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

February 2007

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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 Healthcare Ltd biocide as used in Clinell Universal Sanitising Wipes Batch number: N/A Date of delivery: June 2006 Storage conditions: $20^{o}C$ – $25^{o}C$ 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 (25ºC)
Contact Time Tested	5 (± 10 s) minute.
Test Organisms	*Vibrio cholerae NCTC 11348*
Culture Medium	Tryptone Soya Agar, Lab M
Incubation	Plates were incubated at 37 ºC for 48-60 h
Diluent	MRD, Lab M
Neutraliser	**N**eutraliser, 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 x 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 GAMA Healthcare 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 ºC. After setting, the Petri dishes were incubated at 37 ºC. 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 ºC), 5 minute contact, for *Vibrio cholerae* NCTC 11348, shall demonstrate at least a 5 $\log_{10}$ reduction in viable counts.

Results[1]

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. $\log_{10}$ 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
Vibrio cholerae 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 GAMA Healthcare 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 GAMA Healthcare 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.

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

Table 3. Testing of *Vibrio cholerae* NCTC 11348 the GAMA Healthcare Ltd biocide using BS EN 1276:1997.