

Test Report: EN 14476:2005 Chemical disinfectants and antiseptics - Virucidal quantitative suspension test for chemical disinfectants and antiseptics used in human medicine - Test method and requirements (phase 2/step 1) Modified for Feline Coronavirus

Test Laboratory

BluTest Laboratories Ltd

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Identification of sample

Name of the product
Batch number
Client
Project Code
Date of Delivery
Storage conditions
Active substances

DUOMAX GENERAL PURPOSE CLEANER

N/A
DUOTECH LTD, 11-12 Queens Square, Bristol, BS1 4NT
BT-DUO-19
16 December 2011
Ambient
N/A

Test Method and its validation

Method

1 part interfering substance + 1 part virus suspension + 8 parts biocide were mixed and incubated at the indicated contact temperature for the indicated contact times. Assays were validated by a cytotoxicity control, interference control, neutralization control and a formaldehyde internal standard.

Neutralization

Dilution-neutralization/gel filtration; Dulbecco's modified Eagles medium + 5% v/v foetal bovine serum at 4°C

Experimental Conditions

Period of analysis
Product diluents used
Product test concentrations
Contact times
Test temperature
Interfering substances
Stability of mixture
Temperature of incubation
Identification of virus

31 October to 7 November 2012
Sterile, synthetic hard water
1.0% V/V; 50.0% V/V; 80.0% V/V
5 minutes $\pm$ 10s
20°C $\pm$ 1°C
0.3% g/l bovine serum albumin
Cloudy at 1.0% V/V and 50.0% V/V
37°C $\pm$ 1°C + 5% CO₂
Feline Coronavirus/CRFK Cells

PROTOCOL SUMMARY

The basic virucidal efficacy test is set up with three concentrations of disinfectant and a 5 minute contact time. Virus is exposed to disinfectant in 24-well plates, then neutralized, serially diluted and virus titred in 96-well tissue culture plates to determine the tissue culture infectious dose₅₀ (TCID₅₀) of surviving virus. Poliovirus-1 was assayed. TCID₅₀ is determined by the method of Karber¹.

Cytotoxicity control

The neutralized disinfectant is measured for its effects on the host cells used to propagate the virus, to determine the sensitivity of the assay.

Interference control

The end point titration of the virus is exposed to three different sub-lethal concentrations of neutralized disinfectant to measure the effect of sub-lethal concentrations of disinfectant on virus infectivity in relation to the titre achieved on untreated cells.

Disinfectant suppression control

Virus is added to the highest concentration of disinfectant and then the mixture removed and neutralized. The neutralized virus titre is then determined to assess the efficiency of the neutralization procedure.

Virus recovery control

Virus titre is determined for virus in contact with sterile hard water at t=0 and at t = 60 (or the longest contact time). The virus titre after 60 minutes (or the longest contact time) is then compared to the recovery of disinfectant-treated virus to measure the log reduction in virus titre.

Reference virus inactivation control

Virus is in contact with 0.7% W/V formaldehyde and the recovery of virus determined by TCID₅₀ after 5, 15, 30 and 60 minutes, in order to assess that the test virus has retained reproducible biocide resistance. In addition, the formaldehyde cytotoxicity of neutralized formaldehyde is determined, to measure assay sensitivity.

¹Kärber, G.: Beitrag zur Kollektiven Behandlung Pharmakologischer Reihenversuche. Arch. Exp. Path. Pharmacol. 162 (1931): 480-487.

Suspension test results for the efficacy of DUOMAX GENERAL PURPOSE CLEANER from DUOTECH LTD against Feline Coronavirus under CLEAN CONDITIONS

Exposure Time	Virus Recovery 0 min		Virus Recovery 5 min		Cytotoxicity		Disinfectant Suppression		1.0% (v/v)		50.0% (v/v)		80.0% (v/v)	
	raw data	TCID ₅₀ /ml	raw data	TCID ₅₀ /ml	raw data	TCID ₅₀ /ml	raw data	TCID ₅₀ /ml	raw data	TCID ₅₀ /ml	raw data	TCID ₅₀ /ml	raw data	TCID ₅₀ /ml
t = 5	4.83	2.14E+06	5.00	3.16E+06	3.00	3.16E+04	3.00	3.16E+04	0.00	3.16E+01	3.00	3.16E+04	3.00	3.16E+04
		2.14E+06		3.16E+06		3.16E+04		3.16E+04		3.16E+01		3.16E+04		3.16E+04
log		6.33		6.50		4.50		4.50		1.50		4.50		4.50
log difference								2.00		5.00		2.00		2.00

