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SAMM 1016

Test Report No.: TR-23-0274

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Determination of the Virucidal Activity of **VITASEPT E75 GEL** according to EN 14476:2013+A2:2019

Test Method
EN 14476:2013+A2:2019
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
Goodpoint Chemicals
Urda Tee 2/1
Jälgimäe 76404
Estonia

Testing Laboratory
TECOLAB Sdn. Bhd.
J-2-6, Pusat Komersial Jalan Kuching
No. 115, Jalan Kepayang, Off Jalan Kuching
51200 Kuala Lumpur
Malaysia

Kuala Lumpur, 16 May 2023

Dr Marven Lee Cheng Shoou
Managing Director

IDENTIFICATION OF TESTING LABORATORY

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TECOLAB Sdn. Bhd.
J-2-6, Pusat Komersial Jalan Kuching
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IDENTIFICATION OF CLIENT

Goodpoint Chemicals
Urda Tee 2/1
Jälgimäe 76404
Estonia

IDENTIFICATION OF TEST ITEM

Test item name:	Vitasept E75 Gel
Lab ID:	G007-23-004
Batch no.:	23094
Expiry date:	March 2026
Manufacturer:	Goodpoint Chemicals
Receipt date:	5 April 2023
Storage conditions:	Room temperature away from sunlight
Product diluent recommended by manufacturer:	Not specified
Active substances:	80% w/w Ethanol Quaternary ammonium compounds
Product appearance:	Clear, colourless liquid

TEST METHOD & VALIDATION

Test method:	EN 14476:2013+A2:2019 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)
Titration method:	Quantal tests ($TCID_{50}$ method)
Inactivation method:	Molecular sieving (microspin)

EXPERIMENTAL CONDITIONS

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Date of test:	9 May - 15 May 2023
Product diluent:	Distilled water
Concentration / contact time:	100%* / 30 seconds ± 5 seconds 100%* / 60 seconds ± 5 seconds
Test temperature:	(20 ± 1) °C
Interfering substance:	Clean condition (0.3 g/L bovine serum albumin)
Test organism / passage no.:	Human adenovirus (AdV), strain Adenoid 75, ATCC VR-5 / P4 Murine norovirus (MNV), strain S99 Berlin, FLI RVB-0651 / P3 Poliovirus (PV-1), strain LSc 2ab, NIBSC 01/528 / P4
Cell line / passage no.:	RAW 264.7 ATCC TIB-71 / P20&21 BHK-21 ATCC CCL-10 / P14&15
Growth medium:	DMEM supplemented with 10% foetal bovine serum and 1% penicillin-streptomycin
Incubation temperature:	(37 ± 1) °C, 5% CO_2
Incubation period:	2 to 4 days (PV-1 & AdV) 2 to 3 days (for MNV)
Appearance of the product dilutions:	Clear, colourless liquid
Stability and appearance of product dilutions during test:	Homogenous without any precipitate

* The product can only be tested at a concentration of 80% or less as some dilution is always produced by adding the test organisms and interfering substance.

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CONTROLS AND VALIDATION

Test Organism	Cytotoxicity Effect	Interference Control	Suppression Control	Reference Test
AdV ATCC VR-5	N: 5.63 ± 0.25 CE: 1.50 ± 0.00	A_{PBS}: 7.50 ± 0.00 A_T: 7.50 ± 0.00	B_N: 6.00 ± 0.38 B_T: 5.50 ± 0.00	C_{30}: 3.25 ± 0.50 C_{60}: ≥3.50 ± 0.38
MNV ATCC VR-1937	N: 5.50 ± 0.00 CE: 1.50 ± 0.00	A_{PBS}: 7.00 ± 0.38 A_T: 6.50 ± 0.00	B_N: 5.50 ± 0.00 B_T: 5.00 ± 0.38	C_{30}: 2.25 ± 0.55 C_{60}: 3.00 ± 0.55
PV-1 NIBSC 01/528	N: 5.75 ± 0.33 CE: 1.50 ± 0.00	A_{PBS}: 8.50 ± 0.00 A_T: 8.50 ± 0.00	B_N: 6.13 ± 0.37 B_T: 6.00 ± 0.38	C_{30}: 2.38 ± 0.41 C_{60}: 3.63 ± 0.45

The control and validation tests A, B, and C were within the basic limits:

- The difference between the virus control N and the cytotoxicity effect CE must be ≥4.00 to verify that the cytotoxicity of the product does not affect cell morphology and growth or susceptibility for the test organism which are necessary to demonstrate a 4-log reduction of the virus,
- The difference between the interference effect of PBS control and the product, A_{PBS} and A_T, must be <1.00 to verify that the susceptibility of the cells for the virus infection is not influenced negatively by the treatment with the product test solution,
- The difference between the virus control B_N and the suppression control B_T must be ≤0.50 to validate the inactivation method,
- The reduction of adenovirus in the reference test, C_{30} and C_{60}, must be between 3.00 and 5.00 after 30 minutes, and between 3.50 and 5.50 after 60 minutes,
- The reduction of norovirus in the reference test, C_{30} and C_{60}, must be between 1.00 and 3.00 after 30 minutes, and between 2.00 and 4.00 after 60 minutes, and
- The reduction of poliovirus in the reference test, C_{30} and C_{60}, must be between 0.50 and 2.50 after 30 minutes, and between 2.00 and 4.50 after 60 minutes.

