Vaccinia virus (VR-1549) Elstree strain Test Results

EN14476:2013 + A2:2019 Suspension test for the efficacy of Xtra Protect, BT-AQA-02FT(2) from Aqua Air against Vaccinia virus VR-1549 under Dirty conditions						
Test Results						
Concentration	**1.0% (v/v)**		**4.0% (v/v)**		**8.0% (v/v)**	
Exposure Time	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml
t = 5 mins	2.67	**1.48E+04**	0.00	**3.16E+01**	2.00	**3.16E+03**
Raw Data	664000	**1.48E+04**	000000	**3.16E+01**	660000	**3.16E+03**
log		**4.17**		**1.50**		**3.50**
log difference		**1.50**		**4.17**		**2.17**

EN14476:2013 + A2:2019 Suspension test for the efficacy of Xtra Protect, BT-AQA-02FT(2) from Aqua Air against Vaccinia virus VR-1549 under Dirty conditions									
Summary Table									
Product:	Interfering substance	Concentration	Level of cytotoxicity	lg $TCID_{50}$					>4 lg reduction after 'X' Min
				0 min	5 min	15 min	30 min	60 min	
Xtra Protect	3.0g/l BSA + 3.0ml/l erythrocytes	8.0% (v/v)	3.50	3.50	3.50	n.a	n.a	n.a.	>5 mins
		4.0% (v/v)	3.50	n.a.	1.50	n.a	n.a	n.a.	<5 mins
		1.0% (v/v)	3.50	n.a.	4.17	n.a	n.a	n.a.	>5 mins
	3.0g/l BSA	8.0% (v/v)	3.50	n.a.	3.50	n.a	n.a	n.a.	>5 mins
		4.0% (v/v)	3.50	n.a.	1.50	n.a	n.a	n.a.	<5 mins
		1.0% (v/v)	3.50	n.a.	2.50	n.a	n.a	n.a.	>5 mins
Virus Control	DIRTY			5.67	5.67	5.50	n.a	n.a.	n.a.
Virus Control	CLEAN			5.83	5.83	5.67	n.a	n.a.	n.a.
							5 min	15 min	
Formaldehyde	PBS	0.7% (w/v)	3.50				3.67	3.50	>60 mins

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Controls

Virus Recovery 0 min		Virus Recovery 5 mins		Virus Recovery 15 mins		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
4.17	**4.68E+05**	4.17	**4.68E+05**	4.00	**3.16E+05**	2.00	**3.16E+03**	2.00	**3.16E+03**	4.67	**1.48E+06**
666610	**4.68E+05**	666610	**4.68E+05**	666600	**3.16E+05**	660000	**3.16E+03**	660000	**3.16E+03**	666640	**1.48E+06**
	5.67		**5.67**		**5.50**		**3.50**		**3.50**		**6.17**
									2.17		**-0.50**

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**		2.17	**4.68E+03**	2.00	**3.16E+03**
660000	**3.16E+03**		661000	**4.68E+03**	660000	**3.16E+03**
	3.50	log		**3.67**		**3.50**
		log difference		**1.83**		**2.00**

No column Control

5 mins

raw data	$TCID_{50}$/ml
4.67	**1.48E+06**
666640	**1.48E+06**
	6.17

Interference control	Virus dilution					
	-3	**-4**	**-5**	**-6**	**-7**	**-8**
PBS Control	1	1	1	0.67	0	0
	3.16E+02	3.16E+02	3.16E+02	1.48E+02	3.16E+01	3.16E+01
	2.50	**2.50**	**2.50**	**2.17**	**1.50**	**1.50**
Raw Data	**6**	**6**	**6**	**4**	**0**	**0**
Product	1	1	1	0.67	0	0
	3.16E+02	3.16E+02	3.16E+02	1.48E+02	3.16E+01	3.16E+01
	2.50	**2.50**	**2.50**	**2.17**	**1.50**	**1.50**
Raw Data	**6**	**6**	**6**	**4**	**0**	**0**
Log Difference	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Product Cyt Dilution	-3	-3	-3	-3	-3	-3
PBS Dilution	Neat	Neat	Neat	Neat	Neat	Neat

Stock Virus ($TCID_{50}$)
5.67
6.67E+09
6666631000

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Vaccinia virus (VR-1549) Elstree strain Control Data

Parallel Control Test												
Controls						Test Results						
Virus Recovery 0 min		Virus Recovery 5 mins		Virus Recovery 15 mins		Concentration	1.0% (v/v)		4.0% (v/v)		8.0% (v/v)	
raw data	$TCID_{50}$/ml	raw data	$TCID_{50}$/ml	raw data	$TCID_{50}$/ml	Exposure Time	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml
4.33	**6.76E+05**	4.33	**6.76E+05**	4.17	**4.68E+05**	**t = 5 mins**	1.00	**3.16E+02**	0.00	**3.16E+01**	2.00	**3.16E+03**
666620	**6.76E+05**	666620	**6.76E+05**	666610	**4.68E+05**	Raw data	600000	**3.16E+02**	000000	**3.16E+01**	660000	**3.16E+03**
	5.83		**5.83**		**5.67**	log		**2.50**		**1.50**		**3.50**
						log difference		**3.33**		**4.33**		**2.33**

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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 0.5 and 2.5 after 30 min and between 2.0 and 4.5 after 60 min for poliovirus
   - Between 3.0 and 5.0 after 30 min and between 3.5 and 5.5 after 60 min for adenovirus
   - Between 1.0 and 3.0 after 30 min and between 2.0 and 4.0 after 60 min for murine norovirus
   - Between 0.0 and 2.0 after 30 min and between 0.5 and 2.5 after 60 min for parvovirus
   - 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 8.0% 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, **XtraProtect POSSESSES VIRUCIDAL** activity at a concentration of **4.0% v/v** of the working concentration as tested after **5 MINUTES** at **20°C** under **DIRTY** conditions (3.0 g/l bovine albumin + 3.0 ml/l erythrocytes) against *Vaccinia virus* VR-1549 Elstree strain / Vero cells.

The cytotoxicity of the product has prevented at 4.0 log reduction being observed at 8.0% v/v.

This product therefore is effective against all enveloped viruses as defined in EN 14476:2013 + A2:2019. This therefore includes all coronaviruses and SARS-CoV-2.

Signed

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