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)

Simulated clean conditions:
 0.3 g l⁻¹ bovine albumin (final

concentration)

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

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 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. 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 1-1 bovine albumin) or dirty conditions (3 g l⁻¹ bovine albumin) under the 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 L. monocytogenes (ATCC 7644) viable counts following a 5 minute exposure to the test material.

Referenced Organism	Starting concentration CFU m ¹	Final concentration CFU ml¹clean 0.3 g l¹Bovine Albumin	Final concentration CFU ml ⁻¹ dirty 3.0 g l ⁻¹ Bovine Albumin
Listeria monocytogenes ATCC 7644	1.9 x 10 ⁸ (186,199 ¹ , 23, 19 ²)	Plate count 0, 0. (Actual 6 log ₁₀ reduction)	Plate count 0, 0. (Actual 6 log ₁₀ reduction)

CFU = colony forming units

Table 2. Reductions in L. monocytogenes (ATCC 7644) 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

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:

Dr Paul Humphreys School of Applied Sciences University of Huddersfield

Appendix 1

					VALIDATIONS	SNOL						ľ			ľ			
Test Organism	Bacterial	Experir	Experimental Conditions		alidation	Ne	Neutraliser	ă	Dilution Neutralisation Control	alisation C	ontrol	Pac	Bacterial Test	est		Test Procedure Results	edure	Results
	Suspension		Clean	Dirty	,	Toxic	Toxicity Control		Clean		Dirty	70	suspension	u.		Clean	ŀ	Did
Listeria	Vc 186 199 Vc	Vc 146	9/1 9	172	186	γç	167 195	S/C	179 171	1 172	191	10-6	186	199	V S	15 15	٧	15 15
monocytogenes			35			Į.						10-7	23	10	v	- 37	٧	ų,
ATCC 7644	Nv 1.9E+03 A		1.6E+02	1.8E+02	02	В	1.8E+02	O	1.8E+02	1.8	.8E+02	z	1.9	1 -	^	3.E+05	^	3.F+05
100000000000000000000000000000000000000	Ver	ification o	Verification of Methodology	Non-contract	Passed		Log10 Reductions/cfu/ml	s/cfu/r	lu l	SHOW S		The same of	TOURS.	A. Carlotte	MONORMA	The Party of the P	100	The same of
N is between	N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N =	and 5E+8	3 cfu/ml, N =	1.9E+08	Yes	Clean	5.4							GE TO			W.	
Nv is betwe	Nv is between 6E+2 cfu/ml and 3E+3 cfu/ml, Nv =	and 3E+3	cfu/ml, Nv =	1.9E+03	Yes	Dirty	5.4			1								
0	CLEAN A ≥ 0.05 x Nv when 0.05 x Nv =	x Nv when	0.05 × Nv =	9.6E+01	Yes									97				
	DIRTY A ≥ 0.05 x Nv when 0.05 x Nv =	x Nv when	0.05 x Nv =		Yes							7		N				
	B ≥ 0.05	x Nv when	B ≥ 0.05 x Nv when 0.05 x Nv =	9.6E+01	Yes					The state of				ħ,			OS ME	
	CLEAN C≥	0.5 x B wh	CLEAN C ≥ 0.5 x B when 0.5 x B =	9.1E+01	Yes									7				
	DIRTYCZ	0.5 x B wh	DIRTY C ≥ 0.5 x B when 0.5 x B =	9.1E+01	Yes													

Table 3. Testing of L. monocytogenes (ATCC 7644) the Gama Health Care Ltd biocide using BS EN 1276:1997.

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