



NANEX

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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)

IDENTIFICATION OF SAMPLE

Identification of the substance/mixture and of the company/undertaking

Name of the product
Batch number
Client
Client Address

General Purpose Sanitiser Concentrate
K5093
Nanex company
Kleine Bogaardestraat 57, 9990 Maldegem Belgium

Project Code
Date of Delivery
Storage conditions
Active substances
Appearance
Condition upon receipt

BT-GEN-03
16 April 2020
Ambient
L-Lactic Acid
Liquid
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

EXPERIMENTAL CONDITIONS

Period of analysis
Product diluents used
Product test concentrations

28 April 2020 to 03 May 2020
Sterile, synthetic hard water
10.0% v/v (1-part product plus 9 parts water);
20.0% v/v (1-part product plus 4 parts water); 33.3% v/v (1 part product plus 2 parts water);

Appearance product dilutions
Appearance in test mixture
Contact times (minutes)
Test temperature
Interfering substances

No changes noted- stable
No changes noted- stable
5 ± 10s
20°C ± 1°C
0.3g/l bovine albumin



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Temperature of incubation	37°C ± 1°C + 5% CO ₂
Identification and passage (P) of virus	Vaccinia virus VR-1549 Elstree strain (P9)
Identification and passage (P) of cells	Vero Cells (P49) (<i>Vaccinia Virus</i>)

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 titered 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 products.

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

eVirus titre is determined for virus in contact with sterile hard 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 biocidal activity.



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resistance. In addition, the formaldehyde cytotoxicity of neutralised formaldehyde is determined, to measure assay sensitivity.

Kärber, G.: Beitrag zur Kollektiven Behandlung Pharmakologischer Reihenversuche. Arch. Exp. Path. Pharmacol. 162 (1931): 480-48.

Vaccinia virus (VR-1549) Elstree strain Test Results

EN14476:2013 + A2 : 2019 Suspension test for the efficacy of General Purpose Sanitiser Concentrate, against Vaccinia virus VR- 1549 under CLEAN conditions

Test Results

Concentration	10.0% (v/v)		20.0% (v/v)		33.3% (v/v)	
Exposure Time	data	TCID ₅₀ /ml	data	TCID ₅₀ /ml	data	TCID ₅₀ /ml
t = 5 minutes	1.00	3.16E+02	1.00	3.16E+02	2.00	3.16E+03
Raw Data	600000	3.16E+02	600000	3.16E+02	660000	3.16E+03
log		2.50		2.50		3.50
log difference		4.00		4.00		3.00

EN14476:2013 + A2:2019 Suspension test for the efficacy of General Purpose Sanitiser Concentrate, against Vaccinia virus VR-1549 under CLEAN conditions

Summary Table

Product:	Interfering substance	Concentration	Level of cytotoxicity	lg TCID ₅₀					>4 log reduction after 'X' Min
				0 min	5 min	15 min	30 min	60 min	
General Purpose Sanitiser Concentrate	0.3g/l BSA	33.3% (v/v)	3.50	3.50	3.50	n.a.	n.a.	n.a.	>5 mins
		20.0% (v/v)	3.50	n.a.	2.50	n.a.	n.a.	n.a.	5 mins
		10.0% (v/v)	3.50	n.a.	2.50	n.a.	n.a.	n.a.	5 mins
Virus Control	CLEAN			6.50	6.50	6.50	n.a.	n.a.	n.a.
Formaldehyde	PBS	0.7% (w/v)	3.50				5 min 4.50	15 min 3.67	>15 mins



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Vaccinia virus (VR-1549) Elstree strain Control Data

EN14476:2013 + A2:2019 Suspension test for the efficacy of General Purpose Sanitiser Concentrate, 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
5.00	3.16E+06	5.00	3.16E+06	5.00	3.16E+06	2.00	3.16E+03	2.00	3.16E+03	4.67	1.47E+06
666660	3.16E+06	666660	3.16E+06	666660	3.16E+06	660000	3.16E+03	660000	3.16E+03	666640	1.47E+06
	6.50		6.50		6.50		3.50		3.50		6.17
									3.00		0.33
Formaldehyde reference inactivation controls								No column Control			
Cytotoxicity		Exposure time	0.7% Formaldehyde						5 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	raw data	TCID ₅₀ /ml	raw data	TCID ₅₀ /ml	raw data	TCID ₅₀ /ml
2.00	3.16E+03	3.00	3.16E+04	2.17	4.64E+03	666662	6.81E+06				
660000	3.16E+03	665100	3.16E+04	661000	4.64E+03		6.83				
	3.50	log	4.50	3.67							
		log difference	2.00	2.83							
		Virus dilution						Stock Virus (TCID ₅₀)			
Interference control		-3	-4	-5	-6	-7	-8	6.50			
		1	1	1	1	0.5	0	1.00E+08			
PBS Control		3.16E+02	3.16E+02	3.16E+02	3.16E+02	1.00E+02	3.16E+01	6666663000			
Raw Data		2.50	2.50	2.50	2.50	2.00	1.50				
		6	6	6	6	3	0				
Product		1	1	1	0.83	0.17	0				
		3.16E+02	3.16E+02	3.16E+02	2.14E+02	4.68E+01	3.16E+01				
Raw Data		2.50	2.50	2.50	2.33	1.67	1.50				
		6	6	6	5	1	0				
Log Difference		0.00	0.00	0.00	0.17	0.33	0.00				
Product Cyt Dilution		-3	-3	-3	-3	-3	-3				
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 $4 \log_{10}$.
- 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:
 - 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 $4 \log_{10}$ reduction of the virus.
- e) The interference control result does not show a difference of $< 1.0 \log_{10}$ 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 $0.5 \log_{10}$ indicating rapid irreversible virucidal activity of the disinfectant by dilution at a concentration of 33.3% 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, **General Purpose Sanitiser Concentrate POSSESSES VIRUCIDAL** activity at a concentration of **10.0% v/v** 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.

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 observations 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 B Virus (HBV)
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)

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.

