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

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OBJECTIVE

To determine the effect of prolonged storage on the efficacy of Difficil-S against spores of Clostridium difficile.

The manufacturers claim that the 10 litre drum of disinfectant remains efficacious for a 10 day period after preparation and when transferred to a spray bottle may be kept for 24 hours.

Previous tests carried out by this laboratory established the efficacy of Difficil-S under clean and dirty conditions with test time intervals up to 60 mins. These tests demonstrated a $>3\log_{10}$ reduction in 1 minute. Therefore, for the purpose of these tests only 1, 2 and 5 mins were tested. Tests were carried out on solutions stored in the drum for 10 days and the spray bottle for 24 hours after preperation.

TEST PRODUCT

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: 52012A **Expiry Date:** Feb 2010
Part B: 52012B **Expiry Date:** Jan 2010

STORAGE CONDITIONS

Room temperature (approx 18 to 20°C), in a dry place out of direct sunlight.

Drum 10 litres was prepared on the Monday and used to fill the spray bottle. On Friday the second sample was collected by operating the pump 18 times. On the following Monday and Wednesday the same procedure was followed to collect the test samples.

Spray bottle The first sample was collected by pumping out 80 strokes into a sterile container. Samples were similarly collected at 8 and 24 hours.

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

1, 2 and 5 minutes

TEST TEMPERATURE

20°C

INTERFERING SUBSTANCE

Clean conditions - 0.03 % bovine albumin (final concentration)

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

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 sterile hard water, immediately prior to testing/storage.

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 $\log_{10}$ reduction in 60 minutes.

RESULTS

SPORICIDAL ACTIVITY OF DIFFICILE-S UNDER CLEAN AND DIRTY CONDITIONS

(All tests carried out in duplicate)

Solution stored in a spray bottle for 24 hour period

Test Interval	Contact time	Log10 initial count	Log_{10} reductions obtained	
			Clean conditions	Dirty conditions
Fresh	1 min	6.82	>6.82	4.38
	2 mins		>6.82	4.41
	5mins		>6.82	4.61
8 hours	1 min	6.82	6.19	4.22
	2 mins		>6.82	4.37
	5 mins		>6.82	4.67
24 hours	1 min	6.82	6.33	4.13
	2 mins		5.44	4.59
	5 mins		>6.82	4.79

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SPORICIDAL ACTIVITY OF DIFFICILE-S UNDER CLEAN AND DIRTY CONDITIONS

(All tests carried out in duplicate)

Solution stored in a drum for 10 days

Test Interval	Contact time	Log10 initial count	Log_{10} reductions obtained	
			Clean conditions	Dirty conditions
Fresh – Monday 04.08.08	1 min	7.82	>6.82	4.38
	2 mins		>6.82	4.41
	5 mins		>6.82	4.61
Friday 08.08.08	1 min	7.52	3.71	3.79
	2 mins		4.91	4.21
	5 mins		>6.52	4.38
Monday 11.08.08	1 min	7.52	3.22	3.28
	2 mins		4.82	3.97
	5 mins		>6.52	4.12
Wednesday 13.08.08	1 min	7.47	3.14	3.12
	2 mins		4.14	3.98
	5 mins		5.82	4.02

CONCLUSION

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 with 'DIFFICIL-S' over a 24 hour in a spray bottle and 10 day period in a storage drum. The efficacy did reduce over the storage period of 24 hours and 10 days but a >3 log_{10} reduction was achieved within the test contact times.

It is recommended that when solutions are stored and decanted into containers for use that the container i.e. the drum and/or spray bottle, is fully emptied before being refilled.

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

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