TEST REPORT

Applicant : V-Zap Technology Inc.

Address : 607-1101 rue Saint-Urbain Montreal (Quebec) H2Z1K8 Canada

The following merchandise was (were) submitted and identified by the client as:

Name of Sample :	V-Zap Antibacterial Surface Sanitiser & Protectant
Test Type :	Commission
Analysis No. :	A201021-22
Sample Quantity :	1
Model :	VZ-1008
Brand :	V-Zap Antibacterial Surface Sanitiser & Protectant
Batch No. :	MD205092020
Sample Received :	2020/10/21
Test Period :	2020/10/21-2020/12/11
Test Method :	Please refer to next page(s).
Test Result :	Please refer to next page(s).

Note:

1. Relevant testing items are not within the scope of China Metrology Accreditation, only for the internal use of client.
2. Production date: 2020/09/05.
3. Production Unit : V-Zap Technology Inc.
4. Production Address : 607-1101 rue Saint-Urbain Montreal (Quebec) H2Z1K8 Canada.
5. This report replaces the HG201211-61 issued in 2020/12/11, which is cancelled.

Edited by: _____ Approved by: _____

Checked by: _____ Official Seal: _____

Add: No. 368 Xingke Road, Tianhe District, Guangzhou, P. R .China. E-mail: ate@gic.ac.cn
Tel: 400-119-8299、020-85231290 Website: http://www.cas-test.org

TEST RESULTS:

1. Experimental materials

1.1 Samples tested: V-Zap Antibacterial Surface Sanitiser & Protectant was provided by Smart Sharp Limitd. The sample is a colorless and transparent liquid. Registered trademark: BIO Water; production date: September 5, 2020; Manufacturer: V-Zap Technology Inc. CANADA.

1.2 Cells: L2 cells.

1.3 Virus: Coronavirus MHV-A59, which belongs to the same β-coronavirus genus as the 2019-nCoV,is 66.02% identical at the whole genome lever to 2019-nCoV.

1.4 Reagents: DMEM medium, fetal bovine serum and other reagents were provided by the laboratory of service provider.

2. Experimental principles and methods

2.1Experimental principle: MHV-A59 infection of L2 cells leads to cytopathogenic effect (CPE). The $TCID_{50}$ of the virus after inactivation was measured by CPE in the cell culture, to reflect the inactivation effect of the sample to be tested.

2.2Experimental protocol:

2.2.1Verification of cytotoxic effect

2.2.1.1 L2 cells were inoculated in 96-well cell culture plate;

2.2.1.2 A 0.9 mL V-Zap Antibacterial Surface Sanitiser & Protectant was mixed with 0.1 mL cell culture medium.,then the sample were serially diluted by a gradient of 10 times, with a total of 6 dilutions and 2 duplicate wells for each dilution. 100 μLof each diluted solution was added to L2 cells per well.

2.2.1.3 The cells in 96-well plate were cultured in a carbon dioxide incubator for 72 hours

2.2.1.4 After 72 hours of incubation, 100 μl cell culture medium was removed from each well.

******** TO BE CONTINUE ********

2.2.1.5 Add 100 µl CellTiter-Glo reagent (Promega) to each well of plates. Cover plate with clear adhesive sealing film (PerkinElmer). Mix contents for 5 minutes on an orbital shaker to induce cell lysis.

2.2.1.6 Read luminescence on Enspire(Perkin-Elmer)

2.2.2 Data analysis

The raw data was used for Viability % calculation with following formular:

$$\text{Viability (\%)} = (\text{Raw data})/(\text{Average}_{CC})*100$$

They were further used CC_{50} calculation using software GraphPad Prism 6.

The cytotoxic effect of L2 by V-Zap Antibacterial Surface Sanitiser & Protectant was shown at figure1.

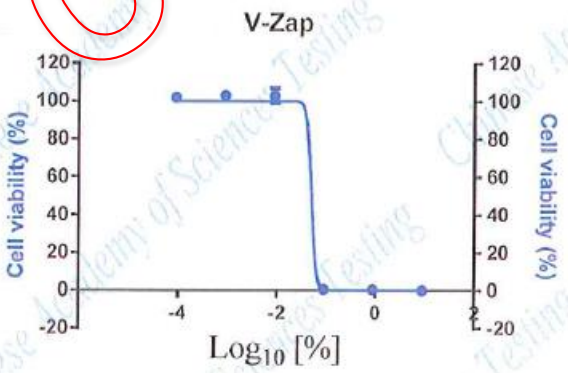

Figure1 The cytotoxic effect of L2 by V-Zap Antibacterial Surface Sanitiser & Protectant. At the concentration of 9%, 0.9%, and 0.09%, the cells were poisoned to death, at 0.009% concentration, no cytotoxicity .

2.2.3The inactivation of MHV-A59 by 0.009%V-Zap Antibacterial Surface Sanitiser & Protectant

2.2.3.1 L2 cells were inoculated in 2 96-well cell culture plates;

******** TO BE CONTINUE ********

2.2.3.2 A 0.9 mL 0.01%V-Zap Antibacterial Surface Sanitiser & Protectant was mixed with 0.1 mL MHV-A59 virus stock solution, followed by incubation at room temperature for 5 minutes, and then used as the sample to test virus titer. At the same time, 0.9 mL PBS and 0.1 mL MHV-A59 virus stock were mixed and incubated at room temperature for 5 minutes, which is used as a untreated control. The sample and the virus control were serially diluted by a gradient of 10 times, with a total of 7 dilutions and 6 duplicate wells for each dilution. 100 μLof each diluted virus solution was used to infect L2 cells per well.

