





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

EN14476:2013 + A2:2019 Suspension test for the efficacy of A-18S RTU, Batch 20-05-36, BT-MRO-02 from McGea-Rohco UK Limited 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 <sub>50</sub> /ml	data	TCID <sub>50</sub> /ml	data	TCID <sub>50</sub> /ml
t = 5 minutes	1.00	3.16E+02	0.17	4.64E+01	0.17	4.64E+01
Raw Data	600000	3.16E+02	100000	4.64E+01	100000	4.64E+01
log		2.50		1.67		1.67
log difference		4.00		4.83		4.83

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Summary Table									
Product:	Interfering substance	Concentration	Level of cytotoxicity	lg TCID <sub>50</sub>					>4 lg reduction after 'X' Min
				0 min	5 min	10 min	15 min	60 min	
A-18S RTU	0.3g/l BSA	80.0% (v/v)	1.50	1.83	1.50	n.a.	n.a.	n.a.	<5 mins
		50.0% (v/v)	1.50	n.a.	1.50	n.a.	n.a.	n.a.	<5 mins
		10.0% (v/v)	1.50	n.a.	1.50	n.a.	n.a.	n.a.	5 mins
Virus Control	CLEAN			6.67	6.50	n.a.	6.50	n.a.	n.a.
							5 min	15 min	
Formaldehyde	PBS	0.7% (w/v)	3.50				4.50	3.50	>15 mins

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

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Controls												
Virus Recovery 0 min		Virus Recovery 5 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	
5.17	4.64E+06	5.00	3.16E+06	5.00	3.16E+06	0.00	3.16E+01	0.33	6.81E+01	5.17	4.64E+06	
666652	4.64E+06	666651	3.16E+06	666651	3.16E+06	000000	3.16E+01	200000	6.81E+01	666661	4.64E+06	
	6.67		6.50		6.50		1.50		1.83		6.67	
									4.67		-0.17	
Formaldehyde reference inactivation controls							No column Control					
Cytotoxicity		Exposure time	0.7% Formaldehyde				5 min		5 min		5 min	
raw data	TCID <sub>50</sub> /ml		5 mins		15 mins		raw data	TCID <sub>50</sub> /ml	raw data	TCID <sub>50</sub> /ml	raw data	TCID <sub>50</sub> /ml
2.00	3.16E+03		3.00	3.16E+04	2.00	3.16E+03	5.33	6.81E+06	666662	6.81E+06		
660000	3.16E+03		665100	3.16E+04	660000	3.16E+03		6.83				
	3.50	log		4.50		3.50						
		log difference		2.00		3.00						
Interference control		Virus dilution						Stock Virus (TCID <sub>50</sub> )				
		-3	-4	-5	-6	-7	-8	6.17				
PBS Control		1	1	1	1	0.17	0	4.68E+07				
		3.16E+02	3.16E+02	3.16E+02	3.16E+02	4.68E+01	3.16E+01	6666661000				
		2.50	2.50	2.50	2.50	1.67	1.50					
Raw Data		6	6	6	6	1	0					
Product		1	1	1	0.67	0.17	0					
		3.16E+02	3.16E+02	3.16E+02	1.48E+02	4.68E+01	3.16E+01					
		2.50	2.50	2.50	2.17	1.67	1.50					
Raw Data		6	6	6	6	1	0					
Log Difference		0.00	0.00	0.00	0.33	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  $10^8$  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 0.75 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.
- 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 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, **A-18S RTU POSSESSES VIRUCIDAL** activity at a concentration of **10.0% v/v** of the working concentration as tested after **5 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: 03 JUNE 2020

### DISCLAIMER

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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