Bactericidal activity of GAMA Healthcare Ltd. Clinell biocide determined using the European Standard Test method BS EN 1276:1997 against: *Campylobacter jejuni* ATCC 33291.

~

Prepared by
Dr Paul Humphreys
Oct '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 Clinell biocide
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 (23-25ºC)

Contact Time Tested

5 (± 10 s) minute.

Test Organisms

Campylobacter jejuni ATCC 33291

Culture Medium

Brain Heart Infusion, Oxoid.

Incubation

Plates were incubated at 42 ºC for 60h under a modified atmosphere (Oxoid Campygen System).

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 Clinell 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 ºC. After setting, the Petri dishes were incubated at 42ºC. Colony forming units were counted after 5 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 (23-25ºC, 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 *Campylobacter jejuni* 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
Campylobacter jejuni ATCC 33291	1.6 x 10^8 (169,132[1], 23, 22[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 *Campylobacter jejuni* viable counts following a 5 minute exposure to the test material.

Interpretation of the Results

When tested against *Campylobacter jejuni* ATCC 33291 with a 5 minute contact time the GAMA Healthcare Ltd Clinell biocide met the requirements of the Standard at ambient temperature (23-25^oC) 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 (23-25^oC) under clean conditions (0.3g/l bovine albumin) and dirty conditions (3g/l bovine albumin) for referenced strain *Campylobacter jejuni* ATCC 33291.

Dr Paul Humphreys
School of Applied Sciences
University of Huddersfield

[1] See Table of results in Appendix 1.

Appendix 1

Test Organism	VALIDATIONS												Test Procedure Results					
	Bacterial Suspension			Experimental Conditions Validation				Neutraliser Toxicity Control			Dilution Neutralisation Control				Bacterial Test Suspension					
				Clean		Dirty					Clean		Dirty					Clean		Dirty
C.jejuni	Vc	169	132	Vc	173	202	218	214	Vc	282	236	Vc	236	206	208	214	10-6	169	132	Vc < 15	15	< 15	15
																	10-7	23	22	Na < 1.5E+02		< 1.5E+02	
	Nv	1.5E+03		A	1.9E+02		2.2E+02		B	2.6E+02		C	2.2E+02		2.1E+02		N	1.6E+08		R > 2.E+05		> 2.E+05	

Verification of Methodology		Passed
N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N =	1.6E+08	
Nv is between 6E+2 cfu/ml and 3E+3 cfu/ml, Nv =	1.5E+03	
CLEAN A ≥ 0.05 x Nv when 0.05 x Nv =	7.5E+01	Yes
DIRTY A ≥ 0.05 x Nv when 0.05 x Nv =	7.5E+01	Yes
B ≥ 0.05 x Nv when 0.05 x Nv =	7.5E+01	Yes
CLEAN C ≥ 0.5 x B when 0.5 x B =	1.3E+02	Yes
DIRTY C ≥ 0.5 x B when 0.5 x B =	1.3E+02	Yes

Log10 Reductions/cfu/ml	
Clean	5.3216
Dirty	5.3216

Table 3. Bactericidal activity of the Gama Healthcare Clinell Biocide when Tested against C*ampylobacter jejuni using the method specified in BSEN 1276*