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Virucidal activity testing of STERIPLANT N

Producer: OBISAN – Institute for Biotechnological Research and Development Murska Sobota

Testing report of virucidal activity following the test method and requirements of the European Standard EN 14476:2013+A2:2019

Ljubljana, 08. January 2020

Testing product: STERIPLANT N, disinfectant

Product received in laboratory for testing: 19.11.2019

Note: the producer delivered 100% testing product in a plastic container, 2x 2 litre volume. The material safety sheet was enclosed (regulation (ES) no. 1907/2006), Version 1.1, revision on 20.04.2018. The testing solution was delivered in original packaging, no visible damage, originally closed.

Testing period: 21.11.2019 – 17.12.2019

Ordered by:
OBISAN – Institute for Biotechnological Research and Development Murska Sobota
Ulica Ivana Regenta 2
9000 Murska Sobota

Producer:
OBISAN – Institute for Biotechnological Research and Development Murska Sobota
Ulica Ivana Regenta 2
9000 Murska Sobota

Testing performed by: Andrej Steyer, BSc, PhD, Senior Research Fellow

Cell culture preparation: Snežana Kramar, sanit. ing.

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1. PURPOSE

Based on the order of OBISAN Institute for Biotechnological Research and Development Murska Sobota, Slovenia (costumer), a disinfectant STERIPLANT N (product) was tested for virucidal activity – focused on antiviral activity against model unenveloped viruses.

2. TESTING PROCEDURE

Testing was performed based on the test method and requirements of the European Standard EN 14476:2013+A2:2019 (1) – Chemical disinfectants and Antiseptics – Quantitative suspension test for the evaluation of virucidal activity in the medical area.

2.1. REQUIREMENTS

The requirement of the costumer was to obtain the testing results for virucidal activity, preferentially against norovirus on surfaces. Testing was consequently adopted with standard procedure requirements for virucidal activity testing on surfaces in clean conditions:

- Limited spectrum virucidal activity, using adenovirus and murine norovirus; this requirements covered virucidal activity against enveloped viruses (e.g. paramyxoviruses, orthomyxoviruses, coronaviruses, herpesviruses,...) and noroviruses, rotaviruses and adenoviruses.
- Test temperature between 4 °C and 30 °C; testing was performed at room temperature, range 22 °C – 23 °C.
- Contact time (virus exposure to product) no longer than 60 minutes; it was agreed with costumer to test the following contact times: 5 minutes, 10 minutes in 30 minutes.
- Interfering substance; testing was performed in clean conditions, according to the standard procedure the interfering substance was 0.3 g/l bovine serum albumin solution (BSA).
- There was no other requirements expressed by costumer.

2.2. MATERIALS AND REAGENTS

TESTING PRODUCT:

STERIPLANT N, disinfectant, delivered by costumer to the Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana on November 19th 2019 at 11:00 AM, 2x 2 litre of testing product in original plastic container, without visible damage. The product was transparent liquid, without colour and smell. A safety document was enclosed (version 1.1., revised 20.04.2018). According to the safety sheet document, the test product is water suspension with the following content (transcript from the safety data document, paragraph 3.2., page 2/7):

Active chlorine – 0.035%; potassium chlorate – 0.0015%; chlorine dioxide – 0.0011%; ozone – 0.00002%

TESTING SYSTEM:

- Adenovirus type (species) C, genotype 2, 4th passage from the origin isolate, isolation on 15.04.2015.
- Murine norovirus strain S99, obtained on 13.04.2016 from Biobank Friedrich-Loeffler Institute, Germany – Cat.no. RVB-0651, batch 19/150609 passage RAW 264.7_{22}, isolation on 25.04.2016.
- Cell culture for adenovirus: cell line A549 (adenocarcinoma human alveolar basal epithelial cells), passage for testing 3-5.
- Cell culture form urine norovirus: cell line RAW 264.7 (monocyte macrophages from mouse (*Mus musculus*, BALB/c inbred line, transformed with Murine Leukemia Virus, MuLV), obtained from Friedrich-Loeffler Institute, Cat.no. CCLV-RIE 996, passage for testing 3-5.

REAGENTS:

- Cell culture media: Dulbecco's Minimal Essential Medium (MEM)

  Producer: Gibco, Cat. no. 21885-025; Lot: 2094710, Expire date: 2020-03-31 and Lot: 2095794, Expire date: 2020-09-30
- Foetal Bovine Serum (FBS)

  Producer: EuroClone®, Cat.no. ECS0180L; Lot: EUS026481, Expire date: 04/2020

- Interfering substance – Bovine Serum Albumin water suspension (BSA) (0.3 g/100 ml), sterile, prepared 20.11.2019; stable for 1 month.

