

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

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Identification of sample

Name of the product	SANI-99
Batch number	07/2020
Client	Scientific Sanitation Solutions (PTY) Limited
Client Address	67 Regency Drive, Route 21 Corporate Park, Irene, Pretoria, South Africa, 0047
Project Code	BT-SSS-01
Date of Delivery	19 August 2020
Storage conditions	Ambient
Active substances	Potassium Peroxymonosulfate
Appearance	Powder
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/gel filtration Eagles Minimum Essential Medium + 5.0% v/v foetal bovine serum at 4°C

Experimental Conditions

Period of analysis	18 September 2020 to 23 September 2020
Product diluents used	Sterile, synthetic hard water
Product test concentrations	3.0g/L; 6.0g/L; 9.0g/L
Appearance product dilutions	No changes noted- stable
Appearance in test mixture	Sedimentation and turbidity observed at all concentrations
Contact times (minutes)	30 seconds; 5 ± 10s; 15 ± 10s
Test temperature	20°C ± 1°C
Interfering substances	0.3g/l bovine albumin
Temperature of incubation	37°C ± 1°C + 5% CO ₂
Identification and passage (P) of virus	Vaccinia virus VR-1549 Elstree strain (P08)
Identification and passage (P) of cells	Vero Cells (P 13)

PROTOCOL SUMMARY

The basic virucidal efficacy test is set up with three concentrations of test product solution and a 30 second, 5 minute and 15 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 dose₅₀ (TCID₅₀) of surviving virus. *Vaccinia virus* VR-1549 Elstree strain / Vero cells are assayed in parallel in each test. TCID₅₀ is determined by the method of Karber¹.

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, synthetic hard water at t=0 and at t =15. The virus titre after 15 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 TCID₅₀ 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.

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

Vaccinia virus (VR-1549) Elstree strain Test Results

EN14476:2013 + A2:2019 Suspension test for the efficacy of SANI-99, BT-SSS-01 from Scientific Sanitation Solutions (PTY) Limited against Vaccinia virus VR-1549 under CLEAN conditions						
Test Results						
Concentration	3.0g/L		6.0g/L		9.0g/L	
Exposure Time	data	TCID ₅₀ /ml	data	TCID ₅₀ /ml	data	TCID ₅₀ /ml
t = 30 secs	0.50	1.00E+02	0.00	3.16E+01	0.00	3.16E+01
Raw Data	300000	1.00E+02	000000	3.16E+01	000000	3.16E+01
log		2.00		1.50		1.50
log difference		3.83		4.33		4.33
Exposure Time	data	TCID ₅₀ /ml	data	TCID ₅₀ /ml	data	TCID ₅₀ /ml
t = 5 mins	0.00	3.16E+01	0.00	3.16E+01	0.00	3.16E+01
Raw Data	000000	3.16E+01	000000	3.16E+01	000000	3.16E+01
log		1.50		1.50		1.50
log difference		4.33		4.33		4.33
Exposure Time	data	TCID ₅₀ /ml	data	TCID ₅₀ /ml	data	TCID ₅₀ /ml
t = 15 mins	0.00	3.16E+01	0.00	3.16E+01	0.00	3.16E+01
Raw Data	000000	3.16E+01	000000	3.16E+01	000000	3.16E+01
log		1.50		1.50		1.50
log difference		4.33		4.33		4.33

Vaccinia virus (VR-1549) Elstree strain Summary Table

EN14476:2013 + A2:2019 Suspension test for the efficacy of SANI-99, BT-SSS-01 from Scientific Sanitation Solutions (PTY) Limited against Vaccinia virus VR-1549 under CLEAN conditions									
Summary Table									
Product:	Interfering substance	Concentration	Level of cytotoxicity	lg TCID ₅₀					>4 lg reduction after 'X' Min
				0 min	30 sec	5 min	15 min	60 min	
SANI-99	0.3g/l BSA	9.0g/L	1.50	2.50	1.50	1.50	1.50	n.a.	<30 sec
		6.0g/L	1.50	n.a.	1.50	1.50	1.50	n.a.	<30 sec
		3.0g/L	1.50	n.a.	2.00	1.50	1.50	n.a.	<5 min
Virus Control	CLEAN			6.00	n.a.	n.a.	5.83	n.a.	n.a.
							5 min	15 min	
Formaldehyde	PBS	0.7% (w/v)	4.50				4.50	4.50	>15 mins

Vaccinia virus (VR-1549) Elstree strain Control Data

EN14476:2013 + A2:2019 Suspension test for the efficacy of SANI-99, BT-SSS-01 from Scientific Sanitation Solutions (PTY) Limited against Vaccinia virus VR-1549 under CLEAN conditions											
Controls											
Virus Recovery 0 min		Virus Recovery 15 min		Cytotoxicity		Disinfectant Suppression VS		Disinfectant Suppression VS2			
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		
4.50	1.00E+06	4.33	6.81E+05	0.00	3.16E+01	1.00	3.16E+02	5.33	6.81E+06		
666621	1.00E+06	666620	6.81E+05	000000	3.16E+01	321000	3.16E+02	666662	6.81E+06		
	6.00		5.83		1.50		2.50		6.83		
							3.33		-1.00		
Formaldehyde reference inactivation controls						No column Control					
Cytotoxicity		Exposure time	0.7% Formaldehyde				15 mins				
			5 mins		15 mins		raw data	TCID ₅₀ /ml			
raw data	TCID ₅₀ /ml		raw data	TCID ₅₀ /ml	raw data	TCID ₅₀ /ml					
3.00	3.16E+04		3.00	3.16E+04	3.00	3.16E+04	4.33	6.81E+05			
666000	3.16E+04		666000	3.16E+04	666000	3.16E+04	666620	6.81E+05			
	4.50	log		4.50		4.50		5.83			
		log difference		1.33		1.33					
Interference control		Virus dilution						Stock Virus (TCID ₅₀)			
		-3	-4	-5	-6	-7	-8				
PBS Control		1	1	1	1	0.17	0	6.50			
		3.16E+02	3.16E+02	3.16E+02	3.16E+02	4.68E+01	3.16E+01	1.00E+08			
		2.50	2.50	2.50	2.50	1.67	1.50	6666630			
Raw Data		6	6	6	6	1	0				
Product		1	1	1	1	0	0				
		3.16E+02	3.16E+02	3.16E+02	3.16E+02	3.16E+01	3.16E+01				
		2.50	2.50	2.50	2.50	1.50	1.50				
Raw Data		6	6	6	6	0	0				
Log Difference		0.00	0.00	0.00	0.00	0.17	0.00				
Product Cyt Dilution		-1	-1	-1	-1	-1	-1				
PBS Dilution		Neat	Neat	Neat	Neat	Neat	Neat				

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 10^8 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 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:
 - 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 log₁₀ reduction of the virus.
- e) The interference control result does not show a difference of > 1.0 log₁₀ 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 log₁₀ indicating rapid irreversible virucidal activity of the disinfectant by dilution at a concentration of 9.0g/L 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, **SANI-99 POSSESSES VIRUCIDAL** activity at concentrations of **6.0g/L and 9.0g/L** as tested after **30 SECONDS** at **20°C** under **CLEAN** conditions (0.3 g/l bovine albumin) against *Vaccinia virus* VR-1549 Elstree strain / Vero cells.

According to EN 14476:2013 + A2:2019, **SANI-99 POSSESSES VIRUCIDAL** activity at concentrations of **3.0g/L, 6.0g/L and 9.0g/L** as tested after **5 MINUTES and 15 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



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Glasgow, UK
Date: 28 SEPTEMBER 2020

DISCLAIMER

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

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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