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**Bactericidal activity of GAMA Healthcare
Ltd. Clinell Universal Wipes 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,
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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 Clinell Universal Wipes

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 (± 1 °C)

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 °C 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 10⁸ 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 Clinell Universal Wipes. 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 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 °C, 5 minute contact, for the selected reference strain), shall demonstrate at least a 5 log₁₀ 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 *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
<i>Mycobacterium smegmatis</i> (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 Clinell Universal Wipes met the requirements of the Standard under simulated clean and dirty conditions.

¹ See Table of results in Appendix 1.



Conclusion

According to EN 1276:1997, Clinell Universal Wipes biocide 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*.

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
<i>Mycobacterium Smegmatis</i> 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
<i>Mycobacteria Smegmatis</i> 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	R > 10 ⁵ (≥ 10 ⁸)

V_c = Viable Count
 N = Number of cfu/ml of bacterial test suspension.
 N_v = Number of cfu/ml of bacterial suspension.
 R = Reduction in viability.
 N_a = 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 Clinell Universal Wipes using a modified version of BS EN 1276:1997.