

**ASSESSMENT OF BACTERICIDAL EFFICACY TESTS  
(EN 1276 - PHASE 2, STEP 1)**

**DIFFICIL-S**

**Clinimax Ltd**

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**TEST PRODUCTS**

DIFFICIL-S Part A:

Ingredients: Sodium chlorite (> 30%), Di-sodium hydrogenorthophosphate,  
Sodium dodecyl sulphate and Sodium carbonate

DIFFICILE-S Part B:

Ingredients: Citric acid monohydrate and Sodium dichlorocyanurate dehydrate.

The test product comprises the mixture of Part A and Part B (sachets of at least 12.5g each) into 10 L water.

**Batch Number:** Part A: 520112      **Expiry Date:** September 2009

**Batch Number:** Part B: 52011      **Expiry Date:** September 2009

**STORAGE CONDITIONS**

At room temperature in a dry place out of direct sunlight.

**TEST ORGANISMS**

<i>Staphylococcus aureus</i>	NCTC 10788
<i>Pseudomonas aeruginosa</i>	NCTC 6749
<i>Escherichia coli</i>	NCTC 10538
<i>Enterococcus hirae</i>	NCTC 12367

## **TEST METHOD AND VALIDATION**

EN 1276 Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas (Phase 2, step 1). Tests for disinfectants for medical establishments not yet ratified.

<b>PRODUCT TEST CONCENTRATION</b>	1.25x supplied
<b>APPEARANCE PRODUCT DILUTION</b>	Clear yellow solution
<b>CONTACT TIMES</b>	1, 2 and 5 minutes
<b>TEST TEMPERATURE</b>	20°C
<b>INTERFERING SUBSTANCE</b>	Bovine albumin 0.03 % albumin (clean condition) 0.3 % albumin (dirty condition)
<b>INHIBITION METHOD</b>	Dilution/neutralization
<b>NEUTRALIZER</b>	Tween 80 (30g/ L), Sodium Lauryl Sulphate (4g/ L), Lecithin (3g/ L).

Tests were performed to establish the suitability of the neutralizer in neutralizing the activity of the disinfectant without being inhibitory to the test organisms (method described in EN 1276). The above neutralizer was found to be suitable.

## **SUMMARY OF TEST METHOD**

The test method is described in EN 1276 Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas (Phase 2, step 1). Copies of EN 1276 are available from BSI, 389 Chiswick High Road, London W4 4AL.

The test method involves mixing 1 ml of the test bacteria with 1 ml of interfering substance (0.3 or 3g/L albumin) and then adding 8 ml of test product. After the required contact time, 1 ml is removed and added to 9 ml of the neutralizer. Following a 5 minute neutralization period, 1 ml is plated to detect surviving test bacteria.

## RESULTS

**Bactericidal Activity of DIFFICIL-S**  
**Following Phase 2 Step 1 Suspension Test Method EN 1276**

**Log<sub>10</sub> counts/reductions achieved in 1, 2, and 5 minutes**

(All tests carried out in duplicate)

Test Organism	Log <sub>10</sub> Initial Count (Challenge)	Contact Time	Log <sub>10</sub> Reduction Achieved					
			Clean Conditions (0.3 % Albumin)			Dirty Conditions (3 % Albumin)		
			Test 1	Test 2	Mean	Test 1	Test 2	Mean
<i>Staphylococcus aureus</i>	8.50	1 min	> 7.50	> 7.50	> <b>7.50</b>	> 7.50	> 7.50	> <b>7.50</b>
		2 mins	> 7.50	> 7.50	> <b>7.50</b>	> 7.50	> 7.50	> <b>7.50</b>
		5 mins	> 7.50	> 7.50	> <b>7.50</b>	> 7.50	> 7.50	> <b>7.50</b>
<i>Pseudomonas aeruginosa</i>	6.67	1 min	> 5.67	> 5.67	> <b>5.67</b>	> 5.67	> 5.67	> <b>5.67</b>
		2 mins	> 5.67	> 5.67	> <b>5.67</b>	> 5.67	> 5.67	> <b>5.67</b>
		5 mins	> 5.67	> 5.67	> <b>5.67</b>	> 5.67	> 5.67	> <b>5.67</b>
<i>Escherichia coli</i>	8.15	1 min	> 7.17	> 7.17	> <b>7.17</b>	> 7.17	> 7.17	> <b>7.17</b>
		2 mins	> 7.17	> 7.17	> <b>7.17</b>	> 7.17	> 7.17	> <b>7.17</b>
		5 mins	> 7.17	> 7.17	> <b>7.17</b>	> 7.17	> 7.17	> <b>7.17</b>
<i>Enterococcus hirae</i>	7.50	1 min	> 7.50	> 7.50	> <b>7.50</b>	> 7.50	> 7.50	> <b>7.50</b>
		2 mins	> 7.50	> 7.50	> <b>7.50</b>	> 7.50	> 7.50	> <b>7.50</b>
		5 mins	> 7.50	> 7.50	> <b>7.50</b>	> 7.50	> 7.50	> <b>7.50</b>

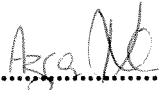
**To meet the requirements of EN 1276 a >5 Log<sub>10</sub> reduction in test bacteria within 5 minutes is required.**

The above results show that DIFFICILE-S demonstrates high activity against the test organisms following the test methods as described in EN 1276.

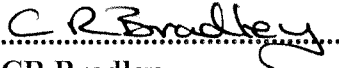
## CONCLUSION


When tested in accordance with EN 1276 (1997), DIFFICIL-S successfully achieved the test criteria, i.e. a  $>5 \text{ Log}_{10}$  (99.999%) reduction was achieved at 1 minute, 2 minutes and 5 minutes at 20°C under both clean (0.03% albumin) and dirty (0.3% albumin) conditions.

To satisfy the requirements of the test, at least a  $> 5 \text{ Log}_{10}$  reduction in specified test organisms is required within 5 minutes when the disinfectant is tested at its intended use dilution. This was successfully achieved and therefore DIFFICILE-S satisfies the requirements of EN 1276.

  
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