- Phosphate buffer saline (PBS), sterile, prepared on 20.11.2019; stable for 3 months.
- Formaldehyde 1.4%, prepared on 21.11.2019; stable for 3 months.
- Hard water – used for test product dilutions, freshly prepared under aseptic conditions on the day of test performance following the standard procedure (used within 12 hours)

3. VIRUS PREPARATION AND DETERMINATION OF $TCID_{50}$ CONCENTRATION

Both testing viruses (murine norovirus and adenovirus) were propagated in susceptible cell lines and isolates were aliquoted in 1 ml vials, stored at -80 °C. Both source isolates were thawed immediately prior to inoculation on cell culture.

The virus concentration was determined before testing the product. The Spearman-Kärber (2) method was used for concentration calculation, as described in the standard procedure EN 14476:2013+A2:2019, Annex C. Virus titration and concentration calculation is described in details in chapter 4 of this report.

Testing virus strains:

- **adenovirus type (species) C, genotype 2.** The isolate was previously propagated in cell culture and characterized in details. Cell line A549 was used with cell culture media Dulbecco's MEM supplemented with 10% FBS (foetal bovine serum). Isolate was titrated and the 50% tissue culture infectious dose per millilitre ($TCID_{50}$/ml) was determined based on the Spearman-Kärber method (2). The source concentration of our isolate was **$10^{8.75}$ $TCID_{50}$/ml** (Table 1). Stock virus suspension was stored at -80 °C.
- **Murine Norovirus, strain S99**. Virus isolate was previously propagated in cell culture and characterized in details. Cell line RAW 264.7 was used with cell culture media Dulbecco's MEM supplemented with 10% FBS. Isolate was titrated and the 50% tissue culture infectious dose per millilitre ($TCID_{50}$/ml) was determined based on the Spearman-Kärber method (2). The source concentration of our isolate was **$10^{8.25}$ $TCID_{50}$/ml** (Table 1). Stock virus suspension was stored at -80 °C.

Table 1: Stock virus concentration used in testing.

Testing virus	Source virus isolate (date of isolation)	Concentration ($log_{10}(TCID_{50}/ml)$)	95% Confidential interval of Log. concentration
Adenovirus	15.04.2015	8.75	±0.33
Murine Norovirus	25.04.2016	8.25	±0.44

4. TESTING METHODS AND PROCEDURE

For testing performance, the standard procedure and methods were followed, based on EN 14476:2013+A2:2019.

Quantal virus titration test on monolayers of cells on microtitre plates was used to determine the concentration of infective viruses. Each dilution of virus suspension was inoculated in 8 well-replicates of 100 µl volume on cell monolayer, incubated for 1 hour at 37 °C, 80% humidity and 5% CO_2 atmosphere. All cell-based tests were incubated at these specific conditions.

The Spearman-Kärber (2) method was used for virus titre determination, using the equation:

$$m = x_k + \frac{d}{2} - d\sum p_i$$

(m – negative decimal logarithm of the titre based on the test volume; x_k – logarithm of lowest dose (dilution level) at which all test objects exhibit a positive reaction; d – logarithm of dilution factor; p_i – observed reaction rate)

The standard error (S_m) for m was calculated as follows:

$$S_m = \sqrt{d^2 \sum \{[p_i(1-p_i)]/(n-1)\}}$$

(S_m – standard error of logarithmic titre; d – logarithm of dilution factor; p_i – observed reaction rate; n – number of test objects per dilution)

95% confidence interval for the difference between the logarithmic virus titre before and after exposure to the product test solution was calculated as follows:

$$K_m = 2 \times \sqrt{S_a^2 + S_b^2}$$

($K_{(m)}$ – 95% confidence interval of the reduction in titre before and after exposure; S_a – standard error of virus titration before the exposure; S_b – standard error of virus titration after the exposure)

For the inhibition of test product, we used suppression of product's activity with 10-times dilution of test system (virus+test product+interfering substance) in ace-cold medium. Medium was prepared in 15 ml tube and incubated in ice-bath prior to use.

In parallel of the virucidal activity, all appropriate controls were included:

- Cells control, included in each test
- Virus control, included in each test
- Virus control for the total contact time of the test
- Suppression efficiency of product's activity
- Interference control – control of cell susceptibility
- Cytotoxic effect control
- Reference test for virus inactivation (Formaldehyde)

5. RESULTS

Raw data is presented in Table 8 and 9 of the Supplement.

5.1. RESULTS OF THE CONTROLS

Cells and virus controls were followed continuously in each test, all controls were OK.

Virus control for the total contact time of the test – virus suspension was incubated in ice-cold media for the maximal contact time in test. This was a control of virus stability in condition of the test performance. Within the time of incubation, no significant reduction of infective virus concentration was observed, not for adenoviruses nor for murine noroviruses. Results are shown in Table 2.

