

**Bactericidal activity of GAMA Healthcare  
Ltd. biocide determined using the  
European Standard Test method BS EN  
1276:1997 against:  
*Campylobacter jejuni* ATCC 33291.**

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**Prepared by  
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Nov '06**



*University of*  
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**DRAFT:**

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## Tests Carried Out By:

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## Microbiological Tests

|  |   |
|--|---|
| <b>Test Method</b>                               | British/European Standard BS EN 1276:1997.<br>Dilution-neutralisation   |
| <b>Test Procedures</b>                           | Full details of all the test and control procedures used are given in the Test Method   |
| <b>Disinfectant</b>                              | GAMA Healthcare Ltd biocide<br>Batch number: N/A<br>Date of delivery: June 2006<br>Storage conditions: 20°C – 25°C<br>Active substances: not specified<br>Appearance product dilutions: colourless, clear product solution.                       |
| <b>Interfering Substance (Organic Challenge)</b> | <ol style="list-style-type: none"><li>1. Simulated clean conditions:<br/>0.3 g l<sup>-1</sup> bovine albumin (final concentration)</li><li>2. Simulated dirty conditions:<br/>3.0 g l<sup>-1</sup> bovine albumin (final concentration)</li></ol> |
| <b>Temperature</b>                               | Ambient (23-25°C)   |
| <b>Contact Time Tested</b>                       | 5 (± 10 s) minute.  |
| <b>Test Organisms</b>                            | <i>Campylobacter jejuni</i> ATCC 33291  |
| <b>Culture Medium</b>                            | Brain Heart Infusion, Oxoid.  |
| <b>Incubation</b>                                | Plates were incubated at 42 °C for 96h under a modified atmosphere (Oxoid Campygen System).   |
| <b>Diluent</b>                                   | MRD, Lab M  |
| <b>Neutraliser</b>                               | Neutraliser, containing 60g/l Tween 80, 60g/l Saponin, 2g/l L-histidine, 2g/l L-cysteine in distilled water.  |



## General Method

A standard suspension of test organisms containing  $1.5 - 5.0 \times 10^8$  cells  $\text{ml}^{-1}$  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 GAMA Healthcare Ltd biocide was added and mixed. 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 42°C. Colony forming units were counted after 4 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  $\text{l}^{-1}$  bovine albumin) or dirty conditions (3 g  $\text{l}^{-1}$  bovine albumin) under the required test conditions (23-25°C, 5 minute contact, for the selected reference strain), shall demonstrate at least a 5  $\log_{10}$  reduction in viable counts.

## Results<sup>1</sup>

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 <sub>10</sub> Reduction Achieved |
|-------------------------------|------------------------|--------------------------------------|
| 0.3 g $\text{l}^{-1}$ (clean) | 5                      | >5 <sup>1</sup>                      |
| 3.0 g $\text{l}^{-1}$ (dirty) | 5                      | >5 <sup>1</sup>                      |

Table 1. Log<sub>10</sub> reductions in *Campylobacter jejuni* viable counts following a 5 minute exposure to the test material.

| Referenced Organism  | Starting concentration CFU $\text{ml}^{-1}$                        | Final concentration CFU $\text{ml}^{-1}$ clean 0.3 g $\text{l}^{-1}$ Bovine Albumin | Final concentration CFU $\text{ml}^{-1}$ dirty 3.0 g $\text{l}^{-1}$ Bovine Albumin |
|--|--|---|---|
| <i>Campylobacter jejuni</i> ATCC 33291   | $1.6 \times 10^8$<br>(169,132 <sup>1</sup> , 23, 22 <sup>2</sup> ) | Plate count 0, 0.<br>(Actual 6 $\log_{10}$ reduction)                               | Plate count 0, 0.<br>(Actual 6 $\log_{10}$ reduction)                               |
| CFU = colony forming units<br><sup>1</sup> viable count of bacterial colonies, 1 ml sample of $10^{-6}$ bacterial suspension<br><sup>2</sup> viable count of bacterial colonies, 1 ml sample of $10^{-7}$ bacterial suspension |  |   |   |

Table 2. Reductions in *Campylobacter jejuni* viable counts following a 5 minute exposure to the test material.

