

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 Batch number

Kitchen Anti-bacterial Cleaner

DDRA01511

Project Code

Date of Delivery Storage conditions

Active substances

Appearance

Condition upon receipt

BT-ORG-02-02 14 September 2020

Ambient

Benzalkonium chloride, Didecyldimethyammonium chloride

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 serum

at 4°C

Experimental Conditions

Period of analysis

Product diluents used

Product test concentrations

Appearance product dilutions
Appearance in test mixture

Contact times (minutes)

Test temperature

Interfering substances

Temperature of incubation

Identification and passage (P) of virus

Identification and passage (P) of cells

17 September 2020 to 22 September 2020

Sterile distilled water

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

No changes noted- stable

No changes noted-stable

 $5 \pm 10s$

20°C + 1°C

3.0 g/l bovine albumin + 3.0 ml/l erythrocytes

37°C + 1°C + 5% CO2

Vaccinia virus VR-1549 Elstree strain (P08)

Vero Cells (P 13)



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

EN14476:20	13 + A2:2019	Suspension to	est for the ef	ficacy of Kito	hen Anti-bac	EN14476:2013 + A2:2019 Suspension test for the efficacy of Kitchen Anti-bacterial Cleaner,
BT-ORG	BT-ORG-02 from		against	Vaccinia viru	against Vaccinia virus VR-1549 under DIRTY	ider DIRTY
			Test Results			
Concentration	10.0%	10.0% (v/v)	20.0%	50.0% (v/v)	80.0	80.0% (v/v)
Exposure Time	data	TCID ₅₀ /ml	data	TCID ₅₀ /ml	data	TCID ₅₀ /ml
t = 5 mins	2.50	1.00E+04	1.00	3.16E+02	0.00	3.16E+01
Raw Data	000899	1.00E+04	000009	3.16E+02	000000	3.16E+01
log		4.00		2.50		1.50
log difference		2.17		3.67		4.67

EN14476:2	EN14476:2013 + A2:2019 Susp	9 Suspension to	est for the e	fficacy of Kite	then Anti-bac	ension test for the efficacy of Kitchen Anti-bacterial Cleaner, BT-ORG-02 from	3T-ORG-02 fro	ш	
			against Vacc	inia virus VR-	1549 under D	against Vaccinia virus VR-1549 under DIRTY conditions			
				Summ	Summary Table				
Product:	Interfering substance	Concentration	Level of cytotoxicity.			Ig TCID ₅₀			>4 lg reduction after 'X' Min
				0 min	5 min	15 min	30 min	60 min	
Kitchen Anti-	-	80.0% (v/v)	1.50	1.50	1.50	n.a.	n.a.	n.a.	<5 mins
bacterial	3.0ml/l	50.0% (v/v)	1.50	n.a.	2.50	n.a.	n.a.	n.a.	>5 min
Cleaner	e i yti i O cyte s	10.0% (v/v)	1.50	n.a.	4.00	n.a.	n.a.	n.a.	>5 min
Kitchen Anti-	3.0g/l BSA	80.0% (v/v)	1.50	n.a.	1.50	n.a.	n.a.	n.a.	<5 mins
bacterial		50.0% (v/v)	1.50	n.a.	2.50	n.a.	n.a.	n.a.	>5 mins
Cleaner		10.0% (v/v)	1.50	n.a.	2.67	n.a.	n.a.	n.a.	>5 mins
Virus Control	DIRTY			5.67	6.17	5.83	n.a.	n.a.	n.a.
Virus Control	CLEAN			5.67	6.17	6.33	n.a.	n.a.	n.a.
							5 min	15 min	
Formaldehyde PBS	PBS	0.7% (w/v)	2.50				4.50	4.50	>15 mins

