BluTest
GLOBAL MICROBIOLOGY EXPERTISE

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) under dirty conditions

Test Laboratory	**BluTest Laboratories Ltd** Robertson Incubator (Level 4) Robertson Building 56 Dumbarton Road Glasgow UK - G11 6NU
Identification of sample	
Name of the product	**SAFE 4 DISINFECTANT CLEANER CONCENTRATE**
Batch number	N/A
Client	**Safe Solutions (Safe4) Ltd**, Bostock Road, Winsford, Cheshire, CW7 3BD
Project Code	BT-SAF-08
Date of Delivery	9 November 2012
Storage conditions	Dry conditions between 0-30^{0}C
Active substances	Halogenated tertiary amines
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. Detection by immunocytochemistry.
Neutralization	Dilution-neutralisation/gel filtration; Modified Eagles medium + 5% V/V foetal bovine serum at 4°C
Experimental Conditions	
Period of analysis	3 to 14 May 2013
Product diluent used	Sterile, synthetic hard water
Product test concentrations	2.0% (1:50); 5.0% (1:20)
Appearance product dilutions	Clear
Contact times (minutes)	30 ± 10
Test temperature	20°C ± 1°C
Interfering substances	0.3g/l bovine serum + 0.3 %V/V sheep erythrocytes
Stability of mixture	Stable
Temperature of incubation	37°C ± 1°C + 5% CO_2
Identification of virus	**Canine parvovirus (VR-953)/ CRFK cells (HPACC 86093002)**

Robertson Incubator, Level 4, Robertson Building, 56 Dumbarton Road, Glasgow, G11 6NU
Email: info@blutest.com Website: www.blutest.com
Company Registration Number: SC364409 VAT Registration Number: GB 979 1131 96

PROTOCOL SUMMARY

The basic virucidal efficacy test is set up with two concentrations of disinfectant and a 30 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 dose$_{50}$ ($TCID_{50}$) of surviving virus. $TCID_{50}$ is determined by the method of Karber[1].

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 = 30 (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 $TCID_{50}$ 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. Pharmak. 162 (1931): 480-487.

Robertson Incubator, Level 4, Robertson Building, 56 Dumbarton Road, Glasgow, G11 6NU
Email: info@blutest.com Website: www.blutest.com
Company Registration Number: SC364409 VAT Registration Number: GB 979 1131 96

BluTest
GLOBAL MICROBIOLOGY EXPERTISE

Suspension test results for the efficacy of SAFE 4 DISINFECTANT CLEANER CONCENTRATE from Safe Solutions (Safe4) Ltd against CANINE PARVOVIRUS-1 under DIRTY CONDITIONS

Exposure Time	Virus Recovery 0 min		Virus Recovery 60 min		Cytotoxicity		Disinfectant Suppression		2.0% (v/v)		5.0% (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
t = 30	4.67	**1.48E+06**	4.83	**2.14E+06**	1.00	**3.16E+02**	3.17	**4.68E+04**	1.17	**4.68E+02**	1.00	**3.16E+02**
		1.48E+06		**2.14E+06**		**3.16E+02**		**4.68E+04**		**4.68E+02**		**3.16E+02**
log		**6.17**		**6.33**		**2.50**		**4.67**		**2.67**		**2.50**
log difference								**1.66**		**3.66**		**3.83**

Suspension test results for the efficacy of SAFE 4 DISINFECTANT CLEANER CONCENTRATE from Safe Solutions (Safe4) Ltd against CANINE PARVOVIRUS-1 under DIRTY CONDITIONS

Product: DISINFECTANT CLEANER CONCENTRATE	Interfering substance	Concentration	Level of cytotoxicity	lg TCID50					>4 lg reduction after .. Min
				0 min	5 min	15 min	30min	60 min	
	3.0g/l BSA + 3.0ml/l erythrocytes	5.0% (v/v)	2.50	6.17	n.a.	n.a.	2.50	n.a.	>5
		2.0% (v/v)	2.50	6.17	n.a.	n.a.	2.67	n.a.	>5
	3.0g/l BSA	5.0% (v/v)	2.50	5.83	n.a.	n.a.	2.50	n.a.	>5
		2.0% (v/v)	2.50	5.83	n.a.	n.a.	3.17	n.a.	>5
Formaldehyde		0.7% (w/v)	3.50	6.17	4.83	4.17	3.83	3.50	>60
Virus Control		n.a.	n.a.	6.17	n.a.	n.a.	n.a.	6.33	n.a.

