MS ISO/IEC 17025
TESTING
SAMM NO. 823

Test Report No.: VX-TR-21-1103
Copy No.: 1

DETERMINATION OF THE VIRUCIDAL ACTIVITY (EN 14476) OF AIRESTEC BIOACTIVE VIRUCIDAL SURFACE DISINFECTANT (BVSD)

Lab No.:	VX-189-21-0001
Sample Name:	**Airestec Bioactive Virucidal Surface Disinfectant (BVSD)**
Method:	EN 14476:2013+A2:2019 (E) Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity in the medical area – Test method and requirements (phase 2, step 1)
Client:	Airestec Innovations Sdn Bhd B-09-10 & 11, Gateway Corporate Suites, Gateway Kiaramas 1 Jalan Desa Kiara, Mont Kiara 50480 Kuala Lumpur Malaysia
Sample Receipt Date:	17 June 2021
Report Date:	16 November 2021

Kuala Lumpur, 16 November 2021

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Dr Syazani Suhaimi
Microbiologist

Viroxy Sdn. Bhd. (1193371-A)
6th Floor · Menara RKT · 36 Jalan Raja Abdullah · 50300 Kuala Lumpur · Malaysia
Phone: +60 (0)3 2630 8888 · Fax: +60 (0)3 2630 8085 · info.kl@viroxylabs.com
www.viroxylabs.com

Materials and Method

Quantitative suspension test for the evaluation of virucidal activity in the medical area according to EN 14476:2013+A2:2019 (E)

1. Testing laboratory identification

Viroxy Sdn. Bhd.
6th Floor, Menara RKT
50300 Kuala Lumpur
Malaysia

2. Sample identification

2.1	Sample name:	Airestec Bioactive Virucidal Surface Disinfectant (BVSD)
2.2	Batch no.:	Not specified
2.3	Product appearance:	Clear, colourless solution
2.4	Manufacturer:	Airestec Innovations Sdn Bhd B-09-10 & 11, Gateway Corporate Suites, Gateway Kiaramas 1 Jalan Desa Kiara, Mont Kiara 50480 Kuala Lumpur Malaysia
2.5	Active substances:	Stabilised hydrogen peroxide
2.6	Sample receipt date:	17 June 2021
2.7	Storage conditions:	Room temperature
2.8	Product diluent:	Distilled water

3. Experimental conditions

3.1	Testing period:	22 Jun – 02 August 2021
3.2	Test organism(s):	*Adenovirus type 5*, strain Adenoid 75, ATCC VR-5 *Murine norovirus*, strain S99 Berlin, FLI-RVB-0651 *Poliovirus type 1*, strain LSc2ab, NIBSC-01/528
3.3	Concentration/contact time:	100.00 %* / 15 and 45 minutes
3.4	Loading:	0.30 g/L bovine albumin solution
3.5	Test temperature:	28 °C ± 1 °C
3.6	Incubation period:	7 days, 36 °C ± 1 °C

4. Test method and its validation

4.1 Testing method: Quantal test

4.2 Inactivation method: Immediate dilution
Molecular sieving using MicroSpin™ S 400 HR

The results of validation tests A, B, and C proved the viability of the method in all cases.

5. Test results

The results are stated in Tables A and B.

6. Conclusion

Airestec Bioactive Virucidal Surface Disinfectant (BVSD) showed the required viral reduction of ≥4.0 $\log_{10}$ against test strains *Adenovirus type 5* ATCC VR-5, *Murine norovirus* FLI-RVB-0651 and *Poliovirus* type 1 NIBSC-01/528 in accordance with EN 14476:2013+A2:2019 (E) at 100.00 %* concentration after 15 and 45 minutes under the stated condition. According to the simple acceptance decision rule†, there is < 50 % risk of false acceptance for *Adenovirus type 5* ATCC VR-5 and *Poliovirus type 1* NIBSC-01/528, and a minimal risk of false acceptance for *Murine norovirus* FLI-RVB-0651.

Kuala Lumpur, 16 November 2021

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Dr Syazani Suhaimi
Microbiologist

7. Note

Virucidal activity – the capability of a product to produce a reduction in the number of viable viruses belonging to reference strains under defined conditions by at least 4 orders (10^4).

$R = V_C/N_a$ = the reduction in viability, or $\lg R = \lg V_C - \lg N_a$

* The product can only be tested at 80.00 % concentration or less, as some dilution always occurs when test organisms and interfering substance are added.

† The decision rule applied is simple acceptance rule with no guard band and up to 50 % risk of false acceptance or rejection. This rule has been determined by the laboratory and agreed with the client prior to testing.

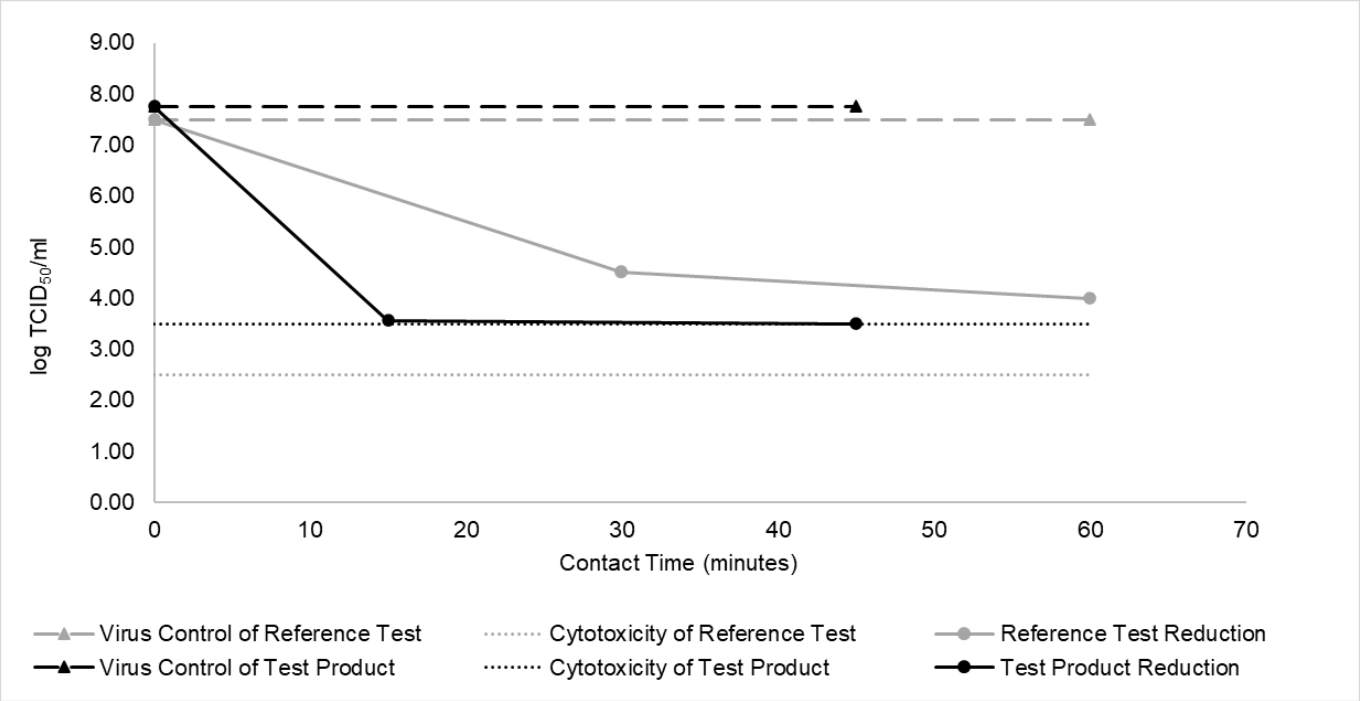
Table A: Evaluation of the virucidal activity of Airestec Bioactive Virucidal Surface Disinfectant (BVSD) on test strains according to EN 14476

