

**Bactericidal activity of GAMA Healthcare Ltd. Clinell biocide determined using the European Standard Test method BS EN 1276:1997 against: *Acinetobacter baumannii* NCIMB 9216.**

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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. 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. Clinell 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.
<b>Interfering Substance (Organic Challenge)</b>	<ol style="list-style-type: none"><li>1. Simulated clean conditions: 0.3 g l<sup>-1</sup> bovine albumin (final concentration)</li><li>2. Simulated dirty conditions: 3.0 g l<sup>-1</sup> bovine albumin (final concentration)</li></ol>
<b>Temperature</b>	Ambient (25°C)
<b>Contact Time Tested</b>	5 (± 10 s) minute.
<b>Test Organisms</b>	<i>Acinetobacter baumannii</i> NCIMB 9216.
<b>Culture Medium</b>	Tryptone Soya Agar, Lab M
<b>Incubation</b>	Plates were incubated at 25 °C for 48 h
<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 MRD.
<b>General Method</b>	

A standard suspension of test organisms containing  $1.5 - 5.0 \times 10^8$  cells ml<sup>-1</sup> 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. Clinell 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 25°C. Colony forming units were counted after 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 l<sup>-1</sup> bovine albumin) or dirty conditions (3 g l<sup>-1</sup> bovine albumin) under the required test conditions (25°C, 5 minute contact, for the selected reference strain), shall demonstrate at least a 5 log<sub>10</sub> 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 l <sup>-1</sup> (clean)	5	>5 <sup>1</sup>
3.0 g l <sup>-1</sup> (dirty)	5	>5 <sup>1</sup>

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

Referenced Organism	Starting concentration CFU ml <sup>-1</sup>	Final concentration CFU ml <sup>-1</sup> clean 0.3 g l <sup>-1</sup> Bovine Albumin	Final concentration CFU ml <sup>-1</sup> dirty 3.0 g l <sup>-1</sup> Bovine Albumin
<i>Acinetobacter baumannii</i> NCIMB 9216	1.9 x 10 <sup>8</sup> (20 <sup>2</sup> , 178 <sup>1</sup> , 23, 20 <sup>2</sup> )	Plate count 0, 0. (Actual 6 log <sub>10</sub> reduction)	Plate count 0, 0. (Actual 6 log <sub>10</sub> reduction)
CFU = colony forming units <sup>1</sup> viable count of bacterial colonies, 1 ml sample of 10 <sup>-6</sup> bacterial suspension <sup>2</sup> viable count of bacterial colonies, 1 ml sample of 10 <sup>-7</sup> bacterial suspension			

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

## Interpretation of the Results

When tested against *A. baumannii* NCIMB 9216 with a 5 minute contact time a full strength GAMA Healthcare Ltd. Clinell biocide met the requirements of the Standard at ambient temperature (25°C) under simulated clean and dirty conditions.

## Conclusion

According to EN 1276:1997, the batch provided of GAMA Healthcare Ltd. Clinell biocide 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 *A. baumannii* NCIMB 9216.

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<sup>1</sup> 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															
<i>A. baumannii</i>	Vc	175	206	193	198	Vc	230	195	Vc	239	222	227	223	10-6	202	178	Vc	<	15	15	<	15	15
	Nv	1.9E+03	A	1.9E+02	2.0E+02		B	2.1E+02		C	2.3E+02		2.3E+02		N	1.9E+08		R	>	3.E+05		>	3.E+05
<b>Verification of Methodology</b>				<b>Pass</b>	<b>Log10 Reductions (cfu/ml)</b>																		
N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N =				1.9E+08	Yes	Clean > 5																	
Nv is between 6E+2 cfu/ml and 3E+3 cfu/ml, Nv =				1.9E+03	Yes	Dirty > 5																	
CLEAN A ≥ 0.05 x Nv when 0.05 x Nv =				9.6E+01	Yes																		
DIRTY A ≥ 0.05 x Nv when 0.05 x Nv =				9.6E+01	Yes																		
B ≥ 0.05 x Nv when 0.05 x Nv =				9.6E+01	Yes																		
CLEAN C ≥ 0.5 x B when 0.5 x B =				1.1E+02	Yes																		
DIRTY C ≥ 0.5 x B when 0.5 x B =				1.1E+02	Yes																		

**Table 3. Testing of *A. baumannii* (NCIMB 9216) the GAMA Healthcare Ltd. Clinell biocide using BS EN 1276:1997.**