Test Report No.: VX-TR-20-0599 Copy No.: 1

DETERMINATION OF THE VIRUCIDAL ACTIVITY (EN 14476) OF ITASH

Lab No.:

Sample Name:

ITASH

Method:

EN 14476:2013+A1:2015 (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:

Sample Receipt Date: 12 August 2020

Report Date:

21 October 2020

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, 21 October 2020

Microbiologist

Lab No.:

Test Period: 9 Oct – 20 Oct 2020 Test Report No.: VX-TR-20-0599 Report Date: 21 October 2020

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Materials and Method

Quantitative suspension test for the evaluation of virucidal activity in the medical area according to EN 14476:2013+A1:2015 (E)

1. Testing laboratory identification

-	THE RESIDENCE STATE		100 000000000		
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2.	Samp	IC IU	CHL	III.ai	IUII

2.1 Sample name:

ITASH

2.2 Batch no.:

iClean IC-S

2.3 Product appearance:

Clear, colourless solution

2.4 Manufacturer:

2.5 Active substances:

Sodium Hypochlorite (NAOCI)

2.6 Sample receipt date:

12 August 2020

2.7 Storage conditions:

Room temperature

2.8 Product diluent:

Distilled water

3. Experimental conditions

3.1 Testing period:

9 October - 20 October 2020

3.2 Test organism(s):

Adenovirus type 5, strain Adenoid 75, ATCC VR-5 Murine norovirus, strain S99 Berlin, FLI-RVB-0651 Human coronavirus, strain 229E, ATCC VR-740 Poliovirus type 1, strain LSc-2ab, NIBSC-01/528 Vaccinia virus, strain Ankara, ATCC VR-1508

3.3 Concentration/contact time:

100.00 %* / 2, 5 and 30 minutes

3.4 Loading:

0.30 g/L bovine albumin solution

3.5 Test temperature:

20 °C ± 1 °C

3.6 Incubation period:

7 days, 36 °C ± 1 °C

Test procedure accredited according to MS ISO/IEC 17025. The test report shall not be reproduced except in full without the written approval of the laboratory. The test result relates only to the sample stated in the test report. The above analysis is based solely on the sample submitted by the customer. Information on measurement uncertainty is available upon request.

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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 (for formaldehyde only)

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

ITASH showed the required virus reduction of ≥4.0 log₁₀ against test strains *Adenovirus type 5* ATCC VR-5, *Murine norovirus* FLI-RVB-0651, *Human coronavirus* ATCC VR-740, *Poliovirus type 1* NIBSC-01/528 and *Vaccinia virus*, strain Ankara, ATCC VR-1508 in accordance with EN 14476:2013+A1:2015 (E) at 100.00 %* concentration after 2, 5 and 30 minutes under the stated condition. According to the simple acceptance decision rule[†], there is a minimal risk of false acceptance.

Dr

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 (10⁴).

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

- * The product can only be tested at 97.00 % 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.

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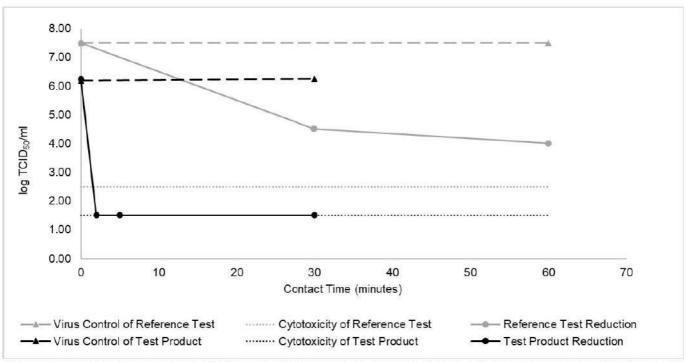
Table A: Evaluation of the virucidal activity of ITASH on test strains according to EN 14476

Product: ITASH 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} : 6.00 ± 0.38	CE ₁ : 1.50 ± 0.00
V_{C2} : 6.50 ± 0.00	CE ₂ : 1.50 ± 0.00

Test concentration (%) / contact time (min)	First assay, N _{a1}	Second assay, N _{a2}	Average reduction
100.00*/2	N _{a1} : ≤1.50 ± 0.00 lg R ₁ : ≥ 4.50 ± 0.38	N _{a2} : ≤1.50 ± 0.00 lg R ₂ : ≥ 5.00 ± 0.00	lg R: ≥4.75 ± 0.27
100.00*/5	N _{a1} : ≤1.50 ± 0.00 lg R ₁ : ≥ 4.50 ± 0.38	N _{a2} : ≤1.50 ± 0.00 Ig R ₂ : ≥ 5.00 ± 0.00	lg R: ≥4.75 ± 0.27
100.00* / 30	N _{a1} : ≤1.50 ± 0.00 lg R ₁ : ≥4.50 ± 0.38	N _{a2} : ≤1.50 ± 0.00 Ig R ₂ : ≥5.00 ± 0.00	lg R: ≥4.75 ± 0.27



^{*} The product can only be tested at 97.00 % concentration or less, as some dilution always occurs when test organisms and interfering substance are added.

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Sample Name: ITASH Batch No.: iClean IC-S

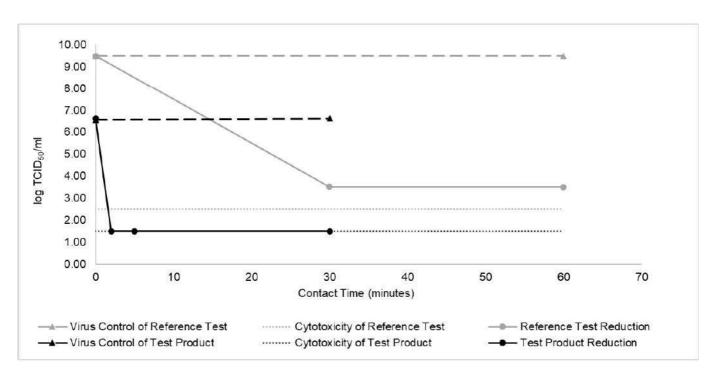
Sample Receipt Date: 12 August 2020

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Test strain: Murine norovirus FLI-RVB-0651

Virus control, V _C	Cytotoxicity effect, CE
V _{C1} : 6.63 ± 0.25	CE ₁ : 1.50 ± 0.00
V _{C2} : 6.63 ± 0.25	CE ₂ : 1.50 ± 0.00

Test concentration (%) / contact time (min)	First assay, N _{a1}	Second assay, N _{a2}	Average reduction
100.00*/2	N _{a1} : ≤1.50 ± 0.00 lg R ₁ : ≥ 5.13 ± 0.25	N_{a2} : $\leq 1.50 \pm 0.00$ lg R_2 : $\geq 5.13 \pm 0.25$	lg R: ≥5.13 ± 0.25
100.00* / 5	N _{a1} : ≤1.50 ± 0.00 lg R ₁ : ≥ 5.13 ± 0.25	N _{a2} : ≤1.50 ± 0.00 Ig R ₂ : ≥ 5.13 ± 0.25	lg R: ≥ 5.13 ± 0.25
100.00* / 30	N _{a1} : ≤1.50 ± 0.00 lg R ₁ : ≥ 5.13 ± 0.25	N _{a2} : ≤1.50 ± 0.00 Ig R ₂ : ≥ 5.13 ± 0.25	lg R: ≥5.13 ± 0.25



^{*} The product can only be tested at 97.00 % concentration or less, as some dilution always occurs when test organisms and interfering substance are added.

