

Vaccinia virus (VR-1549) Elstree strain Test Results

EN14476:2013 + A2:2019 Suspension test for the efficacy of Xtrasan, Batch WT2020001, BT-HYB-01 from Hybrisan WT Surface & Hand Sanitiser against Vaccinia virus VR-1549 under CLEAN conditions						
Test Results						
Concentration	25.0% (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 = 2 min	0.00	3.16E+01	0.00	3.16E+01	0.00	3.16E+01
Raw Data	000000	3.16E+01	000000	3.16E+01	000000	3.16E+01
log		1.50		1.50		1.50
log difference		4.33		4.33		4.33

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Summary Table									
Product:	Interfering substance	Concentration	Level of cytotoxicity	lg TCID ₅₀					>4 lg reduction after 'X' Min
				0 min	2 min	15 min	30 min	60 min	
Hybrisan WT Surface & Hand Sanitiser	0.3g/l BSA	80.0% (v/v)	1.50	2.50	1.50	n.a.	n.a.	n.a.	<2 mins
		50.0% (v/v)	1.50	n.a.	1.50	n.a.	n.a.	n.a.	<2 mins
		25.0% (v/v)	1.50	n.a.	1.50	n.a.	n.a.	n.a.	<2 mins
Virus Control	CLEAN			6.17	5.83	6.00	n.a.	n.a.	n.a.
							5 min	15 min	
Formaldehyde	PBS	0.7% (w/v)	3.50				3.50	2.50	>15 mins

Vaccinia virus (VR-1549) Elstree strain Control Data

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Controls												
Virus Recovery 0 min		Virus Recovery 2 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	
4.67	1.47E+06	4.33	6.81E+05	4.50	1.00E+06	0.00	3.16E+01	1.00	3.16E+02	4.17	4.64E+05	
666640	1.47E+06	666620	6.81E+05	666630	1.00E+06	000000	3.16E+01	600000	3.16E+02	666610	4.64E+05	
	6.17		5.83		6.00		1.50		2.50		5.67	
									3.33		0.17	
Formaldehyde reference inactivation controls												
Cytotoxicity		Exposure time	0.7% Formaldehyde				No column Control		2 mins			
raw data	TCID ₅₀ /ml		5 mins		15 mins		raw data	TCID ₅₀ /ml	raw data	TCID ₅₀ /ml		
2.00	3.16E+03		raw data	TCID ₅₀ /ml	raw data	TCID ₅₀ /ml	4.00	3.16E+05	666600	3.16E+05		
660000	3.16E+03		2.00	3.16E+03	1.00	3.16E+02				5.50		
	3.50	log		3.50		2.50						
		log difference		2.50		3.50						
Interference control		Virus dilution						Stock Virus (TCID ₅₀)				
		-3	-4	-5	-6	-7	-8	5.83				
		1	1	1	0.5	0	0	2.14E+07				
PBS Control		3.16E+02	3.16E+02	3.16E+02	1.00E+02	3.16E+01	3.16E+01	666650				
		2.50	2.50	2.50	2.00	1.50	1.50					
Raw Data		6	6	6	3	0	0					
		1	1	1	0	0	0					
Product		3.16E+02	3.16E+02	3.16E+02	3.16E+01	3.16E+01	3.16E+01					
		2.50	2.50	2.50	1.50	1.50	1.50					
Raw Data		6	6	6	0	0	0					
Log Difference		0.00	0.00	0.00	0.50	0.00	0.00					
Product Cyt Dilution		-1	-1	-1	-1	-1	-1					
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 Ig 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 0.75 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.
- 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 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, **Xtrasan POSSESSES VIRUCIDAL** activity at a concentration of **25.0% 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.

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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Date: 22 APRIL 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.

Amendment 1 BT-HYB-01 EN14476 Report 22 Apr 20 LM CW: Amendment of active substance section. LM 30 April 2020

Amendment 2 BT-HYB-01 A1 EN14476 Report 22 Apr 20 LM CW: Brand name amended into report. LM 30 April 2020

***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