Product: Airestec Bioactive Virucidal Surface Disinfectant (BVSD)
Loading: 0.30 g/L bovine albumin solution

Test strain: *Adenovirus type 5* ATCC VR-5

Virus control, V_C	Cytotoxicity effect, CE
V_{C1}: 7.75 ± 0.33 V_{C2}: 7.75 ± 0.33	CE_1: 3.50 ± 0.00 CE_2: 3.50 ± 0.00

Test concentration (%) / contact time (min)	First assay, N_{a1}	Second assay, N_{a2}	Average reduction
100.00* / 15	N_{a1}: ≤3.50 ± 0.00 lg R_1: **≥4.25 ± 0.33**	N_{a2}: 3.63 ± 0.25 lg R_2: **4.13 ± 0.41**	lg R: **≥4.19 ± 0.37**
100.00* / 45	N_{a1}: ≤3.50 ± 0.00 lg R_1: **≥4.25 ± 0.33**	N_{a2}: ≤3.50 ± 0.00 lg R_2: **≥4.25 ± 0.33**	lg R: **≥4.25 ± 0.33**

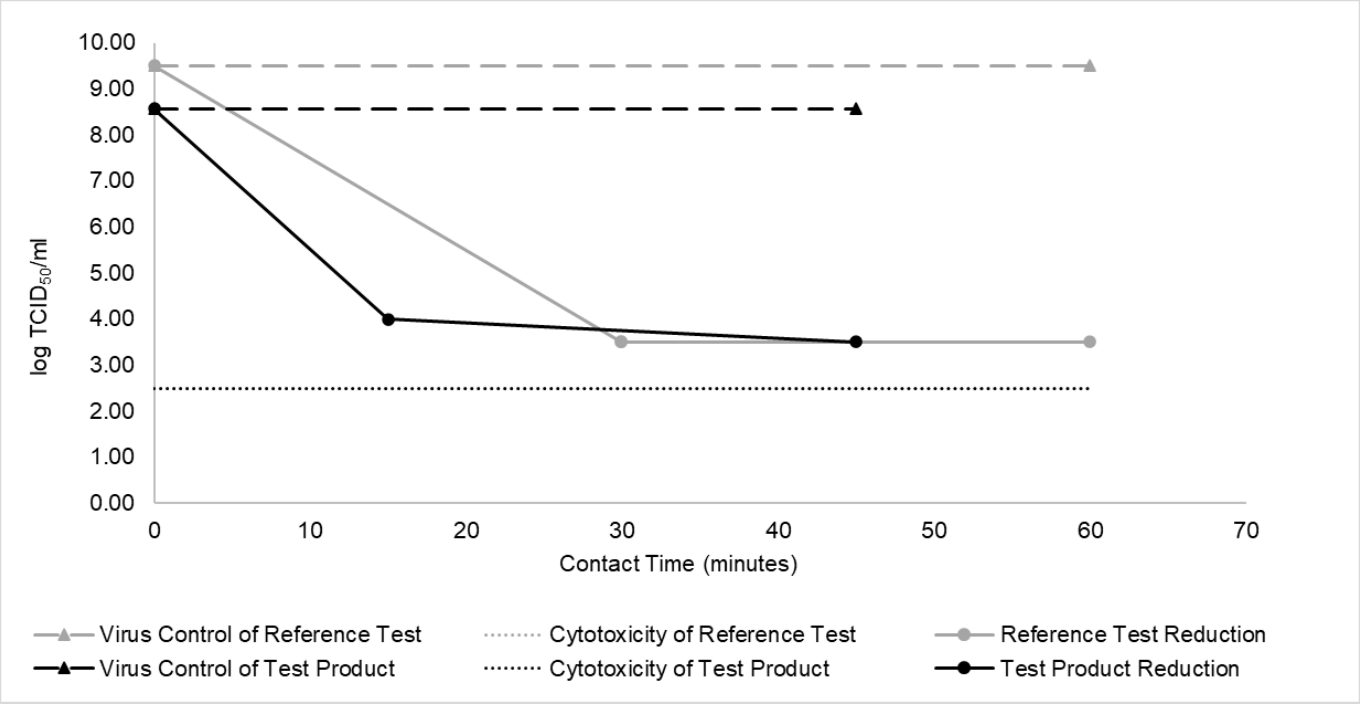


* The product can only be tested at 80.00 %concentration or less, as some dilution always occurs when test organisms and interfering substance are added.

Test strain: *Murine norovirus* FLI-RVB-0651

Virus control, V_C	Cytotoxicity effect, CE
V_{C1}: 8.50 ± 0.00 V_{C2}: 8.63 ± 0.25	CE_1: 3.50 ± 0.00 CE_2: 3.50 ± 0.00

Test concentration (%) / contact time (min)	First assay, N_{a1}	Second assay, N_{a2}	Average reduction
100.00* / 15	N_{a1}: 4.00 ± 0.38 lg R_1: **4.50 ± 0.38**	N_{a2}: 4.00 ± 0.38 lg R_2: **4.63 ± 0.45**	lg R: **4.57 ± 0.42**
100.00* / 45	N_{a1}: 3.50 ± 0.00 lg R_1: **5.00 ± 0.00**	N_{a2}: ≤3.50 ± 0.00 lg R_2: **≥5.13 ± 0.25**	lg R: **≥5.07 ± 0.18**

