





## 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 <sub>50</sub> /ml	data	TCID <sub>50</sub> /ml	data	TCID <sub>50</sub> /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 <sub>50</sub>					>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

### 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 <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
5.33	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								Stock Virus (TCID <sub>50</sub> )			
Cytotoxicity		Exposure time	0.7% Formaldehyde								
			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					
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					
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				

## 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 not greater than 0.5 log<sub>10</sub> 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.**

Authorised signatory



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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