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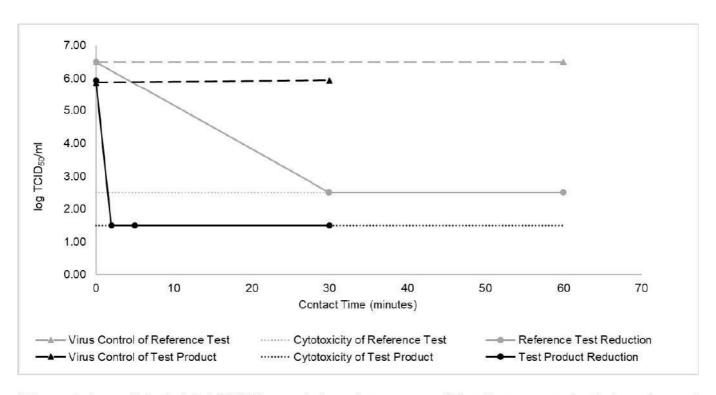
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Test strain: Human coronavirus ATCC VR-740

Virus control, V _C	Cytotoxicity effect, CE
V _{C1} : 5.88 ± 0.37	CE ₁ : 1.50 ± 0.00
V _{C2} : 6.00 ± 0.38	CE ₂ : 1.50 ± 0.00

Test concentration (%) / contact time (min)	First assay, N _{a1}	Second assay, N _{a2}	Average reduction
100.00*/2	N _{a1} : ≤1.50 ± 0.00 lg R ₁ : ≥ 4.38 ± 0.37	N _{a2} : ≤1.50 ± 0.00 Ig R ₂ : ≥4.50 ± 0.38	lg R: ≥4.44 ± 0.38
100.00* / 5	N _{a1} : ≤1.50 ± 0.00 lg R ₁ : ≥4.38 ± 0.37	N _{a2} : ≤1.50 ± 0.00 Ig R ₂ : ≥4.50 ± 0.38	lg R: ≥4.44 ± 0.38
100.00* / 30	N _{a1} : ≤1.50 ± 0.00 lg R ₁ : ≥4.38 ± 0.37	N _{a2} : ≤1.50 ± 0.00 Ig R ₂ : ≥4.50 ± 0.38	lg R: ≥4.44 ± 0.38



^{*} The product can only be tested at 97.00 % concentration or less, as some dilution always occurs when test organisms and interfering substance are added.

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Client Name: Sample Name: ITASH Batch No.: iClean IC-S

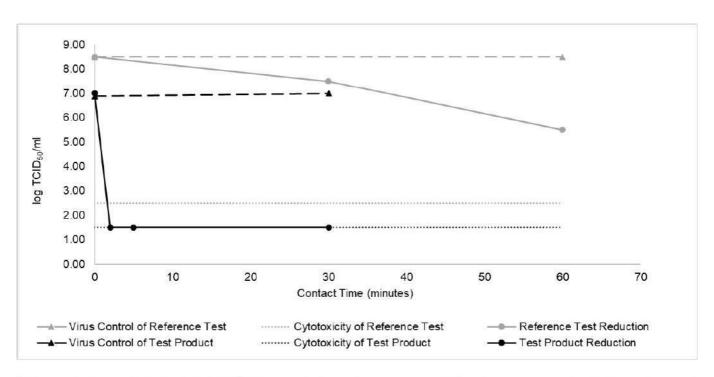
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Test strain: Poliovirus type 1, NIBSC-01/528

Virus control, V _C	Cytotoxicity effect, CE
V _{C1} : 7.00 ± 0.38	CE ₁ : 1.50 ± 0.00
V _{C2} : 7.00 ± 0.38	CE ₂ : 1.50 ± 0.00

Test concentration (%) / contact time (min)	First assay, N _{a1}	Second assay, N _{a2}	Average reduction
100.00*/2	N _{a1} : ≤1.50 ± 0.00 lg R ₁ : ≥5.50 ± 0.38	N _{a2} : ≤1.50 ± 0.00 Ig R ₂ : ≥ 5.50 ± 0.38	lg R: ≥5.50 ± 0.38
100.00* / 5	N _{a1} : ≤1.50 ± 0.00 lg R ₁ : ≥ 5.50 ± 0.38	N _{a2} : ≤1.50 ± 0.00 Ig R ₂ : ≥5.50 ± 0.38	lg R: ≥5.50 ± 0.38
100.00* / 30	N _{a1} : ≤1.50 ± 0.00 lg R ₁ : ≥ 5.50 ± 0.38	N _{a2} : ≤1.50 ± 0.00 Ig R ₂ : ≥5.50 ± 0.38	lg R: ≥5.50 ± 0.38



^{*} The product can only be tested at 97.00 % concentration or less, as some dilution always occurs when test organisms and interfering substance are added.

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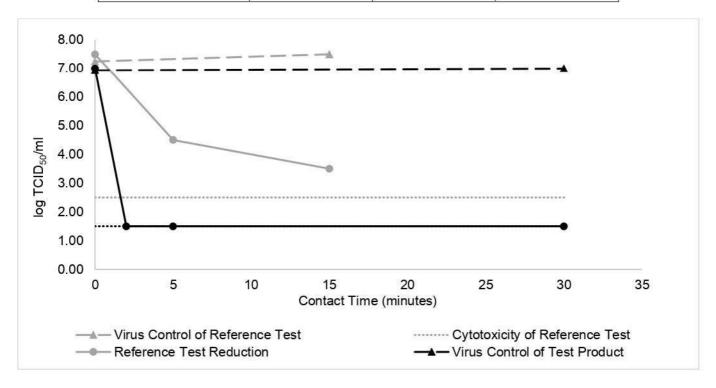
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Test strain: Vaccinia virus, ATCC VR-1508

Virus control, V _C	Cytotoxicity effect, CE
V _{C1} : 5.88 ± 0.37	CE ₁ : 1.50 ± 0.00
V _{C2} : 6.00 ± 0.38	CE ₂ : 1.50 ± 0.00

Test concentration (%) / contact time (min)	First assay, N _{a1}	Second assay, N _{a2}	Average reduction
100.00*/2	Na ₁ : ≤1.50 ± 0.00 lg R ₁ : ≥ 5.38 ± 0.37	N _{a2} : ≤1.50 ± 0.00 lg R ₂ : ≥ 5.63 ± 0.37	lg R: ≥5.51 ± 0.37
100.00*/5	Na₁: ≤1.50 ± 0.00 lg R₁: ≥5.38 ± 0.37	Na ₂ : ≤1.50 ± 0.00 Ig R ₂ : ≥ 5.63 ± 0.37	lg R: ≥5.51 ± 0.37
100.00* / 30	Na ₁ : ≤1.50 ± 0.00 lg R ₁ : ≥5.38 ± 0.37	Na ₂ : ≤1.50 ± 0.00 Ig R ₂ : ≥5.63 ± 0.37	lg R: ≥5.51 ± 0.37



^{*} The product can only be tested at 97.00 % concentration or less, as some dilution always occurs when test organisms and interfering substance are added.