Table 2: Stability of infective viruses within the maximal contact time.

Virus	*Virus titre in 5 minute incubation time ($log_{10}(TCID_{50}/ml)$ ±95% confidence interval)*	*Virus titre in 30 minute incubation time ($log_{10}(TCID_{50}/ml)$ ± 95% confidence interval)*	Titre difference
Adenovirus	9.13±0.37	9.38±0.25	<1
Murine norovirus	8.63±0.53	8.50±0.46	<1

Suppression efficiency of product's activity was performed for each virus and test product concentration. Results are shown in Table 3.

Table 3: Suppression efficiency of product's activity.

Virus	Test product concentration	Virus titre before exposure *($log_{10}(TCID_{50}/ml)$ ± 95% confidence interval)*	Virus titre after exposure *($log_{10}(TCID_{50}/ml)$ ± 95% confidence interval)*	Titre difference
Adenovirus	80%	8.75±0.33	8.75±0.44	<1
	40%		8.00±0.38	<1
Murine norovirus	80%	8.25±0.44	8.13±0.45	<1
	40%		8.13±0.45	<1

The suppression effect of ice-cold media on test product was confirmed. Virus titre was not affected comparing the titre before and after the exposure to test product in ice-cold media. The titre difference did not exceed 1 log concentration.

Interference control – control of cell susceptibility was performed to follow the effect of the test product to cells and how the test product affect the susceptibility of cells for virus, influencing the determination of virus titre. First, cells were exposed to the highest non-cytotoxic concentration of the test product following by virus titration on threated cells. Results of virus titrations on exposed and non-exposed cells are shown in Figure 1 and 2.

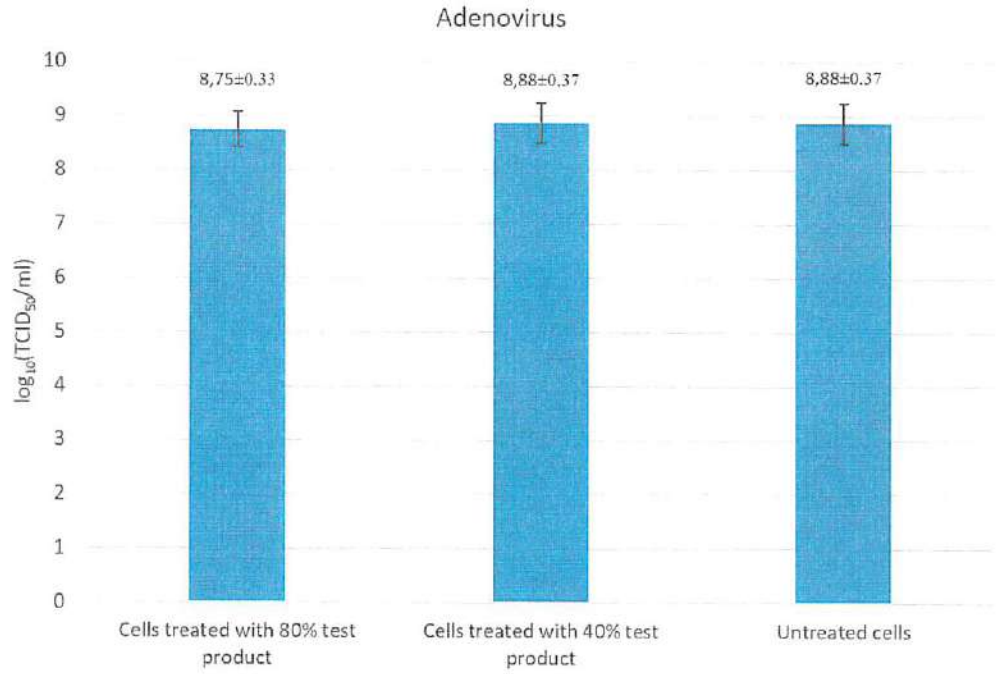

Figure 1: The effect of test product on adenovirus titration.

