M |
| Neutraliser | Neutraliser, containing 60g/l Tween 80, 60g/l Saponin, 2g/l L-histidine, 2g/l L-cysteine in MRD. |
| 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 Gamma 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.

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 *Vibrio cholerae* NCTC 11348, 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 *V. cholerae* 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>Vibrio cholerae</i> NCTC 11348 | 3.1 x 10 ⁸ (273,293 ¹ , 67, 55 ²) | Plate count 0, 0. (Actual 6 log ₁₀ reduction) | Plate count 0, 0. (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 *V. cholerae* viable counts following a 5 minute exposure to the test material.

Interpretation of the Results

When tested against *Vibrio cholerae* NCTC 11348 with a 5 minute contact time a full strength Gamma Health Care Ltd biocide met the requirements of the Standard under simulated clean and dirty conditions. The N_v (Appendix 1) value is slightly higher than that specified in the Standard but not sufficiently to effect the validity of the results.

Conclusion

According to EN 1276:1997, the batch provided of Gamma Health Care 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 *Vibrio cholerae* NCTC 11348.

Signed:

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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 | | | | | | | | | | | |
| <i>V. Cholerae</i> NCTC 11348 | Vc | 293 | 290 | 291 | 281 | Vc | 302 | 286 | Vc | 259 | 282 | 253 | 294 | 10-6 | 273 | 293 | Vc < | 15 | 15 | < | 15 | 15 |
| | Nv | 3.1E+03 | A | 2.9E+02 | 2.9E+02 | B | 2.9E+02 | C | 2.7E+02 | 2.7E+02 | 10-7 | 55 | 67 | N | 3.1E+08 | R > | 4.E+05 | > | 4.E+05 | | | |
| Verification of Methodology N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N = 3.1E+08 Nv is between 6E+2 cfu/ml and 3E+3 cfu/ml, Nv = 3.1E+03 CLEAN A ≥ 0.05 x Nv when 0.05 x Nv = 1.6E+02 Yes DIRTY A ≥ 0.05 x Nv when 0.05 x Nv = 1.6E+02 Yes B ≥ 0.05 x Nv when 0.05 x Nv = 1.6E+02 Yes CLEAN C ≥ 0.5 x B when 0.5 x B = 1.5E+02 Yes DIRTY C ≥ 0.5 x B when 0.5 x B = 1.5E+02 Yes | | | | | | | | | | Passed | | Log10 Reductions/cfu/ml Clean 5.62 Dirty 5.62 | | | | | | | Plate Counts 0 0 | | 0 0 | |

Table 3. Testing of *Vibrio cholerae* NCTC 11348 the Gamma Health Care Ltd biocide using BS EN 1276:1997.