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TEST RESULTS

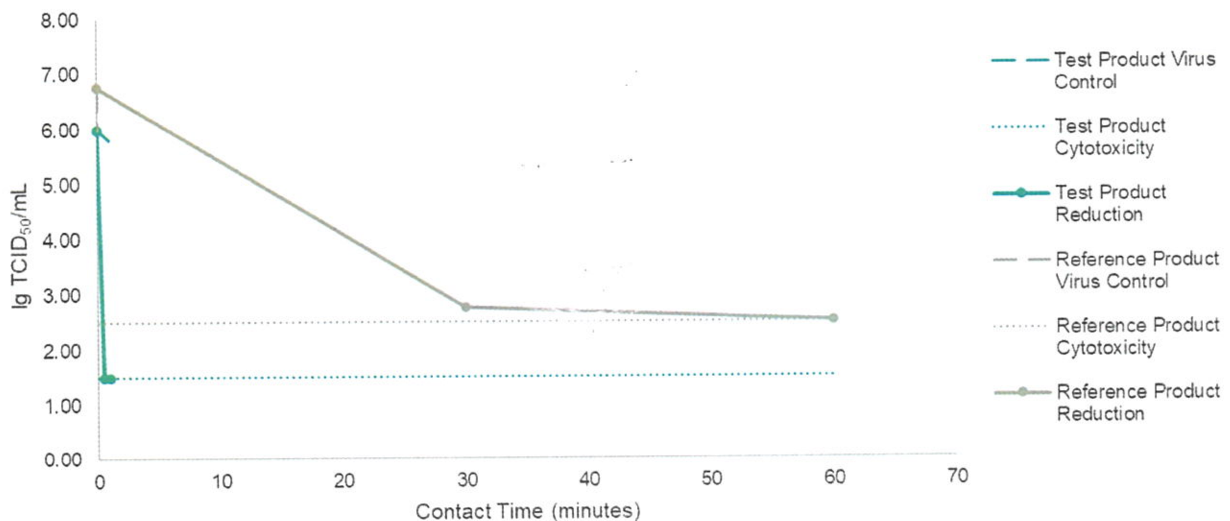
For each product concentration and contact time, the log reduction (lg R) is calculated using the formula lg R = N – Na, in which:

- N is the lg $TCID_{50}$ per mL of the virus control at the end of the contact time, and
- Na is the lg $TCID_{50}$ per mL of the test mixture at the end of the contact time.

Test organism: Human adenovirus (AdV) ATCC VR-5

Virus control, N	N_1: 5.63 ± 0.25 N_2: 6.00 ± 0.38
Cytotoxicity effect, CE	CE_1: 1.50 ± 0.00 CE_2: 1.50 ± 0.00

Concentration / Contact Time	Test, Na	Reduction, lg R = N – Na	Average Reduction, lg R
100%* / 30 seconds	Na_1: ≤1.50 ± 0.00 Na_2: ≤1.50 ± 0.00	lg R_1: ≥4.13 ± 0.25 lg R_2: ≥4.50 ± 0.38	lg R: ≥4.31 ± 0.32 %R: ≥99.995%
100%* / 60 seconds	Na_1: ≤1.50 ± 0.00 Na_2: ≤1.50 ± 0.00	lg R_1: ≥4.13 ± 0.25 lg R_2: ≥4.50 ± 0.38	lg R: ≥4.31 ± 0.32 %R: ≥99.995%



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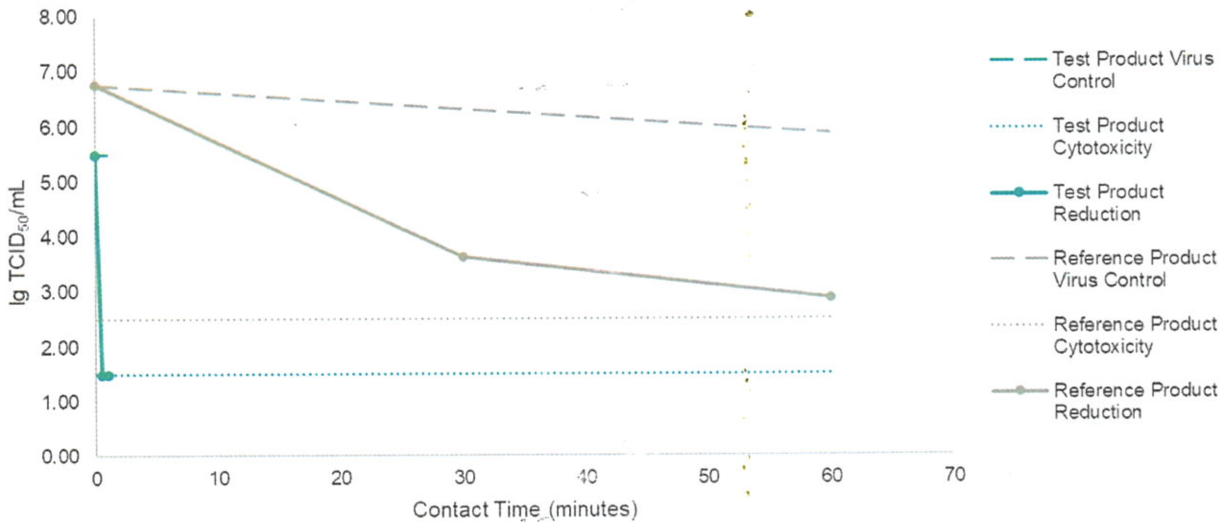
Test organism: Murine norovirus (MNV) FLI RVB-0651

Virus control, N	N_1: 5.50 ± 0.00 N_2: 5.50 ± 0.00
Cytotoxicity effect, CE	CE_1: 1.50 ± 0.00 CE_2: 1.50 ± 0.00