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	rial Cleaner,
	n Anti-bacte
ata_	acy of Kitche
Control Da	for the effica
Vaccinia virus (VR-1549) Elstree strain Contr	ension test
/R-1549) El	EN14476:2013 + A2:2019 Suspension
ia virus (V	476:2013 + /
Vaccir	EN14

against Vaccinia virus VR-

		•			1549 under D	1549 under DIRTY conditions	<u>ب</u>				
					Col	Controls					
Virus Re	Virus Recovery	Virus Recovery	coverv	Virus Re	Virus Recovery	Cytotoxicity	icity	Disinf	Disinfectant	Disinfectant	ectant
0	0 min	5 min	. <u>.</u>	15	15 min			Suppres	Suppression VS	Suppression VS2	sion 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
4.17	4.64E+05	4.67	1.47E+06	4.33	6.81E+05	0.00	3.16E+01	0.00	3.16E+01	5.00	3.16E+06
666610	4.64E+05	666640	1.47E+06	666620	6.81E+05	000000	3.16E+01	000000	3.16E+01	099999	3.16E+06
	5.67		6.17		5.83	×	1.50		1.50		6.50
									4.67		-0.33
	100										
		Formaldehyde	e reference inac	Formaldehyde reference inactivation controls	Si				No column Control	n Control	
Cytot	Cytotoxicity	Exposure time		0.7% Fu	0.7% Formaldehyde				5 mins	ins	
	25		2 8 11	5 mins	15	15 mins			raw data	TCID ₅₀ /ml	
raw data	TCID ₅₀ /ml		raw data	TCID ₅₀ /ml	raw data	TCID ₅₀ /ml			5.33	6.81E+06	
1.00	3.16E+02		3.00	3.16E+04	3.00	3.16E+04			666662	6.81E+06	

Control	INS	TCID ₅₀ /ml	6.81E+06	6.81E+06	6.83	
No column Control	5 mins	raw data	5.33	666662		
						_

3.16E+04 4.50

000999

3.16E+04 4.50 1.33

000999

3.16E+02 2.50

000009

log log difference

Stock Virus (TCID ₅₀) 5.50 1.00E+07	0699999
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			Viru	Virus dilution		
Interference control	-3	4-	-5	9-	-7	8-
	1	1	1	1	0.5	0
PBS Control	3.16E+02	3.16E+02	3.16E+02	3.16E+02	1.00E+02	3.16E+01
	2.50	2.50	2.50	2.50	2.00	1.50
Raw Data	9	9	9	9	3	0
	1	1	1	0.67	0	0
Product	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	9	9	9	4	0	0
Log Difference	0.00	0.00	0.00	0.33	0.50	0.00
Product Cyt Dilution	-1	-1	-1	-1	-1	-1
PBS Dilution	Neat	Neat	Neat	Neat	Neat	Neat



Data	
Control	
Istrop strain	
VR-1549) F	
virus /	
Vaccinia	

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		Controls	trols					_	Test Results			
Virus Re	Virus Recovery	Virus Recovery	ecovery	Virus Re	Virus Recovery	Concentration	10.0% (v/v)	(a/a)	50.0% (v/v)	(a/a)	80.0%	80.0% (v/v)
0.0	0 min	5 min	nin	15 min	nin							ş
raw data	TCID ₅₀ /ml	raw data	TCID _{sc} /ml	raw data	TCID ₅₀ /ml	Exposure Time	data	TCID ₅₀ /ml	data	TCID ₅₀ /ml	data	TCID ₅₀ /
4.17	4.64E+05	4.67	1.47E+06	4.83	2.15E+06	t = 5 mins	1.17	4.64E+02	1.00	3.16E+02	0.00	3.16E+
666610	4.64E+05	666640	1.47E+06	666650	2.15E+06	Raw data	430000	4.64E+02	000009	3.16E+02	000000	3.16E+
,	2.67		6.17		6.33	gol		2.67		2.50		1.50
A CONTRACTOR OF THE PARTY OF TH	36.0					log difference		3.50		3.67		4.67



CONCLUSION

Verification of the methodology

A test is only valid if the following criteria are fulfilled:

- 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.
- 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
- 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.
- 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.
- 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, Kitchen Anti-bacterial Cleaner POSSESSES VIRUCIDAL activity at a concentration of $80.0\% \, v/v$ of the working concentration as tested after 5 MINUTES at 20° C under DIRTY conditions (3.0 g/l bovine albumin + 3.0 ml/l erythrocytes) 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: 28 SEPTEMBER 2020

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.



*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