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**

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									
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

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Vaccinia virus (VR-1549) Elstree strain Control Data

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

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			
			5 mins		15 mins	
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**	1.00	**3.16E+02**
660000	**3.16E+03**		660000	**3.16E+03**	600000	**3.16E+02**
	3.50	log		**3.50**		**2.50**
		log difference		**2.50**		**3.50**

No column Control	
2 mins	
raw data	$TCID_{50}$/ml
4.00	**3.16E+05**
666600	**3.16E+05**
	5.50

Interference control	Virus dilution					
	-3	-4	-5	-6	-7	-8
PBS Control	1	1	1	0.5	0	0
	3.16E+02	3.16E+02	3.16E+02	1.00E+02	3.16E+01	3.16E+01
	2.50	**2.50**	**2.50**	**2.00**	**1.50**	**1.50**
Raw Data	**6**	**6**	**6**	**3**	**0**	**0**
Product	1	1	1	0	0	0
	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

Stock Virus ($TCID_{50}$)
5.83
2.14E+07
6666650

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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.

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 $\log_{10}$ 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

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

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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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