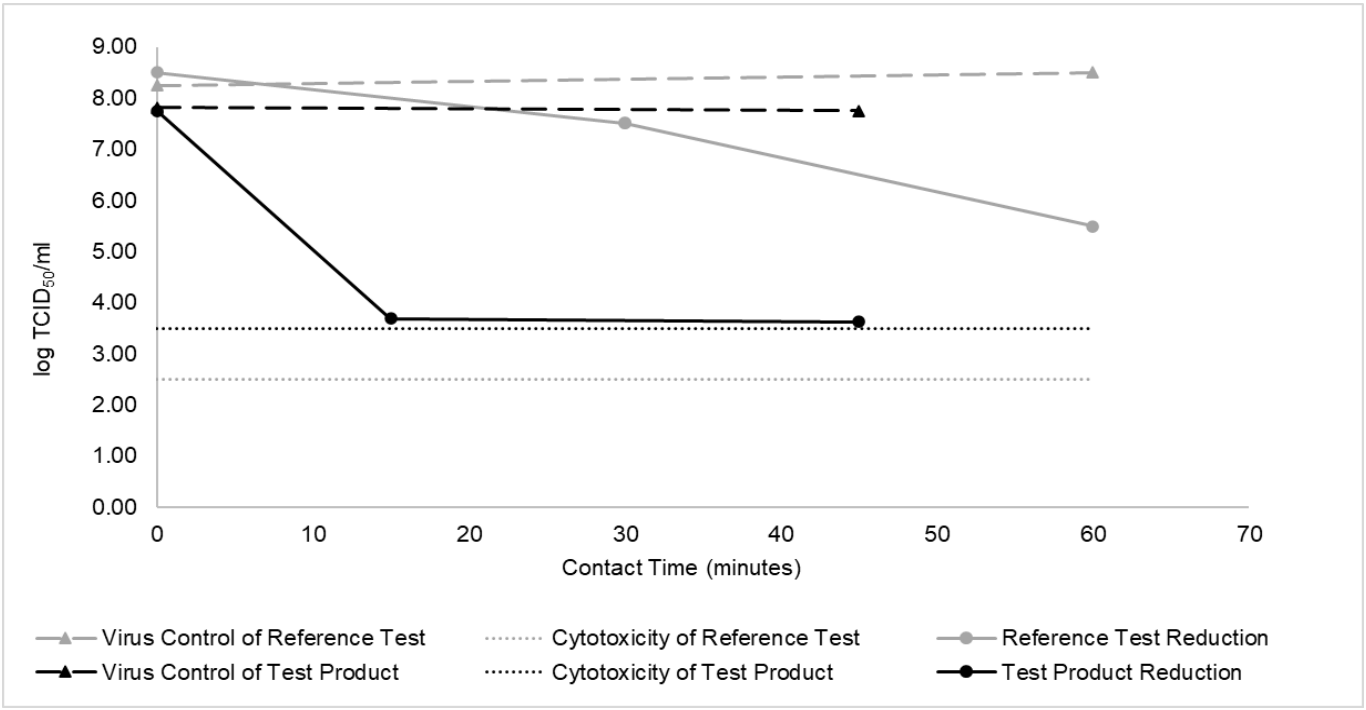


* The product can only be tested at 80.00 %concentration or less, as some dilution always occurs when test organisms and interfering substance are added.

Test strain: *Poliovirus type 1 NIBSC-01/528*

Virus control, V_C	Cytotoxicity effect, CE
V_{C1}: 7.75 ± 0.33 V_{C2}: 7.75 ± 0.33	CE_1: 3.50 ± 0.00 CE_2: 3.50 ± 0.00

Test concentration (%) / contact time (min)	First assay, N_{a1}	Second assay, N_{a2}	Average reduction
100.00* / 15	N_{a1}: 3.75 ± 0.33 lg R_1: **4.00 ± 0.47**	N_{a2}: 3.63 ± 0.25 lg R_2: **4.13 ± 0.41**	lg R: **4.07 ± 0.44**
100.00* / 45	N_{a1}: 3.75 ± 0.33 lg R_1: **4.00 ± 0.47**	N_{a2}: ≤3.50 ± 0.00 lg R_2: **≥4.25 ± 0.33**	lg R: **≥4.13 ± 0.41**



* The product can only be tested at 80.00 %concentration or less, as some dilution always occurs when test organisms and interfering substance are added.

Table B: Control tests and method validation for Table A

Test strain	Cell susceptibility control	Suppression efficiency control	Reference test for virus inactivation
Adenovirus type 5 ATCC VR-5	A: 5.25 ± 0.33 A_{PBS}: 5.75 ± 0.33	B: 5.63 ± 0.25 V_C: 5.88 ± 0.37	C_{30}: 3.00 ± 0.00 C_{60}: 3.50 ± 0.38
Murine norovirus FLI-RVB-0651	A: 9.25 ± 0.33 A_{PBS}: 8.50 ± 0.00	B: 8.25 ± 0.33 V_C: 8.63 ± 0.25	C_{30}: 6.00 ± 0.00 C_{60}: 6.00 ± 0.00
Poliovirus type 1 NIBSC-01/528	A: 8.00 ± 0.38 A_{PBS}: 7.50 ± 0.00	B: 7.75 ± 0.33 V_C: 7.75 ± 0.33	C_{30}: 1.00 ± 0.00 C_{60}: 3.00 ± 0.00

Note

$TCID_{50}$: The dilution of the virus suspension that induces a cytopathic effect (CPE) in 50 % of cell culture units

CPE: The morphological alteration of cells and/or their destruction caused by the cytopathic effect of virus multiplication.

V_C: log_{10} $TCID_{50}$ per ml in the viral test suspension at the beginning and at the maximum contact time

N_a: log_{10} $TCID_{50}$ per ml in the test mixture at the end of the contact time

CE: The morphological alteration of cells caused by the cytotoxicity effect of the product test solution.

A: log_{10} $TCID_{50}$ per ml in the cell susceptibility control as compared to PBS

B: log_{10} $TCID_{50}$ per ml in the suppression efficiency control as compared to the virus control

C: log_{10} $TCID_{50}$ per ml in the reference test for virus inactivation after 30 and 60 minutes (5 and 15 minutes for vaccinia virus)

Table C: Summary of the log reductions of the quantitative suspension test according to EN 14476

Test strain	Test concentration (%) / contact time (minute)	Log reduction ($TCID_{50}$/ml)	Associated risk†
Adenovirus type 5 ATCC VR-5	100.00* / 15	**≥4.19 ± 0.37**	< 50 % risk of false acceptance
	100.00* / 45	**≥4.25 ± 0.33**	< 50 % risk of false acceptance
Murine norovirus FLI-RVB-0651	100.00* / 15	**4.57 ± 0.42**	Minimal risk of false acceptance
	100.00* / 45	**≥5.07 ± 0.18**	Minimal risk of false acceptance
Poliovirus type 1 NIBSC-01/528	100.00* / 15	**4.07 ± 0.44**	< 50 % risk of false acceptance
	100.00* / 45	**≥4.13 ± 0.41**	< 50 % risk of false acceptance

* The product can only be tested at 80.00 % concentration or less, as some dilution always occurs when test organisms and interfering substance are added.

† The decision rule applied is simple acceptance rule with no guard band and up to 50 % risk of false acceptance or rejection. This rule has been determined by the laboratory and agreed with the client prior to testing.

Airestec Innovations Sdn Bhd
B-09-10 & 11, Gateway Corporate Suites, Gateway Kiaramas
1 Jalan Desa Kiara, Mont Kiara
50480 Kuala Lumpur
Malaysia

Efficacy of Airestec Bioactive Virucidal Surface Disinfectant (BVSD) against *Adenovirus type 5*, strain Adenoid 75, ATCC VR-5, *Murine norovirus,* strain S99 Berlin, FLI-RVB-0651 and *Poliovirus type 1*, strain LSc2ab, NIBSC-01/528 in a quantitative suspension test at 28 °C according to EN14476:2013+A2:2019 (E) under clean condition

EXPERT OPINION*

This expert opinion is based on the test report VX-TR-21-1103 dated 16 November 2021.

The virucidal activity of the disinfectant Airestec Bioactive Virucidal Surface Disinfectant (BVSD) of Airestec Innovations Sdn Bhd against *Adenovirus type 5* ATCC VR-5, *Murine norovirus* FLI-RVB-0651 and *Poliovirus type 1* NIBSC-01/528 was investigated by a quantitative suspension test according to EN14476:2013+A2:2019 (E) under clean condition (0.30 g/L bovine albumin solution).