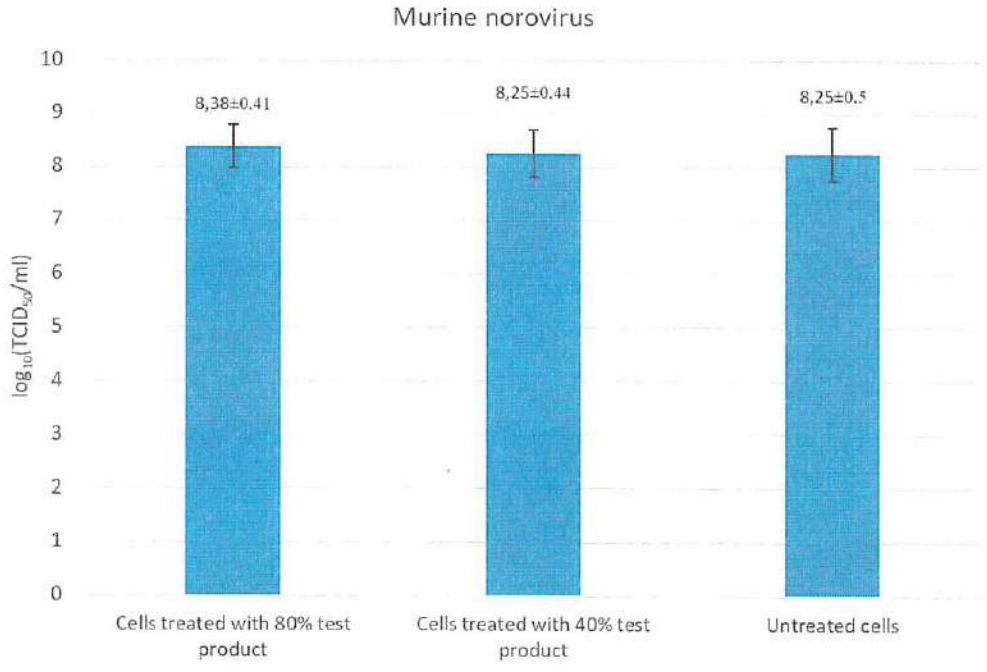

Figure 2: The effect of test product on murine norovirus titration.

As shown in Figure 1 and 2, no effect of test product on virus titration was observed. The accepted chriteria is <1 log concentration difference, which was not reached in our case.

Cytotoxic effect control was tested for all concentrations of the test product, also for the reference test product (Formaldehyde) in both cell lines used in this test.

For the test product, no cytotoxic effect was observed in the range of 10^{-1} to 10^{-10} dilutions of the source test product. Within this tested dilutions no effect on cell morphology nor cell monolayer destructions were observed.

Contrarily, in both cell lines cytotoxic effect was observed for the reference inactivation test with 0.7% formaldehyde for dilutions 10^{-1} to 10^{-3}. The observed cytotoxic effect was considered in results interpretation of the reference inactivation test (Cytotoxic level, Figures 3-6).

Reference test for virus inactivation in 0.7% formaldehyde was included as the testing system control, where acceptance criteria according to EN 14476:2013+A2:2019 must be fulfilled for viruses included in testing scheme.

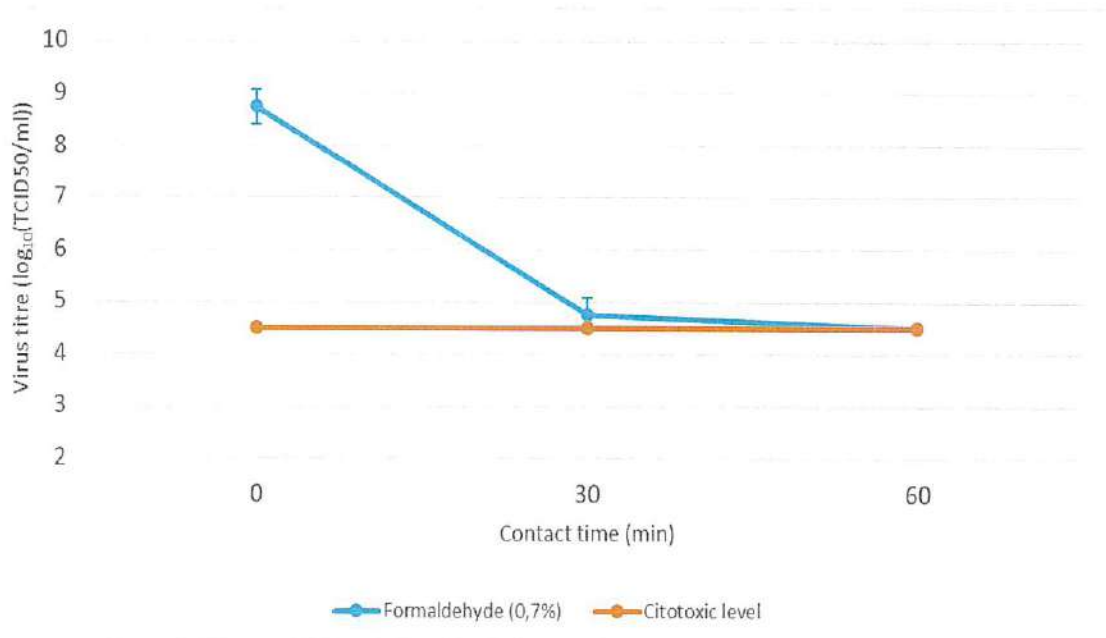

Figure 3: Reference inactivation test for adenovirus.