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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: 6.50 ± 0.00	B: 5.88 ± 0.37	C ₃₀ : 3.00 ± 0.00
	A _{PBS} : 6.25 ± 0.33	Vc: 6.00 ± 0.38	C ₆₀ : 3.50 ± 0.38
Murine norovirus FLI-RVB-0651	A: 7.00 ± 0.38	B: 6.75 ± 0.33	C _{30:} 6.00 ± 0.00
	A _{PBS} : 6.75 ± 0.33	V _C : 6.63 ± 0.25	C _{60:} 6.00 ± 0.00
Human coronavirus ATCC VR-740	A: 6.25 ± 0.33	B: 5.75 ± 0.33	C _{30:} ≥4.00 ± 0.00
	A _{PBS} : 5.75 ± 0.33	V _C : 5.88 ± 0.37	C _{60:} ≥4.00 ± 0.00
Poliovirus type 1, NIBSC-01/528	A: 7.25 ± 0.33	B: 7.00 ± 0.38	C ₃₀ : 1.00 ± 0.00
	A _{PBS} : 7.50 ± 0.00	V _C : 7.00 ± 0.38	C ₆₀ : 3.00 ± 0.00
Vaccinia virus, ATCC VR-1508	A: 7.50 ± 0.00	B: 6.38 ± 0.25	C _{5:} 3.00 ± 0.00
	A _{PBS} : 7.50 ± 0.00	V _C : 6.88 ± 0.37	C _{15:} 4.00 ± 0.00

Note

TCID50: 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.

Vc: log₁₀ TCID₅₀ per mI in the viral test suspension at the beginning and at the maximum contact time

log₁₀ TCID₅₀ per ml in the test mixture at the end of the contact time Na:

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

log₁₀ TCID₅₀ per ml in the cell susceptibility control as compared to PBS A:

B: log₁₀ TCID₅₀ per ml in the suppression efficiency control as compared to the virus control

C: log₁₀ TCID₅₀ per mI in the reference test for virus inactivation after 30 and 60 minutes (5 and 15 minutes for vaccinia virus)

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

Test strain	Test concentration (%) / contact time (min)	Log reduction (TCID50/ml)	Associated risk [†]
	100.00* / 2	≥4.75 ± 0.27	Minimal risk of false acceptance
Adenovirus type 5 ATCC VR-5	100.00* / 5	≥4.75 ± 0.27	Minimal risk of false acceptance
	100.00* / 30	≥4.75 ± 0.27	Minimal risk of false acceptance
	100.00* / 2	≥5.13 ± 0.25	Minimal risk of false acceptance
Murine norovirus FLI-RVB-0651	100.00* / 5	≥5.13 ± 0.25	Minimal risk of false acceptance
	100.00* / 30	≥5.13 ± 0.25	Minimal risk of false acceptance
	100.00* / 2	≥4.44 ± 0.38	Minimal risk of false acceptance
Human coronavirus ATCC VR-740	100.00* / 5	≥4.44 ± 0.38	Minimal risk of false acceptance
	100.00* / 30	≥4.44 ± 0.38	Minimal risk of false acceptance
	100.00* / 2	≥5.50 ± 0.38	Minimal risk of false acceptance
Poliovirus type 1, NIBSC-01/528	100.00* / 5	≥5.50 ± 0.38	Minimal risk of false acceptance
	100.00* / 30	≥5.50 ± 0.38	Minimal risk of false acceptance
	100.00* / 2	≥5.51 ± 0.37	Minimal risk of false acceptance
Vaccinia virus, ATCC VR-1508	100.00* / 5	≥5.51 ± 0.37	Minimal risk of false acceptance
	100.00* / 30	≥5.51 ± 0.37	Minimal risk of false acceptance

^{*} The product can only be tested at 97.00 % 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.

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Efficacy of ITASH against Adenovirus type 5, strain Adenoid 75, ATCC VR-5, Murine norovirus, strain S99 Berlin, FLI-RVB-0651, Human coronavirus, strain 229E ATCC VR-740, Poliovirus type 1, strain LSc-2ab NIBSC-01/528 and Vaccinia virus, strain Ankara, ATCC VR-1508 in a quantitative suspension test at 20 °C according to EN14476:2013+A1:2015 (E) under clean condition

EXPERT OPINION*

This expert opinion is based on the test report

-0599 dated 21 October 2020.

The virucidal activity of the disinfectant ITASH of Family Products Sdn. Bhd. against *Adenovirus type 5 ATCC VR-5*, *Murine norovirus* FLI-RVB-0651, *Human coronavirus* ATCC VR-740, *Poliovirus type 1* NIBSC-01/528 and *Vaccinia virus*, ATCC VR-1508 was investigated by a quantitative suspension test according to EN14476:2013+A1:2015 (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 $\geq 4 \log_{10}$ (inactivation ≥ 99.99 %) within the recommended exposure period.

ITASH was examined at 20 °C at the concentration of 100.00 %** for the exposure times of 2, 5, and 30 minutes. After the exposure time, the viral reduction exceeded 4 log₁₀-steps in all assays. According to the simple acceptance decision rule[†], there is a minimal risk of false acceptance. Therefore, virucidal activity against *Adenovirus type 5* ATCC VR-5, *Murine norovirus* FLI-RVB-0651, *Human coronavirus* ATCC VR-740, *Poliovirus type 1* NIBSC-01/528 and *Vaccinia virus*, ATCC VR-1508 was measured as follows:

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^{*} Opinions and interpretations expressed here are outside the scope of accreditation.

^{**} The product can only be tested at 97.00 % 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.

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Appendix 1

QAU CERTIFICATE*

The results stated in test report .0599 dated 21 October 2020 were compared to the raw data of the tests and checked for correct transfer. No deviations were detected.

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Appendix 2 Raw data

Test Method		EN	14476:2013+A1	:2015		Titration Method	Quantal t	est
Product		Disir	nfectant Aerosol	Spray		Batch No.	iClean IC	-S
Product Diluent			Distilled water	•		Lab No.	, A	
Test Organism		Adenovirus,	strain Adenoid 7	5, ATCC VR-5		Passage No.	5	
Cell Line		Verd	cells, ATCC C	CL-81		Passage No.	6	
Interfering Substance		0.30 g/l	bovine albumii	n solution	1114	Inactivation Method	I Immedi	ate dilution
Test Temperature (°C)	2	0	Incubation Te	mperature (°C)	36	Dilution Method	Mo	odified
First Assay Test Date	21/08/2020	Second Assa	y Test Date	25/08/2020	Analyzed By		Verified By	

Validation and Control Procedures

>	Product	Dilution		Dilution (log ₁₀)									log ₁₀	ΔTCID ₅₀
# = =	Concentration	Dildion	1	2	3	4	5	6	7	8	9	10	TCID ₅₀ /ml	< 1 lg
Cell sceptibility Control	PBS	Without	PARTICULAR PROPERTY.	Comment of the state of the sta						0000	n a	n.d	6.25 ± 0.33	Pass?
Sus	100.00%	1:10	14E-02005 85	13 B B S		H31 20 36 3	D 555000 155 155 155			0000	n.d	n.d	6.50 ± 0.00	Yes

_	Product	Contact Time		Dilution (log ₁₀)										TCID ₅₀ - V _C
sion or	Concentration	(minutes)	1	2	3	4	5	6	7	8	9	10	TCID ₅₀ /ml	≤ 0.5 lg
Suppress Efficien Confro	100.00%	30								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	Pass?
Su	Virus Control (V _C)	30						Te 170 (576) (70	1970/1975 - 5710/179	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	n d	n.d	6.00 ± 0.38	Yes

