

**VIRUCIDAL EFFICACY TESTS  
EN 14476 (PHASE 2, STEP 1)**

**DIFFICIL-S**

**CLINIMAX LTD**

**HOSPITAL INFECTION RESEARCH LABORATORY  
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**MANUFACTURER**

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**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

**TEST VIRUSES**

**Non-enveloped RNA virus** Picornavirus group – poliovirus type 1, LSc-2ab

**Non-enveloped DNA virus** Adenovirus group – adenovirus type 5, strain Adenoid  
75, ATCC VR-5

**TEST METHOD AND VALIDATION**

EN 14476:(2005) Chemical disinfectants and antiseptics – Virucidal quantitative suspension test for chemical disinfectants used in the human medicine. (Phase 2, step 1).

Copies available from BSI, 389 Chiswick High Road, London W4 4AL.

## **REQUIREMENT**

The test product when tested in accordance with the test methodology described under simulated clean and dirty conditions shall demonstrate at least a 4 log<sub>10</sub> reduction in the obligatory contact time of 60 mins. Additional contact times may be selected.

## **PRODUCT TEST CONCENTRATION**

Tested at the concentration supplied, taking into account the dilution that occurs during the test.

## **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. The cytotoxic effect to the cells was determined as described in section 6.6.4.

## SUMMARY OF TEST METHOD

EN 14476: - This test method involves mixing 1 ml of the test virus with 1 ml of soil (0.3% albumin – clean conditions or 3% albumin with 3% freshly triple washed and spun sheep erythrocytes – dirty conditions), and then adding 8 ml of the test disinfectant. After the required contact time, 0.5 ml is removed to 4.5 ml of Eagles Minimal Essential Medium (MEM) plus 2% foetal calf serum which is then sampled to detect surviving test virus.

## RESULTS

### VIRUCIDAL ACTIVITY

#### USING PHASE 2 STEP 1 SUSPENSION TEST EN 14476

**Log<sub>10</sub> counts/reductions achieved in 5, 10, 15 and 60 minutes at 20°C**

(Tests carried out in duplicate – figures expressed are the mean of two test results)

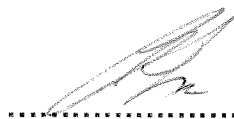
		log TCID <sub>50</sub> after							
Test virus		Clean conditions (0.03% albumin)				Dirty conditions (0.3% albumin/0.3% sheep erythrocytes)			
		5min	10 min	15 min	60min	5min	10 min	15 min	60min
Adenovirus	Pre count	5.5	5.7	5.7	5.5	5.5	5.7	5.7	5.5
	Post count	1.9	<1.5	<1.5	<1.5	2.1	0.7	0.9	<0.5
	Log RF	3.6	>4.2	>4.2	>4.2	3.4	>4.2	>4.2	>4.2
Poliovirus	Pre count	6.1	5.9	5.9	5.9	6.1	5.9	5.9	5.9
	Post count	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
	Log RF	>4.6	>4.4	>4.4	>4.4	>4.6	>4.4	>4.4	>4.4

## **CONCLUSION**

When tested in accordance with EN 14476 (2005), Difficil-S successfully satisfies the requirements of the test ie possesses virucidal activity at 20°C when tested under clean conditions (0.03% albumin) and dirty conditions (0.3% albumin and 0.3% sheep erythrocytes). A  $>4 \log_{10}$  (99.999%) reduction was achieved with both test viruses ie adenovirus and poliovirus within the mandatory contact time of 60 mins.

A  $>4 \log_{10}$  reduction with adenovirus was achieved in 10 mins under clean and dirty conditions and a 5 min contact time with poliovirus under clean and dirty conditions.

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

  
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**Dr Adam P Fraise**  
**Director**

  
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**Christina Bradley**  
**Laboratory Manager**