Bactericidal activity of GAMA Healthcare Ltd. handrub (Clinell) product for post contamination treatment of hands determined using the European Standard Test method BS prEN 12054:1995

January 2005

Author: P. Humphreys

University of HUDDERSFIELD

Tests Carried Out By:	University of Huddersfield**.** School of Applied Sciences, Queensgate, Huddersfield HD1 3DH
Date:	January 2005
Microbiological Tests	
Test Method	British/European Standard BS prEN 12054:1995
Test Procedures	Full details of all the test and control procedures used are given in the Test Method
Handrub	GAMA Healthcare Ltd. (Clinell)
Batch Tested	17012005
Temperature	20 ºC (± 1 ºC)
Contact Time Tested	1 minute
Test Organisms	*Escherichia coli* 8879 (NCIMB), *Enterococcus hirae* 8192 (NCIMB), *Pseudomonas aeruginosa* 10421 (NCIMB), *Staphylococcus aureus* 9518 (NCIMB) National Collections of Industrial, Food and Marine Bacteria, Aberdeen.
Culture Medium	Tryptone Soya Agar, LabM
Incubation	Plates were incubated at 37 ºC for 24 - 48 h
Counting procedure	Pour plate
Diluent	Tryptone Sodium Chloride Solution
Neutraliser	"Universal" neutraliser containing polysorbate 80, L-histidine, lecithin and sodium thiosulphate in diluent. Tests were carried out to verify that this neutraliser was satisfactory (see Annex 1 validation results tables A2 and A3).

General Method

A standard suspension of test organisms containing 1.5 – 5.0 x 108 cells ml-1 of bacteria was prepared. 9 ml of hygienic handrub solution was pipetted into a sterile universal bottle. 1 ml of test organism suspension was added, mixed and left for a contact time of 1 min ± 5 s in a temperature controlled water bath (20 ºC ± 1 ºC). At the contact time 1 ml of the mixture was pipetted into a tube containing 8 ml of neutraliser and 1.0 ml of water. After a neutralisation time of 1 min ± 5 s a series of ten-fold dilutions (to 10-3) was prepared in the diluent. 1 ml samples in duplicate of the 100 (undiluted neutralised sample), 10-2 and 10-3 dilutions of neutralised mixture was inoculated into sterile 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 48 h incubation and the fraction of surviving organisms calculated.

Results

Table 1 Bactericidal activity of hygienic handrub GAMA Healthcare Ltd. (Clinell)

Test Organism	Contact Time (min)	Colony Counts per plate cfu ml^{-1}
Escherichia coli	1	< 3 x 10^{2*}
Enterococcus hirae	1	< 3 x 10^{2*}
Pseudomonas aeruginosa	1	< 3 x 10^{2*}
Staphylococcus aureus	1	< 3 x 10^{2*}

* No visible bacterial growth on any plate; 10^{0}, 10^{-2}, 10^{-3})

Summary and Conclusion

Tests were carried out according to BS EN 12054:1995

Requirements of this standard

Hygienic handrub products which demonstrate a reduction in viable counts from 1 x 10^{7} to 3 x 10^{7} cfu ml^{-1} to no more than 3 x 10^{2} cfu ml^{-1} within 1 min at 20 ºC when the test organisms are *Escherichia coli*, *Enterococcus hirae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* are deemed to have passed the test.

Interpretation of the Results

GAMA Healthcare Ltd handrub (Clinell) when tested against this standard passed the test and can be described as a bactericidal preparation for hygienic hand disinfection.

Annex 1

Method Validation

A1. Validation of non-toxicity of the neutralisation medium (control)
To 9 ml of neutralisation medium 1 ml of bacterial test suspension diluted to 1 x 10^3 to 3 x10^3 is added, mixed and left in the water bath at 20 ºC ± 1 ºC for a contact time of 1 min ± 5 s. At the end of the contact time 1 ml samples are taken in duplicate and pour plates prepared, after incubation at 37 ºC for 48 h. The average plate count N' of the neutralisation medium is determined.

A2. Validation of the inactivation by the dilution neutralisation method (test)
To 1 ml of the bacterial diluent 9 ml of the handrub product is added, mixed and left in a water bath at 20 ºC ± 1 ºC for 5 min ± 10 s. 1 ml of the mixture is then transferred to 8 ml of neutralising medium, maintained at 20 ºC ± 1 ºC and left for 5 min ± 10 s. 1 ml of bacterial test suspension diluted to 1 x 10^3 to 3 x10^3 is added, mixed and left for a contact time of 1 min ± 5 s. At the end of the contact time 1 ml samples are taken in duplicate and pour plates prepared, after incubation at 37 ºC for 48 h. The average plate count n' of the dilution neutralisation method is determined.

Table 2A Validation of dilution-neutralisation method

Test Organism	**Bacterial test suspension (cfu ml^{-1})**	**Validation of neutralisation**	
		A1. Control	**A2. Test**
Escherichia coli	117, 108 (N=113)	105, 99 (N'=102)	89, 96 (n'= 93)
Enterococcus hirae	250, 244 (N=247)	236,252 (N'=244)	225,215 (n'=220)
Pseudomonas aeruginosa	165, 166 (N=166)	155,153 (N'=154)	150,155 (n'=153)
Staphylococcus aureus	127,111 (N=119)	96, 83 (N'=90)	88, 79 (n'=84)

Results
For the strains tested:

N and N' are between 100 and 300 cfu

N' is greater than 0.5 times N

n' is greater than 0.5 times N'

The neutralisation is validated with the neutralisation medium tested.