	Product	Contact Time					Dilution	(log ₁₀)					log ₁₀	lgR=
	Concentration	(minutes)	- 1	2	3	4	5	6	7	8	9	10	TCID ₅₀ /ml	V _c - Na
st	0.70 %	30	ttt	t 4 4 4 4 t 4 4 4 4	TAG TOWNER OF	F6.11799/F885		200 THE REST	n d	n.d	n.d	n.d	4.50 ± 0.00	3.00 ± 0.00
ce Te	Formaldehyde	60	ttt	t 4 4 4 4 t 4 4 4 4					n d	n.d	n.d	n.d	4.00 ± 0.38	3.50 ± 0.38
Reference Test	Vinus Control (V.)	0	15.00000	1 4 4 4 4	U42300000 40	S 3	1000 E 15 SE				n.d	n. d	7.50 ± 0.00	
_ œ	Virus Control (V _C)	60	110000000000000000000000000000000000000	1 4 4 4 4	100000000000000000000000000000000000000	and the second			G 1 G 1 G 1 G	200000000000000000000000000000000000000	n.d	n.d	7.50 ± 0.00	
	Cytotoxicity Effect (CE)	8		t 0 0 0 0 t 0 0 0 0			# # # # # # # # # # # # # # # # # # #	n d	n.d	n.d	n.d	n.d	2.50 ± 0.00	

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Appendix 2 Raw data

Test Procedure

	Product	Contact Time					Dilution	(log ₁₀)					log ₁₀	
	Concentration	(minutes)	1	2	3	4	5	6	7	8	9	10	TCID ₅₀ /ml	
5247	100.00%	2	1	00000		1			na	n.d	n.d	n.d	1.50 ± 0.00	
(Na ₁)	100.00%	5	(2000-100) (CO)	00000			2004 000 1000 1000		l na	n.d	n.d	n.d	1.50 ± 0.00	V _{C1} - CE ≥
Assay	100.00%	30	PERSON NO. 12	00000			E-105 2 2		l na	n.d	n.d	n.d	1.50 ± 0.00	Pass?
First As	Virus Control	0		1 4 4 4 4							n.d	n.d	6.00 ± 0.38	Yes
	(V _{C1})	30	300303	1 4 4 4 4	1 3 EU 3 SUNA 15	1108 82 83 33	D4005 E E		2 2 2 2	72,424,224,32	n.d	n.d	6.00 ± 0.38	
	Cytotoxicity Effect (CE)	1001	10000000000000000000000000000000000000	00000				n.d	n.d	n.d	n.d	n.d	1.50 ± 0.00	[

	Product	Contact Time					Dilution	(log ₁₀)					log ₁₀	
	Concentration	(minutes)	1	2	3	4	5	6	7	8	9	10	TCID ₅₀ /ml	
2)	100.00%	2		00000	[2]::::::::::::::::::::::::::::::::::::				n d	n.d	n.d	n. d	1.50 ± 0.00	
say (Na ₂)	100.00%	5		00000					n d	n.d	n.d	n.d	1.50 ± 0.00	V _{C2} - CE ≥ 4
As	100.00%	30		00000			450 KEN KEN 150		n d	n.d	n.d	n. d	1.50 ± 0.00	Pass?
Second	Virus Control	0	30000000	1 4 4 4 4		[13] Et 31 31	0.750202 105 250			72.02.02.02	n.d	n.d	6.38 ± 0.25	Yes
0)	(V _{C2})	30		1 4 4 4 4			7727037 37 - 17				n.d	n.d	6.50 ± 0.00	
	Cytotoxicity Effect (CE)	(44)	SERVE (SE	00000			TE (1) 2 2 2	n.d	n.d	n.d	n.d	n.d	1.50 ± 0.00	

-	Product	Contact Time	First As	say (Na ₁)	Second A	ssay (Na ₂)	Average Reduction
duction)	Concentration	(minutes)	log ₁₀ TCID ₅₀ /ml	Ig R ₁ = V _{C1} - Na ₁	log ₁₀ TClD ₅₀ /ml	Ig R ₂ = V _{C2} - Na ₂	(lg R)
\$ ℃	100.00%	2	≤1.50 ± 0.00	≥4.50 ± 0.38	≤1.50 ± 0.00	≥5.00 ± 0.00	≥4.75 ± 0.27
Average F (lg	100.00%	5	≤1.50 ± 0.00	≥4.50 ± 0.38	≤1.50 ± 0.00	≥5.00 ± 0.00	≥4.75 ± 0.27
Av	100.00%	30	≤1.50 ± 0.00	≥4.50 ± 0.38	≤1.50 ± 0.00	≥5.00 ± 0.00	≥4.75 ± 0.27

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Test Method		EN 1	14476:2013+A1	:2015		Titration Metho	d	Quantal tes	t
Product			ITASH			Batch No.	*	iClean IC-S	8
Product Diluent			Distilled water			Lab No.	02		
Test Organism		Murine norovirus,	, strain S99 Ber	din, FLI-RVB-065	1	Passage No.		6	
Cell Line		RAW 2	64.7 cells, ATC	C TIB-71		Passage No.	0:	11	
Interfering Substance		0.30 g/L	bovine albumir	n solution	217.	Inactivation Me	thod	Immediate	e dilution
Test Temperature (°C)	2	0	Incubation Te	mperature (°C)	36	Dilution Method	ı	Modi	fied
First Assay Test Date	09/10/2020	Second Assay	Test Date	13/10/2020	Analyzed By	7	Verified	Ву	

Validation and Control Procedures

>	Product	Dilution		Dilution (log ₁₀)										ΔTCID ₅₀
 	Concentration	Dilation	1	2	3	4	5	6	7	8	9	10	TCID ₅₀ /ml	< 1 lg
Cell Susceptibility Control	PBS	Without	0.7500.7500.7500	4 4 4 4 4 4 4 4 4 4	P 1000000000 - 10					100000000000000000000000000000000000000	(5) - (5) (5) (5) (5)	n.d	6.75 ± 0.33	Pass?
Sns	100.00%	1:10	100000	4 4 4 4 4 4 4 4 4 4	1 1000000000000000000000000000000000000	188 89 86 3						n.d	7.00 ± 0.38	Yes

	Product	Contact Time	j	Dilution (log ₁₀)									log ₁₀	[TCID ₅₀ - V _C]
sion or ol	Concentration	(minutes)	1	2	3	4	5	6	7	8	9	10	TCID ₅₀ /ml	≤ 0.5 lg
Suppression Efficiency Control	100.00%	30								0000		n d	6.75 ± 0.33	Pass?
Su	Virus Control (V _C)	30						Fig. 1750 (576) (576)	1970 HZ 570 TS	0000	RS 2017 24 (22)	n d	6.63 ± 0.25	Yes

	Product	Contact Time					Dilution	(log ₁₀)					log ₁₀	lgR=
	Concentration	(minutes)	1	2	3	4	5	6	7	8	9	10	TCID ₅₀ /ml	V _C - Na
st	0.70 %	30	tttt	4 4 4 4	######################################				na	n.d	n.d	n.d	3.50 ± 0.00	6.00 ± 0.00
ice Te	Formaldehyde	60	1000FT 100 BY	4 4 4 4					n d	n.d	n.d	n.d	3.50 ± 0.00	6.00 ± 0.00
Reference Test	Vinus Control (V.)	0	120000000000000000000000000000000000000	4 4 4 4	- [전기(유)/본 - 급인					10 65 1055		n.d	9.50 ± 0.00	
œ	Virus Control (V _C)	60		4 4 4 4	100000000000000000000000000000000000000					100000000000000000000000000000000000000	6 . 6 . 5 . 5 .	n.d	9.50 ± 0.00	
	Cytotoxicity Effect (CE)	*		0000			4710170 tr - 171	l nd	n.d	n.d	n.d	n. d	2.50 ± 0.00	

