

LABOR ENDERS

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Expert Opinion

on the efficacy of

SETASAN Desinfizierende Waschlotion

against the

**Modified vaccinia virus Ankara
(MVA)**

according to

DIN EN 14476:2019-10

PD Dr. Maren Eggers

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SETASAN care GmbH

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2021-10-29

Expert opinion

The efficacy of **SETASAN Desinfizierende Waschlotion** against the Modified vaccinia virus Ankara was tested in a suspension test according to the European standard DIN EN 14476:2019-10. The effectiveness of the disinfectant was evaluated under dirty conditions (3.0 g/l BSA + 3.0 ml/l erythrocytes) as interfering substance. **SETASAN Desinfizierende Waschlotion** was tested as a 10.0%, 20.0% and 50.0% solution. The exposure times were 15, 30 and 60 s. This expert opinion refers to the test report LI-021-359.

In conclusion, the 50.0% concentration of SETASAN Desinfizierende Waschlotion is effective against the Modified vaccinia virus Ankara at room temperature under dirty conditions (3.0 g/l BSA + 3.0 ml/l erythrocytes) with an application time of 15 seconds.

According to the Guidance on the Biocidal Products Regulation, Volume II, Parts B & C from April 2018, an efficacy against the Modified vaccinia virus Ankara enables to claim an efficacy against all enveloped viruses.

Moreover, Coronaviruses being included in the group of enveloped viruses as described in Annex A of the EN 14476:2019-10, it can be concluded that SETASAN Desinfizierende Waschlotion is effective against Coronavirus SARS-CoV-2.



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29.10.2021

Gutachterliche Stellungnahme

Das Prüfprodukt **SETASAN Desinfizierende Waschlotion** wurde auf seine virusinaktivierenden Eigenschaften gegenüber dem Modified Vacciniavirus Ankara untersucht. Die Prüfung erfolgte gemäß der Europäischen Norm DIN EN 14476:2019-10. Die Wirksamkeit des Desinfektionsmittels wurde unter hoher Belastung (3,0 g/l BSA + 3,0 ml/l erythrocytes) evaluiert. Das Produkt wurde in einer 10,0%igen, 20,0%igen und 50,0%igen Produktprüfkonzentration eingesetzt. Folgende Einwirkzeiten wurden geprüft: 15, 30 und 60 s. Diese gutachterliche Stellungnahme bezieht sich auf den Prüfbericht LI-021-359.

Zusammenfassend kann gesagt werden, dass SETASAN Desinfizierende Waschlotion in einer 50,0%igen Produktprüfkonzentration unter hoher Belastung (3,0 g/l BSA + 3,0 ml/l erythrocytes) nach 15 Sekunden das Modified Vacciniavirus Ankara inaktiviert.

Gemäß der Guidance on the Biocidal Products Regulation, Volume II, Parts B & C vom April 2018, entspricht eine Wirksamkeit gegen das Modified Vacciniavirus Ankara einer Wirksamkeit gegen alle behüllten Viren. Da die Coronaviren gemäß Anhang A der EN 14476:2019-10 in die Gruppe der behüllten Viren gehören, kann der Schluss gezogen werden, dass SETASAN Desinfizierende Waschlotion gegen Coronavirus SARS-CoV-2 wirksam ist.



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Leitung der Desinfektionsmittelprüfung

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Test report

on the efficacy of

SE-20-200

against the

**Modified vaccinia virus Ankara
(MVA)**

according to

DIN EN 14476:2019-10

SETASAN care GmbH
Hermann-Beuttenmüller-Straße 14
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2021-10-29

Test report

The efficacy of SE-20-200 against the Modified vaccinia virus Ankara in the virucidal quantitative suspension test for chemical disinfectants and antiseptics.

SE-20-200 was tested for its efficacy against the Modified vaccinia virus Ankara in a suspension test according to the European standard DIN EN 14476:2019-10. Under this standard, the product performance is tested against model viruses under defined test conditions, including temperature, contact time, or interfering substances, and the product should demonstrate at least a four $\lg_{10}$ reduction in the titre of the test strain. **SE-20-200** was examined as a 10.0%, 20.0% and 50.0% solution under dirty conditions (3.0 g/l BSA + 3.0 ml/l erythrocytes). The contact times were 15, 30 and 60 s.

