

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

Name of the product

Sanitiser Disinfectant Spray

Client

Orcagel Ltd

Client Address

Blackhouse Circle, Blackhouse Ind Estate, Peterhead, AB42 1BN

Project Code

BT-ATC-01

Date of Delivery

06 April 2020

Storage conditions

Ambient

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/gel filtration

Eagles Minimum Essential Medium + 5.0% v/v foetal bovine serum at 4°C

Experimental Conditions

Period of analysis

16 April 2020 to 21 April 2020

Product diluents used

Sterile distilled water

Product test concentrations

10.0% v/v; 50.0%; 80.0% v/v

Appearance product dilutions

No changes noted- stable

Appearance in test mixture

Turbidity observed at 80.0% and 50.0%

Contact times (minutes)

5 ± 10s

Test temperature

20°C ± 1°C

Interfering substances

0.3g/l bovine albumin

Temperature of incubation

37°C ± 1°C + 5% CO₂

Identification and passage (P) of virus

Vaccinia virus VR-1549 Elstree strain (P10)

Identification and passage (P) of cells

Vero Cells (P 46) (*Vaccinia Virus*)

PROTOCOL SUMMARY

The basic virucidal efficacy test is set up with three concentrations of test product solution 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 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 distilled water at t=0, t = 5 and at t =15. 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 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.

1Kärber, 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 , Batch 042020, BT-ATC-01 from against Vaccinia virus VR-1549 under CLEAN conditions						
Test Results						
Concentration	10.0% (v/v)		50.0% (v/v)		80.0% (v/v)	
Exposure Time	data	TCID ₅₀ /ml	data	TCID ₅₀ /ml	data	TCID ₅₀ /ml
t = 5 mins	3.00	3.16E+04	0.00	3.16E+01	0.00	3.16E+01
Raw Data	665100	3.16E+04	000000	3.16E+01	000000	3.16E+01
log		4.50		1.50		1.50
log difference		1.33		4.33		4.33

EN14476:2013 + A2:2019 Suspension test for the efficacy of , Batch 042020, BT-ATC-01 from 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	5 min	15 min	30 min	60 min	
EnviroSafe	0.3g/l BSA	80.0% (v/v)	2.50	2.67	1.50	n.a.	n.a.	n.a.	<5 mins
		50.0% (v/v)	2.50	n.a.	1.50	n.a.	n.a.	n.a.	<5 mins
		10.0% (v/v)	2.50	n.a.	4.50	n.a.	n.a.	n.a.	>5 mins
Virus Control	CLEAN			6.17	5.83	6.00	n.a.	n.a.	n.a.
							5 min	15 min	
Formaldehyde	PBS	0.7% (w/v)	3.50				3.50	2.50	>15 mins

Vaccinia virus (VR-1549) Elstree strain Control Data

EN14476:2013 + A2:2019 Suspension test for the efficacy of , Batch 042020, BT-ATC-01 from against Vaccinia virus VR-1549 under CLEAN conditions												
Controls												
Virus Recovery 0 min		Virus Recovery 5 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	raw data	TCID ₅₀ /ml	
4.67	1.47E+06	4.33	6.81E+05	4.50	1.00E+06	1.00	3.16E+02	1.17	4.64E+02	4.50	1.00E+06	
666640	1.47E+06	666530	6.81E+05	666630	1.00E+06	600000	3.16E+02	610000	4.64E+02	666630	1.00E+06	
	6.17		5.83		6.00		2.50		2.67		6.00	
									3.17		-0.17	
Formaldehyde reference inactivation controls												
Cytotoxicity		Exposure time	0.7% Formaldehyde				No column Control		5 mins			
raw data	TCID ₅₀ /ml		5 mins		15 mins		raw data	TCID ₅₀ /ml	raw data	TCID ₅₀ /ml		
2.00	3.16E+03		raw data	TCID ₅₀ /ml	raw data	TCID ₅₀ /ml	4.67	1.47E+06	666640	1.47E+06		
660000	3.16E+03		2.00	3.16E+03	1.00	3.16E+02				6.17		
	3.50	log		3.50		2.50						
		log difference		2.50		3.50						
Interference control		Virus dilution						Stock Virus (TCID ₅₀)				
		-3	-4	-5	-6	-7	-8	5.83				
PBS Control		1	1	1	0.5	0	0	2.14E+07				
		3.16E+02	3.16E+02	3.16E+02	1.00E+02	3.16E+01	3.16E+01	6666650				
		2.50	2.50	2.50	2.00	1.50	1.50					
Raw Data		6	6	6	3	0	0					
Product		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					
Raw Data		6	6	6	4	0	0					
Log Difference		0.00	0.00	0.00	-0.17	0.00	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.
- 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 greater than 0.5 log₁₀ indicating rapid irreversible virucidal activity of the disinfectant by dilution at a concentration of 80.0% 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, **POSSESSES VIRUCIDAL** activity at a concentration of **50.0% v/v** of the working concentration as tested after **5 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: 22 APRIL 2020

DISCLAIMER

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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