According to this suspension test, a disinfectant or a disinfectant solution at a particular concentration is considered as having virucidal activity if the virus titre is reduced by ≥4 $\log_{10}$ (inactivation ≥99.99 %) within the recommended exposure period.

Airestec Bioactive Virucidal Surface Disinfectant (BVSD) was examined at 28 °C at the concentration of 100.00 %** for the exposure times of 15 and 45 minutes. After the exposure times, the viral reduction exceeded 4 $\log_{10}$-steps in all assays. According to the simple acceptance decision rule†, there is < 50 % risk of false acceptance for *Adenovirus type 5* ATCC VR-5 and *Poliovirus type 1* NIBSC-01/528, and a minimal risk of false acceptance for *Murine norovirus* FLI-RVB-0651. Therefore, **a virucidal activity against *Adenovirus type 5* ATCC VR-5, *Murine norovirus* FLI-RVB-0651 and *Poliovirus type 1* NIBSC-01/52**8 was measured as follows:

Clean condition	100.00 %**	15 minutes
Clean condition	100.00 %**	45 minutes

After evaluation with *Adenovirus type 5* ATCC VR-5, *Murine norovirus* FLI-RVB-0651 and *Poliovirus type 1* NIBSC-01/528 the disinfectant Airestec Bioactive Virucidal Surface Disinfectant (BVSD) can be declared as having "**virucidal activity**" according to EN 14476:2013+A2:2019.

Therefore, after successful experiments with the three above mentioned non-enveloped viruses the test product is also effective against the so-called blood-borne viruses including HBV, HCV and HIV as well as against members of other virus families such as orthomyxoviridae (incl. all human and animal influenza viruses like H5N1 and H1N1),**coronaviridae (like MERS-CoV, SARS-CoV-1 and SARS-CoV-2)** and filoviridae including Ebola virus (see list at the Appendix 5).

Kuala Lumpur, 16 November 2021

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Dr Syazani Suhaimi
Microbiologist

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Wei Keat Tan
Microbiologist

* Opinions and interpretations expressed here are outside the scope of SAMM (Laboratory Accreditation Scheme of Malaysia) accreditation.

** The product can only be tested at 80.00 % concentration or less, as some dilution always occurs when test organisms and interfering substance are added.

† The decision rule applied is simple acceptance rule with no guard band and up to 50 % risk of false acceptance or rejection. This rule has been determined by the laboratory and agreed with the client prior to testing.

Appendix 1

QAU CERTIFICATE*

The results stated in test report VX-TR-21-1103 dated 16 November 2021 were compared to the raw data of the tests and checked for correct transfer. No deviations were detected.

Kuala Lumpur, 16 November 2021

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Wei Keat Tan
Microbiologist

* Opinions and interpretations expressed here are outside the scope of SAMM (Laboratory Accreditation Scheme of Malaysia) accreditation.

Appendix 2 Raw data

Test Method	EN 14476:2013+A2:2019				**Titration Method**	Quantal test	
Product	Airestec Bioactive Virucidal Surface Disinfectant (BVSD)				**Batch No.**	N/A	
Product Diluent	Distilled water				**Lab No.**	VX-189-21-0001	
Test Organism	Adenovirus, strain Adenoid 75, ATCC VR-5				**Passage No.**	3	
Cell Line	Vero cells, ATCC CCL-81				**Passage No.**	23	
Interfering Substance	0.30 g/L bovine albumin solution				**Inactivation Method**	Immediate dilution	
Test Temperature (°C)	28	**Incubation Temperature (°C)**		36	**Dilution Method**	Standard	
First Assay Test Date	02/07/2021	**Second Assay Test Date**	06/07/2021	**Analyzed By**	WTA	**Verified By**	SSU

Validation and Control Procedures

	Product Concentration	Dilution	Dilution (log_{10}) 1	2	3	4	5	6	7	8	9	10	log_{10} $TCID_{50}$/ml	$\Delta TCID_{50}$ < 1 lg
Cell Susceptibility Control	PBS	Without	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 0 0 0 4 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	5.75 ± 0.33	Pass? **Yes**
	100.00 %	1:10000	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 0 4 4 3 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	5.25 ± 0.33	

	Product Concentration	Contact Time (minutes)	Dilution (log_{10}) 1	2	3	4	5	6	7	8	9	10	log_{10} $TCID_{50}$/ml	$\lvert TCID_{50} - V_C \rvert$ ≤ 0.5 lg
Suppression Efficiency Control	100.00 %	30	t t t t t t t t	t t t t t t t t	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	5.63 ± 0.25	Pass? **Yes**
	Virus Control (V_C)	30	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 3 0 0 4 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	5.88 ± 0.37	

	Product Concentration	Contact Time (minutes)	Dilution (log_{10}) 1	2	3	4	5	6	7	8	9	10	log_{10} $TCID_{50}$/ml	lg R = V_C - Na
Reference Test	0.70 % Formaldehyde	30	t t t t t t t t	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	n.d	n.d	4.50 ± 0.00	3.00 ± 0.00
		60	t t t t t t t t	4 4 4 4 4 4 4 4	4 4 0 0 4 4 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	n.d	n.d	4.00 ± 0.38	3.50 ± 0.38
	Virus Control (V_C)	0	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 3 3 2 2	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	7.50 ± 0.00	
		60	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 3 3	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	7.50 ± 0.00	
	Cytotoxicity Effect (CE)	-	t t t t t t t t	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	n.d	n.d	n.d	2.50 ± 0.00	

Appendix 2 Raw data

Test Procedure

	Product Concentration	Contact Time (minutes)	Dilution (log_{10}) 1	2	3	4	5	6	7	8	9	10	log_{10} $TCID_{50}$/ml	
First Assay (Na_1)	100.00 %	15	t t t t t t t t	t t t t t t t t	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	n.d	n.d	3.50 ± 0.00	V_{C1} - CE ≥ 4 Pass? **Yes**
	100.00 %	45	t t t t t t t t	t t t t t t t t	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	n.d	n.d	3.50 ± 0.00	
	Virus Control (V_{C1})	0	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	3 0 0 0 3 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	7.75 ± 0.33	
		45	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 3	3 0 0 0 3 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	7.75 ± 0.33	
	Cytotoxicity Effect (CE)	-	t t t t t t t t	t t t t t t t t	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d.	n.d.	n.d.	n.d	n.d	3.50 ± 0.00	

	Product Concentration	Contact Time (minutes)	Dilution (log_{10}) 1	2	3	4	5	6	7	8	9	10	log_{10} $TCID_{50}$/ml	
Second Assay (Na_2)	100.00 %	15	t t t t t t t t	t t t t t t t t	3 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	n.d	n.d	3.63 ± 0.25	V_{C2} - CE ≥ 4 Pass? **Yes**
	100.00 %	45	t t t t t t t t	t t t t t t t t	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	n.d	n.d	3.50 ± 0.00	
	Virus Control (V_{C2})	0	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	3 0 0 0 3 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	7.75 ± 0.33	
		45	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 3	3 0 0 0 3 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	7.75 ± 0.33	
	Cytotoxicity Effect (CE)	-	t t t t t t t t	t t t t t t t t	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d.	n.d.	n.d.	n.d	n.d	3.50 ± 0.00	

