

**Bactericidal activity of Gama Health Care Ltd.  
biocide determined using the European Standard  
Test method BS EN 1276:1997 against: *Listeria  
monocytogenes* ATCC 7644.**

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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 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<sup>-1</sup> bovine albumin (final  
concentration)
2. Simulated dirty conditions:  
3.0 g l<sup>-1</sup> bovine albumin (final  
concentration)

**Temperature**      Ambient (25°C)

**Contact Time Tested**      5 (± 10 s) minute.

**Test Organisms**      *Listeria monocytogenes* ATCC 7644

**Culture Medium**      Columbia Blood Agar, Lab M

**Incubation**      Plates were incubated at 35 °C for 24 - 48 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  $\text{ml}^{-1}$  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 disinfectant 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 to 47 °C. After setting, the Petri dishes were incubated at 35°C. Colony forming units were counted after 1-2 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 test conditions (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 *L. monocytogenes* (ATCC 7644) 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>Listeria monocytogenes</i> ATCC 7644	$1.9 \times 10^8$ (186,199 <sup>1</sup> , 23, 19 <sup>2</sup> )	Plate count 0, 0. (Actual 6 $\log_{10}$ reduction)	Plate count 0, 0. (Actual 6 $\log_{10}$ reduction)

CFU = colony forming units

<sup>1</sup> viable count of bacterial colonies, 1 ml sample of  $10^{-6}$  bacterial suspension

<sup>2</sup> viable count of bacterial colonies, 1 ml sample of  $10^{-7}$  bacterial suspension

**Table 2. Reductions in *L. monocytogenes* (ATCC 7644) viable counts following a 5 minute exposure to the test material.**

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



### **Interpretation of the Results**

When tested against *L. monocytogenes* (ATCC 7644) with a 5 minute contact time a full strength Gama Health Care Ltd biocide met the requirements of the Standard under simulated clean and dirty conditions.

### **Conclusion**

According to EN 1276:1997, the batch of Gama Health Care biocide provided possesses bactericidal activity in 5 minutes at ambient temperature (25°C) under clean conditions (0.3g/l bovine albumin) and dirty conditions (3g/l bovine albumin) for referenced strain *L. monocytogenes* (ATCC 7644).

### **Signed:**

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