

Bactericidal activity of GAMA Healthcare Ltd. Clinell biocide determined using the European Standard Test method BS EN 1276:1997 against: *Salmonella typhimurium* ATCC 14028.

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Commercial in Confidence

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 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.
Interfering Substance (Organic Challenge)	<ol style="list-style-type: none">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>Salmonella typhimurium</i> ATCC 14028
Culture Medium	Tryptone Soya Agar, Lab M
Incubation	Plates were incubated at 37 °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 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. In this case the disinfectant was the GAMA Healthcare Ltd Clinell biocide. 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 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 l⁻¹ bovine albumin) or dirty conditions (3 g l⁻¹ bovine albumin) under the required test conditions (25°C, 5 minute contact, for the selected reference strain), 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 *S. typhimurium* 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>Salmonella typhimurium</i> ATCC14028	1.7 x 10 ⁸ (171,160 ¹ , 20, 22 ²)	Plate count 3, 3. (Actual 6 log ₁₀ reduction)	Plate count 5, 2. (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 *S. typhimurium* viable counts following a 5 minute exposure to the test material.

Interpretation of the Results

When tested against *S. typhimurium* (ATCC 14028) 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 *S. typhimurium* (ATCC 14028).

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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>S. typhimurium</i>	Vc	138	130	124	122	Vc	166	158	Vc	203	169	164	161	10-6	171	160	Vc	<	15	15	<	15	15	
	Nv	1.7E+03	A	1.3E+02	1.2E+02	B	1.6E+02	C	1.9E+02	1.6E+02	10-7	20	22	Na	<	1.5E+02	<	1.5E+02	<	1.5E+02	<	1.5E+02	<	1.5E+02
												N	1.7E+08	R	>	2.E+05	>	2.E+05	>	2.E+05	>	2.E+05	>	2.E+05
Verification of Methodology				Pass	Log10 Reductions (cfu/ml)																			
N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N =		1.7E+08		Yes	Clean	>	5																	
Nv is between 6E+2 cfu/ml and 3E+3 cfu/ml, Nv =		1.7E+03		Yes	Dirty	>	5																	
CLEAN A ≥ 0.05 x Nv when 0.05 x Nv =		8.5E+01		Yes																				
DIRTY A ≥ 0.05 x Nv when 0.05 x Nv =		8.5E+01		Yes																				
B ≥ 0.05 x Nv when 0.05 x Nv =		8.5E+01		Yes																				
CLEAN C ≥ 0.5 x B when 0.5 x B =		8.1E+01		Yes																				
DIRTY C ≥ 0.5 x B when 0.5 x B =		8.1E+01		Yes																				

Table 3. Testing of *S. typhimurium* (ATCC 14028) the GAMA Healthcare Ltd Clinell biocide using BS EN 1276:1997.