2.2.3.3 Virus replication in each well was detected 72 hours after infection. The $TCID_{50}$ of the sample to be tested and the control sample was calculated according to the technical specification for disinfection, 2002 edition.

2.2.3.4 Results

The inactivation of MHV-A59 by 0.009%V-Zap Antibacterial Surface Sanitiser & Protectant was shown at Table 1

Table 1. The inactivation of MHV-A59 by 0.009%V-Zap Antibacterial Surface Sanitiser & Protectant

MHV-A59 5minutes	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	Cell control	Virus titer ($TCID_{50}$/mL)
Virus group	+	+	+	+	-	-	-	-	$10^{5.5}$
	+	+	+	+	+	-	-	-	
	+	+	+	+	-	-	-	-	
	+	+	+	-	-	-	-	-	
	+	+	+	+	-	-	-	-	
	+	+	+	+	-	-	-	-	
0.009%V-Zap Antibacterial Surface Sanitiser & Protectant group	+	+	+	+	-	-	-	-	$10^{5.5}$
	+	+	+	+	-	-	-	-	
	+	+	+	+	-	-	-	-	
	+	+	+	+	-	-	-	-	
	+	+	+	+	-	-	-	-	
	+	+	+	+	-	-	-	-	

******** TO BE CONTINUE ********

2.2.4Summary

The V-Zap Antibacterial Surface Sanitiser & Protectant diluted 10000 times was not toxic to cells and had no inactivation effect on viruses.

2.2.5Test procedure

2.2.5.1L2 cells were inoculated in 38 48-well cell culture plates.

2.2.5.2A 0.9 mL V-Zap Antibacterial Surface Sanitiser & Protectant was mixed with 0.1 mL MHV-A59 virus stock solution, followed by incubation at room temperature for 5 minutes, and then used as the sample to test virus titer. At the same time, 0.9 mL PBS and 0.1 mL MHV-A59 virus stock were mixed and incubated at room temperature for 5 minutes, which is used as a untreated control. The sample and the virus control were serially diluted by a gradient of 10 times, with a total of 7 dilutions and 6 duplicate wells for each dilution.

2.2.5.3 Prepare 6 100mL glass bottles,added 99.9ml cell culture medium to each glass bottles.

2.2.5.4 Transfer 100μLof the first dilution to each glass bottles, Mixed well ,then,added all the 600ml diluent to 12 48-well cell culture plates,1.042mL each well.

2.2.5.5 Prepare 6 15mL centrifuge tubes,added 9.9ml cell culture medium to each centrifuge tube.

2.2.5.6 Transfer 100μL of the second dilution to each centrifuge tubes, Mixed well ,then,added all the 60ml diluent to 6 48-well cell culture plates,0.2083ml each well.

2.2.5.7 The remaining 5 dilutions, 100 μLof each diluted virus solution was used to infect L2 cells per well.

2.2.5.8 Virus replication in each well was detected 72 hours after infection. The $TCID_{50}$ of the sample to be tested and the control sample was calculated according to the technical specification for disinfection, 2002 edition.

******** TO BE CONTINUE ********

3. Results

The inactivation of MHV-A59 by V-Zap Antibacterial Surface Sanitiser & Protectant was shown at Table 2.

Table 2. The inactivation of MHV-A59 by V-Zap Antibacterial Surface Sanitiser & Protectant

MHV-A59 5minutes	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	Cell control	Virus titer ($TCID_{50}$/mL)
Virus group	+	+	+	+	-	-	-	-	$10^{5.5}$
	+	+	+	+	-	-	-	-	
	+	+	+	-	-	-	-	-	
	+	+	+	+	-	-	-	-	
	+	+	+	+	+	-	-	-	
	+	+	+	+	-	-	-	-	
V-Zap Antibacterial Surface Sanitiser & Protectant group	-	-	-	-	-	-	-	-	$\leq 10^{1.5}$
	-	-	-	-	-	-	-	-	
	-	-	-	-	-	-	-	-	
	-	-	-	-	-	-	-	-	
	-	-	-	-	-	-	-	-	
	-	-	-	-	-	-	-	-	

Note: "+" means there is at least one CPE locus caused by virus replication, and "-" means there is no CPE locus in the well. TCID50 was calculated by Spearman–Karber method.

******** TO BE CONTINUE ********

4. Conclusion

In this test, the inactivation effect of V-Zap Antibacterial Surface Sanitiser & Protectant on MHV-A59 was detected at room temperature for 5 minutes. The test results showed that the titer was $10^{5.5}TCID_{50}$/mL in the untreated virus control and less than or equal to $10^{1.5}TCID_{50}$ /mL in the V-Zap Antibacterial Surface Sanitiser & Protectant group. Compared with the virus group, the titer of MHV-A59 was decreased by greater than or equal to 4 logs at room temperature for 5minutes, and the inactivation rate was greater than or equal to 99.99%.

The above test results are the results of a single experiment, it can only be used for research purpose.

******** END OF REPORT ********