

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	Biochem Non Alcohol Hand Sanitiser and Protectant
Batch number	Alpha One
Client	Biochem Solutions Ltd
Client Address	Unit 2, 28 Maich Road, Manurewa, Auckland, New Zealand
Project Code	BT-VHS-02-02
Date of Delivery	21 April 2020
Storage conditions	Ambient
Active substances	Benzethonium chloride USP
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 NCTC media + 10.0% v/v horse serum at 4°C

Experimental Conditions

Period of analysis	05 June 2020 to 08 June 2020
Product diluents used	Sterile, synthetic hard water
Product test concentrations	5.0% v/v; 10.0% v/v; 20.0% v/v
Appearance product dilutions	No changes noted- stable
Appearance in test mixture	Sedimentation and turbidity observed at all concentrations
Contact times (minutes)	1 minute ± 5s
Test temperature	20°C ± 1°C
Interfering substances	3.0 g/l bovine albumin + 3.0 ml/l erythrocytes
Temperature of incubation	37°C ± 1°C + 5% CO ₂
Identification and passage (P) of virus	Murine coronavirus A59 ATCC VR-764 (P8)
Identification and passage (P) of cells	NCTC clone 1469 cells (P29)

PROTOCOL SUMMARY

The basic virucidal efficacy test is set up with three concentrations of test product solution and a 1-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. *Murine coronavirus* A59 ATCC VR-764/ NCTC clone 1469 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, t = 1 and at t =60. The virus titre after 1 minute is then compared to the recovery of disinfectant-treated virus to measure the log reduction in virus titre. The virus titre at 60 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, 15, 30 and 60 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.

Murine coronavirus (A59) Test Results

EN14476:2013 + A2:2019 Suspension test for the efficacy of Biochem Non Alcohol Hand Sanitiser and Protectant, Batch Alpha 1, BT-VHS-02-02 from Biochem Solutions Ltd against Murine coronavirus (A59) ATCC VR-764 under DIRTY conditions						
Test Results						
Concentration	5.0% (v/v)		10.0% (v/v)		20.0% (v/v)	
Exposure Time	data	TCID ₅₀ /ml	data	TCID ₅₀ /ml	data	TCID ₅₀ /ml
t = 1 minute	1.00	3.16E+02	0.00	3.16E+01	0.00	3.16E+01
Raw Data	600000	3.16E+02	000000	3.16E+01	000000	3.16E+01
log		2.50		1.50		1.50
log difference		3.00		4.00		4.00

EN14476:2013 + A2:2019 Suspension test for the efficacy of Biochem Non Alcohol Hand Sanitiser and Protectant, Batch Alpha 1, BT-VHS-02-02 from Biochem Solutions Ltd against Murine coronavirus (A59) ATCC VR-764 under DIRTY conditions									
Summary Table									
Product:	Interfering substance	Concentration	Level of cytotoxicity	lg TCID ₅₀					>4 lg reduction after 'X' Min
				0 min	1 min	15 min	30 min	60 min	
Biochem Non Alcohol Hand Sanitiser and Protectant	3.0g/l BSA + 3.0ml/l erythrocytes	20.0% (v/v)	1.50	1.50	1.50	n.a.	n.a.	n.a.	<1 minute
		10.0% (v/v)	1.50	n.a.	1.50	n.a.	n.a.	n.a.	<1 minute
		5.0% (v/v)	1.50	n.a.	2.50	n.a.	n.a.	n.a.	>1 minute
	3.0g/l BSA	20.0% (v/v)	1.50	n.a.	1.50	n.a.	n.a.	n.a.	<1 minute
		10.0% (v/v)	1.50	n.a.	1.50	n.a.	n.a.	n.a.	<1 minute
		5.0% (v/v)	1.50	n.a.	2.50	n.a.	n.a.	n.a.	>1 minute
Virus Control	DIRTY			5.50	5.50	n.a.	n.a.	5.33	n.a.
Virus Control	CLEAN			5.50	5.50	n.a.	n.a.	5.50	n.a.
				5 min	15 min	30 min	60 min		
Formaldehyde	PBS	0.7% (w/v)	3.50		3.50	3.50	3.50	3.50	>60 mins

