

# 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	Alcohol Free Sanitizer
Batch number	Not supplied
Client	Cleenol Group Limited
Client Address	Neville House, Beaumont Road, Banbury, OX16 1RB
Project Code	BT-CNL-03FT(2)-02
Date of Delivery	10 February 2020
Storage conditions	Ambient
Active substances	Not supplied
Appearance	Liquid

## 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	21 February 2020 to 26 February 2020
Product diluents used	Sterile distilled water
Product test concentrations	80.0% v/v; 50.0%; 10.0% v/v
Appearance product dilutions	No changes noted- stable
Appearance in test mixture	Turbidity observed at 10.0% v/v
Contact times (minutes)	2 ± 10s
Test temperature	20°C ± 1°C
Interfering substances	0.3g/l bovine albumin
Temperature of incubation	37°C ± 1°C + 5% CO <sub>2</sub>
Identification and passage (P) of virus	<b>Vaccinia virus VR-1549 Elstree strain (P10)</b>
Identification and passage (P) of cells	Vero Cells (P 30) ( <i>Vaccinia Virus</i> )

## 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.

### Vaccinia virus (VR-1549) Elstree strain Test Results

EN14476:2013 + A2:2019 Suspension test for the efficacy of Alcohol Free Sanitizer, BT-CNL-03-02 from Cleenol Group Limited against Vaccinia ATCC VR-1549 under Clean conditions						
Test Results						
Concentration	10% (v/v)		50% (v/v)		80% (v/v)	
Exposure Time	data	TCID <sub>50</sub> /ml	data	TCID <sub>50</sub> /ml	data	TCID <sub>50</sub> /ml
t = 2 min	0.00	3.16E+01	2.00	3.16E+03	2.00	3.16E+03
Raw Data	000000	3.16E+01	660000	3.16E+03	660000	3.16E+03
log		1.50		3.50		3.50
log difference		4.50		2.50		2.50

EN14476:2013 + A2:2019 Suspension test for the efficacy of Alcohol Free Sanitizer, BT-CNL-03-02 from Cleenol Group Limited against Vaccinia ATCC VR-1549 under Clean conditions									
Summary Table									
Product:	Interfering substance	Concentration	Level of cytotoxicity	lg TCID <sub>50</sub>					>4 lg reduction after 'X' Min
				0 min	2 min	15 min	30 min	60 min	
Alcohol Free Sanitizer	0.3g/l BSA	80% (v/v)	3.50	3.50	3.50	n.a.	n.a.	n.a.	>2 min
		50% (v/v)	3.50	n.a.	3.50	n.a.	n.a.	n.a.	>2 min
		10% (v/v)	3.50	n.a.	1.50	n.a.	n.a.	n.a.	<2 min
Virus Control	CLEAN			6.00	6.00	6.17	n.a.	n.a.	n.a.
							5 min	15 min	
Formaldehyde	PBS	0.7% (w/v)	2.50			4.67		2.50	>60 mins

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

EN14476:2013 + A2:2019 Suspension test for the efficacy of Alcohol Free Sanitizer, BT-CNL-03-02 from Cleenol Group Limited against Vaccinia ATCC VR-1549 under Clean conditions											
Controls											
Virus Recovery 0 min		Virus Recovery 2 min		Virus Recovery 15 min		Cytotoxicity		Disinfectant Suppression VS		Disinfectant Suppression VS2	
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	raw data	TCID <sub>50</sub> /ml
4.50	1.00E+06	4.50	1.00E+06	4.67	1.48E+06	2.00	3.16E+03	2.00	3.16E+03	4.00	3.16E+05
666630	1.00E+06	666630	1.00E+06	666640	1.48E+06	660000	3.16E+03	660000	3.16E+03	666600	3.16E+05
	6.00		6.00		6.17		3.50		3.50		5.50
									2.50		0.50
Formaldehyde reference inactivation controls										No column Control	
Cytotoxicity		Exposure time	0.7% Formaldehyde				2 mins				
			5 min		15 min		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					
1.00	3.16E+02		3.17	4.68E+04	1.00	3.16E+02		5.00	3.16E+06		
600000	3.16E+02		666100	4.68E+04	600000	3.16E+02		666660	3.16E+06		
	2.50	log		4.67		2.50			6.50		
		log difference		1.50		3.67					
Interference control		Virus dilution						Stock Virus (TCID <sub>50</sub> )			
		-3	-4	-5	-6	-7	-8				
PBS Control		1	1	1	0.83	0.33	0	6.50			
		3.16E+02	3.16E+02	3.16E+02	2.14E+02	6.76E+01	3.16E+01	1.00E+08			
		2.50	2.50	2.50	2.33	1.83	1.50	6666663000			
Raw Data		6	6	6	5	2	0				
Product		1	1	1	0.83	0	0				
		3.16E+02	3.16E+02	3.16E+02	2.14E+02	3.16E+01	3.16E+01				
		2.50	2.50	2.50	2.33	1.50	1.50				
Raw Data		6	6	6	5	0	0				
Log Difference		0.00	0.00	0.00	0.00	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  $10^8$  TCID<sub>50</sub> /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 0.5 and 2.5 after 30 min and between 2.0 and 4.5 after 60 min for poliovirus
  - Between 3.0 and 5.0 after 30 min and between 3.5 and 5.5 after 60 min for adenovirus
  - Between 1.0 and 3.0 after 30 min and between 2.0 and 4.0 after 60 min for murine norovirus
  - Between 0.0 and 2.0 after 30 min and between 0.5 and 2.5 after 60 min for parvovirus
  - 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.
- 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<sub>10</sub> 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, **Alcohol Free Sanitizer POSSESSES VIRUCIDAL** activity at a concentration of **10.0% 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.

The cytotoxicity of the product has prevented at 4.0 log reduction being observed at 80.0% v/v and 50.0% v/v.

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

Signed



Dr Chris Woodall, Director  
BluTest Laboratories Ltd  
Glasgow, UK  
Date: 27 February 2020.

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