Average Reduction (lg R)	Product Concentration	Contact Time (minutes)	First Assay (Na_1) log_{10} $TCID_{50}$/ml	lg R_1 = V_{C1} - Na_1	Second Assay (Na_2) log_{10} $TCID_{50}$/ml	lg R_2 = V_{C2} - Na_2	Average Reduction (lg R)
	100.00 %	15	≤3.50 ± 0.00	≥4.25 ± 0.33	3.63 ± 0.25	4.13 ± 0.41	≥4.19 ± 0.37
	100.00 %	45	≤3.50 ± 0.00	≥4.25 ± 0.33	≤3.50 ± 0.00	≥4.25 ± 0.33	≥4.25 ± 0.33

Appendix 2 Raw data

Test Method	EN 14476:2013+A2:2019			**Titration Method**	Quantal test		
Product	Airestec Bioactive Virucidal Surface Disinfectant (BVSD)			**Batch No.**	N/A		
Product Diluent	Distilled water			**Lab No.**	VX-189-21-0001		
Test Organism	Murine norovirus, strain S99 Berlin, FLI-RVB-0651			**Passage No.**	3		
Cell Line	RAW 264.7 cells, ATCC TIB-71			**Passage No.**	19		
Interfering Substance	0.30 g/L bovine albumin solution			**Inactivation Method**	Immediate dilution		
Test Temperature (°C)	28	**Incubation Temperature (°C)**	36	**Dilution Method**	Standard		
First Assay Test Date	29/06/2021	**Second Assay Test Date**	02/07/2021	**Analyzed By**	WTA	**Verified By**	SSU

Validation and Control Procedures

	Product Concentration	Dilution	Dilution (log_{10}) 1	2	3	4	5	6	7	8	9	10	log_{10} $TCID_{50}$/ml	$\Delta TCID_{50}$ < 1 lg
Cell Susceptibility Control	PBS	Without	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	0 0 0 0 0 0 0 0	n.d	n.d	8.50 ± 0.00	Pass? **Yes**
	100.00 %	1:10000	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 3 3 0 3 3 3 0	n.d	n.d	9.25 ± 0.33	

	Product Concentration	Contact Time (minutes)	Dilution (log_{10}) 1	2	3	4	5	6	7	8	9	10	log_{10} $TCID_{50}$/ml	$\lvert TCID_{50} - V_C \rvert$ ≤ 0.5 lg
Suppression Efficiency Control	100.00 %	30	t t t t 4 4 4 4	t t t t t t t t	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 3 3 0 4 3 3 0	0 0 0 0 0 0 0 0	n.d	n.d	8.25 ± 0.33	Pass? **Yes**
	Virus Control (V_C)	30	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	3 0 0 0 0 0 0 0	n.d	n.d	8.63 ± 0.25	

	Product Concentration	Contact Time (minutes)	Dilution (log_{10}) 1	2	3	4	5	6	7	8	9	10	log_{10} $TCID_{50}$/ml	lg R = V_C - Na
Reference Test	0.70 % Formaldehyde	30	t t t t t t t t	4 4 4 4 4 4 4 4	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	n.d	n.d	3.50 ± 0.00	6.00 ± 0.00
		60	t t t t t t t t	4 4 4 4 4 4 4 4	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	n.d	n.d	3.50 ± 0.00	6.00 ± 0.00
	Virus Control (V_C)	0	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 3 3	0 0 0 0 0 0 0 0	n.d	9.50 ± 0.00	
		60	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 3	0 0 0 0 0 0 0 0	n.d	9.50 ± 0.00	
	Cytotoxicity Effect (CE)	-	t t t t t t t t	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	n.d	n.d	n.d	2.50 ± 0.00	

Appendix 2 Raw data

Test Procedure

	Product Concentration	Contact Time (minutes)	Dilution (log_{10}) 1	2	3	4	5	6	7	8	9	10	log_{10} $TCID_{50}$/ml	
First Assay (Na_1)	100.00 %	15	t t t t t t t t	t t t t t t t t	3 2 0 0 2 2 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	n.d	n.d	4.00 ± 0.38	V_{C1} - CE ≥ 4 Pass? **Yes**
	100.00 %	45	t t t t t t t t	t t t t t t t t	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	n.d	n.d	3.50 ± 0.00	
	Virus Control (V_{C1})	0	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	0 0 0 0 0 0 0 0	n.d	n.d	8.50 ± 0.00	
		45	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	0 0 0 0 0 0 0 0	n.d	n.d	8.50 ± 0.00	
	Cytotoxicity Effect (CE)	-	t t t t t t t t	t t t t t t t t	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	n.d	n.d	n.d	3.50 ± 0.00	

	Product Concentration	Contact Time (minutes)	Dilution (log_{10}) 1	2	3	4	5	6	7	8	9	10	log_{10} $TCID_{50}$/ml	
Second Assay (Na_2)	100.00 %	15	t t t t t t t t	t t t t t t t t	3 3 0 0 3 3 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	n.d	n.d	4.00 ± 0.38	V_{C2} - CE ≥ 4 Pass? **Yes**
	100.00 %	45	t t t t t t t t	t t t t t t t t	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	n.d	n.d	3.50 ± 0.00	
	Virus Control (V_{C2})	0	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 0 0 0 0 0 0 0	n.d	n.d	8.63 ± 0.25	
		45	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 3	3 0 0 0 0 0 0 0	n.d	n.d	8.63 ± 0.25	
	Cytotoxicity Effect (CE)	-	t t t t t t t t	t t t t t t t t	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	n.d	n.d	n.d	3.50 ± 0.00	

Average Reduction (lg R)	Product Concentration	Contact Time (minutes)	First Assay (Na_1) log_{10} $TCID_{50}$/ml	First Assay (Na_1) lg $R_1 = V_{C1} - Na_1$	Second Assay (Na_2) log_{10} $TCID_{50}$/ml	Second Assay (Na_2) lg $R_2 = V_{C2} - Na_2$	Average Reduction (lg R)
	100.00 %	15	4.00 ± 0.38	4.50 ± 0.38	4.00 ± 0.38	4.63 ± 0.45	4.57 ± 0.42
	100.00 %	45	≤3.50 ± 0.00	≥5.00 ± 0.00	≤3.50 ± 0.00	≥5.13 ± 0.25	≥5.07 ± 0.18

Appendix 2 Raw data

Test Method	EN 14476:2013+A2:2019			**Titration Method**	Quantal test		
Product	Airestec Bioactive Virucidal Surface Disinfectant (BVSD)			**Batch No.**	N/A		
Product Diluent	Distilled water			**Lab No.**	VX-189-21-0001		
Test Organism	Poliovirus, strain LSc 2ab			**Passage No.**	4		
Cell Line	Vero cells, ATCC CCL-81			**Passage No.**	23		
Interfering Substance	0.30 g/L bovine albumin solution			**Inactivation Method**		Immediate dilution	
Test Temperature (°C)	28	**Incubation Temperature (°C)**	36	**Dilution Method**		Standard	
First Assay Test Date	25/06/2021	**Second Assay Test Date**	02/07/2021	**Analyzed By**	WTA	**Verified By**	SSU