Laboratory

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Identification of the sample

Laboratory project identification number	LI-021-359
Name of the product	SE-20-200
Batch number	201201HW1500
Manufacturer	SETASAN care GmbH
Appearance of the undiluted product	clear, liquid, green
Date of manufacture	2020-10
Expiry date	2023-10
Date of delivery	2021-10-15
Opened on	2021-10-18
Storage conditions	20.0 °C, dark
pH value undiluted (ion-selective electrode, VWR)	pH 5.84
Active compounds in 100 g	According to the manufacturer: 63.0 g Denatured Ethanol 96%, 0.90 g Sodium laurylEther Sulphate, 2.0 g Sodium chloride, 1.03 g Menthol B.P, 4.80 g Perfume, 190.20 g Purified Water B.P

Experimental conditions

Test period	2021-10-20 – 2021-10-27
Test temperature	20.0 °C ± 1.0 °C
Product test concentrations, pH value (ion-selective electrode, VWR)	10.0% (pH 7.09), 20.0% (pH 6.79), 50.0% (pH 6.62)
Contact times	15, 30 and 60 seconds
Interfering substance	dirty conditions (3.0 g/l BSA + 3.0 ml/l erythrocytes)
Diluent used for product test solution	hard water
Appearance of product dilutions	10.0%: slightly turbid
Stability and appearance of the mixture during procedure	10.0% and 20.0%: slightly turbid 50.0%: strongly turbid
Temperature of incubation	37.0 °C ± 1.0 °C, CO ₂ incubator (5.0% CO ₂)
Virus	Modified vaccinia virus Ankara
Virus source	Institute of Animal Hygiene and Veterinary Public Health in the Centre of Veterinary Public Health of the University Leipzig
Virus charge	070721
Virus, number of passage	n+4
Cell line	BHK-21 cells
Cell line, source	Friedrich Loeffler Institute
Cell line, number of passage	88 / 17

Test strain virus and cell culture line

Modified vaccinia virus Ankara was used as the test virus. BHK-21 cells, a cell line established from fibroblasts of newborn hamster kidneys, were used for virus cultivation and the suspension test. The host were cultivated at 37.0 °C in a humid atmosphere under 5.0% CO₂. The cells were fed with Dulbeccos Minimum Essential Medium (D-MEM) supplemented with heat-inactivated fetal calf serum (FCS) and non-essential amino acids. For the virus cultivation, confluent monolayers with a maximum age of 2 days were used.

The stock virus suspension was produced according to the directive. Cell debris was separated by low speed centrifugation. Aliquots of the virus suspension were stored at -70 °C.

Inactivation assay

The inactivation tests were run at 20.0 °C ± 1.0 °C. The virus suspension was added to the test solution of the product under dirty conditions (3.0 g/l BSA + 3.0 ml/l erythrocytes) as interfering substance.

The test assays were mixed in the following way:

1 part	(0.1 ml)	virus suspension
1 part	(0.1 ml)	3.0 g BSA/97 ml water + 3.0 ml erythrocytes (3.0 g/l BSA + 3.0 ml/l erythrocytes in the test mixture; dirty conditions)
8 parts	(0.8 ml)	12.5% dilution of the test product (10.0% in the test mixture) 25.0% dilution of the test product (20.0% in the test mixture) 62.5% dilution of the test product (50.0% in the test mixture)

**Due to the addition of test virus suspension and interfering substance an 80.0% solution resulted.*

virus control

1 part	(0.1 ml)	virus suspension
1 part	(0.1 ml)	3.0 g BSA/97 ml water + 3.0 ml erythrocytes (3.0 g/l BSA + 3.0 ml/l erythrocytes in the test mixture; dirty conditions)
8 parts	(0.8 ml)	water

cytotoxicity test

1 part	(0.1 ml)	water
1 part	(0.1 ml)	3.0 g BSA/97 ml water + 3.0 ml erythrocytes (3.0 g/l BSA + 3.0 ml/l erythrocytes in the test mixture; dirty conditions)
8 parts	(0.8 ml)	12.5% dilution of the test product (10.0% in the test mixture) 25.0% dilution of the test product (20.0% in the test mixture) 62.5% dilution of the test product (50.0% in the test mixture)

**Due to the addition of test virus suspension and interfering substance an 80.0% solution resulted.*

Determination of the residual virus titre by the end-point titration method

The test product **SE-20-200** was examined as a 10.0%, 20.0% and 50.0% solution. After the specified contact time, the virucidal activity was immediately suppressed by dilution with nine volumes of ice-cold medium (D-MEM + 2.0% FCS) and without delay the assay was serially diluted 10-fold. Due to the immediate titration, no aftereffect of the product could occur.

The infectivity was determined as an end point titration as follows: Six wells of a microtitre plate containing a confluent monolayer of BHK-21 cells were inoculated with 0.1 ml of each dilution, and the cells were incubated at 37.0 °C in a humidified atmosphere under 5.0% CO₂.