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	Product	Contact Time					Dilution	(log ₁₀)					log ₁₀	
	Concentration	(minutes)	1	2	3	4	5	6	7	8	9	10	TCID ₅₀ /ml	
	100.00%	2	1	0000		1			l na	n.d	n.d	n.d	1.50 ± 0.00	
(Na ₁)	100.00%	5		0000					l na	n.d	n.d	n.d	1.50 ± 0.00	V _{C1} - CE ≥ 4
Assay	100.00%	30	CENTRAL (1997)	0000					i na	n.d	n.d	n.d	1.50 ± 0.00	Pass?
First As	Virus Control	0		4 4 4 4 4 4 4 4 4								n.d	6.50 ± 0.00	Yes
	(V _{C1})	30	1000000 O	4 4 4 4 4 4 4 4 4	13003003 25	HR 20 20 3	0.400.65 0.5 157	110000000000000000000000000000000000000		50,000,000	2 2 3 2	n.d	6.63 ± 0.25	
	Cytotoxicity Effect (CE)	155		0000				n d	n.d	n.d	n.d	n.d	1.50 ± 0.00	

	Product	Contact Time					Dilution	(log ₁₀)					log ₁₀	
	Concentration	(minutes)	1	2	3	4	5	6	7	8	9	10	TCID ₅₀ /ml	
2)	100.00%	2		0000			170 BON 1888 1988	THE STATE OF THE STATE OF	n d	n.d	n.d	n.d	1.50 ± 0.00	
ıy (Na2)	100.00%	5		0000					n d	n.d	n.d	n.d	1.50 ± 0.00	V _{C2} - CE ≥
l Assay	100.00%	30		0000					n d	n.d	n.d	n.d	1.50 ± 0.00	Pass?
Second	Virus Control	0	3.000483755 75	4 4 4 4	13 000 4500 550	100 20 00 0	DARKE 15 15			72.52.52		n.d	6.63 ± 0.25	Yes
0)	(V _{C2})	30		4 4 4 4			78793745-15					n.d	6.63 ± 0.25	
	Cytotoxicity Effect (CE)	8(4)	SERVER (SE)	0000				n a	n.d	n.d	n.d	n.d	1.50 ± 0.00	

	Product	Contact Time	First As	say (Na ₁)	Second A	ssay (Na ₂)	Average Reduction
duction	Concentration	(minutes)	log ₁₀ TCID ₅₀ /ml	Ig R ₁ = V _{C1} - Na ₁	log ₁₀ TClD ₅₀ /ml	Ig R ₂ = V _{C2} - Na ₂	(lg R)
& &	100.00%	2	≤1.50 ± 0.00	≥5.13 ± 0.25	≤1.50 ± 0.00	≥5.13 ± 0.25	≥5.13 ± 0.25
Average F (lg	100.00%	5	≤1.50 ± 0.00	≥5.13 ± 0.25	≤1.50 ± 0.00	≥5.13 ± 0.25	≥5.13 ± 0.25
Av	100.00%	30	≤1.50 ± 0.00	≥5.13 ± 0.25	≤1.50 ± 0.00	≥5.13 ± 0.25	≥5.13 ± 0.25

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Test Method		EN	14476:2013+A1	:2015		Titration Method	Quantal te	est		
Product			ITASH			Batch No.	iClean IC-	-S		
Product Diluent			Distilled water			Lab No.				
Test Organism		Human coronav	irus, strain 229	E, ATCC VR-740	8	Passage No.	3			
Cell Line		MRC-	5 cells, ATCC C	CL-171		Passage No.	6			
Interfering Substance		0.30 g/L	bovine albumir	solution	240	Inactivation Method	Immedia	ate dilution		
Test Temperature (°C)	2	0	Incubation Te	mperature (°C)	36	Dilution Method	Modified			
First Assay Test Date	09/10/2020	Second Assay	y Test Date	13/10/2020	Analyzed By		Verified By			

Validation and Control Procedures

>	Product	Dilution					Dilution	(log ₁₀)		W			log ₁₀	ΔTCID ₅₀
1 = -	Concentration	Dilation	1	2	3	4	5	6	7	8	9	10	TCID ₅₀ /mi	< 1 lg
Cell Susceptibility Control	PBS	Without			277E0327643 305		130 Maria 115 100			0000	n a	n.d.	6.25 ± 0.33	Pass?
Sns	100.00 %	1:10	177 J. 350 J. S. C. C.		370.030.05 63	133 89 36 3		2002/02/02/02/02	2 2 2 3	0000	n a	n.d.	5.75 ± 0.33	Yes

_	Product	Contact Time					Dilutio	n (log ₁₀)		, -			log ₁₀	TCID ₅₀ - V _C
C 50	Concentration	(minutes)	1	2	3	4	5	6	7	8	9	10	TCID ₅₀ /ml	≤ 0.5 lg
Suppression Efficiency Control	100.00 %	30								0000	n d	n.d.	5.75 ± 0.33	Pass?
Sul	Virus Control (V _C)	30			-22000000000000000000000000000000000000		[- 10 HT - 17]	111 111 111 111 111		0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	n a	n.d.	5.88 ± 0.37	Yes

	Product	Contact Time					Dilution	n (log ₁₀)					log ₁₀	IgR=
	Concentration	(minutes)	1	2	3	4	5	6	7	8	9	10	TCID ₅₀ /ml	V _C - Na
sst	0.70 %	30	15990 File 8	0000		76 UPA 5465		F645000000000	n d	n.d.	n.d.	n.d.	2.50 ± 0.00	≥4.00 ± 0.00
Reference Test	Formaldehyde	60	5981 77 3	0000	# 10 H - 10 H		#170015 - 1구 - 1구		n d	n.d.	n.d.	n.d.	2.50 ± 0.00	≥4.00 ± 0.00
eferer	Vinus Control (V)	0	150000000000000000000000000000000000000	4 4 4 4	U200000 30	E 4 6 2	Opt 50 00	50.505000	5500G R S		n.d.	n.d.	6.50 ± 0.00	
<u> </u>	Virus Control (V _C)	60	0.0000000000000000000000000000000000000	4 4 4 4	120002188				[15] 시민국 (미국) (미국)		n.d.	n.d.	6.50 ± 0.00	
	Cytotoxicity Effect (CE)	-	20122144	0000					n d	n.d.	n.d.	n.d.	2.50 ± 0.00	

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Test Procedure

	Product	Contact Time					Dilution	(log ₁₀)					log ₁₀	
	Concentration	(minutes)	1	2	3	4	5	6	7	8	9	10	TCID ₅₀ /ml	
	100.00%	2		00000					l nd	n.d.	n.d.	n.d.	1.50 ± 0.00	
(Na ₁)	100.00%	5		00000					n d	n.d.	n.d.	n.d.	1.50 ± 0.00	V _{C1} - CE ≥
Assay	100.00%	30		00000					n d	n.d.	n.d.	n.d.	1.50 ± 0.00	Pass?
First A	Virus Control	0		4 4 4 4 4 4 4 4 4 4							n.d.	n.d.	5.75 ± 0.33	Yes
	(V _{C1})	30	ADDITIONS OF	4 4 4 4 4 4 4 4 4 4		1 132 227 331 44				041, 421, 432, 432	n.d.	n.d.	5.88 ± 0.37	
	Cytotoxicity Effect (CE)	1870		0 0 0 0 0				n.d.	n.d.	n.d.	n.d.	n.d.	1.50 ± 0.00	

