



# Test Report: BS EN 14476:2013 + A2:2019 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity in the medical 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 BT-NVU-02

Name of the product Ready to use RM001 for Sanigone Ltd

disinfectants

Batch number 00042

Client Sanigone Ltd

Client Address 86-90 Paul Street, London, United Kingdom, EC2A 4NE

Project Code BT-NVU-02 Date of Delivery 23-Sep-20

Storage conditions Ambient; Protected from light

Active substances Didecyl dimethyl ammonium chloride; Tetrasodium EDTA alkyl;

dimethylbenzyl ammonium chloride

Appearance Liquid
Condition upon receipt Undamaged

**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

Eagles Minimum Essential Medium + 5.0% v/v foetal bovine serum

at 4°C

**Experimental Conditions** 

Period of analysis 27-Dec-20 to 01-Jan-21
Product diluents used Sterile, distilled water
Product test concentrations 10% v/v; 50% v/v; 100% v/v
Appearance product dilutions No changes noted- stable

Appearance in test mixture Test mixture becomes cloudy at 50% and 100% concentrations

Contact times (minutes)  $2 \pm 10s$ Test temperature  $20^{\circ}\text{C} + 1^{\circ}\text{C}$ 

Interfering substances 0.3g/l bovine albumin Temperature of incubation  $37^{\circ}\text{C} \pm 1^{\circ}\text{C} + 5\% \text{ CO}_2$ 

Identification and passage (P) of virus Vaccinia virus VR-1549 Elstree strain (P9)





### PROTOCOL SUMMARY

The basic virucidal efficacy test is set up with three concentrations of test product solution and a 2 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<sub>50</sub> (TCID<sub>50</sub>) of surviving virus. *Vaccinia virus* VR-1549 Elstree strain / Vero cells are assayed in parallel in each test. TCID<sub>50</sub> is determined by the method of Karber<sup>1</sup>.

### Cytotoxicity control

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

### Interference control

The effect of the cells after treatment of the test product solution are verified to ensure the cells can show susceptibility for virus infection. This is compared against cells that have not been treated with test product.

### Disinfectant suppression control VS1

Virus is added to the highest concentration of test product solution and then the mixture immediately removed and neutralised. The neutralised virus titre is then determined to assess the efficiency of the neutralisation procedure.

### Disinfectant suppression control VS2

Internal control which adds virus to neutralised test product solution to assess the efficiency of the neutralisation procedure.

### **No column Control**

Internal control on the highest contact time to assess any impact of the Microspin™ S 400 HR columns.

### Virus recovery control

Virus titre is determined for virus in contact with sterile, distilled water at t=0, t= 2 and at t =15. The virus titre after 2 minutes is then compared to the recovery of disinfectant-treated virus to measure the log reduction in virus titre. The virus titre at 15 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<sub>50</sub> after 5 and 15 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 (VR-1549) Elstree strain Test Results

EN1446:2013 +A2:2019 Suspension test for the efficacy of Ready to use RM001 for Sanigone surface disinfectants, Batch 00042, BT-NVU-02 from Sanigone Ltd. against Vaccinia virus VR-1549 under clear conditions

			<b>Test Results</b>			
Concentration		10%	35	20%	11	100%
Exposure Time	data	TCID <sub>50</sub> /ml	data	TCID <sub>50</sub> /ml	data	TCID <sub>50</sub> /ml
t = 2 mins	3.83	2.15E+05	0.83	2.15E+02	0.00	3.16E+01
Raw Data	666410	2.15E+05	500000	2.15E+02	000000	3.16E+01
log		5.33		2.33		1.50
log difference		0.50		3.50		4.33

EN14.	EN1446:2013 +A2:2019	019 Suspension	on test for the	e efficacy of	Ready to use	A2:2019 Suspension test for the efficacy of Ready to use RM001 for Sanigone surface disinfectants, Batch 00042 RT-NVILO2 from Sanigons 14d against Vaccinia virus VR-1549 under clear conditions	gone surface	disinfectants	, Batch
				Simple Simulation	Summary Table				8
					and I apple				
Product:	Interfering	Concentration	Level of			Ig TCID <sub>50</sub>			>4 lg reduction
	substance		cytotoxicity						after 'X' Min
						0			
				0 min	5 min	15 min	30 min	60 min	
RM001 for	0.3g/I BSA			1000					
Aqueos,		100%	1.50	1.50	1.50	n.a.	n.a.	n.a.	<2 mins
continu &					700000000000000000000000000000000000000				
saloncide		20%	1.50	n.a.	2.33	n.a.	n.a.	n.a.	>2 mins
surface		-			913.30.000	***************************************			
disinfectants		10%	1.50	n.a.	5.33	n.a.	n.a.	n.a.	>2 mins
Virus Control	Clean			6.17	5.83	6.00	n.a.	n.a.	n.a.
							5 min	15 min	
Formaldehyde PBS	PBS	0.7% (w/v)	3.50				4.67	3.50	>15 mins