Robertson Incubator, Level 4, Robertson Building, 56 Dumbarton Road, Glasgow, G11 6NU
Email: info@blutest.com Website: www.blutest.com
Company Registration Number: SC364409 VAT Registration Number: GB 979 1131 96

BluTest
GLOBAL MICROBIOLOGY EXPERTISE

Control Data for CANINE PARVOVIRUS-1

Parallel control test

Exposure Time	Virus Recovery		Virus Recovery		2.0% (v/v)		5.0% (v/v)	
	raw data	$TCID_{50}$/ml	raw data	$TCID_{50}$/ml	raw data	$TCID_{50}$/ml	raw data	$TCID_{50}$/ml
t = 30	4.33	6.76E+05	4.50	1.00E+06	1.67	1.48E+03	1.00	3.16E+02
		6.76E+05		1.00E+06		1.48E+03		3.16E+02
log		5.83		6.00		3.17		2.50
log difference						2.83		3.50

Stock Virus ($TCID_{50}$)	5.17	4.68E+06

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	4.67	1.48E+06	4.83	2.14E+06	2.00	3.16E+03	3.33	6.76E+04	2.67	1.48E+04	2.33	6.76E+03	2.00	3.16E+03
		1.48E+06		2.14E+06		3.16E+03		6.76E+04		1.48E+04		6.76E+03		3.16E+03
log		6.17		6.33		3.50		4.83		4.17		3.83		3.50
log difference								1.50		2.16		2.50		2.83

No Column Control

Virus Recovery t min	
raw data	$TCID_{50}$/ml
4.67	1.48E+06
	1.48E+06
	6.17

Interference control

	Cytoxicity dilution			
	-1	-2	-3	Mock
Virus dilution -5	NA	2	NA	3
-6	NA	1	NA	1
-7	NA	0	NA	0

Robertson Incubator, Level 4, Robertson Building, 56 Dumbarton Road, Glasgow, G11 6NU
Email: info@blutest.com Website: www.blutest.com
Company Registration Number: SC364409 VAT Registration Number: GB 979 1131 96

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 $\log_{10}$ reduction of the virus titre.

b) Detectable titre reduction is at least 4 $\log_{10}$.

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 $\log_{10}$ 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. This was slightly elevated at 1.66 $\log_{10}$ at a concentration of 5.0% v/v.

f) A difference of <0.5 $\log_{10}$ 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 EN 14476: 2005, SAFE 4 DISINFECTANT CLEANER CONCENTRATE from Safe Solutions (Safe4) Ltd achieves a virucidal activity of > 3.83 $\log_{10}$ reduction against against CANINE PARVOVIRUS-1 at 20ºC following 30 MINUTES CONTACT UNDER DIRTYCONDITIONS at a concentration of 5.0% V/V (1:20).

Signed

Dr Chris Woodall, Director
BluTest Laboratories Ltd
Glasgow, UK
21 May 2013

DISCLAIMER

The results in this test report only pertain to the sample supplied.
BluTest (BT) has performed the testing detailed in this report using reasonable skill and care and has used reasonable endeavours to carry out the testing in accordance with a modified EN 14476 protocol. All forecasts, recommendations and results contained in this report are submitted in good faith. However, other than as expressly set out in this report, no warranty is given (i) in relation to the testing or the use(s) to which any results or deliverables produced in the course of the testing are or may be put by the Client or their fitness or suitability for any particular purpose or under any special conditions notwithstanding that any such purpose or conditions may have been made known to BT or (ii) that the intended results or deliverables from the testing can be achieved or (iii) that the Client can freely make use of the results or the deliverables without infringing any third party intellectual property rights and the Client will be deemed to have satisfied itself in this regard. BT shall have no liability (which is hereby excluded to the fullest extent permissible by law) in respect of any loss, liability or damage, including without limitation any indirect and/or consequential loss such as loss of profit or loss of business, market or goodwill, that the Client may suffer directly or indirectly as a result of or in connection with: (i) the performance of the testing; (ii) the use of any materials, samples or other information provided by the Client for use in the testing; and (iii) the Client's reliance upon or use of any results or deliverables provided as part of the testing.

Robertson Incubator, Level 4, Robertson Building, 56 Dumbarton Road, Glasgow, G11 6NU
Email: info@blutest.com Website: www.blutest.com
Company Registration Number: SC364409 VAT Registration Number: GB 979 1131 96