Vaccinia virus (VR-1549) Elstree strain Test Results

EN14476:2013 + A2:2019 Suspension test for the efficacy of STERIZAR, Batch 9720, BT-CSS-03 from Creative Supply Solutions against Vaccinia virus ATCC 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 minute	0.00	**3.16E+01**	0.00	**3.16E+01**	0.00	**3.16E+01**
Raw Data		**3.16E+01**		**3.16E+01**		**3.16E+01**
log		**1.50**		**1.50**		**1.50**
log difference		**5.00**		**5.00**		**5.00**

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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	
STERIZAR	0.3 g/l bovine albumin	80.0% (v/v)	1.50	1.50	1.50	1.50	n.a.	n.a.	< 1 minute
		50.0% (v/v)	1.50	n.a.	1.50	1.50	n.a.	n.a.	< 1 minute
		10.0% (v/v)	1.50	n.a.	1.50	1.50	n.a.	n.a.	< 1 minute
Virus Control	CLEAN			6.50	6.50	6.50	6.50	6.50	n.a.
							5 min	15 min	
Formaldehyde	PBS	0.7% (w/v)	3.50				4.67	3.50	>15 mins

BLUTEST LABORATORIES LIMITED, 5 Robroyston Oval, Nova Business Park, Glasgow, UK, G33 1AP
Tel: +44 (0)141 558 2782. Email: info@blutest.com. Web site: www.blutest.com. Company No.: SC364409. VAT No.: GB 979 1131 96. UKAS No.: 4597

Vaccinia virus (VR-1549) Elstree strain Control Data

EN14476:2013 + A2:2019 Suspension test for the efficacy of STERIZAR, Batch 9720, BT-CSS-03 from Creative Supply Solutions against Vaccinia virus ATCC VR-1549 under CLEAN conditions

Controls

Virus Recovery 0 min		Virus Recovery 1 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
5.00	**3.16E+06**	5.00	**3.16E+06**	5.00	**3.16E+06**	0.00	**3.16E+01**	0.00	**3.16E+01**	4.67	**1.48E+06**
	3.16E+06		**3.16E+06**		**3.16E+06**		**3.16E+01**		**3.16E+01**		**1.48E+06**
	6.50		**6.50**		**6.50**		**1.50**		**1.50**		**6.17**
									5.00		**0.33**

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**		3.17	**4.68E+04**	2.00	**3.16E+03**
	3.16E+03			**4.68E+04**		**3.16E+03**
	3.50	log		**4.67**		**3.50**
		log difference		**1.83**		**3.00**

No column Control

1 min

raw data	$TCID_{50}$/ml
5.33	**6.76E+06**
	6.76E+06
	6.83

Interference control	Virus dilution					
	-3	**-4**	**-5**	**-6**	**-7**	**-8**
PBS Control	1	1	1	1	0	0
	3.16E+02	3.16E+02	3.16E+02	3.16E+02	3.16E+01	3.16E+01
	2.50	**2.50**	**2.50**	**2.50**	**1.50**	**1.50**
Raw Data	**6**	**6**	**6**	**6**	**0**	**0**
Product	1	1	1	1	0.17	0
	3.16E+02	3.16E+02	3.16E+02	3.16E+02	4.68E+01	3.16E+01
	2.50	**2.50**	**2.50**	**2.50**	**1.67**	**1.50**
Raw Data	**6**	**6**	**6**	**6**	**1**	**0**
Log Difference	**0.00**	**0.00**	**0.00**	**0.00**	**-0.17**	**0.00**
Product Cyt Dilution	-1	-1	-1	-1	-1	-1
PBS Dilution	Neat	Neat	Neat	Neat	Neat	Neat

Stock Virus ($TCID_{50}$)
6.17
4.68E+07

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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 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 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. 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.% v/v.

According to EN 14476:2013 + A2:2019, **STERIZAR POSSESSES VIRUCIDAL** activity at a concentration of **10.0, 50.0 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

Dr Chris Woodall, Director
BluTest Laboratories Ltd
Glasgow, UK
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BLUTEST LABORATORIES LIMITED, 5 Robroyston Oval, Nova Business Park, Glasgow, UK, G33 1AP
Telephone: +44 (0)141 558 2782. Email: info@blutest.com. Web site: www.blutest.com.
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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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