Concentration / Contact Time	Test, Na	Reduction, lg R = N – Na	Average Reduction, lg R
100%* / 30 seconds	Na_1: ≤1.50 ± 0.00 Na_2: ≤1.50 ± 0.00	lg R_1: ≥4.00 ± 0.00 lg R_2: ≥4.00 ± 0.00	lg R: ≥4.00 ± 0.00 %R: ≥99.990%
100%* / 60 seconds	Na_1: ≤1.50 ± 0.00 Na_2: ≤1.50 ± 0.00	lg R_1: ≥4.00 ± 0.00 lg R_2: ≥4.00 ± 0.00	lg R: ≥4.00 ± 0.00 %R: ≥99.990%



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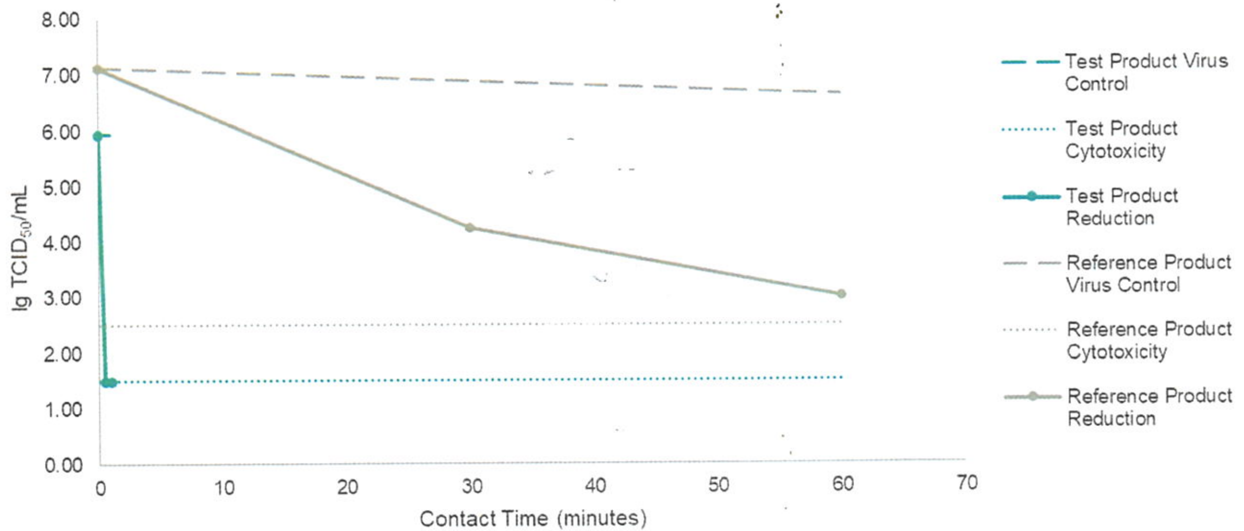
Test organism: Poliovirus (PV-1) NIBSC 01/528

Virus control, N	N_1: 5.75 ± 0.33 N_2: 6.13 ± 0.37
Cytotoxicity effect, CE	CE_1: 1.50 ± 0.00 CE_2: 1.50 ± 0.00

Concentration / Contact Time	Test, Na	Reduction, lg R = N – Na	Average Reduction, lg R
100%* / 30 seconds	Na_1: ≤1.50 ± 0.00 Na_2: ≤1.50 ± 0.00	lg R_1: ≥4.25 ± 0.33 lg R_2: ≥4.63 ± 0.37	lg R: ≥4.44 ± 0.35 %R: ≥99.996%
100%* / 60 seconds	Na_1: ≤1.50 ± 0.00 Na_2: ≤1.50 ± 0.00	lg R_1: ≥4.25 ± 0.33 lg R_2: ≥4.63 ± 0.37	lg R: ≥4.44 ± 0.35 %R: ≥99.996%



* The product can only be tested at a concentration of 80% or less as some dilution is always produced by adding the test organisms and interfering substance.

CONCLUSION

The test item achieved a reduction of ≥4.00 log against the test organisms Human adenovirus (AdV) ATCC VR-5, Murine norovirus (MNV) FLI RVB-0651, and Poliovirus NIBSC 01/528 under the tested conditions.

Therefore, **Vitasept E75 Gel** has demonstrated a full virucidal activity according to EN 14476:2013+A2:2019 under the following conditions:

Concentration	Contact Time	Test Temperature	Soiling
100%*	30 seconds	20 °C	Clean condition
100%*	60 seconds	20 °C	Clean condition

Kuala Lumpur, 16 May 2023

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Chan Yanqi
Microbiologist

* The product can only be tested at a concentration of 80% or less as some dilution is always produced by adding the test organisms and interfering substance.

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EXPERT OPINION

This expert opinion is based on the test report TR-23-0274 dated 16 May 2023. Opinions and interpretations expressed herein are outside the scope of the Laboratory Accreditation Scheme of Malaysia (SAMM).

The product **Vitasept E75 Gel** was tested according to EN 14476:2013+A2:2019 against Human adenovirus (AdV), strain Adenoid 75, ATCC VR-5, Murine norovirus (MNV), strain S99 Berlin, FLI RVB-0651, and Poliovirus (PV-1), strain LSc 2ab, NIBSC 01/528. These organisms are the minimum test organisms and they have been chosen as a representative species for viruses, taking into account their relative resistance, relevance to practical use, handling properties, and microbiological safety.

