







### Murine coronavirus (A59) Control Data

EN14476:2013 + A2:2019 Suspension test for the efficacy of Crebisol, Batch 300919, BT-CRB-01 from Crebisol Limited against Murine hepatitis virus (A59) under DIRTY conditions											
Controls											
Virus Recovery 0 min		Virus Recovery 5 min		Virus Recovery 60 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
4.00	3.16E+05	4.00	3.16E+05	3.83	2.15E+05	0.00	3.16E+01	0.00	3.16E+01	3.50	1.00E+05
666600	3.16E+05	666600	3.16E+05	666500	2.15E+05	000000	3.16E+01	000000	3.16E+01	666210	1.00E+05
	5.50		5.50		5.33		1.50		1.50		5.00
									4.00		0.50
Formaldehyde reference inactivation controls											
Cytotoxicity		0.7% Formaldehyde									
		Exposure time	5 mins		15 mins		30 mins		60 mins		
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	
2.00	3.16E+03		2.00	3.16E+03	2.00	3.16E+03	2.00	3.16E+03	2.00	3.16E+03	
660000	3.16E+03		660000	3.16E+03	660000	3.16E+03	660000	3.16E+03	660000	3.16E+03	
	3.50	log		3.50		3.50		3.50		3.50	
		log difference		1.83		1.83		1.83		1.83	
Interference control		Virus dilution						No column Control			
		-3	-4	-5	-6	-7	-8	5 mins			
PBS Control		1	1	1	0.33	0	0	raw data	TCID <sub>50</sub> /ml		
		3.16E+02	3.16E+02	3.16E+02	6.76E+01	3.16E+01	3.16E+01	4.17	4.64E+05		
		2.50	2.50	2.50	1.83	1.50	1.50	666610	4.64E+05		
									5.67		
Raw Data		6	6	6	2	0	0				
Product		1	1	1	0.5	0	0				
		3.16E+02	3.16E+02	3.16E+02	1.00E+02	3.16E+01	3.16E+01				
		2.50	2.50	2.50	2.00	1.50	1.50				
Raw Data		6	6	6	3	0	0				
Log Difference		0.00	0.00	0.00	-0.17	0.00	0.00	Stock Virus (TCID <sub>50</sub> )			
								5.50			
								1.00E+07			
								6666630000			
Product Cyt Dilution		-1	-1	-1	-1	-1	-1				
PBS Dilution		Neat	Neat	Neat	Neat	Neat	Neat				

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Parallel Control Test												
Controls						Test Results						
Virus Recovery 0 min		Virus Recovery 5 min		Virus Recovery 60 min		Concentration	1.0% (v/v)		2.0% (v/v)		5.0% (v/v)	
raw data	TCID <sub>50</sub> /ml	raw data	TCID <sub>50</sub> /ml	raw data	TCID <sub>50</sub> /ml	Exposure Time	data	TCID <sub>50</sub> /ml	data	TCID <sub>50</sub> /ml	data	TCID <sub>50</sub> /ml
4.00	3.16E+05	4.00	3.16E+05	4.00	3.16E+05	t = 2 mins	2.00	3.16E+03	1.00	3.16E+02	0.00	3.16E+01
666600	3.16E+05	666600	3.16E+05	666600	3.16E+05	Raw data	660000	3.16E+03	600000	3.16E+02	000000	3.16E+01
	5.50		5.50		5.50	log		3.50		2.50		1.50
						log difference		2.00		3.00		4.00
						Exposure Time	data	TCID <sub>50</sub> /ml	data	TCID <sub>50</sub> /ml	data	TCID <sub>50</sub> /ml
						t = 5 mins	2.00	3.16E+03	1.00	3.16E+02	0.00	3.16E+01
						Raw data	660000	3.16E+03	600000	3.16E+02	000000	3.16E+01
						log		3.50		2.50		1.50
						log difference		2.00		3.00		4.00

## 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 0.5 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<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 5.0% 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, **Crebisol POSSESSES VIRUCIDAL** activity at a concentration of **5.0% v/v** as tested after **2 MINUTES** at **20°C** under (**DIRTY** conditions (3.0 g/l bovine albumin + 3.0 ml/l erythrocytes) against *Murine coronavirus* (A59) ATCC VR-764/ NCTC clone 1469 cells, a surrogate for SARS-CoV-1,2 and MERS CoV.

**Murine coronavirus (also known as murine hepatitis virus) as a surrogate for SARS-CoV-2/Covid-19 is the type species of the Betacoronavirus genus that includes SARS-CoV-1&2; MERS-CoV.**

**Genus Betacoronavirus; Type species: Murine coronavirus**

**Species: Betacoronavirus 1, Human coronavirus HKU1, Murine coronavirus, Pipistrellus bat coronavirus HKU5, Rousettus bat coronavirus HKU9, Severe acute respiratory syndrome-related coronavirus 1, Severe acute respiratory syndrome-related coronavirus-2, Tylonycteris bat coronavirus HKU4, Middle East respiratory syndrome-related coronavirus, Human coronavirus OC43, Hedgehog coronavirus 1 (EriCoV)**

**This genus includes (source) bat coronaviruses, pre-existing identified human coronaviruses not associated with severe acute respiratory distress, SARS-CoV 1,2 and MERS-CoV.**

Authorised signatory



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Date: 09 JUNE 2020

**DISCLAIMER**

The results in this test report only pertain to the sample supplied.

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