SOP 11000 SOP 8003 EN14476 Vaccinia REPORT TEMPLATE V01 Effective Date: 23 March 2020



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# Vaccinia virus (VR-1549) Elstree strain Control Data

EN1446:2013 +A2:2019 Suspension test for the efficacy of Ready to use RM001 for Sanigone surface disinfectants, Batch 00042, BT-NVU-02 from Sanigone Ltd. against Vaccinia virus VR-1549 under clear conditions

Distribution of the converty of the c						Ō	Controls					
O min         2 min         15 min         Suppression of the control of t	Virus R	ecovery	Virus Re	covery	Virus R		Make	cicity	Disin	ectant	Disinfectant	ectant
	0 .	nin	2 m	nin	15	min			Suppre	ssion VS	Suppression VS2	sion VS2
147F+06   648E+05   648E	raw data	TCI D <sub>50</sub> /ml	raw data	TCID <sub>50</sub> /ml	raw data	TCID <sub>50</sub> /ml	raw data	TCID <sub>50</sub> /ml	raw data	TCID <sub>50</sub> /ml	raw data	TCID <sub>50</sub> /ml
1.47F+06   666620   6.81E+05   666630   1.00E+06   000000   3.15E+01   0000000   3.15E+01   000000   0.3   0000000000000000000000	4.67	1.47E+06	4.33	6.81E+05	4.50	1.00E+06	0.00	3.16E+01	0.00	3.16E+01	4.33	6.81E+05
Formaldehyde reference inactivation controls   Formaldehyde reference inactivation controls   Formaldehyde reference inactivation controls   Smins   Smins   TICD <sub>20</sub> /ml   Faposure time   Smins   TICD <sub>20</sub> /ml   Taw data   Taw da	666640	1.47E+06	666620	6.81E+05	666630	1.00E+06	000000	3.16E+01	0000000	3.16E+01	666620	6.81E+05
Todoxicity   Exposure time   Formaldehyde reference inactivation controls   Formaldehyde reference inactivation controls     Todosc/mi		6.17		5.83		9.00		1.50	76	1.50		5.83
										4.33		0.00
Formaldehyde reference inactivation controls   Formaldehyde reference inactivation controls   Faposure time   S mins   Family   Faposure time   Faposure time   Family   Faposure time   Faposure t												
TCDD_g/m    Exposure time   S mins   S mins   S mins   S mins   S mins   S mins   TCDD_g/m    Taw data   TCDD_g/m    Taw data   TCDD_g/m			Formaldehyde	reference inac						No column Control	n Control	
TCDD_SVM	Cytot	oxicity	Exposure time		0.7% Fo	ırmaldehyde				2 mins	ins	
TCDD <sub>30</sub> /ml   raw data   TCD <sub>20</sub> /ml   raw data				n 2	suju	15	mins			raw data	TCID <sub>50</sub> /ml	
3.16E+03   3.17   4.64E+04   2.00   3.16E+03   9.66   9.66   9.10   9.66   9.10   9.10   9.66   9.10   9.66   9.10   9.	raw data	TCI D <sub>50</sub> /ml	22	raw data	TCID <sub>50</sub> /ml	raw data	TCID <sub>50</sub> /ml			4.83	2.15E+06	
3.16E+0.3   1.0g   4.64E+0.4   660000   3.16E+0.3   3.50   1.0g difference   1.33   2.50	2.00	3.16E+03		3.17	4.64E+04	2.00	3.16E+03			666650	2.15E+06	
3.50   10g difference   4.67   3.50	000099	3.16E+03		666100	4.64E+04	000099	3.16E+03				6.33	
log difference   1.33   2.50		3.50	log		4.67		3.50					
Sample   S			log difference		1.33		2.50					
Sample   S												
1	Interferen	lostuco est			Viru	s dilution				Stock Virus (TCID <sub>50</sub> )	us (TCID <sub>50</sub> )	
1		2011100	÷	-4	ç.	9-	-7	8-		6.1	6.17	
Salicate   3.16E+02			1	1	1	1	0.17	0		4.68E+07	E+07	
Neat	PBS (	Control	3.16E+02	3.16E+02	3.16E+02	3.16E+02	4.68E+01	3.16E+01		6666661000	61000	
Neat			2.50	2.50	2.50	2.50	1.67	1.50				
Product         1         1         1         0           Raw Data         3.16E+02         3.16E+02         3.16E+02         3.16E+02         3.16E+01           Raw Data         6         6         6         6         6         0           se         0.00         0.00         0.00         0.017         0.17           Dilution         -1         -1         -1         -1           Neat         Neat         Neat         Neat         Neat	Raw	Data	9	9	9	9	1	0				
Product         3.16E+02         3.16E+02         3.16E+02         3.16E+02         3.16E+02         3.16E+01         3.16E+01           Raw Data         6         6         6         6         6         0         0           ce         0.00         0.00         0.00         0.00         0.17         -1         -1         -1         -1           Neat         Neat         Neat         Neat         Neat         Neat         Neat         Neat			1	1	1	1	0	0				
Raw Data         2.50         2.50         2.50         2.50         1.50           ce         6         6         6         6         0           ollution         -1         -1         -1         -1           Neat         Neat         Neat         Neat         Neat	Pro	duct	3.16E+02	3.16E+02	3.16E+02	3.16E+02	3.16E+01	3.16E+01				
Raw Data         6         6         6         6         0         0           re         0.00         0.00         0.00         0.17           Dilution         -1         -1         -1         -1           Neat         Neat         Neat         Neat         Neat			2.50	2.50	2.50	2.50	1.50	1.50				
ce         0.00         0.00         0.00         0.17           Dilution         -1         -1         -1         -1           Neat         Neat         Neat         Neat         Neat	Raw	Data	9	9	9	9	0	0	ron			
Neat Neat Neat Neat Neat	Log Difference		0.00	0.00	0.00	0.00	0.17	0.00				
Neat Neat Neat Neat	Product Cyt Diluti	ion	-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) The titre of the test suspension of at least 10<sup>8</sup> TCID50 /ml is sufficiently high to at least enable a titre reduction of 4 lg to verify the method.
- b) Detectable titre reduction is at least 4 log<sub>10</sub>.
- 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:
  - Between 0.75 and 3.5 after 5 min and between 2.0 and 4.0 after 15 min for Vaccinia 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<sub>10</sub> reduction of the virus.
- e) The interference control result does not show a difference of > 1.0 log<sub>10</sub> of virus titre for test product treated cells in comparison to the non-treated cells.
- f) 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 greater than 0.5 log<sub>10</sub> indicating rapid irreversible virucidal activity of the disinfectant by dilution at a concentration of 100% v/v for VS1. This neutralisation validation has been verified by VS2, which shows the product has been successfully neutralised.