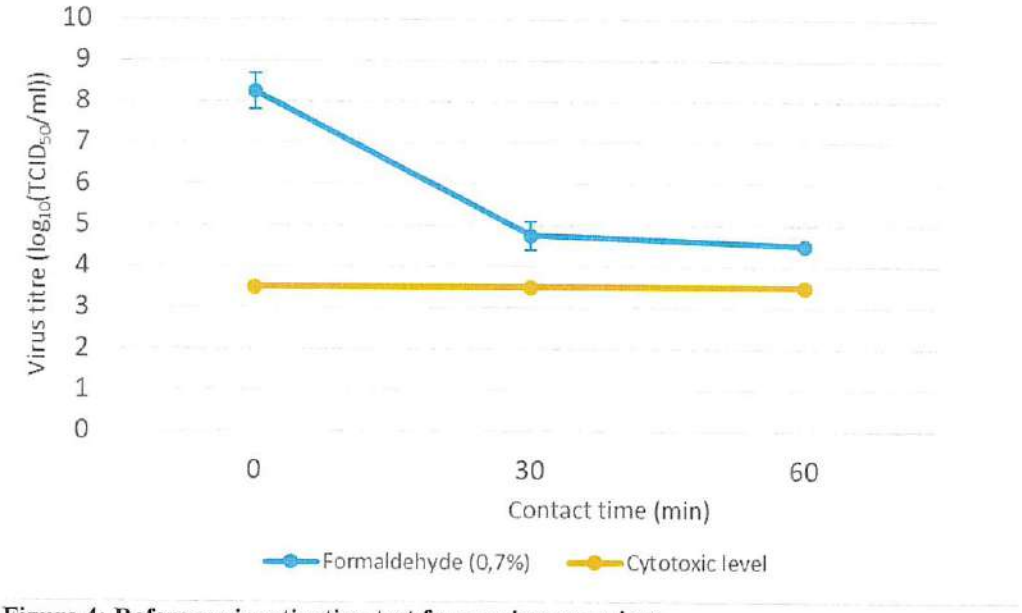

Figure 4: Reference inactivation test for murine norovirus.

As shown in Figures 3 and 4 and in tables 4-7 (Supplement) more than 4 log reduction of concentration of infective viruses was achieved by the reference inactivation test with 0.7% formaldehyde.

5.2. TEST PRODUCT EFFECTIVENESS IN VIRUS INACTIVATION

For test product, three contact times were tested, 5 minutes, 10 minutes and 30 minutes and in two concentrations, 80% and 40% of the original product. Results are shown in Figures 5 and 6 and numerically in tables 4-7 (Supplementary data).