## Interpretation of the Results

When tested against *Campylobacter jejuni* ATCC 33291 with a 5 minute contact time the GAMA Healthcare Ltd biocide met the requirements of the Standard at ambient temperature (23-25°C) under simulated clean and dirty conditions.

## Conclusion

According to EN 1276:1997, the batch provided of GAMA Healthcare Ltd 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 *Campylobacter jejuni* ATCC 33291.

<sup>1</sup> See Table of results in Appendix 1.





**Signed:**

A handwritten signature in blue ink, appearing to be 'P. Humphreys', written in a cursive style.

Dr Paul Humphreys  
School of Applied Sciences  
University of Huddersfield



# Appendix 1

| Test Organism  | VALIDATIONS                        |         |       |    |                              |     |                                 |     |    |         | Bacterial Test Suspension |         | Test Procedure Results |     |         |       |       |         |     |    |   |    |    |   |    |    |   |         |   |         |   |        |   |        |  |  |  |
|--|------------------------------------|---------|-------|----|------------------------------|-----|---------------------------------|-----|----|---------|---------------------------|---------|------------------------|-----|---------|-------|-------|---------|-----|----|---|----|----|---|----|----|---|---------|---|---------|---|--------|---|--------|--|--|--|
|  | Experimental Conditions Validation |         |       |    | Neutraliser Toxicity Control |     | Dilution Neutralisation Control |     |    |         |                           |         |                        |     |         |       |       |         |     |    |   |    |    |   |    |    |   |         |   |         |   |        |   |        |  |  |  |
|  | Bacterial Suspension               |         | Clean |    | Dirty                        |     | Vc                              | B   | Vc | C       | Clean                     | Dirty   | Vc                     | Na  | R       | Clean | Dirty |         |     |    |   |    |    |   |    |    |   |         |   |         |   |        |   |        |  |  |  |
| C.jejuni   | Vc                                 | 169     | 132   | Vc | 173                          | 202 | 218                             | 214 | Vc | 282     | 236                       | Vc      | 236                    | 206 | 208     | 214   | 10-6  | 169     | 132 | Vc | < | 15 | 15 | < | 15 | 15 | < | 1.5E+02 | < | 1.5E+02 | > | 2.E+05 | > | 2.E+05 |  |  |  |
|  | Nv                                 | 1.5E+03 | A     |    | 1.9E+02                      |     | 2.2E+02                         |     | B  | 2.6E+02 | C                         | 2.2E+02 |                        |     | 2.1E+02 |       | N     | 1.6E+08 |     |    |   |    |    |   |    |    |   |         |   |         |   |        |   |        |  |  |  |
| <b>Verification of Methodology</b><br>Passed<br>N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N = 1.6E+08<br>Nv is between 6E+2 cfu/ml and 3E+3 cfu/ml, Nv = 1.5E+03<br>CLEAN A ≥ 0.05 x Nv when 0.05 x Nv = 7.5E+01 Yes<br>DIRTY A ≥ 0.05 x Nv when 0.05 x Nv = 7.5E+01 Yes<br>B ≥ 0.05 x Nv when 0.05 x Nv = 7.5E+01 Yes<br>CLEAN C ≥ 0.5 x B when 0.5 x B = 1.3E+02 Yes<br>DIRTY C ≥ 0.5 x B when 0.5 x B = 1.3E+02 Yes |                                    |         |       |    |                              |     |                                 |     |    |         |                           |         |                        |     |         |       |       |         |     |    |   |    |    |   |    |    |   |         |   |         |   |        |   |        |  |  |  |

Table 3. Bactericidal activity of the Gamma Healthcare Biocide when Tested against Campylobacter jejuni using the method specified in BSEN 1276