According to EN 14476:2013 + A2:2019 Ready to use RM001 for Sanigone surface disinfectants

POSSESSES VIRUCIDAL activity at a concentration of 100 % v/v of the working concentration as tested after 2

MINUTES at 20°C under CLEAN conditions (0.3 g/l bovine albumin) against *Vaccinia virus* VR-1549 Elstree strain / Vero cells.

This product therefore is effective against all enveloped viruses as defined in EN 14476:2013 + A2:2019 Annex A\*. This therefore includes all coronaviruses and SARS-CoV-2.

Authorised signatory

Dr Chris Woodall, Director BluTest Laboratories Ltd Glasgow, UK

Date: 07 JANUARY 2021

### 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 an 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.

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### \*EN 14476 2013 + A2 2019 Annex A (informative – Enveloped viruses)

Poxviridae

Herpesviridae

Filoviridae (e.g. Ebola, Marburg)

**Flavivirus** 

Hepatitis C Virus (HCV)

Hepatitis Delta Virus (HDV)

Influenza Virus

Paramyxoviridae

Rubella Virus

Measles Virus

Rabies Virus

Coronavirus (e.g. SARS, MERS)

Human Immunodeficiency Virus (HIV)

Human T Cell Leukemia Virus (HTLV)

Hepatitis B virus (HBV)

Reference: Van Regenmortel MHV et al., Eds.: Virus Taxonomy, Classification and Nomenclature of Viruses, seventh report of the international committee on taxonomy of viruses. Academic Press, San Diego, 2000