Virucidal activity is defined as a capability of a product or active substance to produce a reduction in the number of infectious virus particles of relevant test organisms under defined conditions. According to EN 14476, a surface disinfectant is considered to possess a full virucidal activity if it demonstrates a reduction of ≥4.00 log against the minimum spectrum of test organisms within 60 minutes (or 5 minutes for surfaces in contact with patient or medical staff) when tested at 4 to 30 °C under clean (0.3 g/L bovine serum albumin) or dirty (3.0 g/L bovine serum albumin and 3.0 mL/L sheep erythrocytes) condition.

When tested under the following conditions, **Vitasept E75 Gel** achieved a reduction of ≥4.00 log against Human adenovirus (AdV) ATCC VR-5, Murine norovirus (MNV) FLI RVB-0651, and Poliovirus NIBSC 01/528:

Concentration	Contact Time	Test Temperature	Soiling
100%*	30 seconds	20 °C	Clean condition
100%*	60 seconds	20 °C	Clean condition

Therefore, **Vitasept E75 Gel** has demonstrated a full virucidal activity according to EN 14476:2013+A2:2019 under the aforementioned conditions.

Kuala Lumpur, 16 May 2023

Dr Marven Lee Cheng Shoou
Managing Director

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INFORMATION ON MEASUREMENT UNCERTAINTY & DECISION RULE

The statement of conformity given by EN 14476:2013+A2:2019 states that the test product shall be considered to have passed EN 14476 if it demonstrates ≥4.00 log reduction under the defined conditions.

The laboratory employs the simple acceptance decision rule to account for the measurement uncertainty when stating the statement of conformity. The measurement uncertainty and conformance probability are shown in the raw data and are summarized as follows:

Test Organism	Concentration / Contact Time	Log Reduction	Conformance	Conformance Probability†
AdV ATCC VR-5	100%* / 30 seconds	≥4.31 ± 0.32	Yes	≤16.472% chance of false acceptance
	100%* / 60 seconds	≥4.31 ± 0.32	Yes	≤16.472% chance of false acceptance
MNV ATCC VR-1937	100%* / 30 seconds	≥4.00 ± 0.00	Yes	≤50.000% chance of false acceptance
	100%* / 60 seconds	≥4.00 ± 0.00	Yes	≤50.000% chance of false acceptance
PV-1 NIBSC 01/528	100%* / 30 seconds	≥4.44 ± 0.35	Yes	≤10.381% chance of false acceptance
	100%* / 60 seconds	≥4.44 ± 0.35	Yes	≤10.381% chance of false acceptance

* The product can only be tested at a concentration of 80% or less as some dilution is always produced by adding the test organisms and interfering substance.

† The conformance probability follows a normal distribution. Therefore, the percentage of conformance can never be zero or 100% due to the asymptotic tails.

RAW DATA

Test Method: EN 14476:2013+A2:2019 | Dilution Method: Standard (80%) | Passing Criteria (lg): 4.00
Product Name: Vitasept E75 Gel | Batch No.: 23094 | Lab ID: G007-23-004
Product Diluent: Distilled water | Appearance of Product Dilutions: Clear, colourless liquid | Titration Method: Quantal test
Inactivation: Molecular sieving (microspin) | Interfering Substance: 0.3 g/L bovine albumin solution
Test Organism: Human adenovirus, strain Adenoid 75, ATCC VR-5 | Passage No.: 4 | Test Temperature (°C): 20
Cell Line: Vero cells, ATCC CCL-31 | Passage No.: 14 & 15 | Incubation Temperature (°C): 36
First Assay Testing Period: 09/05/2023 | Second Assay Testing Period: 11/05/2023 | Tested By: CYQ | Verified By: CSE

Validation & Controls

	Product Concentration	Dilution	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	$\lvert A_{PBS} - A_T \rvert$ = 0.00
Interference Control (A)	PBS	N/A	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	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				7.50 ± 0.00	
	100%	10^-2	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	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				7.50 ± 0.00	Limit: $\lvert A_{PBS} - A_T \rvert$ < 1

	Product Concentration	Contact Time (seconds)	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	$\lvert B_N - B_T \rvert$ = 0.50
Suppression Control (B)	Virus Control (N)	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	0 4 4 0 0 0 4 4	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				6.00 ± 0.38	
	100%	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	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				5.50 ± 0.00	Limit: $\lvert B_N - B_T \rvert$ ≤ 0.5

	Product Concentration	Contact Time (minutes)	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	lg $R_C = C_N - C_T$
Reference Test (C)	0.7% Formaldehyde	30	t t t t t t t t	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	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				2.75 ± 0.33	3.25 ± 0.50
		60	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	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				≤2.50 ± 0.00	≥3.50 ± 0.38
	Virus Control (N)	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 3 4 4 4 4 4 4	4 1 1 4 2 2 2 2	1 0 0 0 0 1 0 0	0 0 0 0 0 0 0 0				6.75 ± 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	0 0 4 0 0 1 1 1	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				6.00 ± 0.38	
	Cytotoxicity Effect (CE)	N/A	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	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				2.50 ± 0.00	

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Test Procedure

	Product Concentration	Contact Time (seconds)	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	lg $R_1 = N_1 - Na_1$
First Assay (Na_1)	100%	30	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 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				≤1.50 ± 0.00	≥4.13 ± 0.25
		60	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 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				≤1.50 ± 0.00	≥4.13 ± 0.25
	Virus Control (N)	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	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				5.50 ± 0.00	$N_1 - CE_1$ = 4.13 Limit: $N_1 - CE_1 \geq 4$
		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	0 0 0 0 0 0 4 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				5.63 ± 0.25	
	Cytotoxicity Effect (CE)	N/A	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 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				1.50 ± 0.00	

