Bactericidal activity of GAMA Healthcare Ltd. handrub (Clinell) determined using the European Standard Test method BS EN 1276:1997 (modified) against: Mycobacterium smegmatis (NCIMB 133116)

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Tests Carried Out By:	University of Huddersfield**.** School of Applied Sciences, Queensgate, Huddersfield HD1 3DH
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Microbiological Tests	
Test Method	British/European Standard BS EN 1276:1997 (modified). Dilution-neutralisation
Test Procedures	Full details of all the test and control procedures used are given in the Test Method
Disinfectant	GAMA Healthcare Ltd Handrub (Clinell)
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 ºC (± 1 ºC)
Contact Time Tested	5 (± 10 s) minute.
Test Organisms	*Mycobacterium smegmatis* (NCIMB 133116)
Culture Medium	Nutrient Agar, Lab M
Incubation	Plates were incubated at 37 ºC for 24 - 48 h
Diluent	Tryptone Sodium Chloride Solution
Neutraliser	"Universal" neutraliser, containing polysorbate 80, L-histidine, lecithin, saponin and sodium thiosulphate in diluent. Tests were carried out to verify that this neutraliser was satisfactory.

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 disinfectant was added and mixed. In this case the disinfectant was a 50% dilution of the GAMA Healthcare Ltd biocide, (Clinell). 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 37 ºC. Colony forming units were counted after 1-2 days incubation and the fraction of surviving organisms calculated

Modifications to BS EN 1276:1997

Tests were carried out according to a modified version of BS EN 1276:1997. These modifications were that a 50% dilution of the test material was used, *M. smegmatis* was the test organism and nutrient agar was employed as growth medium. A 50% dilution of the test material was necessary in order to ensure an accurate contact time. It was not possible to produce an accurate contact time with the full strength product due to incomplete neutralisation.

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 (20 ºC, 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 *M. smegmatis* viable counts following a 5 minute exposure to a 50% dilution of 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
Mycobacterium smegmatis (NCIMB 133116)	3.2 x 10^8 (340,300)[1]	Plate count 0, 0. (Actual 8 log_{10} reduction)	Plate count 0, 0. (Actual 8 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 *M. smegmatis* viable counts following a 5 minute exposure to a 50% dilution of the test material.

Interpretation of the Results

When tested against *Mycobacterium smegmatis* (NCIMB 133116) with a 5 minute contact time a 50% dilution of the GAMA Healthcare Ltd biocide (Clinell) met the requirements of the Standard under simulated clean and dirty conditions.

Conclusion

According to EN 1276:1997, GAMA Healthcare biocide (Clinell) when diluted at 50% (V/V) in hard water, possesses bactericidal activity in 5 minutes at 20°C under clean conditions (0.3g/l bovine albumin) and dirty conditions (3g/l bovine albumin) for referenced strain *Mycobacterium smegmatis.*

[1] See Table of results in Appendix 1.

Appendix 1

Test Organism	Validation Test				Bacterial Test Suspension	Test Procedure at Concentration %(V/V)
	Bacterial Suspension	Experimental Conditions (A)	Neutraliser Toxicity control (B)	Dilution-Neutralisation Control (C)		50% solution biocide
Mycobacterium Smegmatis **Clean**	V_c 340; 300 *N*v: 3.2 x 10^3	V_c 183; 176 A: 3.2 x 10^3	V_c 317, 329 A: 3.2 x 10^3	V_c 120; 209 A: 3.2 x 10^3	10^{-6}: 340, 300 10^{-7}: 31, 26 3.2 x 10^8	**V_c >300, 300** **N_a > 3 x 10^3** **R > 10^5 (≥ 10^8)**
Mycobacteria Smegmatis **Dirty**	V_c 340; 300 *N*v: 3.2 x 10^3	V_c 278; 264 A: 3.2 x 10^3	V_c 317; 329 A: 3.2 x 10^3	V_c 131; 160 A: 3.2 x 10^3	10^{-6}: 340, 300 10^{-7}: 31, 26 3.2 x 10^8	

Vc = Viable Count
N = Number of cfu/ml of bacterial test suspension.
Nv= Number of cfu/ml of bacterial suspension.
R = Reduction in viability.
Na = Number of cfu/ml in the test mixture.
A = Number of cfu/ml of the experimental conditions validation.
B = Number of cfu/ml of the neutraliser toxicity validation.
C = Number of cfu/ml of the dilution-neutralisation validation.

Table 3. Testing of *Mycobacteria smegmatis* (NCIMB 133116) against a 50% dilution of the GAMA Healthcare Handrub (Clinell) using a modified version of BS EN 1276:1997.