Vaccinia virus (ATCC VR-1549) Test Results

EN14476:2013 + A2:2019 Suspension test for the efficacy of Alcohol Free Hand Sanitiser, Batch L503, BT-SAF-22 from Safe Solutions against Vaccinia virus VR-1549 under CLEAN conditions						
Test Results						
Concentration	**10.0% (v/v)**		**50.0% (v/v)**		**100.0% (v/v)**	
Exposure Time	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml
t = 2 mins	1.67	**1.47E+03**	1.00	**3.16E+02**	0.00	**3.16E+01**
Raw Data	640000	**1.47E+03**	600000	**3.16E+02**	000000	**3.16E+01**
log		**3.17**		**2.50**		**1.50**
log difference		**3.50**		**4.17**		**5.17**

Vaccinia virus VR-1549 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**	
Alcohol Free Hand Sanitiser	0.3g/l BSA	100.0% (v/v)	1.50	6.83	1.50	n.a	n.a.	n.a.	< 2 min
		50.0% (v/v)	1.50	n.a.	2.50	n.a	n.a.	n.a.	< 2 min
		10.0% (v/v)	1.50	n.a.	3.17	n.a	n.a.	n.a.	> 2 min
Virus Control	CLEAN			6.83	6.67	6.83	n.a.	n.a.	n.a.
							5 min	15 min	
Formaldehyde	PBS	0.7% (w/v)	2.50				5.67	4.83	> 15 min

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Vaccinia virus (ATCC VR-1549) Control Data

Vaccinia virus VR-1549 Controls											
Virus Recovery 0 min		Virus Recovery 2 min		Virus Recovery 15 min		Cytotoxicity		Disinfectant Suppression VS		No column Control	
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
5.33	**6.81E+06**	5.17	**4.64E+06**	5.33	**6.81E+06**	0.00	**3.16E+01**	5.33	**6.81E+06**	5.50	**1.00E+07**
666662	**6.81E+06**	666661	**4.64E+06**	666653	**6.81E+06**	000000	**3.16E+01**	666662	**6.81E+06**	666663	**1.00E+07**
	6.83		**6.67**		**6.83**		**1.50**		**6.83**		**7.00**
									-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
1.00	**3.16E+02**		4.17	**4.64E+05**	3.33	**6.81E+04**
600000	**3.16E+02**		666610	**4.64E+05**	666200	**6.81E+04**
	2.50	log		**5.67**		**4.83**
		log difference		**1.17**		**2.00**

Stock Virus ($TCID_{50}$)
6.67
1.48E+08
6666664

Interference control	Virus dilution					
	-3	**-4**	**-5**	**-6**	**-7**	**-8**
PBS Control	1	1	1	1	0.83	0
	3.16E+02	3.16E+02	3.16E+02	3.16E+02	2.14E+02	3.16E+01
	2.50	**2.50**	**2.50**	**2.50**	**2.33**	**1.50**
Raw Data	**6**	**6**	**6**	**6**	**5**	**0**
Product	1	1	1	1	0.83	0
	3.16E+02	3.16E+02	3.16E+02	3.16E+02	2.14E+02	3.16E+01
	2.50	**2.50**	**2.50**	**2.50**	**2.33**	**1.50**
Raw Data	**6**	**6**	**6**	**6**	**5**	**0**
Log Difference	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Product Cyt Dilution	-1	-1	-1	-1	-1	-1
PBS Dilution	Neat	Neat	Neat	Neat	Neat	Neat

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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.
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 not greater than 0.5 $\log_{10}$ indicating effective neutralisation of the virucidal activity of the disinfectant by dilution at a concentration of 100.0% v/v.

According to EN 14476:2013 + A2:2019, **Alcohol Free Hand Sanitiser POSSESSES VIRUCIDAL** activity at concentrations of **50.0% v/v and 100.0% 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.

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Date: 24 SEPTEMBER 2021

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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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