	Product Concentration	Contact Time (seconds)	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	lg $R_2 = N_2 - Na_2$
Second Assay (Na_2)	100%	30	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 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				≤1.50 ± 0.00	≥4.50 ± 0.38
		60	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 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				≤1.50 ± 0.00	≥4.50 ± 0.38
	Virus Control (N)	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 0 1 4 0 4 4	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				6.25 ± 0.33	$N_2 - CE_2$ = 4.50 Limit: $N_2 - CE_2 \geq 4$
		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	0 4 4 0 0 0 4 4	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				6.00 ± 0.38	
	Cytotoxicity Effect (CE)	N/A	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 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				1.50 ± 0.00	

Product Concentration	Contact Time (seconds)	First Assay (Na_1) lg $TCID_{50}$/mL	First Assay (Na_1) lg $R_1 = N_1 - Na_1$	Second Assay (Na_2) lg $TCID_{50}$/mL	Second Assay (Na_2) lg $R_2 = N_2 - Na_2$	Average Reduction (lg R)	Conformance Probability
100%	30	≤1.50 ± 0.00	≥4.13 ± 0.25	≤1.50 ± 0.00	≥4.50 ± 0.38	≥4.31 ± 0.32 ≥99.995%	≥83.528%
	60	≤1.50 ± 0.00	≥4.13 ± 0.25	≤1.50 ± 0.00	≥4.50 ± 0.38	≥4.31 ± 0.32 ≥99.995%	≥83.528%

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Test Method:	EN 14476:2013+A2:2019	Dilution Method:	Standard (80%)	Passing Criteria (lg):	4.00
Product Name:	Vitasept E75 Gel	Batch No.:	23094	Lab ID:	G007-23-004
Product Diluent:	Distilled water	Appearance of Product Dilutions:	Clear, colourless liquid	Titration Method:	Quantal test
Inactivation:	Molecular sieving (microspin)	Interfering Substance:	0.3 g/L bovine albumin solution		
Test Organism:	Murine norovirus, strain S99 Berlin, FLI RVB-0651	Passage No.:	3	Test Temperature (°C):	20
Cell Line:	RAW 264.7 cells, ATCC TIB-71	Passage No.:	20 & 21	Incubation Temperature (°C):	36
First Assay Testing Period:	09/05/2023	Second Assay Testing Period:	11/05/2023	Tested By: CYQ	Verified By: CSE

Validation & Controls

Interference Control (A)	Product Concentration	Dilution	Dilution 10^-1	10^-2	10^-3	10^-4	10^-5	10^-6	10^-7	10^-8	10^-9	10^-10	lg $TCID_{50}$/mL	$\lvert A_{PBS} - A_T \rvert$ = 0.50
	PBS	N/A	4 4 4 4	4 4 4 4	4 4 4 4	4 4 4 4	1 4 1 1	1 1 0 0	0 0 0 0				7.00 ± 0.38	
			4 4 4 4	4 4 4 4	4 4 4 4	4 4 4 4	1 4 1 1	0 1 1 0	0 0 0 0					
	100%	10^-2	4 4 4 4	4 4 4 4	4 4 4 4	1 4 4 4	1 1 1 1	0 0 0 0	0 0 0 0				6.50 ± 0.00	Limit: $\lvert A_{PBS} - A_T \rvert$ < 1
			4 4 4 4	4 4 4 4	4 4 4 4	1 4 4 4	1 1 1 1	0 0 0 0	0 0 0 0					

Suppression Control (B)	Product Concentration	Contact Time (seconds)	Dilution 10^-1	10^-2	10^-3	10^-4	10^-5	10^-6	10^-7	10^-8	10^-9	10^-10	lg $TCID_{50}$/mL	$\lvert B_N - B_T \rvert$ = 0.50
	Virus Control (N)	60	4 4 4 4	4 4 2 4	2 2 4 2	1 1 1 1	0 0 0 0	0 0 0 0	0 0 0 0				5.50 ± 0.00	
			4 4 4 4	2 4 4 4	2 2 2 4	1 1 1 1	0 0 0 0	0 0 0 0	0 0 0 0					
	100%	60	4 4 4 4	1 4 4 4	1 1 4 1	1 1 0 0	0 0 0 0	0 0 0 0	0 0 0 0				5.00 ± 0.38	Limit: $\lvert B_N - B_T \rvert$ ≤ 0.5
			4 4 4 4	4 1 4 4	1 1 1 4	1 0 0 1	0 0 0 0	0 0 0 0	0 0 0 0					

Reference Test (C)	Product Concentration	Contact Time (minutes)	Dilution 10^-1	10^-2	10^-3	10^-4	10^-5	10^-6	10^-7	10^-8	10^-9	10^-10	lg $TCID_{50}$/mL	lg $R_C = C_N - C_T$
	0.7% Formaldehyde	30	t t t t	4 3 1 0	1 1 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0				3.63 ± 0.41	2.25 ± 0.55
			t t t t	2 2 2 2	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0					
		60	t t t t	1 0 0 1	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0				2.88 ± 0.41	3.00 ± 0.55
			t t t t	0 0 0 0	1 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0					
	Virus Control (N)	0	4 4 4 4	4 4 4 4	4 4 4 4	1 1 2 3	1 1 1 1	1 0 0 0	0 0 0 0				6.75 ± 0.33	
			4 4 4 4	4 4 4 4	4 4 4 4	3 3 3 3	2 1 1 1	0 1 0 0	0 0 0 0					
		60	4 4 4 4	4 4 4 4	4 4 4 4	3 4 1 4	0 1 0 0	0 0 0 0	0 0 0 0				5.88 ± 0.37	
			4 4 4 4	4 4 4 4	4 4 4 4	3 4 1 2	0 1 1 0	0 0 0 0	0 0 0 0					
	Cytotoxicity Effect (CE)	N/A	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				2.50 ± 0.00	
			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					