Murine coronavirus (A59) Control Data

EN14476:2013 + A2:2019 Suspension test for the efficacy of Biochem Non Alcohol Hand Sanitiser and Protectant, Batch Alpha 1, BT-VHS-02-0 from Biochem Solutions Ltd against Murine coronavirus (A59) ATCC VR-764 under DIRTY conditions											
Controls											
Virus Recovery 0 min		Virus Recovery 1 min		Virus Recovery 60 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
4.00	3.16E+05	4.00	3.16E+05	3.83	2.15E+05	0.00	3.16E+01	0.00	3.16E+01	3.67	1.47E+05
666600	3.16E+05	666600	3.16E+05	666500	2.15E+05	000000	3.16E+01	000000	3.16E+01	666310	1.47E+05
	5.50		5.50		5.33		1.50		1.50		5.17
									4.00		0.33
Formaldehyde reference inactivation controls											
Cytotoxicity		Exposure time	0.7% Formaldehyde								
			5 mins		15 mins		30 mins		60 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	
2.00	3.16E+03		2.00	3.16E+03	2.00	3.16E+03	2.00	3.16E+03	2.00	3.16E+03	
660000	3.16E+03		660000	3.16E+03	660000	3.16E+03	660000	3.16E+03	660000	3.16E+03	
	3.50	log		3.50		3.50		3.50		3.50	
		log difference		1.83		1.83		1.83		1.83	
Interference control		Virus dilution						Stock Virus (TCID ₅₀)			
		-3	-4	-5	-6	-7	-8	5.50			
PBS Control		1	1	1	0.33	0	0	1.00E+07			
		3.16E+02	3.16E+02	3.16E+02	6.76E+01	3.16E+01	3.16E+01	6666630000			
		2.50	2.50	2.50	1.83	1.50	1.50				
Raw Data		6	6	6	2	0	0				
Product		1	1	1	0.5	0	0	No column Control			
		3.16E+02	3.16E+02	3.16E+02	1.00E+02	3.16E+01	3.16E+01	1 min			
		2.50	2.50	2.50	2.00	1.50	1.50	raw data	TCID ₅₀ /ml		
Raw Data		6	6	6	3	0	0	4.17	4.64E+05		
Log Difference		0.00	0.00	0.00	-0.17	0.00	0.00	666610	4.64E+05		
Product Cyt Dilution		-1	-1	-1	-1	-1	-1		5.67		
PBS Dilution		Neat	Neat	Neat	Neat	Neat	Neat				

Murine coronavirus (A59) Control Data

Parallel Control Test												
Controls						Test Results						
Virus Recovery 0 min		Virus Recovery 1 min		Virus Recovery 60 min		Concentration	5.0% (v/v)		10.0% (v/v)		20.0% (v/v)	
raw data	TCID ₅₀ /ml	raw data	TCID ₅₀ /ml	raw data	TCID ₅₀ /ml	Exposure Time	data	TCID ₅₀ /ml	data	TCID ₅₀ /ml	data	TCID ₅₀ /ml
4.00	3.16E+05	4.00	3.16E+05	4.00	3.16E+05	t = 1 minute	1.00	3.16E+02	0.00	3.16E+01	0.00	3.16E+01
666600	3.16E+05	666600	3.16E+05	666600	3.16E+05	Raw data	600000	3.16E+02	000000	3.16E+01	000000	3.16E+01
	5.50		5.50		5.50	log		2.50		1.50		1.50
						log difference		3.00		4.00		4.00

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 0.5 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 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 20.0% 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, **BioChem Non Alcohol Hand Sanitiser and Protectant POSSESSES VIRUCIDAL** activity at a concentration of **10.0% v/v** as tested after **1 MINUTE** at **20°C** under **DIRTY** conditions (3.0 g/l bovine albumin + 3.0 ml/l erythrocytes) against *Murine coronavirus* (A59) ATCC VR-764/ NCTC clone 1469 cells, a surrogate for SARS-CoV-1,2 and MERS CoV.

Murine coronavirus (also known as murine hepatitis virus) as a surrogate for SARS-CoV-2/Covid-19 is the type species of the Betacoronavirus genus that includes SARS-CoV-1&2; MERS-CoV.

Genus Betacoronavirus; Type species: Murine coronavirus

Species: Betacoronavirus 1, Human coronavirus HKU1, Murine coronavirus, Pipistrellus bat coronavirus HKU5, Rousettus bat coronavirus HKU9, Severe acute respiratory syndrome-related coronavirus 1, Severe acute respiratory syndrome-related coronavirus-2, Tylonycteris bat coronavirus HKU4, Middle East respiratory syndrome-related coronavirus, Human coronavirus OC43, Hedgehog coronavirus 1 (EriCoV)

This genus includes (source) bat coronaviruses, pre-existing identified human coronaviruses not associated with severe acute respiratory distress, SARS-CoV 1,2 and MERS-CoV.

Authorised signatory



Dr Chris Woodall, Director
BluTest Laboratories Ltd
Glasgow, UK.
Date: 09 JUNE 2020

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