Determination of infectivity

After 7 days the cell cultures were stained with 50 µl crystal violet per well. The cells were microscopically examined for cytopathic effects (CPE). The cell culture results were recorded as "0" for no CPE and "1" (25.0% CPE) to "4" (100% CPE) depending on the degree of cell damage. The viral titre was calculated using the Spearman-Kärber-method (Br. J. Psychol. 2 (1908): 227-42, Arch. exp. Path. Pharmac. 162 (1931): 480-87).

Calculation of the virucidal activity of the products

The virucidal activity was determined by the difference of the logarithmic titre of the virus control minus the logarithmic titre of the test virus ($\Delta \log_{10} \text{TCID}_{50}/\text{ml}$).

Cytotoxic effect

To check for possible morphological alterations of BHK-21 cells caused by the test product **SE-20-200**, eight parts of the test product were diluted with one part of the interfering substance and one part of water. In the same way as for determining virus infectivity, serial dilutions (1:10) were prepared in culture medium and 100 μl were inoculated onto confluent BHK-21 cell monolayers in a 96 well plates.

Comparative virus titration on cells pre-treated with the test mixture

The comparative virus titration was performed on cells that had been treated with disinfectants to check the reduction in the sensitivity to the virus as follows: Cells were incubated for 1 h with 100 μl of a 10^{-3} dilution of the 50.0% solution of **SE-20-200**. Based on the results of the cytotoxicity test, only the lowest apparently non-cytotoxic dilution of the test mixture could be used. In parallel to the test mixture, cells were also incubated for one hour with PBS as a control. After 1 h at 37.0 °C the test solution was removed and the cells were infected with the titrated control virus. The assay is only valid if the virus control of mock-treated cells (no disinfectant) minus the virus control of cells pre-treated with **SE-20-200** resulted in less than a log difference.

Control of efficiency of suppression of product's activity

A control of efficacy for suppression of test product's activity was included according to EN 14476 (5.5.5.1). This so called aftereffect control should be conducted if, especially after short contact times, an uncontrolled aftereffect of the disinfectant due to methodology beyond contact time cannot be excluded. Generally, the time elapsed between the completion of the contact time and the preparation of the dilution series for titration shall not exceed 15–30 s. One part of test mixture according to EN 14476 (5.5.2) is mixed with nine parts ice-cold culture medium and incubated in an ice-cold bath for 30 min $\pm$ 10 s . After this period of time, a dilution series is prepared from the suspension in order to determine the titre. The disinfectant shows a negligible or non-existing aftereffect if the difference of this titre in comparison to the virus control is $\leq 0.5 \log_{10}$.

Reference virus inactivation assay

As a validity control of the test system, formaldehyde was selected for inactivation of the reference virus. The contact times were 5 min, 15 min, 30 min and 60 min. The test assays were mixed as follows:

1 part	(0.2 ml)	virus suspension
4 parts	(0.8 ml)	PBS
5 parts	(1.0 ml)	1.4% formaldehyde solution

To control the cytotoxicity of the formaldehyde test solution, 1.0 ml of 1.4% formaldehyde solution was added to 0.8 ml of PBS and 0.2 ml water. The infectivity of the virus control was determined at 0 min and 60 min. As a control, the formaldehyde solution was substituted with water. Immediately following the contact time, 0.2 ml of the test mixture was pipetted into a tube containing 1.8 ml ice-cold D-MEM + 2.0% FCS, which was then serially diluted ten-fold.

Results

Validity of the test

The test product dilutions caused cytotoxic effects as shown in Table 1. In Table 2, the comparative virus titration on cells treated with test mixture dilution or without resulted in a difference of less than one log.

The aftereffect control, which measures the efficiency of suppression of product's activity, shall be ≤ 0.50 lg. As indicated in Table 3, the control was 0.50 lg.

The results of the reference virus inactivation using formaldehyde are given in Table 4. The 0.7% formaldehyde solution was toxic for BHK-21 cells at the 1:100 dilution. The difference of the logarithmic titre of the virus control minus the test virus was $\geq 3.50 \pm 0.56 \log_{10}$ after 60 min.

Test results

The data of the virucidal efficacy is presented in Table 5. **SE-20-200** was tested as 10.0%, 20.0% and 50.0% solution under dirty conditions, and following exposure times of 15, 30 and 60 s, the reduction of virus titre was measured. With the 10.0% and 20.0% solution of **SE-20-200** no virucidal efficacy was obtained. However, the 50.0% solution of **SE-20-200** showed excellent virucidal activity against the Modified vaccinia virus Ankara under dirty conditions following a 15 seconds exposure time. Even the 20.0% solution was active within 60 s contact time.