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Test Procedure

	Product Concentration	Contact Time (seconds)	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	lg $R_1 = N_1 - Na_1$
First Assay (Na_1)	100%	30	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 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				≤1.50 ± 0.00	≥4.00 ± 0.00
		60	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 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				≤1.50 ± 0.00	≥4.00 ± 0.00
	Virus Control (N)	0	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	3 3 2 3 3 2 2 2	1 1 1 1 1 1 1 1	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				5.50 ± 0.00	$N_1 - CE_1 = 4.00$
		60	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	2 3 3 2 2 2 2 2	1 1 1 1 1 1 1 1	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				5.50 ± 0.00	
	Cytotoxicity Effect (CE)	N/A	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 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				1.50 ± 0.00	Limit: $N_1 - CE_1 \geq 4$

	Product Concentration	Contact Time (seconds)	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	lg $R_2 = N_2 - Na_2$
Second Assay (Na_2)	100%	30	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 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				≤1.50 ± 0.00	≥4.00 ± 0.00
		60	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 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				≤1.50 ± 0.00	≥4.00 ± 0.00
	Virus Control (N)	0	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	2 4 2 2 2 2 2 2	1 1 1 1 1 1 1 1	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				5.50 ± 0.00	$N_2 - CE_2 = 4.00$
		60	4 4 4 4 4 4 4 4	4 4 2 4 2 4 4 4	2 2 4 2 2 2 2 4	1 1 1 1 1 1 1 1	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				5.50 ± 0.00	
	Cytotoxicity Effect (CE)	N/A	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 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0				1.50 ± 0.00	Limit: $N_2 - CE_2 \geq 4$

Product Concentration	Contact Time (seconds)	First Assay (Na_1)		Second Assay (Na_2)		Average Reduction (lg R)	Conformance Probability
		lg $TCID_{50}$/mL	lg $R_1 = N_1 - Na_1$	lg $TCID_{50}$/mL	lg $R_2 = N_2 - Na_2$		
100%	30	≤1.50 ± 0.00	≥4.00 ± 0.00	≤1.50 ± 0.00	≥4.00 ± 0.00	≥4.00 ± 0.00 ≥99.99%	≥50.000%
	60	≤1.50 ± 0.00	≥4.00 ± 0.00	≤1.50 ± 0.00	≥4.00 ± 0.00	≥4.00 ± 0.00 ≥99.99%	≥50.000%

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Test Method:	EN 14476:2013+A2:2019	Dilution Method:	Standard (80%)	Passing Criteria (lg):	4.00
Product Name:	Vitasept E75 Gel	Batch No.:	23094	Lab ID:	G007-23-004
Product Diluent:	Distilled water	Appearance of Product Dilutions:	Clear, colourless liquid	Titration Method:	Quantal test
Inactivation:	Molecular sieving (microspin)	Interfering Substance:	0.3 g/L bovine albumin solution		
Test Organism:	Poliovirus, strain LSc 2ab, NIBSC 01/528	Passage No.:	4	Test Temperature (°C):	20
Cell Line:	Vero cells, ATCC CCL-31	Passage No.:	14 & 15	Incubation Temperature (°C):	36
First Assay Testing Period:	09/05/2023	Second Assay Testing Period:	11/05/2023	Tested By: CYQ	Verified By: CSE

Validation & Controls

	Product Concentration	Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	
Interference Control (A)	PBS	N/A	4 4 4 4	4 4 4 4	4 4 4 4	4 4 4 4	4 4 4 4	4 4 4 4	4 4 4 2	0 0 0 0			8.50 ± 0.00	$\lvert A_{PBS} - A_T \rvert$ = 0.00
			4 4 4 4	4 4 4 4	4 4 4 4	4 4 4 4	4 4 4 4	4 4 4 4	4 1 1 2	0 0 0 0				
	100%	10^-2	4 4 4 4	4 4 4 4	4 4 4 4	4 4 4 4	4 4 4 4	4 4 4 4	4 1 4 2	0 0 0 0			8.50 ± 0.00	Limit: $\lvert A_{PBS} - A_T \rvert$ < 1
			4 4 4 4	4 4 4 4	4 4 4 4	4 4 4 4	4 4 4 4	4 4 4 4	1 2 4 4	0 0 0 0				

	Product Concentration	Contact Time (seconds)	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	
Suppression Control (B)	Virus Control (N)	60	4 4 4 4	4 4 4 4	4 4 4 4	4 4 4 4	0 4 4 0	0 0 0 0	0 0 0 0	0 0 0 0			6.13 ± 0.37	$\lvert B_N - B_T \rvert$ = 0.13
			4 4 4 4	4 4 4 4	4 4 4 4	4 4 4 4	2 4 1 0	0 0 0 0	0 0 0 0	0 0 0 0				
	100%	60	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			6.00 ± 0.38	Limit: $\lvert B_N - B_T \rvert$ ≤ 0.5
			4 4 4 4	4 4 4 4	4 4 4 4	4 4 4 4	2 4 1 4	0 0 0 0	0 0 0 0	0 0 0 0				