Suspension test results for the efficacy of DUOMAX GENERAL PURPOSE CLEANER from DUOTECH LTD against Feline Coronavirus under CLEAN CONDITIONS

Product:	Interfering substance	Concentration	Level of cytotoxicity	lg TCID ₅₀					>4 lg reduction after .. Min
				0 min	5 min	15 min	30min	60 min	
DUOMAX General Purpose Cleaner	0.3g/l BSA	80.0% v/v	4.50	6.33	4.50	n.a.	n.a.	1.50	<5
		50.0% v/v	4.50	6.33	4.50	n.a.	n.a.	4.50	<5
		1.0% v/v	4.50	6.33	1.50	n.a.	n.a.	4.50	5
Formaldehyde		0.7% (w/v)	2.50	6.33	4.33	3.50	3.50	3.50	>60
Virus Control		n.a.	n.a.	6.33	n.a.	n.a.	n.a.	6.50	n.a.

Control Data for Feline Coronavirus

Stock Virus (TCID₅₀)

6.83 2.14E+08

Formaldehyde reference inactivation
control

Exposure time	Virus recovery 0 min		Virus recovery 60 min		Cytotoxicity		0.7% Formaldehyde							
							5		15		30		60	
	raw data	TCID ₅₀ /ml	raw data	TCID ₅₀ /ml	raw data	TCID ₅₀ /ml	raw data	TCID ₅₀ /ml	raw data	TCID ₅₀ /ml	raw data	TCID ₅₀ /ml	raw data	TCID ₅₀ /ml
60 min log log difference	4.83	2.14E+06	5.17	4.68E+06	1.00	3.16E+02	2.83	2.14E+04	2.00	3.16E+03	2.00	3.16E+03	2.00	3.16E+03
		2.14E+06		4.68E+06		3.16E+02		2.14E+04		3.16E+03		3.16E+03		3.16E+03
		6.33		6.67		2.50		4.33		3.50		3.50		3.50
								2.34		3.17		3.17		3.17

No Column Control

Virus Recovery t min	
(PC Control)	
raw data	TCID ₅₀ /ml
4.83	2.14E+06
	2.14E+06
	6.33

Interference control

Virus dilution

Cytotoxicity dilution

	-1	-2	-3	Mock
-5	0	0	0	3
-6	0	0	0	3
-7	0	0	0	2

CONCLUSION

Verification of the methodology

A test is only valid if the following criteria are fulfilled:

- a) Test virus suspension has at least a concentration which allows the determination of a 4 log₁₀ reduction of the virus titre.
- b) Detectable titre reduction is at least 4 log₁₀.
- c) Difference of the logarithmic titre of the virus control minus the logarithmic titre of the test virus in the reference inactivation test is between – 0.5 and – 2.5 after 30 min and between – 2 and – 4.5 after 60 min for poliovirus.
- d) Cytotoxicity of the product solution does not affect cell morphology and growth or susceptibility for the test virus in the dilutions of the test mixtures which are necessary to demonstrate a 4 log reduction of the virus.
- e) The interference control result does not show a difference of < 1.0 log₁₀ of virus titre in comparison to the virus recovery control; dilutions of disinfectant to sub-acute levels did not interfere in the generation of viral cytopathic effect.
- e) Neutralisation validation. This is called the disinfectant suppression test in this protocol. The difference for poliovirus is 2.0 log₁₀ indicating rapid virucidal activity of the disinfectant by dilution at a concentration of 80.0% v/v.
- f) A difference of <0.5 log₁₀ is not observed between virus recovered directly from the virus recovery control at 60 minutes and virus from the same control recovered through an Illustra Microspin S-400 HR column

According to a modified EN 14476: 2005, DUOMAX GENERAL PURPOSE CLEANER from DUOTECH LTD DOES possess virucidal activity (5.0 log₁₀ reduction) against Feline coronavirus at 20°C following 5 MINUTES CONTACT UNDER CLEAN CONDITIONS at a concentration of 1.0% V/V.

Signed



Dr Chris Woodall, Director, BluTest Laboratories Ltd, Glasgow, UK, 9 November 2012

DISCLAIMER

The results in this test report only pertain to the sample supplied.

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