	Product	Contact Time					Dilution	(log ₁₀)					log ₁₀	
	Concentration	(minutes)	1	2	3	4	5	6	7	8	9	10	TCID ₅₀ /ml	
5)	100.00%	2		0000				THE STATE OF THE STATE OF	n a	n.d.	n.d.	n.d.	1.50 ± 0.00	
ıy (Na2)	100.00%	5		0000					l na	n.d.	n.d.	n.d.	1.50 ± 0.00	V _{C2} - CE ≥ 4
l Assay	100.00%	30		0000					n d	n.d.	n.d.	n.d.	1.50 ± 0.00	Pass?
Second	Virus Control	0		4 4 4 4 4 4 4 4							n.d.	n.d.	6.00 ± 0.38	Yes
0)	(V _{C2})	30		4 4 4 4 4						10.000	n.d.	n.d.	6.00 ± 0.38	
¥.	Cytotoxicity Effect (CE)	(84)	CENTRAL (1947 E.)	0 0 0 0 0 0 0 0 0 0 0 0			12 (1) 2 2 2 E	n a	n.d.	n.d.	n.d.	n.d.	1.50 ± 0.00	

-	Product	Contact Time	First As	say (Na ₁)	Second A	ssay (Na ₂)	Average Reduction
ction	Concentration	(minutes)	log ₁₀ TCID ₅₀ /ml	Ig R ₁ = V _{C1} - Na ₁	log ₁₀ TCID ₅₀ /ml	Ig R ₂ = V _{C2} - Na ₂	(Ig R)
	100.00%	2	≤1.50 ± 0.00	≥4.38 ± 0.37	≤1.50 ± 0.00	≥4.50 ± 0.38	≥4.44 ± 0.38
rerage Redu (lg R)	100.00%	5	≤1.50 ± 0.00	≥4.38 ± 0.37	≤1.50 ± 0.00	≥4.50 ± 0.38	≥4.44 ± 0.38
Av	100.00%	30	≤1.50 ± 0.00	≥4.38 ± 0.37	≤1.50 ± 0.00	≥4.50 ± 0.38	≥4.44 ± 0.38

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Test Method		EN 1	14476:2013+A1	:2015		Titration Method		Quantal test	
Product			ITASH			Batch No.	2	iClean IC-S	
Product Diluent			Distilled water	Š.		Lab No.	0.2	J1 10	100
Test Organism		Polic	ovirus, strain LS	Passage No.		3			
Cell Line		Vero	cells, ATCC C	CL-81		Passage No.	0.	20	
Interfering Substance		0.30 g/L	bovine albumin	solution	-10-	Inactivation Met	hod	Immediate	dilution
Test Temperature (°C)	20	E	Incubation Ter	mperature (°C)	36	Dilution Method	Mod		ed
First Assay Test Date	09/10/2020	Second Assay	/ Test Date	13/10/2020	Analyzed By	J J	Verified B	y	

Validation and Control Procedures

>	Product	Dilution				11.5	Dilution	1 (log ₁₀)				01-	log ₁₀	ΔTCID ₅₀
# _	Concentration	Dilution	1	2	3	4	5	6	7	8	9	10	TCID ₅₀ /ml	< 1 lg
Cell sceptibility Control	PBS	Without			277E0327643 305		#30Y0W3 ~ V5	PARTY AND DESCRIPTION OF THE PARTY AND PARTY.		0000	n a	n.d	7.50 ± 0.00	Pass?
Sus	100.00%	1:10	100,000,000		1100000000 cg	138 80 36 3	151155 SS SS	138 SB MESES	2 2 3 2	0 0 0 0	n d	n.d	7.25 ± 0.33	Yes

	Product	Contact Time	į				Dilutio	1 (log ₁₀)					log ₁₀	[TCID ₅₀ - V _C]
sion ncy ol	Concentration	(minutes)	1	2	3	4	5	6	7	8	9	10	TCID ₅₀ /ml	≤ 0.5 lg
Suppress Efficien Contro	100.00%	30	4 4 4 4							0000	n d	n.d	7.00 ± 0.38	Pass?
Su	Virus Control (V _C)	30		NAME OF TAXABLE PARTY.						0000	n d	n.d	7.00 ± 0.38	Yes

	Product	Contact Time					Dilutio	(log ₁₀)					log ₁₀	IgR=
	Concentration	(minutes)	1	2	3	4	5	6	7	8	9	10	TCID ₅₀ /ml	V _C - Na
Test	0.70 %	30	15986 FIE		TANK TOWNERS OF		A CONTRACTOR OF		0000	n.d	n.d	n.d	7.50 ± 0.00	1.00 ± 0.00
ice Te	Formaldehyde	60	5281 OV 0	A STATE OF THE STA					0000	n.d	n.d	n.d	5.50 ± 0.00	3.00 ± 0.00
Reference	Vinus Control (V.)	0	F-10000 - 15			6 9 6	10 mars 25 35	S . S . S . N	4 4 4 4 4 4 4 4 3 2		n.d	n.d	8.50 ± 0.00	
œ	Virus Control (V _C)	60			100000000000000000000000000000000000000		1000000		4 4 4 4 4		n.d	n.d	8.50 ± 0.00	
	Cytotoxicity Effect (CE)	*					0000	l nd	n.d	n.d	n.d	n.d	2.50 ± 0.00	1

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1,2	Product	Contact Time	5				Dilutio	n (log ₁₀)					log ₁₀	
	Concentration	(minutes)	1	2	3	4	5	6	7	8	9	10	TCID ₅₀ /ml	
	100.00%	2					1	0000	na	n.d	n.d	n. d	1.50 ± 0.00	
(Na ₁)	100.00%	5	2000 CO (1000 CO)		\$30 BESCHARKSO = 72.6		10.00 (0.00 (a.50) = 12.6	0000	n n	n.d	n.d	n.d	1.50 ± 0.00	V _{C1} - CE ≥ 4
Assay	100.00%	30	PERSONAL PROPERTY.	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1		-8 955	DESCRIPTION OF THE PERSON OF T	0000	l no	n.d	n.d	n.d	1.50 ± 0.00	Pass?
First,	Virus Control	0								0000	n.d	n.d	6.88 ± 0.37	Yes
	(V _{C1})	30	300/38/23 03	18 B 80 S	1 SED 1500 250	1131 20 30 3	DESCRIPTION 105 105	10 300000	8 8 8 8	0000	n.d	n.d	7.00 ± 0.38	
	Cytotoxicity Effect (CE)	858				0000		n d	n.d	n.d	n.d	n.d	1.50 ± 0.00]

	Product	Contact Time					Dilution	(log ₁₀)					log ₁₀	
	Concentration	(minutes)	1	2	3	4	5	6	7	8	9	10	TCID ₅₀ /ml	
2)	100.00%	2		0000	[CHANG 15]		173 FEBRUAR 170		n d	n.d	n.d	n.d	1.50 ± 0.00	
ay (Na2)	100.00%	5		0000					n d	n.d	n.d	n.d	1.50 ± 0.00	V _{C2} - CE ≥
Assay	100.00%	30		0000	[CHANG 15]				n d	n.d	n.d	n.d	1.50 ± 0.00	Pass?
Second	Virus Control	0	380036755 05	4 4 4 4 4 4 4 4 4		H32 20 30 3	155,000 155 155				n.d	n.d	6.88 ± 0.37	Yes
0)	(V _{C2})	30		4 4 4 4 4			72743 - 15 - 15				n.d	n.d	7.00 ± 0.38	
	Cytotoxicity Effect (CE)	(44)	SEA SEA SEA	0000			13(02 2 3	n a	n.d	n.d	n.d	n.d	1.50 ± 0.00	