Validation and Control Procedures

	Product Concentration	Dilution	Dilution (log_{10}) 1	2	3	4	5	6	7	8	9	10	log_{10} $TCID_{50}$/ml	$\Delta TCID_{50}$ < 1 lg
Cell Susceptibility Control	PBS	Without	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 3	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	7.50 ± 0.00	Pass? Yes
	100.00 %	1:10000	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 0 0 4 4 0 0	0 0 0 0 0 0 0 0	n.d	n.d	8.00 ± 0.38	

	Product Concentration	Contact Time (minutes)	Dilution (log_{10}) 1	2	3	4	5	6	7	8	9	10	log_{10} $TCID_{50}$/ml	$\lvert TCID_{50} - V_C \rvert$ ≤ 0.5 lg
Suppression Efficiency Control	100.00 %	30	t t t t t t t t	t t t t t t t t	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 0 0 0 3 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	7.75 ± 0.33	Pass? Yes
	Virus Control (V_C)	30	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	3 0 0 0 3 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	7.75 ± 0.33	

	Product Concentration	Contact Time (minutes)	Dilution (log_{10}) 1	2	3	4	5	6	7	8	9	10	log_{10} $TCID_{50}$/ml	lg R = V_C - Na
Reference Test	0.70 % Formaldehyde	30	t t t t t t t t	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	0 0 0 0 0 0 0 0	n.d	n.d	n.d	7.50 ± 0.00	1.00 ± 0.00
		60	t t t t t t t t	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	n.d	5.50 ± 0.00	3.00 ± 0.00
	Virus Control (V_C)	0	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 0 4 4 3 0	0 0 0 0 0 0 0 0	n.d	n.d	8.25 ± 0.33	
		60	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 3	0 0 0 0 0 0 0 0	n.d	n.d	8.50 ± 0.00	
	Cytotoxicity Effect (CE)	-	t t t t t t t t	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	n.d	n.d	n.d	2.50 ± 0.00	

Appendix 2 Raw data

Test Procedure

	Product Concentration	Contact Time (minutes)	Dilution (log_{10}) 1	2	3	4	5	6	7	8	9	10	log_{10} $TCID_{50}$/ml	
First Assay (Na_1)	100.00 %	15	t t t t t t t t	t t t t t t t t	3 0 0 0 3 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	n.d	n.d	3.75 ± 0.33	V_{C1} - CE ≥ 4 Pass? **Yes**
	100.00 %	45	t t t t t t t t	t t t t t t t t	3 0 0 0 2 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	n.d	n.d	3.75 ± 0.33	
	Virus Control (V_{C1})	0	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	3 3 0 0 3 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	7.88 ± 0.37	
		45	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	3 0 0 0 3 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	7.75 ± 0.33	
	Cytotoxicity Effect (CE)	-	t t t t t t t t	t t t t t t t t	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	n.d	n.d	n.d	3.50 ± 0.00	

	Product Concentration	Contact Time (minutes)	Dilution (log_{10}) 1	2	3	4	5	6	7	8	9	10	log_{10} $TCID_{50}$/ml	
Second Assay (Na_2)	100.00 %	15	t t t t t t t t	t t t t t t t t	4 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	n.d	n.d	3.63 ± 0.25	
	100.00 %	45	t t t t t t t t	t t t t t t t t	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	n.d	n.d	3.50 ± 0.00	V_{C2} - CE ≥ 4 Pass? **Yes**
	Virus Control (V_{C2})	0	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	3 0 0 0 3 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	7.75 ± 0.33	
		45	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	3 0 0 0 3 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	7.75 ± 0.33	
	Cytotoxicity Effect (CE)	-	t t t t t t t t	t t t t t t t t	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	n.d	n.d	n.d	n.d	n.d	3.50 ± 0.00	

	Product Concentration	Contact Time (minutes)	First Assay (Na_1) log_{10} $TCID_{50}$/ml	First Assay (Na_1) lg R_1 = V_{C1} - Na_1	Second Assay (Na_2) log_{10} $TCID_{50}$/ml	Second Assay (Na_2) lg R_2 = V_{C2} - Na_2	Average Reduction (lg R)
Average Reduction (lg R)	100.00 %	15	3.75 ± 0.33	4.00 ± 0.47	3.63 ± 0.25	4.13 ± 0.41	4.07 ± 0.44
	100.00 %	45	3.75 ± 0.33	4.00 ± 0.47	≤3.50 ± 0.00	≥4.25 ± 0.33	≥4.13 ± 0.41

Note

$TCID_{50}$: The dilution of the virus suspension that induces a CPE in 50 % of cell culture units

CPE: The morphological alteration of cells and/or their destruction caused by the cytopathic effect of virus multiplication. '0' denotes no CPE and '1' (approximately 25 % of cells) to '4' (all cells) denotes the degree of CPE per cell culture units.

V_C: log_{10} $TCID_{50}$ per ml in the viral test suspension at the beginning and at the maximum contact time

N_a: log_{10} $TCID_{50}$ per ml in the test mixture at the end of the contact time

CE: The morphological alteration of cells caused by the cytotoxicity effect of the product test solution. 't' denotes the presence of cytotoxicity per cell culture units.

A: log_{10} $TCID_{50}$ per ml in the cell susceptibility control as compared to PBS

B: log_{10} $TCID_{50}$ per ml in the suppression efficiency control as compared to the virus control

C: log_{10} $TCID_{50}$ per ml in the reference test for virus inactivation after 30 and 60 minutes (5 and 15 minutes for vaccinia virus)

Appendix 3 Summary of test description

1. Virus and cells

1.1. *Adenovirus type 5*, strain Adenoid 75, ATCC VR-5
- 1.1.1. Passage no.: 3
- 1.1.2. Cell line: Vero cells, ATCC CCL-81
- 1.1.3. Cell line passage no.: 23
- 1.1.4. Culture medium: EMEM

1.2. *Murine norovirus,* strain S99 Berlin, FLI-RBI-0651
- 1.2.1. Passage no.: 3
- 1.2.2. Cell line: Raw 264.7 cells, ATCC TIB-71
- 1.2.3. Cell line passage no.: 19
- 1.2.4. Culture medium: DMEM

1.3. *Poliovirus type 1*, strain LSc2ab, NIBSC-01/528
- 1.3.1. Passage no.: 4
- 1.3.2. Cell line: Vero cells, ATCC CCL -81
- 1.3.3. Cell line passage no.: 23
- 1.3.4. Culture medium: EMEM

2 Materials and reagents

2.1 Eagle's Minimal Essential Medium (EMEM, Sigma, catalogue no. M3024)
2.2 Dulbecco's Modified Eagle Medium (DMEM, Sigma, catalogue no. D7777)
2.3 Fetal Bovine Serum (FBS, Sigma, catalogue no. F7524)
2.4 Formaldehyde (Merck, catalogue no. 1.0.4003.2500)
2.5 Dulbecco's Phosphate Buffered Saline (PBS, Sigma, catalogue no. P3813)
2.6 Bovine albumin fraction V (Merck, catalogue no. K49238418733)

3 Apparatus and glassware

3.1 CO_2 incubator (Memmert, model ICO 105)
3.2 Cooling water bath (Memmert, model WNB7 with CDP115)
3.3 Inverted microscope (Optika, IM-2)
3.4 Vortex® mixer (Biosan model Biosan V-1 Plus)
3.5 Microtitre plate (NEST)
3.6 Tissue culture flask (JET Biofil)

4 Test procedure

4.1 Preparation of test virus suspension

4.1.1 Cell monolayers shall be >90 % confluent before inoculation. Cell lines are selected in accordance with their sensitivity to the test organisms.

