

**Bactericidal activity of Gama Health Care Ltd.
biocide determined using the European Standard
Test method BS EN 1276:1997 against: *Klebsiella
pneumoniae* NCTC 13368 (ATCC 700603)**

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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)	<ol style="list-style-type: none">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 - 25°C)
Contact Time Tested	5 (± 10 s) minute.
Test Organisms	<i>Klebsiella pneumoniae</i> NCTC 13368
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.

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 2-3 days incubation and the fraction of surviving organisms calculated.

Test Organism

The test organism *Klebsiella pneumoniae* NCTC 13368 (ATCC 700603) is a multi-drug resistant strain which:

- has intermediate resistance to ceftriaxone and gentamicin,
- is used as a control organism for extended-spectrum beta-lactamase production and produces beta-lactamase SHV-18,
- is resistant to ampicillin, aztreonam, ceftazidime, ceftazidime, chloramphenicol, piperacillin and tetracycline.

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 ambient temperature (23 to 25 °C), 5 minute contact, for *Klebsiella pneumoniae* NCTC 13368 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 *Klebsiella pneumoniae* NCTC 13368 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>Klebsiella pneumoniae</i> NCTC 13368	1.6×10^8 (150,164 ¹ , 15, 16 ²)	Plate count 0, 0. (Actual 6 \log_{10} reduction)	Plate count 0, 4. (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 *Klebsiella pneumoniae* NCTC 13368 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 *Klebsiella pneumoniae* NCTC 13368 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 provided of Gama Health Care biocide possesses bactericidal activity in 5 minutes at ambient temperature (23-25°C) under clean conditions (0.3g/l bovine albumin) and dirty conditions (3g/l bovine albumin) for referenced strain *Klebsiella pneumoniae* NCTC 13368.

Signed:

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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	Clean	Dirty																
<i>Klebsiella pneumoniae</i>	Vc	178	187	193	192	Vc	158	181	Vc	173	182	158	177	10-6	150	164	Vc	<	15	15	<	15	15			
	Nv	1.6E+03	A	1.8E+02	1.9E+02		B	1.7E+02	C	1.8E+02	1.7E+02		10-7	15	16	N	1.6E+08	Na	<	1.5E+02	<	1.5E+02	R	>	2.E+05	>
Verification of Methodology				Passed				Log10 Reductions/cfu/ml																		
N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N =				1.6E+08				Clean		5.32																
Nv is between 6E+2 cfu/ml and 3E+3 cfu/ml, Nv =				1.6E+03				Dirty		5.32																
CLEAN A ≥ 0.05 x Nv when 0.05 x Nv =				7.8E+01																						
DIRTY A ≥ 0.05 x Nv when 0.05 x Nv =				7.8E+01																						
B ≥ 0.05 x Nv when 0.05 x Nv =				7.8E+01																						
CLEAN C ≥ 0.5 x B when 0.5 x B =				8.5E+01																						
DIRTY C ≥ 0.5 x B when 0.5 x B =				8.5E+01																						

Table 3. Testing of *Klebsiella pneumoniae* NCTC 13368 the Gama Health Care Ltd biocide using BS EN 1276:1997.