

**Bactericidal activity of Gama Health Care Ltd. Clinell biocide determined using the European Standard Test method BS EN 1276:1997 against:
Vancomycin Resistant
Enterococcus faecalis (ATCC 51299)**



University of
HUDDERSFIELD

**Bactericidal activity of Gama Health Care Ltd.
Clinell biocide determined using the European
Standard Test method BS EN 1276:1997 against:
Vancomycin Resistant *Enterococcus faecalis*
(ATCC 51299)**

6 March 2008

Author: P. Humphreys Signature:  Date: 6/3/08
 Checked by: ~~Kevin Lisle~~ Signature:  Date: 6/3/08
 Authorised by: Kevin Lisle Signature:  Date: 6/3/08
 P. Humphreys

HMS Report	HMS/Gama/1/08
ISSUE:	Date:
Draft for Comment	19/2/08
Version 1	3/3/08

Tests Carried Out By:

University of Huddersfield

Huddersfield Microbiology services
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	Clinell Gama Health Care Ltd biocide Batch number: N/A Date of delivery: Feb 2007 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	20°C
Contact Time Tested	5 (± 10 s) minute.
Test Organisms	Vancomycin Resistant <i>Enterococcus faecalis</i> (ATCC 51299)
Culture Medium	Tryptone Soya Agar, Lab M
Incubation	Plates were incubated at 37 °C for 24-48h
Diluent	MRD, Lab M
Neutraliser	Neutraliser, containing 60g/l Tween 80, 60g/l Saponin, 2g/l L-histidine, 2g/l L-cysteine in MRD.

Whilst these analyses have been carried out carefully and have been checked, no liabilities can be accepted for consequential or indirect damages.

General Method

A standard suspension of test organisms containing $1.5 - 5.0 \times 10^8$ 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 Health Care 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 °C. After setting, the Petri dishes were incubated at 37 °C. Colony forming units were counted after 24-48 hours incubation and the fraction of surviving organisms calculated.

Test Organism

The test organism was the Vancomycin Resistant strain of *Enterococcus faecalis* (ATCC 51299).

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 20°C and a 5 minute contact time shall demonstrate at least a $5 \log_{10}$ 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	>5 ¹
3.0 g l ⁻¹ (dirty)	5	>5 ¹

Table 1. Log₁₀ reductions in *Enterococcus faecalis* (ATCC 51299) 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
<i>Enterococcus faecalis</i> (ATCC 51299)	1.6×10^8 (164,163 ¹)	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

Table 2. Reductions in Vancomycin Resistant *Enterococcus faecalis* (ATCC 51299) viable counts following a 5 minute exposure to the test material.

Interpretation of the Results

When tested against Vancomycin Resistant *Enterococcus faecalis* (ATCC 51299) with a 5 minute contact time a full strength Clinell Gama Health Care Ltd biocide met the requirements of the Standard under simulated clean and dirty conditions.

Conclusion

According to EN 1276:1997, the Gama Health Care biocide Clinell possesses bactericidal activity in 5 minutes at 20°C under clean (0.3g/l bovine albumin) and dirty conditions (3g/l bovine albumin) for the Vancomycin resistant strain of *Enterococcus faecalis* (ATCC 51299).

Signed:



Dr Paul Humphreys, School of Applied Sciences, University of Huddersfield

¹ See Table of results in Appendix 1.

Whilst these analyses have been carried out carefully and have been checked, no liabilities can be accepted for consequential or indirect damages.

Appendix 1

Test Organism	VALIDATIONS															
	Bacterial Suspension	Experimental Conditions Validation		Neutraliser Toxicity	Dilution Neutralisation Control		Bacterial Test Suspension	Test Procedure Results								
		Clean	Dirty		Clean	Dirty		Clean	Dirty							
<i>E. faecalis</i>	Vc 146	136	146	138	Vc 150	170	Vc 128	130	123	125	10-6	164	163	Vc < 15	15	15
Nv 1.6E+03	A	1.4E+02	1.4E+02	1.4E+02	B	1.6E+02	C	1.3E+02	1.2E+02	10-7	N	1.6E+08	R	Na < 1.5E+02	< 1.5E+02	> 2.E+05
Verification of Methodology Passed N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N = 1.6E+08 Yes Nv is between 6E+2 cfu/ml and 3E+3 cfu/ml, Nv = 1.6E+03 Yes CLEAN A ≥ 0.05 x Nv when 0.05 x Nv = 8.2E+01 Yes DIRTY A ≥ 0.05 x Nv when 0.05 x Nv = 8.2E+01 Yes B ≥ 0.05 x Nv when 0.05 x Nv = 8.2E+01 Yes CLEAN C ≥ 0.5 x B when 0.5 x B = 8.0E+01 Yes DIRTY C ≥ 0.5 x B when 0.5 x B = 8.0E+01 Yes Log10 Reductions/cfu/ml Clean 5.3 Dirty 5.3																

Table 3. Testing of *Enterococcus faecalis* (ATCC 51299) the Gama Health Care Ltd biocide using BS EN 1276:1997.

