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	**TX-5 Disinfectant & Deodorant**
Batch number	TX-5-N1/N2
Client	Treasure Purification Technology (HK) Limited
Client address	Room C, 4th Floor, China Insurance Building, 48 Cameron Road, Tsim Sha Tsui, Kowloon, Hong Kong
Project code	BT-TPT-01
Date of delivery	24 May 2022
Storage conditions	Ambient
Active substances	Quaternary Ammonium Salt / Hypochlorous acid
Appearance	Liquid
Condition upon receipt	Undamaged

Test Method and Neutralisation

Internal SOP Number	SOP 8003
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, neutralization control and a formaldehyde internal standard.
Neutraliser	Dilution-neutralization/gel filtration/ Enhanced neutralisation; Eagles Minimum Essential Medium + 10% v/v foetal bovine serum at 4°C

Experimental Conditions

Period of analysis	03 June 2022 to 08 June 2022
Product diluent used	Sterile distilled water
Product test concentrations	**10.0% v/v; 50.0% v/v; 80.0% v/v**
Appearance product dilutions	No changes noted- stable
Appearance in test mixture	No changes noted- stable
Contact time	t = 1 min ± 5 s
Test temperature	20°C ± 1°C
Interfering substance	0.3g/l bovine albumin
Temperature of incubation	37°C ± 1°C + 5% CO2

Test Organism(s)

Identification and passage (P) of virus	Vaccinia virus ATCC VR-1549 Elstree strain (P 05)
Identification and passage (P) of cells	Vero cells (Vaccinia Virus) (P 42)

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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 dose$_{50}$ ($TCID_{50}$) of surviving virus. Vaccinia virus VR-1549 Elstree strain / Vero cells are assayed in parallel in each test. $TCID_{50}$ is determined by the method of Karber[1].

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
Virus is added to a neutralized test product solution. The neutralised virus titre is then determined 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 = 1 and at t = 15. 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 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 $TCID_{50}$ 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.

[1]Kärber, G.: Beitrag zur Kollektiven Behandlung Pharmakologischer Reihenversuche. Arch. Exp. Path. Pharmak. 162 (1931): 480-483.

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Vaccinia virus (VR-1549) Elstree strain Test Results

EN14476:2013 + A2:2019 Suspension test for the efficacy of TX-5 Disinfectant & Deodorant, Batch TX-5-N1/N2, BT-TPT-01 from Treasure Purification Technology (HK) Ltd 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 = 1 min	5.17	**4.64E+06**	0.33	**6.81E+01**	0.00	**3.16E+01**
Raw Data	666661	**4.64E+06**	200000	**6.81E+01**	000000	**3.16E+01**
log		**6.67**		**1.83**		**1.50**
log difference		**0.33**		**5.17**		**5.50**

Vaccinia virus VR-1549 Summary Table									
Product:	**Interfering substance**	**Concentration**	**Level of cytotoxicity**	**lg $TCID_{50}$**					**>4 lg reduction after 'X' Min**
				0 min	**1 min**	**15 min**	**15 min**	**60 min**	
TX-5 Disinfectant & Deodorant	0.3g/l BSA	80.0% (v/v)	1.50	7.50	1.50	n.a	n.a.	n.a.	< 1 min
		50.0% (v/v)	1.50	n.a.	1.83	n.a	n.a.	n.a.	< 1 min
		10.0% (v/v)	1.50	n.a.	6.67	n.a	n.a.	n.a.	> 1 min
Virus Control	CLEAN			6.83	7.00	n.a.	6.83	n.a.	n.a.
Formaldehyde	PBS	0.7% (w/v)	2.50	n.a.	n.a.	5.00	2.50	n.a.	> 15 mins

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Vaccinia virus (VR-1549) Elstree strain Control Data

Stock Virus ($TCID_{50}$)		
	data	$TCID_{50}$/ml
	6.83	**2.14E+08**
raw data	6666665000	**2.14E+08**
log		**8.33**

Vaccinia virus VR-1549 Controls											
Virus Recovery 0 min		**Virus Recovery 1 min**		**Virus Recovery 15 min**		**Cytotoxicity**		**No column Control**		**Disinfectant Suppression VS**	
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
5.33	**6.81E+06**	5.50	**1.00E+07**	5.33	**6.81E+06**	0.00	**3.16E+01**	5.67	**1.47E+07**	6.00	**3.16E+07**
666662	**6.81E+06**	666663	**1.00E+07**	666662	**6.81E+06**	000000	**3.16E+01**	666664	**1.47E+07**	666666	**3.16E+07**
	6.83		**7.00**		**6.83**		**1.50**		**7.17**		**7.50**
								log difference	**-0.17**	log difference	**-0.50**
								diff < 0.5lg?	**yes**	**diff < 0.5lg?**	**yes**

Formaldehyde reference inactivation controls							Interference Control				
Cytotoxicity		**Exposure time**	**0.7% Formaldehyde**					**PBS Control**		**Product**	
			5 mins		**15 mins**			Dilution:	Neat	Dilution:	-1
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
1.00	**3.16E+02**		3.50	**1.00E+05**	1.67	**1.47E+03**		5.17	**4.64E+06**	5.17	**4.64E+06**
600000	**3.16E+02**		666210	**1.00E+05**	640000	**1.47E+03**		666661	**4.64E+06**	666661	**4.64E+06**
	2.50	log		**5.00**		**3.17**	log		**6.67**		**6.67**
		log difference		**1.83**		**3.67**	log difference		**0.00**	**diff < 1.0lg?**	**yes**
		validation	**0.75 < lg D < 3.5?**	**yes**	**2.0 < lg D < 4.0?**	**yes**					

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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.

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 not greater than 0.5 $\log_{10}$ indicating effective neutralisation of the virucidal activity of the disinfectant by dilution at a concentration of 80.0% v/v.

According to EN 14476:2013 + A2:2019, **TX-5 Disinfectant & Deodorant POSSESSES VIRUCIDAL** activity at concentrations of **50.0% v/v and 80.0% v/v** of the working concentration as tested after **1 MINUTE** 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

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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

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