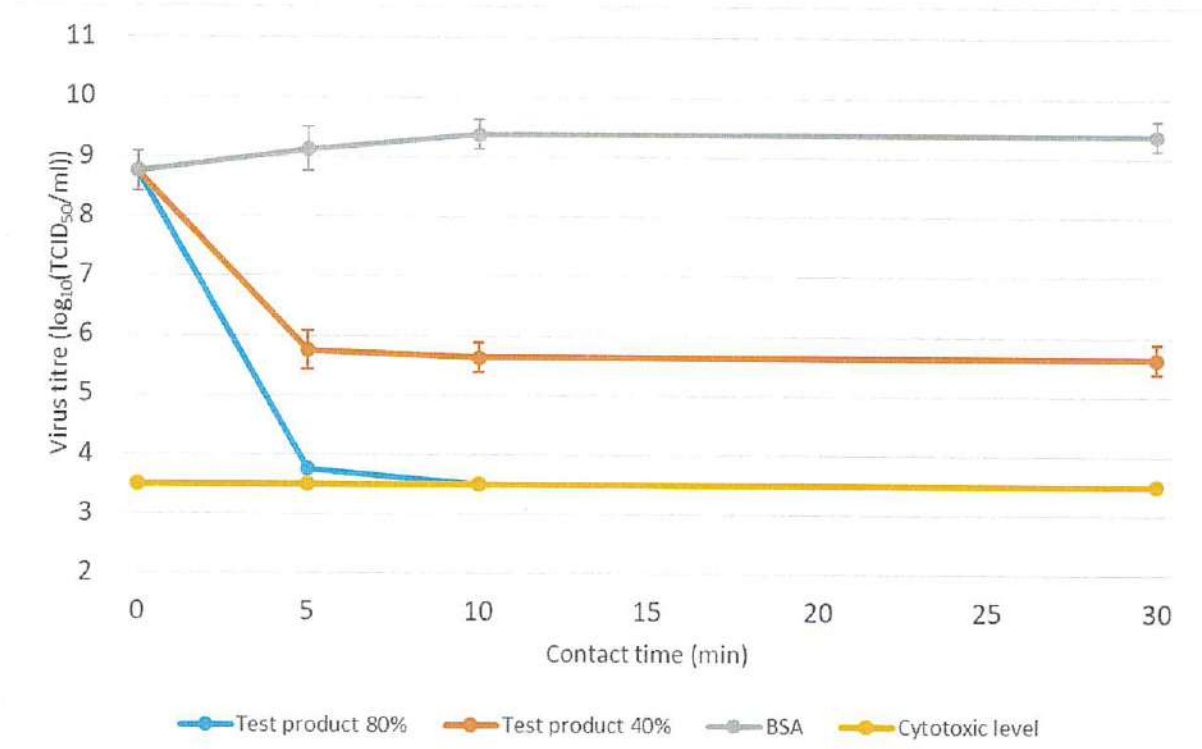

Figure 5: Adenovirus exposure to test product.

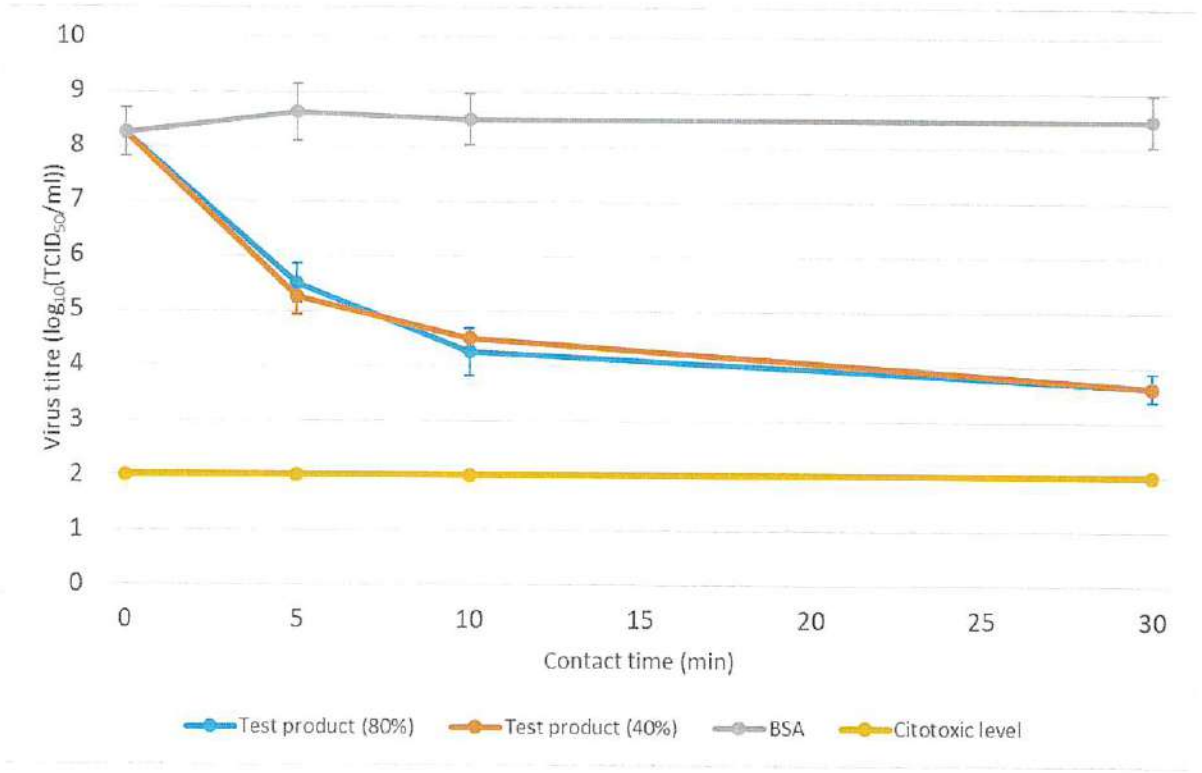

Figure 6: Murine norovirus exposure to test product.

For adenovirus, a rapid decline in virus concentration was achieved after exposure of virus to the 80% test product for only 5 minutes, showing a 5 log reduction in infective virus concentration. However, only 3 log concentration reduction was shown in 5 minutes for 40% test product, without considerable changes after 10 and 30 minutes.