Table 1: Cytotoxic factor of SE-20-200

Concentration	Interfering substance	Dilution (log ₁₀)						
		10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷
10.0%	dirty conditions	+	+	–	–	–	–	–
20.0%	dirty conditions	+	+	–	–	–	–	–
50.0%	dirty conditions	+	+	–	–	–	–	–

Table 2: Comparative virus titration on cells treated with the test mixture dilution

Concentration	Interfering substance	Titre of the virus control (log ₁₀ TCID ₅₀ /ml) PBS	Titre of the comparative virus titration (log ₁₀ TCID ₅₀ /ml)	Difference in virus titre (log ₁₀ TCID ₅₀ /ml)
50.0%	dirty conditions	7.00 +/- 0.45	7.17 +/- 0.42	0.17

Table 3: Control of efficiency of suppression of product's activity

Concentration	Titre of the virus control (log ₁₀ TCID ₅₀ /ml)	Titre of the aftereffect control titration (log ₁₀ TCID ₅₀ /ml)	Difference in virus titre (log ₁₀ TCID ₅₀ /ml)
50.0%	6.67 +/- 0.33	6.17 +/- 0.42	0.50

Table 4: Reference virus inactivation of Modified vaccinia virus Ankara with 0.7% formaldehyde

Cyto- toxicity	Titre of the virus control (0 min) (log ₁₀ TCID ₅₀ /ml) with 95.0% confidence interval	Titre of the virus control (60 min) (log ₁₀ TCID ₅₀ /ml) with 95.0% confidence interval	Titre of the “residual virus” inactivation (log ₁₀ TCID ₅₀ /ml) with 95.0% confidence interval				Reduction factor with 95.0% confidence interval			
			5 min	15 min	30 min	60 min	5 min	15 min	30 min	60 min
3.50 +/- 0.00	7.33 +/- 0.54	7.17 +/- 0.56	5.50 +/- 0.00	3.83 +/- 0.42	≤ 3.50 +/- 0.00	≤ 3.50 +/- 0.00	1.67 +/- 0.56	3.33 +/- 0.70	≥ 3.67 +/- 0.56	≥ 3.67 +/- 0.56

Table 5: Virucidal activity of SE-20-200 against the Modified vaccinia virus Ankara

Concentration	Interfering substance	Titre of the virus control (log ₁₀ TCID ₅₀ /ml) with 95.0% confidence interval	Level of cyto-toxicity	Titre of the reference virus inactivation (log ₁₀ TCID ₅₀ /ml) with 95.0% confidence interval			Reduction factor with 95.0% confidence interval		
				15 s	30 s	60 s	15 s	30 s	60 s
10.0%	dirty conditions	7.00 +/- 0.45	3.50	6.83 +/- 0.42	6.33 +/- 0.33	5.50 +/- 0.47	0.17 +/- 0.61	0.67 +/- 0.56	1.50 +/- 0.65
20.0%	dirty conditions	7.00 +/- 0.45	3.50	5.17 +/- 0.56	5.17 +/- 0.42	4.67 +/- 0.33	1.83 +/- 0.71	1.83 +/- 0.61	2.33 +/- 0.56
50.0%	dirty conditions	7.00 +/- 0.45	3.50	4.17 +/- 0.42	4.00 +/- 0.45	3.83 +/- 0.42	2.83 +/- 0.61	3.00 +/- 0.63	3.17 +/- 0.61

n.d. not done

Conclusion

The 50.0% solution of **SE-20-200** efficiently inactivates the Modified vaccinia virus Ankara at room temperature under dirty conditions within 15 seconds exposure time.

The following concentration and exposure times were active against enveloped viruses in the quantitative suspension test:

Dirty conditions	50.0%	15 seconds
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Archiving: The raw data with respect to this test and a copy of the report will be stored in the archive of Labor Enders MVZ.

Information: The test results exclusively refer to the samples described above. Account of extracts of this test report is only possible by written approval from Labor Enders MVZ.

The assessment of medical devices is not covered by accreditation.