	Product Concentration	Contact Time (minutes)	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	lg $R_C = C_N - C_T$
Reference Test (C)	0.7% Formaldehyde	30	t t t t	4 4 4 4	1 4 0 1	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0			4.25 ± 0.33	2.38 ± 0.41
			t t t t	4 4 4 4	1 2 0 1	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0				
		60	t t t t	1 3 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			3.00 ± 0.38	3.63 ± 0.45
			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				
	Virus Control (N)	0	4 4 4 4	4 4 4 4	4 4 4 4	4 4 4 4	4 4 4 4	4 0 0 4	0 0 0 0	0 0 0 0			7.13 ± 0.37	
			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				
		60	4 4 4 4	4 4 4 4	4 4 4 4	4 2 3 3	4 2 2 4	4 0 0 0	0 0 0 0	0 0 0 0			6.63 ± 0.25	
			4 4 4 4	4 4 4 4	4 4 4 4	4 3 4 4	2 2 4 4	0 0 0 0	0 0 0 0	0 0 0 0				
	Cytotoxicity Effect (CE)	N/A	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			2.50 ± 0.00	
			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				

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Test Procedure

First Assay (Na_1)	Product Concentration	Contact Time (seconds)	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	lg $R_1 = N_1 - Na_1$
	100%	30	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 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			≤1.50 ± 0.00	≥4.25 ± 0.33
		60	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 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			≤1.50 ± 0.00	≥4.25 ± 0.33
	Virus Control (N)	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	1 1 1 1 4 4 2 2	0 0 0 0 1 1 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			5.75 ± 0.33	$N_1 - CE_1$ = 4.25
		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	1 2 4 1 4 1 1 2	0 1 0 1 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			5.75 ± 0.33	
	Cytotoxicity Effect (CE)	N/A	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 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			1.50 ± 0.00	Limit: $N_1 - CE_1 \geq 4$

Second Assay (Na_2)	Product Concentration	Contact Time (seconds)	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	lg $R_2 = N_2 - Na_2$
	100%	30	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 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			≤1.50 ± 0.00	≥4.63 ± 0.37
		60	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 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			≤1.50 ± 0.00	≥4.63 ± 0.37
	Virus Control (N)	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	0 4 4 0 0 2 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			6.13 ± 0.37	$N_2 - CE_2$ = 4.63
		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	0 4 4 0 2 4 1 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			6.13 ± 0.37	
	Cytotoxicity Effect (CE)	N/A	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 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			1.50 ± 0.00	Limit: $N_2 - CE_2 \geq 4$

Product Concentration	Contact Time (seconds)	First Assay (Na_1) lg $TCID_{50}$/mL	First Assay (Na_1) lg $R_1 = N_1 - Na_1$	Second Assay (Na_2) lg $TCID_{50}$/mL	Second Assay (Na_2) lg $R_2 = N_2 - Na_2$	Average Reduction (lg R)	Conformance Probability
100%	30	≤1.50 ± 0.00	≥4.25 ± 0.33	≤1.50 ± 0.00	≥4.63 ± 0.37	≥4.44 ± 0.35 ≥99.996%	≥89.619%
	60	≤1.50 ± 0.00	≥4.25 ± 0.33	≤1.50 ± 0.00	≥4.63 ± 0.37	≥4.44 ± 0.35 ≥99.996%	≥89.619%

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TEST PROCEDURE

1. Test Procedure *Na*: Determination of Virucidal Concentrations

1.1 100 µL of interfering substance was pipetted into a tube. 100 µL of virus test suspension was added to the tube and mixed.

1.2 800 µL of the product test solution was added to tube. The tube was mixed and the stopwatch was started at once. The tube was placed in a cooled incubator controlled at the chosen test temperature θ for the contact time t. For ready-to-use products, the product can be tested at a higher concentration using a modified method in which the ratio of interfering substance, virus test suspension, and product test solution was changed to 20 µL, 10 µL, and 970 µL.

1.3 Immediately at the end of t, the tube was mixed and the activity of the product test solution was inactivated or removed using one of the following methods:

1.3.1 Dilution in ice-cold maintenance medium (growth media with 2% serum). 50 µL of the mixture was transferred into 450 µL ice-cold maintenance medium and put in an ice bath. Within 30 minutes of product inactivation, a series of ten-fold dilutions of the inactivated mixture was prepared in ice-cold maintenance medium. If the cytotoxicity of the product test solution is too high,

1.3.2 Ultrafiltration using MicroSpin™ S-400 HR columns. 100 µL of the mixture is transferred to the column and the residual virus was obtained as per manufacturer's instructions. Within 30 minutes of product inactivation, a series of ten-fold dilutions of the inactivated mixture was prepared in ice-cold maintenance medium, or

1.3.3 Large-volume plating (LVP). The procedure was done according to Section 1.3.1. However, the series of ten-fold dilutions of the inactivated mixture was immediately prepared in ice-cold maintenance medium up to the lowest apparently non-cytotoxic dilution (determined from the cytotoxicity effect test *CE*) of the product test solution. For this non-cytotoxic dilution, the dilution was prepared in large volume sufficient to be added to a defined number of microtitre plates, with each well requiring 100 µL of the mixture.

1.4 The virus titre for *Na* was determined using quantal test (endpoint titration). 100 µL of each dilution was transferred into 8 wells of a microtitre plate containing a confluent (>90%) cell monolayer without any medium. In the case of LVP, 100 µL of only the lowest non-cytotoxic dilution was transferred into the required number of wells. In any case, 100 µL of maintenance medium was added to the last row of wells to serve as the cell control.