The dynamic of the test product effect on murine norovirus was different compared to adenovirus. After 5 minutes of virus exposure to 80% test product only a 2.75 log reduction in concentration was observed and after 10 minutes, the concentration reduction was 4 log. In 40% test product, very similar dynamic was observed, with 4 log concentration reduction after more than 10 minutes (between 10 and 30 minutes).

6. CONCLUSIONS

The test product, STERIPLANT N (Obisan, Institute for Biotechnological Research and Development Murska Sobota), has according to our results virucidal activity with a demanding 4 log reduction in virus concentration, reached after 10 minutes of virus exposure to 80% test product. Testing was performed with two model viruses, adenovirus and murine norovirus. According to our testing conditions, it is possible to confirm, that STERIPLANT N has virucidal activity against enveloped viruses (e.g. paramyxoviruses, orthomyxoviruses, coronaviruses, herpesviruses,...), noroviruses, rotaviruses and adenoviruses.

Comment: Based on producer's information, STERIPLANT N is used with fogging. According to this, it would be very informative to test the product with fogging in a controlled environment with viruses inoculated on a defined area of a sterile carrier on the surface. Although this can result in an extensive additional testing, it would give us comparable results, close to real conditions. Testing with standard procedure EN 14476:2013+A2:2019 is for antiviral testing in suspensions.

Testing of STERIPLANT N was performed following the test method and requirements of the European Standard EN 14476:2013+A2:2019 and is relating only to the test conditions and usage of the test product in clean conditions for disinfection of surfaces.

Total experimental procedure in this report was performed at the Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana (IMI). IMI is certified from the Ministry of Health of the Republic of Slovenia with allowance to perform analytical laboratory diagnostics in

medicine, fulfilling the demands of the regulations in the field of laboratory medicine in Slovenia (Ur. L. RS 64/2004), which is based on ISO 15189 Medical laboratories — Requirements for quality and competence. Since 2009, IMI possess also a certificate ISO 9001 and is yearly involved in external audit. All the equipment and machines are evidently checked and personal is constantly trained and is competent to perform analytical work.

7. REFERENCES

1. 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); EN 14476:2013+A2:2019.
2. Kärber G. *Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Arch. Exp. Path. Pharmak. 1931, Vol.162 pp. 480-487. Springer-Verlag* doi:10.1007/BF01863914

8. SUPPLEMENTAL MATERIAL

Table 4: Results of virus (adenovirus species C, genotype 2) exposure to the test product.

	Concentration	Interfering substance	Cytotoxic level	Log_{10} $TCID_{50}$/ml with 95% confidence interval for the contact time					≥4 log_{10}/ml reduction at contact time
				0 min	5 min	10 min	30 min	60 min	
TEST PRODUCT	80 %	3.0 g/l BSA	2.0	8.75±0.33	≤3.75	≤3.5	≤3.5	NT	≤5 min
TEST PRODUCT	40 %	3.0 g/l BSA	2.0	8.75±0.33	5.75±0.33	5.63±0.25	5.63±0.25	NT	>30 min
Formaldehyde	0.7 %	PBS	4.5	8.75±0.33	NT	NT	≤4.75	≤4.5	≥30 min
Virus control	/	3.0 g/l BSA	/	8.75±0.33	9.13±0.37	NT	9.38±0.25	NT	/

BSA – Bovine Serum Albumin; PBS –Phosphate Buffer Saline; NT – not tested;

Table 5: Results of the virus (adenovirus species C, genotype 2) concentration reduction after the exposure to the test product.

	Concentration	Interfering substance	Cytotoxic level	Log_{10} $TCID_{50}$/ml with 95% confidence interval for the contact time					≥4 log_{10}/ml reduction at contact time
				0 min	5 min	10 min	30 min	60 min	
TEST PRODUCT	80 %	3.0 g/l GSA	2.0	/	≥5.00	≥5.25	≥5.25	NT	≤5 min
TEST PRODUCT	40 %	3.0 g/l GSA	2.0	/	3.00±0.47	3.12±0.41	3.12±0.41	NT	>30 min
Formaldehyde	0.7 %	PBS	4.5	/	NT	NT	>4.00	>4.25	≤30 min

BSA – Bovine Serum Albumin; PBS –Phosphate Buffer Saline; NT – not tested;

Table 6: Results of virus (adenovirus species C, genotype 2) exposure to the test product.