Raw data for SE-20-200 tested against the Modified vaccinia virus Ankara under dirty conditions (quantal test; 6 wells)

2021-10-20 – 2021-10-27

Product	Con- centra- tion	Interfering substance	Contact time	Dilution (log ₁₀)							
				1	2	3	4	5	6	7	8
SE-20-200	10.0%	dirty conditions	15 s	xxx xxx	xxx xxx	444 444	444 444	444 334	404 000	000 000	000 000
SE-20-200	10.0%	dirty conditions	30 s	xxx xxx	xxx xxx	444 444	444 444	444 440	000 000	000 000	000 000
SE-20-200	10.0%	dirty conditions	60 s	xxx xxx	xxx xxx	444 444	344 404	000 004	000 000	000 000	000 000
Virus control		dirty conditions	60 s	444 444	444 444	444 444	444 444	444 444	303 004	000 000	000 000
Cytotoxicity	10.0%	dirty conditions		xxx xxx	xxx xxx	000 000	000 000	000 000	000 000	000 000	000 000
SE-20-200	20.0%	dirty conditions	15 s	xxx xxx	xxx xxx	444 444	440 040	400 000	000 000	000 000	000 000
SE-20-200	20.0%	dirty conditions	30 s	xxx xxx	xxx xxx	444 444	043 044	000 000	000 000	000 000	000 000
SE-20-200	20.0%	dirty conditions	60 s	xxx xxx	xxx xxx	444 444	400 000	000 000	000 000	000 000	000 000
Virus control		dirty conditions	60 s	444 444	444 444	444 444	444 444	444 444	303 004	000 000	000 000
Cytotoxicity	20.0%	dirty conditions		xxx xxx	xxx xxx	000 000	000 000	000 000	000 000	000 000	000 000

1–4 virus present, degree of CPE in cell culture units (6 wells of microtitre plates)

0 no virus present

x cytotoxic

Raw data for SE-20-200 tested against the Modified vaccinia virus Ankara under dirty conditions (quantal test; 6 wells)

2021-10-20 – 2021-10-27

Product	Con- centra- tion	Interfering substance	Contact time	Dilutions (log ₁₀)							
				1	2	3	4	5	6	7	8
SE-20-200	50.0%	dirty conditions	15 s	xxx xxx	xxx xxx	004 444	000 000	000 000	000 000	000 000	000 000
SE-20-200	50.0%	dirty conditions	30 s	xxx xxx	xxx xxx	020 404	000 000	000 000	000 000	000 000	000 000
SE-20-200	50.0%	dirty conditions	60 s	xxx xxx	xxx xxx	000 032	000 000	000 000	000 000	000 000	000 000
Virus control		dirty conditions	60 s	444 444	444 444	444 444	444 444	444 444	303 004	000 000	000 000
Cytotoxicity	50.0%	dirty conditions		xxx xxx	xxx xxx	000 000	000 000	000 000	000 000	000 000	000 000
Cell sensitivity to virus after preincubation with the test product				444 444	444 444	444 444	444 444	444 444	233 300	000 000	000 000
Cell sensitivity to virus after preincubation with PBS				444 444	444 444	444 444	444 444	444 444	000 114	000 000	000 000
Aftereffect control without product			30 min	444 444	444 444	444 444	444 444	444 334	000 200	000 000	000 000
Aftereffect control with product			30 min	xxx xxx	xxx xxx	444 444	444 444	244 040	000 000	000 000	000 000

1–4 virus present, degree of CPE in cell culture units (6 wells of microtitre plates)

0 no virus present

x cytotoxic

Raw data for 0.7% formaldehyde tested against the Modified vaccinia virus Ankara (quantal test; 6 wells)

2021-10-20 – 2021-10-27

Product	Con- centra- tion	Interfering substance	Con- tact time	Dilution (log ₁₀)								
				1	2	3	4	5	6	7	8	9
Formaldehyde	0.7%	PBS	5 min	xxx xxx	xxx xxx	444 444	343 424	000 000	000 000	000 000	000 000	000 000
Formaldehyde	0.7%	PBS	15 min	xxx xxx	xxx xxx	020 003	000 000	000 000	000 000	000 000	000 000	000 000
Formaldehyde	0.7%	PBS	30 min	xxx xxx	xxx xxx	000 000	000 000	000 000	000 000	000 000	000 000	000 000
Formaldehyde	0.7%	PBS	60 min	xxx xxx	xxx xxx	000 000	000 000	000 000	000 000	000 000	000 000	000 000
Virus control		PBS	0 min	444 444	444 444	444 444	444 444	444 444	004 344	000 040	000 000	000 000
Virus control		PBS	60 min	444 444	444 444	444 444	444 444	444 444	004 033	004 000	000 000	000 000
Cytotoxicity		PBS		xxx xxx	xxx xxx	000 000	000 000	000 000	000 000	000 000	000 000	000 000

1–4 virus present, degree of CPE in cell culture units (6 wells of microtitre plates)

0 no virus present

x cytotoxic