

**ASSESSMENT OF THE ACTIVITY OF DIFFICIL-S
AGAINST *CLOSTRIDIUM DIFFICILE***

Clinimax Ltd

**HOSPITAL INFECTION RESEARCH LABORATORY
CITY HOSPITAL
DUDLEY ROAD
BIRMINGHAM B18 7QH**

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MANUFACTURER

Clinimax Ltd
Shepards Grove West,
Stanton,
Bury St. Edmonds,
Suffolk.
IP31 2AR

TEST PRODUCTS

DIFFICIL-S Part A:

Ingredients – Sodium chlorite (>30%), Disodium hydrogenorthophosphate, Sodium dodecyl sulphate and Sodium carbonate.

DIFFICIL-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 10L water.

Lot number: Part A: 50221A

Expiry Date: 09/2009

Part B: 50221B

Expiry Date: 09/2009

STORAGE CONDITIONS

Room temperature, in a dry place out of direct sunlight.

TEST ORGANISMS

Clostridium difficile NCTC 11209

TEST METHOD AND VALIDATION

No European Phase 2/Step 1 test has as yet been described to establish sporicidal activity of chemical disinfectants against *Clostridium difficile* in the medical area. The only Phase 2/Step 1 test published to date is EN 13704 which is designated for food, industrial, domestic and industrial areas. This test looks for a $> 3 \log_{10}$ reduction in 60 minutes under clean conditions only. The test product DIFFICILE-S was tested against *Clostridium difficile* spores following the test conditions described in other Phase 2/Step 1 tests for the medical area.

PRODUCT TEST CONCENTRATION

1.25x supplied

APPEARANCE PRODUCT

Clear green solution

CONTACT TIMES

5, 10, 15 and 60 minutes

TEST TEMPERATURE

20°C

INTERFERING SUBSTANCE

Bovine albumin:-

Clean conditions - 0.03 % albumin (final concentration)

Dirty conditions - 0.3 % albumin (final concentration) plus 3% washed sheep erythrocytes

INHIBITION METHOD

Dilution/neutralization

NEUTRALIZER

Double strength Nutrient Broth

Tests were performed to establish the suitability of this neutralizer in inhibiting the activity of the disinfectant without being toxic to the test organisms (method described in EN 14348).

SUMMARY OF TEST METHOD

The disinfectant was prepared in accordance with EN14348, in sterile hard water, immediately prior to testing.

A suspension of *Clostridium difficile* was prepared, containing at least 10^7 viable spores/ml. The EN 14348 test method involves mixing 1 ml of the test bacteria with 1 ml of soil (0.3% albumin or 3% albumin plus 3% sheep erythrocytes) and then adding 8 ml of test disinfectant. After the required contact time, 1 ml is removed to 9 ml of recovery broth (8ml neutralizer and 1ml diluent). Surviving test bacteria were detected by plating onto blood agar and incubated anaerobically for 3 -5 days.

REQUIREMENT

The test requirements for EN 13704 (Phase2 Step 1 Sporicidal test) is for a 3 log₁₀ reduction in 60 minutes.

RESULTS

SPORICIDAL ACTIVITY OF DIFFICILE-S UNDER CLEAN AND DIRTY CONDITIONS

(All tests carried out in duplicate)

Log ₁₀ Initial Count (Challenge)	Contact Time	Log ₁₀ Reduction Achieved					
		Clean Conditions (0.03 % Albumin)			Dirty Conditions (0.3 % Albumin)		
		Test 1	Test 2	Mean	Test 1	Test 2	Mean
7.29	1 mins	>6.29	5.27	5.78	4.33	4.37	4.35
	2 mins	>6.29	>6.29	>6.29	4.37	4.61	4.49
	5 mins	>6.29	>6.29	>6.29	4.56	4.67	4.61
	10 mins	>6.29	>6.29	>6.29	4.68	4.76	4.72
	15 mins	>6.29	>6.29	>6.29	4.78	4.85	4.82
	30 mins	>6.29	>6.29	>6.29	4.86	5.01	4.93
	60 mins	>6.29	>6.29	>6.29	5.17	5.34	5.25

CONCLUSION

'DIFFICIL-S' demonstrates sporicidal activity at 20°C under clean (0.03% albumin) and dirty (0.3% albumin/0.3% sheep erythrocytes) conditions. The presence of organic matter has an effect on the activity of 'DIFFICIL-S', however a $> 4 \log_{10}$ (99.99%) reduction was achieved with the test organism, *Clostridium difficile*.

Other published EN tests for sporicidal activity have a requirement for a $3 \log_{10}$ reduction in 60 minutes. This was achieved after a contact time of 1 minute under both clean and dirty conditions; therefore 'DIFFICIL-S' meets the test criteria and passes the test.

Testing by the Hospital Infection Research Laboratory does not imply approval or endorsement of this product.



CR Bradley
Laboratory Manager



K Chana
Biomedical Scientist



Dr AP Fraise
Director