	Concentration	Interfering substance	Cytotoxic level	Log_{10} $TCID_{50}$/ml with 95% confidence interval for the contact time					≥4 log_{10}/ml reduction at contact time
				0 min	5 min	10 min	30 min	60 min	
TEST PRODUCT	80 %	3.0 g/l BSA	2.0	8.25±0.44	5.50±0.35	4.25±0.44	3.63±0.25	NT	10 min
TEST PRODUCT	40 %	3.0 g/l BSA	2.0	8.25±0.44	5.25±0.33	≤4.50	≤3.63	NT	10-30 min
Formaldehyde	0,7 %	PBS	4.5	8.25±0.44	NT	NT	4.75±0.33	≤4.5	≥60 min
Virus control	/	3.0 g/l BSA	/	8.25±0.44	8.63±0.53	NT	8.50±0.46	NT	/

BSA – Bovine Serum Albumin; PBS –Phosphate Buffer Saline; NT – not tested;

Table 7: Results of the virus (murine norovirus strain S99) concentration reduction after the exposure to the test product.

	Concentration	Interfering substance	Cytotoxic level	Log_{10} $TCID_{50}$/ml with 95% confidence interval for the contact time					≥4 log_{10}/ml reduction at contact time
				0 min	5 min	10 min	30 min	60 min	
TEST PRODUCT	80 %	3.0 g/l BSA	2.0	/	2.75±0.56	4.00±0,62	4.62±0.51	NT	10 min
TEST PRODUCT	40 %	3.0 g/l BSA	2.0	/	3.00±0.55	≥3.75	≥4.62	NT	10-30 min
Formaldehyde	0.7 %	PBS	4.5	/	NT	NT	3.50±0.55	>3.75	>60 min

BSA – Bovine Serum Albumin; PBS –Phosphate Buffer Saline; NT – not tested;

Table 8: Raw data for adenovirus (species C, genotype 2) on cell line A549.

Testing material	Concentration of testing material	Interfering substance	Contact time (min)	dilutions (log_{10})[a]								
				2	3	4	5	6	7	8	9	10
Virus control	/	0.3 g/l BSA	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0002 0300	0000 0000	0000 0000
		0.3 g/l BSA	30	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3444 3344	1310 3222	0000 0000	0000 0000
Test product (STERIPLANT N)	80%	0.3 g/l BSA	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0002 0300	0000 0000	0000 0000
		0.3 g/l BSA	5	/	0301 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
		0.3 g/l BSA	10	/	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
		0.3 g/l BSA	30	/	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	40%	0.3 g/l BSA	0	/	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0002 0300	0000 0000	0000 0000
		0.3 g/l BSA	5	/	4444 4444	3112 3411	0010 0100	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
		0.3 g/l BSA	10	/	4444 4444	3241 1211	0000 0100	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
		0.3 g/l BSA	30	/	4444 4444	3331 1222	0000 0100	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
STERIPLANT N (cytotoxic test)	80%	0.3 g/l BSA	/	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	40%	0.3 g/l BSA	/	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
Formaldehyde	0.7% (w/v)	PBS	30	/	/	0101 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
		PBS	60	/	/	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
Formaldehyde (cytotoxic test)		PBS	/	4444 4444	3333 3333	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000

Table 9: Raw data for murine norovirus on cell line RAW 264.7.

Testing material	Concentration of testing material	Interfering substance	Contact time (min)	Dilutions (log_{10})[a]								
				2	3	4	5	6	7	8	9	10
Virus control	/	0.3 g/l BSA	0	4444 4444	4444 4444	4444 4444	4444 3434	4444 4344	0400 1311	0000 0100	0000 0000	0000 0000
	/	0.3 g/l BSA	30		4444 4444	4444 4444	4444 4444	4444 4444	4343 1001	0100 0010	0000 0000	0000 0000
Test product (STERIPLANT N)	80%	0.3 g/l BSA	0	4444 4444	4444 4444	4444 4444	4444 3434	4444 4344	0400 1311	0000 0100	0000 0000	0000 0000
		0.3 g/l BSA	5		2443 4443	2304 3234	0040 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
		0.3 g/l BSA	10		4403 0043	0000 0300	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
		0.3 g/l BSA	30		0000 4000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	40%	0.3 g/l BSA	0	4444 4444	4444 4444	4444 4444	4444 3434	4444 4344	0400 1311	0000 0100	0000 0000	0000 0000
		0.3 g/l BSA	5		4434 4344	4344 0203	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
		0.3 g/l BSA	10		2404 0344	0000 3200	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
		0.3 g/l BSA	30		0000 0001	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
STERIPLANT N (cytotoxic test)	80%	0.3 g/l BSA	/	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	40%	0.3 g/l BSA	/	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
Formaldehyde	0.7% (w/v)	PBS	30	/	/	0040 0001	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
		PBS	60	/	/	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
Formaldehyde (cytotoxic test)		PBS	/	4444 4444	4444 4444	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000

a)

1-4	range of cytopathic effect in each well of microtiter plate
0	no cytopathic effect observed

b)

PBS – Phosphate Buffer Saline (pH 7,4)

BSA – Bovine Serum Albumin