4.1.2 The test organisms and their stock cultures shall be prepared and kept in accordance with EN 12353:2013 (E).

4.1.3 The stock virus suspension is multiplied in an appropriate cell line that produces high titres of infectious viruses for 1 hour at 36 °C with intermittent tilting every 15 minutes.

4.1.4 The cells are subjected to 3 freeze/thaw cycles once cytopathic effect (CPE) is observed in 80 % of the cell population.

4.1.5 Separate the cells debris is by centrifugation at 400 g_N for 15 minutes.

4.1.6 Aliquot the supernatant containing the test virus suspension and store at -80 °C.

4.2 Test Na – Determination of virucidal concentrations

4.2.1 Pipette 1 ml of interfering substance into a container of suitable capacity for appropriate mixing.

4.2.2 Add 1 ml of the virus test suspension to the container, carefully avoiding the upper part of the sides. Mix well.

4.2.3 Add 8 ml of the product test solution to the container.

4.2.4 Mix, start a stopwatch at once, and place the container in a water bath controlled at the chosen test temperature.

4.2.5 Immediately at the end of the chosen contact time, mix, pipette 0.5 ml of the test mixture (virus suspension, interfering substance, and product test solution) into 4.5 ml ice-cold maintenance medium and put into an ice bath.

4.2.6 Within 30 minutes, prepare a series of ten-fold dilutions of this mixture (text mixture and maintenance medium).

4.2.7 Transfer 0.1 ml of each dilution into six or eight wells of a microtitre plate containing a confluent (>90 %) cell monolayer without any medium.

4.2.8 The last row of six or eight wells will receive 0.1 ml of culture medium and will serve as the cell control.

4.2.9 After 1 hour of incubation at 37 °C, 0.1 ml of cell culture medium is added to each well.

4.2.10 After incubation, the virus titre is calculated. The reduction of virus infectivity is determined from differences of $\log_{10}$ virus titres before and after treatment with the product.

4.3 Cytotoxicity effect – determination of the morphological alteration of cells caused by the product test solution

4.3.1 Mix 1 part of hard water and 1 part of interfering substances with 8 parts of the product test solution.

4.3.2 Serial dilutions are prepared in the culture medium and are inoculated into cell monolayers.

4.3.3 This test is done in parallel with Section 4.2.

4.3.4 Any microscopic changes in the cells are recorded when reading the tests for CPE.

4.3.5 If the cytotoxicity is so great that the residual infectivity titre is smaller than the required $\log_{10}$ $TCID_{50}$, special techniques have to be used, such as molecular sieving or ultrafiltration. Follow the instructions of the manufacturer.

4.4 Cell susceptibility control A – Verification of the susceptibility of the cells for virus infection is not influenced negatively by the treatment with the product test solution

4.4.1 Comparative virus titrations are performed on cells that have or have not been treated with product test solution to check the reduction of the sensitivity to viruses.

4.4.2 0.1 ml of the lowest apparently non-cytotoxic dilution (no microscopic alteration) of the product test solution or PBS and 0.1 ml of culture medium are distributed onto each of 6 established cell cultures in 96-well microtitre plates.

4.4.3 After 1 hour of incubation at 37 °C, the supernatant is discarded.

4.4.4 The virus is diluted from 10^{-1} to 10^{-10} and titrated on the treated or untreated cells.

4.4.5 Verify according to Section 4.8.

4.5 Suppression efficiency control B – Immediate dilution method validation

4.5.1 Immediately after preparation of the test mixture in Section 4.2, pipette 0.5 ml of the test mixture (virus suspension, interfering substance, and product test solution) into 4.5 ml of ice-cold maintenance medium.

4.5.2 Mix again and start the clock. Incubate the mixture in the ice bath for 30 minutes ± 10 seconds.

4.5.3 Immediately prepare dilutions up to 10^{-8} and titrate the virus.

4.5.4 This control is performed in parallel to the test.

4.5.5 Verify according to Section 4.8.

4.6 Reference test for virus inactivation C – Validation of the test system

4.6.1 2 ml of the test suspension shall be mixed with 8 ml of PBS and 10 ml of 1.4 % (w/v) formaldehyde.

4.6.2 Contact times are 30 and 60 minutes.

4.6.3 Immediately at the end of the contact time, mix and pipette 0.2 ml of the test mixture into a tube containing 1.8 ml ice-cold maintenance medium followed by a further 10-fold dilution.

4.6.4 Leave the mixture in the ice bath.

4.6.5 Dilutions up to 10^{-6} are prepared by pipetting the diluted test mixture into another tube containing ice-cold maintenance medium in the ice bath.

4.6.6 In exceptional cases, smaller volumes of the reagents and of the test suspension could be used, ensuring that the relative proportions are maintained.

4.6.7 The cytotoxic control of the formaldehyde shall be performed according to Section 4.3 whereby 8 ml of 1.4 % (w/v) formaldehyde is used instead of the product.

4.6.8 The mixture is further diluted to 10^{-5} in an ice bath.

4.6.9 Verify according to Section 4.8.

4.7 Titration of the virus control

4.7.1 The infectivity of the test suspension shall be determined under test conditions at the beginning of the contact time and at the maximum contact time used in the test.

4.7.2 Section 4.2 is repeated by substituting the product test solution with hard water or water for ready-to-use products.

4.7.3 Verify according to Section 4.8.

4.8 Verification of methodology

4.8.1 The titre of the test suspension (virus control) of at least 10^8 $TCID_{50}$/mL is sufficiently high to at least enable a titre reduction of 4 log to verify the method. The detectable titre reduction shall be at least 4 log.

4.8.2 Cytotoxicity of the product test solution does not affect cell morphology and growth or susceptibility for the test organism in the dilutions of the test mixtures which are necessary to demonstrate a 4-log reduction of the virus.

4.8.3 Comparative virus titration on cells cultures treated with test mixture dilutions and in parallel with PBS (cell susceptibility control) result in a difference of <1 log of virus titre.

4.8.4 The difference to the test suspension in the control of efficiency for suppression of products' activity shall be ≤0.5 log.

4.8.5 The difference between the logarithmic titre of the virus control and the logarithmic titre of the test organism in the reference inactivation test is:

4.8.5.1 Between -0.5 and -2.5 after 30 minutes and between -2 and -4.5 after 60 minutes for poliovirus

4.8.5.2 Between -3 and -5 after 30 minutes and between -3.5 and -5.5 after 60 minutes for adenovirus

4.8.5.3 Between 0.0 and -2.0 after 30 minutes and between -0.5 and -2.5 after 60 minutes for parvovirus

4.8.5.4 Between -0.75 and -3.5 after 5 minutes and between -2.0 and ≥-4.0 after 15 minutes for vaccinia virus.

5 Literature

5.1 EN 14476:2013+A2:2019 (E): Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity in the medical area – Test method and requirements (phase 2, step 1)

5.2 EN 14885:2015 (E): Chemical disinfectants and antiseptics – Application of European Standards for chemical disinfectants and antiseptics

5.3 EN 12353:2013 (E): Chemical disinfectants and antiseptics – Preservation of test organisms used for the determination of bactericidal (including Legionella), mycobactericidal, sporicidal, fungicidal and virucidal (including bacteriophages) activity

Appendix 4 Introduction to Airestec Group

Airestec Group started its journey in Australia in 1990 and set its foot in Kuala Lumpur, Malaysia through the establishment of Airestec Sdn Bhd in 2002 and Airestec Innovations Sdn Bhd in 2011. We are the market leader in **Deep Cleaning / Decontamination & Treatment**, providing exceptional products, solutions and services across diverse industries worldwide to remove biofilms, bacteria, fungi and mould. Our solutions are safe, non-toxic, non-hazardous and readily biodegradable, complementing water table and the environment.