	Product	Contact Time	First As	say (Na ₁)	Second A	ssay (Na ₂)	Average Reduction
duction	Concentration	(minutes)	log ₁₀ TCID ₅₀ /ml	Ig R ₁ = V _{C1} - Na ₁	log ₁₀ TCID ₅₀ /ml	Ig R ₂ = V _{C2} - Na ₂	(lg R)
8 8	100.00%	2	≤1.50 ± 0.00	≥5.50 ± 0.38	≤1.50 ± 0.00	≥5.50 ± 0.38	≥5.50 ± 0.38
Average F (lg	100.00%	5	≤1.50 ± 0.00	≥5.50 ± 0.38	≤1.50 ± 0.00	≥5.50 ± 0.38	≥5.50 ± 0.38
Av	100.00%	30	≤1.50 ± 0.00	≥5.50 ± 0.38	≤1.50 ± 0.00	≥5.50 ± 0.38	≥5.50 ± 0.38

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Test Method		EN:	14476:2013+A1	2015	•	Titration Method	Quanta	test
Product			ITASH			Batch No.	iClean	IC-S
Product Diluent			Distilled water	į.		Lab No.		
Test Organism		Vaccinia virus,	strain Ankara,	ATCC VR-1508		Passage No.	3	
Cell Line		BHK-2	21 cells, ATCC	CCL-10		Passage No.	9	
Interfering Substance		0.30 g/L	bovine albumin	solution	-10-	Inactivation Method	I Imme	diate dilution
Test Temperature (°C)	20	Ē	Incubation Ter	mperature (°C)	36	Dilution Method	1	/lodified
First Assay Test Date	29/04/2020	Second Assay	/ Test Date	30/04/2020	Analyzed By	7	Verified By	

Validation and Control Procedures

>	Product	Dilution		A1		11.5	Dilution	(log ₁₀)	,			01-	log ₁₀	ΔTCID ₅₀
# -	Concentration	Dilution	1	2	3	4	5	6	7	8	9	10	TCID ₅₀ /ml	< 1 lg
Cell sceptibility Control	PBS	Without	Participation of the Control of the		277E0327643 305		#30%(A) #49.00 - 3.00			0000	n a	n.d.	7.50 ± 0.00	Pass?
Sus	100.00 %	1:10	370.030000 355		1100000000 cg	138 80 36 3		138 SB ARCTO		0000	n.d.	n.d.	7.50 ± 0.00	Yes

_	Product	Contact Time					Dilution	1 (log ₁₀)					log ₁₀	TCID ₅₀ - V _C
sion or	Concentration	(minutes)	1	2	3	4	5	6	7	8	9	10	TCID ₅₀ /mI	≤ 0.5 lg
Suppress Efficien Confro	100.00 %	30	tttt							0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	n d	n.d.	6.38 ± 0.25	Pass?
Su	Virus Control (V _C)	30		NAME OF THE OWNER, OF THE OWNER, OF THE OWNER, OF THE OWNER, OWNER, OWNER, OWNER, OWNER, OWNER, OWNER, OWNER,	-25000000000000000000000000000000000000					0000	n d	n.d.	6.88 ± 0.37	Yes

	Product	Contact Time					Dilution	(log ₁₀)					log ₁₀	lgR=
	Concentration	(minutes)	- 1	2	3	4	5	6	7	8	9	10	TCID ₅₀ /ml	V _c - Na
st	0.70 %	5		4 4 4 4	PARTITION AND				ı na	n.d.	n.d.	n.d.	4.50 ± 0.00	3.00 ± 0.00
ice Te	Formaldehyde	15	5987 87 37	4 4 4 4			하겠었다고 하고 그것!		l nd	n.d.	n.d.	n.d.	3.50 ± 0.00	4.00 ± 0.00
Reference Test	Vi 0 (AV.)	0	200	4 4 4 4						0000	n.d.	n.d.	7.25 ± 0.33	
α.	Virus Control (V _C)	15		4 4 4 4	100000000000000000000000000000000000000				G 10 0 10	0000	n.d.	n.d.	7.50 ± 0.00	
	Cytotoxicity Effect (CE)	-		0000					l na	n.d.	n.d.	n.d.	2.50 ± 0.00	

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Appendix 2 Raw data

Test Procedure

	Product	Contact Time	0				Dilution	(log ₁₀)					log ₁₀	
	Concentration	(minutes)	1	2	3	4	5	6	7	8	9	10	TCID ₅₀ /ml	
-24	100%	2		0000					n d	n.d.	n.d.	n.d.	1.50 ± 0.00	
(Na ₁)	100%	5	2000 DEC 100 SERVICE (1885)	0000	200 DESCRIPTION - 12.6		2008/000/05/00 = 22.6		i na	n.d.	n.d.	n.d.	1.50 ± 0.00	V _{C1} - CE ≥ 4
Assay	100%	30	SERVER (1982 SE	0000		3 74 55	FF()7 2 2 2		l nd	n.d.	n.d.	n.d.	1.50 ± 0.00	Pass?
First As	Virus Control	0		4 4 4 4							n.d.	n.d.	6.88 ± 0.37	Yes
	(V _{C1})	30	300303	4 4 4 4 4	13 00/35005 250	108 22 81 3	0.4852-25 124 25	18000 0000000		200	n.d.	n.d.	6.88 ± 0.37	
	Cytotoxicity Effect (CE)	870		0000				n d	n.d.	n.d.	n.d.	n.d.	1.50 ± 0.00	

	Product	Contact Time					Dilution	(log ₁₀)					log ₁₀	
	Concentration	(minutes)	1	2	3	4	5	6	7	8	9	10	TCID ₅₀ /ml	
5)	100%	2		0000	(T) (T) (T)		170 F 186 H 186 186 186 186 186 186 186 186 186 186		n d	n.d.	n.d.	n.d.	1.50 ± 0.00	
say (Na ₂)	100%	5		0000					l nd	n.d.	n.d.	n.d.	1.50 ± 0.00	V _{C2} - CE ≥ 4
As	100%	30		0000	(T): (T)				n d	n.d.	n.d.	n.d.	1.50 ± 0.00	Pass?
Second	Virus Control	0	300000000	4 4 4 4 4		[13] 22 S) 3	DARKER 125 25				n.d.	n.d.	7.00 ± 0.38	Yes
S)	(V _{C2})	30		4 4 4 4 4			727030000000000000000000000000000000000				n.d.	n.d.	7.13 ± 0.37	
	Cytotoxicity Effect (CE)	849	SEALE (1997 E	0000			1000 A A	n a	n.d.	n.d.	n.d.	n.d.	1.50 ± 0.00	

	Product	Contact Time	First As	say (Na ₁)	Second A	ssay (Na ₂)	Average Reduction
duction)	Concentration	(minutes)	log ₁₀ TCID ₅₀ /ml	Ig R ₁ = V _{C1} - Na ₁	log ₁₀ TCID ₅₀ /ml	Ig R ₂ = V _{C2} - Na ₂	(lg R)
8 8	100%	2	≤1.50 ± 0.00	≥5.38 ± 0.37	≤1.50 ± 0.00	≥5.63 ± 0.37	≥5.51 ± 0.37
Average F (lg	100%	5	≤1.50 ± 0.00	≥5.38 ± 0.37	≤1.50 ± 0.00	≥5.63 ± 0.37	≥5.51 ± 0.37
Av	100%	30	≤1.50 ± 0.00	≥5.38 ± 0.37	≤1.50 ± 0.00	≥5.63 ± 0.37	≥5.51 ± 0.37

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Note

Vc:

