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

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Author: P. Humphreys **Signature:** RP Humphreys **Date:** 25/7/06
Authorised by: P. Humphreys **Signature:** RP Humphreys **Date:** 25/7/06

University of
HUDDERSFIELD

Tests Carried Out By:

University of Huddersfield

School of Applied Sciences
Queensgate
Huddersfield
HD1 3DH

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)

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

Acinetobacter baumanii NCIMB 9216.

Culture Medium

Tryptone Soya Agar, Lab M

Incubation

Plates were incubated at 25 °C for 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 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 GAMA Healthcare Ltd 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^{-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 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 *A. baumanii* 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
Acinetobacter baumani NCIMB 9216	1.9 x 10^8 (202,178[1], 23, 20[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 *A. baumanii* viable counts following a 5 minute exposure to the test material.

[1] See Table of results in Appendix 1.

Interpretation of the Results

When tested against *A. baumanii* NCIMB 9216 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 *A. baumanii* NCIMB 9216.

Signed:

Dr Paul Humphreys
School of Applied Sciences
University of Huddersfield

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
A. baumanii		Vc 175 206	193 198	Vc 230 195	Vc 239 222	227 223	10-6 202 178	Vc < 15 15	< 15 15
							10-7 23 20	Na < 1.5E+02	< 1.5E+02
	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

Verification of Methodology		Pass
N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N =	1.9E+08	Yes
Nv is between 6E+2 cfu/ml and 3E+3 cfu/ml, Nv =	1.9E+03	Yes
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

Log10 Reductions (cfu/ml)		
Clean	>	5
Dirty	>	5

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