With more than 20 years' experiences in this field, we have been awarded with numerous certifications such as **BioNexus Status, SIRIM Eco-Label, GreenTech MyHijau Mark, HALAL** and proudly **Malaysian Made Products (Buatan Malaysia).** We have also collaborated with local and international universities as well as government agencies such as GreenRE, Malaysian Bioeconomy Development Corporation (MBDC) and Malaysia Productivity Corporation (MPC).

As a Life & Environmental Sciences company, Airestec Group is always committed to protect the environment by minimizing environmental impacts concerning our activities, products and services. Our aim is to provide high quality products without causing detrimental effect to human health, asset, equipment and the environment. We are an **ISO 14001:2015 Environmental Management System** certified company.

As the world has been hit by Coronavirus SARS-CoV-2 / COVID-19 pandemic, people are becoming more concern and aware of a hygienic environment, especially with the emergence of highly contagious Delta variant. People are expecting to have a safe and germ-free environment, not only in their home, but also in healthcare facilities, air-conditioning systems, as well as public & commercial buildings such as restaurants, schools, gyms, shopping centers, hotels, offices, etc. This is because germs including viruses can persist on surfaces for hours or even days and when people touch these contaminated surfaces, they are at risk of getting infected.

As our core business is related to decontamination & treatment, we feel the need to address people's concern on hygienic environment. In order to curb the transmission of this disease, it is crucial to use a verified and credible product.

At Airestec, through meticulous R&D, we have developed a virucidal surface disinfectant, namely **Airestec Bioactive Virucidal Surface Disinfectant (BVSD),** applicable on all types of **Frequently Touched Surfaces (Furniture, Tabletop, Work Stations, Walls, Public Transportation, Chairs, etc.) HVAC/Air-Conditioning, Ducting and Refrigeration Systems,** across all industrial, commercial and residential sectors. BVSD is formulated with **eco-friendly, non-hazardous and scientifically proven** active ingredients, with guidelines provided by Centers for Disease Control and Prevention (CDC), World Health Organization

(WHO) and Environmental Protection Agency (EPA). This product is applied by our trained and professional engineers and technicians according to a strict SOP, via misting for total area disinfection. It can also be applied as a spray for surface disinfection.

The virucidal activity of the product has been tested according to **EN14476** European Standard. Based on the results obtained, the product was found to be **fully virucidal** and it is effective against both enveloped and non-enveloped virus, including the Coronavirus, SARS-CoV-2. With this validation, Airestec is now able to offer disinfection solution for all types of industries and buildings / facilities, covering all area including **Frequently Touched Common Surfaces (Furniture, Tabletop, Work Stations, Walls, Public Transportation, Chairs, etc.) HVAC/Air-Conditioning, Ducting and Refrigeration Systems, across all sectors.**

Airestec Group has tremendous experience in designing, manufacturing and providing Decontamination & Treatment products and services since 1990. Our claims have remained the same since the beginning (except that we have gradually and consistently improved our products significantly over time), keeping in mind that we are an “Environmental and Life Sciences Company”. Airestec Group has been developing and manufacturing a wide range of Decontamination products that can cater to different kinds of buildings to personal goods for years.

The product is effective of eradicating 99.99% of all types of viruses in 10 – 15 minutes contact time, including:

- **Coronavirus (including SARS-CoV-2 that causes COVID-19 infection and its variants)**
- **Influenza**
- **Rhinovirus**
- **Enterovirus (viruses that cause Hand, Foot and Mouth / HFMD infection)**
- **Measles virus**
- **Rotavirus**
- **Feline calicivirus**
- **Parvovirus**

*****Clarification on EN14476 Standard*****

EN14476 Standard: Quantitative suspension test for the evaluation of virucidal activity of disinfectants is an internationally recognized Standard. Non-enveloped viruses are more resistant to disinfectant compared to enveloped virus (e.g: Influenza, Coronavirus, etc.). Poliovirus is the most resistant among non-enveloped virus. For a disinfectant to be fully virucidal and recognized as capable of eradicating all enveloped and non-enveloped viruses, it must be effective against Adenovirus, Norovirus and Poliovirus.

EN14476 Standard covers all forms of viruses. If the test results show the capability of the product to eradicate the test viruses, it can be officially stated that the product is active and successful in eradicating all viruses, including Coronaviruses. This is because each strain of viruses tested in the EN14476 test represents a whole family of viruses. All known viruses belong to one of these virus families, so it can be stated that other viruses in this family are eradicated in the same way as the tested viruses. With that being said, the product is expected to be effective against current and future emerging pandemic caused by viruses.

Appendix 5 EN 14476:2013+A2:2019 Annex A

List of viruses from different parts of human body, which may contaminate hands, surgical instruments, surfaces and textiles.

Note: Enveloped viruses are in bold. This list is not exhaustive.

Blood

Enterovirus	**Hepatitis C virus (HCV)**
Filoviridae	**Hepatitis Delta virus (HDV)**
Flavivirus	**Human Immunodeficiency Virus (HIV)**
Herpesviridae	**Human T Cell Leukemia Virus (HTLV)**
Hepatitis A Virus (HAV)	Parvovirus B 19
Hepatitis B virus (HBV)	

Respiratory tract

Adenovirus (Mast-)	**Influenza Virus**
Coronavirus	**Paramyxoviridae**
Enterovirus	Rhinovirus
Herpesviridae	**Rubella Virus**

Neuronal tissue, ear & nose, eye

Adenovirus (Mast-)	**Human Immunodeficiency Virus (HIV)**
Enterovirus	Polyomavirus
Herpesviridae	**Rabies Virus**
Measles Virus	**Rubella Virus**

Gastro-intestinal

Adenovirus(Mast-)	Enterovirus
Caliciviridae	Hepatitis A Virus (HAV)
Coronavirus	Hepatitis E Virus (HEV)
Astrovirus	Rotavirus

Skin, breast and/or milk

Enterovirus

Herpesviridae

Human Immunodeficiency Virus (HIV)

Human T Cell Leukemia Virus (HTLV)

Papillomavirus

Poxviridae

Spleen and lymph nodes (see also “Blood“)

Human T Cell Leukemia Virus (HTLV)

Human Immunodeficiency Virus (HIV)

Dental procedure

Adenovirus(Mast-)

Enterovirus

Herpesviridae

Hepatitis B virus (HBV)

Hepatitis C Virus (HCV)

Hepatitis Delta Virus (HDV)

Human Immunodeficiency Virus (HIV)

Urogenital tract

Hepatitis B Virus (HBV)

Herpesviridae

Human Immunodeficiency Virus (HIV)

Human T Cell Leukemia Virus (HTLV)

Papillomavirus

Polyomavirus

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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