1.5 After 1 hour incubation at 37 °C, 100 µL of maintenance medium was added to each well.

1.6 The cells were incubated for the appropriate incubation period until cytopathic effect (CPE; morphological alteration of cells and/or their destruction as a consequence of virus multiplication) was observed. The results were recorded as '0' for no CPE, or '1' to '4' for approximately 25%, 50%, 75%, and 100% CPE, respectively.

1.7 The virus titre was calculated using the Spearman-Kärber method. For LVP, the titre was calculated using the Poisson distribution if no virus was observed, and using the Taylor series formula if low amount of viruses were detected.

1.8 The virus titre was expressed as lg $TCID_{50}$/mL, i.e., the 50% infecting dose of a virus suspension that induces a CPE in 50% of cell culture units.

2. Virus Control *N*

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2.1 The virus control *N* was performed in parallel to the test *Na* at two contact times: at 0 minute and the longest contact time used in the test *Na*. The product test solution was substituted with hard water (distilled water for ready-to-use products).

2.2 The inactivation method chosen must be the same as the one chosen in *Na*. For LVP, the inactivation method was done as per dilution in ice-cold maintenance medium method.

2.3 A series of ten-fold dilutions of the inactivated mixture was prepared in ice-cold maintenance medium.

2.4 The virus titre for *N* was determined using quantal test according to Sections 1.4 to 1.8.

3. Cytotoxicity Effect *CE*: Verification for Possible Morphological Alteration of Cells by the Test Product

3.1 100 µL of hard water (distilled water for ready-to-use products) and 100 µL of interfering substance were mixed with 800 µL of the product test solution.

3.2 The product test solution was inactivated or removed using the same method as the one chosen in *Na*. For LVP, the inactivation method was done as per dilution in ice-cold maintenance medium method.

3.3 A series of ten-fold dilutions of the inactivated mixture was prepared in ice-cold maintenance medium.

3.4 The cytotoxicity of the product test solution was determined using quantal test according to Sections 1.4 to 1.8.

3.5 The results were recorded as 't' for cytotoxicity, i.e., the morphological alteration of cells and/or their destruction or their reduced sensitivity to virus multiplication caused by the product.

4. Interference Control *A*: Verification that the Susceptibility of the Cells for the Virus Infection is Not Influenced Negatively by the Treatment with the Test Product

4.1 To check the reduction of the sensitivity of the cells to virus, comparative virus titrations were performed in cells that have or have not been treated with product test solution.

4.2 For the test A_T, 100 µL of the lowest apparently non-cytotoxic dilution (determined from the cytotoxicity effect test *CE*) of the product test solution and 100 µL of maintenance medium were transferred into each 8 wells of a microtitre plate containing a confluent (>90%) cell monolayer without any medium.

4.3 In parallel, the negative interference control A_{PBS} was performed using PBS instead of the product test solution.

4.4 After 1 hour incubation at 37 °C, the supernatant was discarded. A series of ten-fold dilutions of the virus test suspension was prepared in maintenance medium. 100 µL of each dilution was titrated to each well. The virus titre for A_T and A_{PBS} was determined using quantal test according to Sections 1.4 to 1.8.

5. Suppression Control *B*: Validation of the Inactivation Method

5.1 100 µL of interfering substance was pipetted into a tube. 100 µL of maintenance medium was added to the tube and mixed.

5.2 800 µL of the product test solution of the highest concentration used in the test *Na* was added to tube and mixed.

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5.3 The activity of the product test solution was inactivated or removed using the same method employed for the test *Na* using one of the following methods:

5.3.1 Dilution in ice-cold maintenance medium or LVP. 50 µL of the mixture was transferred into 400 µL ice-cold maintenance medium. 50 µL of the virus test suspension was added to the mixture. The tube was mixed and put in an ice bath for 30 minutes ± 10 seconds, or if the cytotoxicity of the product test solution is too high,

5.3.2 Ultrafiltration using MicroSpin™ S-400 HR columns. 100 µL of the mixture is transferred to the column and the eluate was obtained as per manufacturer's instructions. 50 µL of the eluate was transferred into 400 µL ice-cold maintenance medium. 50 µL of the virus test suspension was added to the mixture. The tube was mixed and put in an ice bath for 30 minutes ± 10 seconds.

5.4 At the end of the 30 minutes incubation, a series of ten-fold dilutions of the inactivated mixture B_T was prepared in ice-cold maintenance medium.

5.5 The virus titre for B_T was determined using quantal test according to Sections 1.4 to 1.8.

6. Reference Test *C*: Verification of Virus Inactivation

6.1 200 µL of the virus test suspension and 800 µL of PBS were mixed with 1 mL of 1.4% (w/v) formaldehyde.

6.2 The tube was mixed and the stopwatch was started at once. The tube was placed in a cooled incubator controlled at the chosen test temperature θ for the contact time t.

6.3 Immediately at the end of t, the tube was mixed and the activity of the product test solution was inactivated or removed using one of the following methods:

6.3.1 Dilution in ice-cold maintenance medium. 20 µL of the mixture was transferred into 180 µL ice-cold maintenance medium and put in an ice bath, or if the cytotoxicity of the formaldehyde is too high,

6.3.2 Ultrafiltration using MicroSpin™ S-400 HR columns. 100 µL of the mixture is transferred to the column and the residual virus was obtained as per manufacturer's instructions.

6.4 Within 30 minutes of product inactivation, a series of ten-fold dilutions of the inactivated mixture was prepared in ice-cold maintenance medium.

6.5 The virus titre for *C* was determined using quantal test according to Sections 1.4 to 1.8.

6.6 The cytotoxicity effect for formaldehyde was determined according to Section 3, using the same inactivation method chosen for the reference test *C*.

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