TCID50: The dilution of the virus suspension that induces a CPE in 50 % of cell culture units

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

log₁₀ TCID₅₀ per ml in the viral test suspension at the beginning and at the maximum contact time

Na: log₁₀ TCID₅₀ 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: log₁₀ TCID₅₀ per ml in the cell susceptibility control as compared to PBS

B: log₁₀ TCID₅₀ per ml in the suppression efficiency control as compared to the virus control

C: log₁₀ TCID₅₀ per ml in the reference test for virus inactivation after 30 and 60 minutes (5 minutes for vaccinia virus)

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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.: 5
 - 1.1.2. Cell line: Vero cells, ATCC CCL-81
 - 1.1.3. Cell line passage no.: 6
 - 1.1.4. Culture medium: EMEM
- 1.2. Murine norovirus, strain S99 Berlin, FLI-RBI-0651
 - 1.2.1. Passage no.: 6
 - 1.2.2. Cell line: Raw 264.7 cells, ATCC TIB-71
 - 1.2.3. Cell line passage no.: 11
 - 1.2.4. Culture medium: DMEM
- 1.3 Human coronavirus, strain 229E, ATCC VR-740
 - 1.3.1 Passage no.: 3
 - 1.3.2 Cell line: MRC-5 cells, ATCC CCL-171
 - 1.3.3 Cell line passage no.: 6
 - 1.3.4 Culture medium: EMEM
- 1.4 Poliovirus type 1, NIBSC-01/528, ATCC VR-740
 - 1.4.1 Passage no.: 3
 - 1.4.2 Cell line: Vero cells, ATCC CCL-81
 - 1.4.3 Cell line passage no.: 20
 - 1.4.4 Culture medium: EMEM
- 1.5 Vaccinia virus, strain Ankara, ATCC VR-1508
 - 1.5.1 Passage no.: 3
 - 1.5.2 Cell line: BHK-21 cells, ATCC CCL-10
 - 1.5.3 Cell line passage no.: 9
 - 1.5.4 Culture medium: EMEM

2 Materials and reagents

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

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3 Apparatus and glassware

- 3.3 CO2 incubator (Memmert, model ICO 105)
- 3.4 Cooling water bath (Memmert, model WNB7 with CDP115)
- 3.5 Inverted microscope (Optika, IM-2)
- 3.6 Vortex® mixer (Biosan model Biosan V-1 Plus)
- 3.7 Microtitre plate (NEST)
- 3.8 Tissue culture flask (JET Biofil)

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Test procedure

4.3 Preparation of test virus suspension

- Cell monolayers shall be >90 % confluent before inoculation. Cell lines are selected in accordance with their sensitivity to the test organisms.
- 4.3.2 The test organisms and their stock cultures shall be prepared and kept in accordance with EN 12353:2013 (E).
- 4.3.3 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.
- 4.3.4 The cells are subjected to 3 freeze/thaw cycles once cytopathic effect (CPE) is observed in 80 % of the cell
- 4.3.5 Separate the cells debris is by centrifugation at 400 g_N for 15 minutes.
- 4.3.6 Aliquot the supernatant containing the test virus suspension and store at -80 °C.

4.4 Test Na - Determination of virucidal concentrations

- 4.4.1 Pipette 1 ml of interfering substance into a container of suitable capacity for appropriate mixing.
- 4.4.2 Add 1 ml of the virus test suspension to the container, carefully avoiding the upper part of the sides. Mix well.
- 4.4.3 Add 8 ml of the product test solution to the container.
- 4.4.4 Mix, start a stopwatch at once, and place the container in a water bath controlled at the chosen test temperature.
- 4.4.5 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.
- 446 Within 30 minutes, prepare a series of ten-fold dilutions of this mixture (text mixture and maintenance medium).
- 4.4.7 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.
- 4.4.8 The last row of six or eight wells will receive 0.1 ml of culture medium and will serve as the cell control.
- 4.4.9 After 1 hour of incubation at 37 °C, 0.1 ml of cell culture medium is added to each well.
- 4.4.10 After incubation, the virus titre is calculated. The reduction of virus infectivity is determined from differences of log10 virus titres before and after treatment with the product.

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

- 4.5.1 Mix 1 part of hard water and 1 part of interfering substances with 8 parts of the product test solution.
- 4.5.2 Serial dilutions are prepared in the culture medium and are inoculated into cell monolayers.
- 4.5.3 This test is done in parallel with Section 4.2.
- 4.5.4 Any microscopic changes in the cells are recorded when reading the tests for CPE.
- 4.5.5 If the cytotoxicity is so great that the residual infectivity titre is smaller than the required log10 TCID50, special techniques have to be used, such as molecular sieving or ultrafiltration. Follow the instructions of the manufacturer.

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4.6 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

- 4.6.1 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.
- 4.6.2 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.
- 4.6.3 After 1 hour of incubation at 37 °C, the supernatant is discarded.
- 4.6.4 The virus is diluted from 10⁻¹ to 10⁻¹⁰ and titrated on the treated or untreated cells.
- 4.6.5 Verify according to Section 4.8.

4.7 Suppression efficiency control B – Immediate dilution method validation

- 4.7.1 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.
- 4.7.2 Mix again and start the clock. Incubate the mixture in the ice bath for 30 minutes ± 10 seconds.
- 4.7.3 Immediately prepare dilutions up to 10⁻⁸ and titrate the virus.
- 4.7.4 This control is performed in parallel to the test.
- 4.7.5 Verify according to Section 4.8.

4.8 Reference test for virus inactivation C - Validation of the test system

- 4.8.1 2 ml of the test suspension shall be mixed with 8 ml of PBS and 10 ml of 1.4 % (w/v) formaldehyde.
- 4.8.2 Contact times are 30 and 60 minutes.
- 4.8.3 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.
- 4.8.4 Leave the mixture in the ice bath.
- 4.8.5 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.
- 4.8.6 In exceptional cases, smaller volumes of the reagents and of the test suspension could be used, ensuring that the relative proportions are maintained.
- 4.8.7 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.
- 4.8.8 The mixture is further diluted to 10⁻⁵ in an ice bath.
- 4.8.9 Verify according to Section 4.8.

4.9 Titration of the virus control

- 4.9.1 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.
- 4.9.2 Section 4.2 is repeated by substituting the product test solution with hard water or water for ready-to-use products.
- 4.9.3 Verify according to Section 4.8.

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4.10Verification of methodology

- The titre of the test suspension (virus control) of at least 108 TCID50/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.
- 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.
- 4.10.3 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.
- 4.10.4 The difference to the test suspension in the control of efficiency for suppression of products' activity shall be ≤0.5
- 4.10.5 The difference between the logarithmic titre of the virus control and the logarithmic titre of the test organism in the reference inactivation test is:
 - 4.10.5.1 Between -0.5 and -2.5 after 30 minutes and between -2 and -4.5 after 60 minutes for poliovirus
 - 4.10.5.2 Between -3 and -5 after 30 minutes and between -3.5 and -5.5 after 60 minutes for adenovirus
 - 4.10.5.3 Between 0.0 and -2.0 after 30 minutes and between -0.5 and -2.5 after 60 minutes for parvovirus
 - 4.10.5.4 Between -0.75 and -3.5 after 20 and 30 minutes and between -2.0 and ≥-4.0 after 120 and 30 minutes for vaccinia virus.

5 Literature

- 5.3 EN 14476:2013+A1:2015 (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)
- 5.4 EN 14885:2015 (E): Chemical disinfectants and antiseptics Application of European Standards for chemical disinfectants and antiseptics
- 5.5 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