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

July 2006

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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

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)

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

 $5 (\pm 10 \text{ s})$ minute.

Test Organisms

Salmonella typhimurium 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 x 10⁸ 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 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	>51
3.0 g l ⁻¹ (dirty)	5	>51

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 mΓ¹clean 0.3 g Γ¹ Bovine Albumin	Final concentration CFU m\(\Gamma^1\) dirty 3.0 g \(\Gamma^1\) Bovine Albumin
Salmonella typhimurium 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

Table 2. Reductions in S. typhimurium viable counts following a 5 minute exposure to the test material.

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

¹ See Table of results in Appendix 1.



Interpretation of the Results

When tested against *S. typhimurium* (ATCC 14028) with a 5 minute contact time a full strength GAMA Healthcare Ltd 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 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).

Signed:

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Appendix 1

						ALIDA	VALIDATIONS												100	9	- 10
Test Organism	Bacterial		xperim	ental Co	Experimental Conditions Validation	ition	Neutra	Neutraliser Toxicity	lity	ă	Dilution Neutralisation Control	Neutrali Control	sation	Bac	Bacterial Test Suspension	st		Test	Test Procedure Results	ure Re	sults
0	Suspension		Clean	_	Dirty			Control	Q.	0	Clean		Dirty					Clean	ue		Dirty
S. typhimurium		2	138	138 130	124	122	2	166	158	Vc 2	203 1	169	164 161	10-6	171	160	2	< 15	15	v	15 15
														10-7	20	22	BN	1.1	1.5E+02	٧	1.5E+02
	Nv 1.7E+03 A	A 8		1.3E+02	1.2E+02	576	В	1.6E+02		O	1.9E+02	_	1.6E+02	z	1.7E+08		œ	> 2	2.E+05	۸	2.E+05
	,	/erific	ation of	Verification of Methodology	fology	Pass	Log10	Log10 Reductions (cfu/ml)	s (cfu	(ml)	Charles Control		The state of the s								
N is between Nv is betwee	N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N = Nv is between 6E+2 cfu/ml and 3E+3 cfu/ml. Nv	and 5E and 3	E+3 cful	ml, N=	1.7E+08	Yes	Clean	^	ω												
				11	1.7E+03	Yes	Dirty	٨	5												
CLE	CLEAN A > 0.05 x Nv when 0.05 x Nv =	Nv wh	en 0.05	= N ×	8.5E+01	Yes															
PIC	DIRTY A ≥ 0.05 x Nv when 0.05 x Nv =	Nv wh	en 0.05	× Nv =	8.5E+01	Yes															
	B ≥ 0.05 x Nv when 0.05 x Nv =	Nv wh	en 0.05	= NX ×	8.5E+01	Yes															
	CLEAN C ≥ 0.5 x B when 0.5 x B =	5 x B	when 0.	5 x B =	8.1E+01	Yes	N.														
	DIRTY C ≥ 0.5 x B when 0.5 x B =	5 x B	when 0.	5 x B =	8.1E+01	Yes	1														

Table 3. Testing of S. typhimurium (ATCC 14028) the Gamma Health Care Ltd biocide using BS EN 1276:1997.

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