Test Report EN 14675:2015 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in the veterinary area- Test method and requirements (Phase 2/Step 1)

Test Laboratory	**BluTest Laboratories Ltd** 5 Robroyston Oval, Nova Business Park, Glasgow, G33 1AP
Identification of sample	
Name of the product	Safe4 Disinfectant Cleaner
Batch number	3060
Client	Safe Solutions (Safe4) Limited
Client Address	Bostock Road, Wharton Green, Winsford, CW7 3BD
Project Code	BT-SAF-19
Date of Delivery	02 September 2019
Storage conditions	Ambient
Active substances	Benzyl Alkyldimethyl chloride, polyhexamethylene biguanide hydrochloric acid
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, neutralisation control and a formaldehyde internal standard.
Neutralisation	Dilution-neutralisation/Enhanced neutralisation gel filtration Eagles minimum essential medium + 5% v/v foetal bovine serum at 4°C
Experimental Conditions	
Period of analysis	17 September 2019 to 22 September 2019
Product diluents used	Sterile, synthetic hard water
Product test concentrations	1:10; 1:50; 1:500
Appearance product dilutions	No changes noted- stable
Appearance in test mixture	No changes noted- stable
Contact times (minutes)	5 ± 10s
Test temperature	10°C ± 1°C
Interfering substances	3.0 g/l bovine albumin
Temperature of incubation	37°C ± 1°C + 5% CO_2
Identification of virus	**Vaccinia virus Elstree Strain VR-1549/ VERO Cells**

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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 neutralised, serially diluted and virus titred in 96-well tissue culture plates to determine the tissue culture infectious dose$_{50}$ ($TCID_{50}$) of surviving virus. Vaccinia virus Elstree Strain VR-1549/ VERO Cells are assayed in parallel in each test. $TCID_{50}$ is determined by the method of Karber[1].

Cytotoxicity control
The neutralised 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 neutralised 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 immediately removed and neutralised. The neutralised virus titre is then determined to assess the efficiency of the neutralisation procedure.

Virus recovery control
Virus titre is determined for virus in contact with sterile hard water at t=0, t = 5 and at t =60. The virus titre after 5 minutes is then compared to the recovery of disinfectant-treated virus to measure the log reduction in virus titre. The virus titre at 60 minutes is compared to the reference virus inactivation control.

Reference virus inactivation control
Virus is exposed to 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 neutralised formaldehyde is determined, to measure assay sensitivity.

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

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Vaccinia virus Elstree Strain VR-1549/ VERO Cells

SOP 10000 V02 EN14675 Suspension test results for the efficacy of Safe4 Disinfectant Cleaner, BT-SAF-19 from Safe Solutions (Safe4) Ltd against Vaccinia													
Virus Recovery 0 min		**Virus Recovery 5 min**		**Cytotoxicity**		**Disinfectant Suppression**		**Exposure Time**	**1:500**		**1:50**		**1:10**	
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
5.17	**4.68E+06**	5.00	**3.16E+06**	0.00	**3.16E+01**	5.17	**4.68E+06**	**t = 5 min**	3.17	**4.68E+04**	0.00	**3.16E+01**	0.00	**3.16E+01**
	4.68E+06		**3.16E+06**		**3.16E+01**		**4.68E+06**			**4.68E+04**		**3.16E+01**		**3.16E+01**
	6.67		**6.50**		**1.50**		**6.67**	log		**4.67**		**1.50**		**1.50**
							0.00	log difference		**1.83**		**5.00**		**5.00**

Summary table of results of virucidal activity against Vaccinia under CLEAN conditions for Safe4 Disinfectant Cleaner, BT-SAF-19 from Safe Solutions (Safe4) Ltd									
Product:	Interfering substance	Concentration	Level of cytotoxicity	lg $TCID_{50}$					>4 lg reduction after 'X' Min
				0 min	1 min	3 min	5 min	60 min	
Safe4 Disinfectant Cleaner	3.0g/l BSA	1:10	1.50	n.a.	n.a.	n.a.	1.50	n.a.	<5 min
		1:50	1.50	n.a.	n.a.	n.a.	1.50	n.a.	<5 min
		1:500	1.50	n.a.	n.a.	n.a.	4.67	n.a.	>5 min
Virus Control	CLEAN	n.a.	n.a.	6.67	n.a.	n.a.	6.50	6.67	n.a.
				0 min	5 min	15 min	30 min	60 min	
Formaldehyde	PBS	0.7% (w/v)	2.50	n.a.	5.50	4.50	3.50	3.50	>60 min

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Control Data

Stock Virus ($TCID_{50}$)	6.33	6.76E+07

Formaldehyde reference inactivation control

Virus recovery 0 min		Virus recovery 60 min		Cytotoxicity		Exposure time	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
5.17	**4.68E+06**	5.17	**4.68E+06**	1.00	**3.16E+02**	**60 min**	4.00	**3.16E+05**	3.00	**3.16E+04**	2.00	**3.16E+03**	2.00	**3.16E+03**
	4.68E+06		**4.68E+06**		**3.16E+02**			**3.16E+05**		**3.16E+04**		**3.16E+03**		**3.16E+03**
	6.67		**6.67**		**2.50**	log		**5.50**		**4.50**		**3.50**		**3.50**
						log difference		**1.17**		**2.17**		**3.17**		**3.17**

No Column Control

Virus Recovery	
60	**min**
raw data	$TCID_{50}$/ml
5.17	**4.68E+06**
	4.68E+06
	6.67

Interference control

Interference control	Virus dilution					
	-3	**-4**	**-5**	**-6**	**-7**	**-8**
PBS Control	1	1	1	0.67	0	0
	3.16E+02	3.16E+02	3.16E+02	1.48E+02	3.16E+01	3.16E+01
	2.50	**2.50**	**2.50**	**2.17**	**1.50**	**1.50**
Product	1	1	1	0.83	0.5	0
	3.16E+02	3.16E+02	3.16E+02	2.14E+02	1.00E+02	3.16E+01
	2.50	**2.50**	**2.50**	**2.33**	**2.00**	**1.50**
Log Difference	**0.00**	**0.00**	**0.00**	**-0.16**	**-0.50**	**0.00**
Product Cyt Dilution	-1	-1	-1	-1	-1	-1
PBS Dilution	Neat	Neat	Neat	Neat	Neat	Neat

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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 virus.
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 $\log_{10}$ 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 does not interfere in the generation of viral cytopathic effect.
e) Neutralisation validation. This is called the disinfectant suppression test in this protocol. The disinfectant was neutralised by column chromatography through an Illustra Microspin S-400 HR column to achieve the best possible neutralisation available for this test. The difference for virus is not greater than 0.5 $\log_{10}$ indicating effective neutralisation of the virucidal activity of the disinfectant by dilution at a concentration of 1:10.
f) A difference of <0.5 $\log_{10}$ should be 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 14675:2015, **Safe4 Disinfectant Cleaner POSSESSES VIRUCIDAL** activity at a concentration of **1:50** as tested after **5 MINUTES** at **10°C** under **CLEAN** conditions (3.0 g/l bovine albumin) against **Vaccinia virus Elstree Strain VR-1549/ VERO Cells** that is a surrogate for **ENVELOPED VIRUSES**.

Signed

Dr Chris Woodall, Director
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Date: 01 October 2019

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