LABOR ENDERS

PD Dr. Maren Eggers

c/o Labor Prof. Dr. G. Enders MVZ GbR

Rosenbergstraße 85 • 70193 Stuttgart

Expert Opinion

on the efficacy of

Desinfektions-Handgel

against

Modified vaccinia virus Ankara (MVA)

PD Dr. Maren Eggers

c/o Labor Prof. Dr. G. Enders MVZ GbR
Rosenbergstraße 85
70193 Stuttgart

LABOR ENDERS

Akkreditiert nach DIN EN ISO/IEC 17025

DAkkS Deutsche Akkreditierungsstelle D-PL-17120-01-00

Labor Prof. Dr. G. Enders MVZ GbR • Rosenbergstraße 85 • 70193 Stuttgart

NEMADIA GmbH
Landwehrstraße 55

64293 Darmstadt

2020-07-07

The efficacy of the product **Desinfektions-Handgel** against Modified vaccinia virus Ankara was tested in a suspension test according to the European standard DIN EN 14476:2019-10. In the amendment of this standard, Modified vaccinia virus Ankara is the new test virus for the claim “virucidal efficacy against enveloped viruses”. The effectiveness of the disinfectant was evaluated under clean conditions (0.3 g/l BSA) as interfering substance. **Desinfektions-Handgel** was tested as a 20.0%, 40.0% and 80.0% concentration. The exposure times were 15, 30 and 60 seconds.

In conclusion, the 80.0% concentration of the test product Desinfektions-Handgel is effective against the Modified vaccinia virus Ankara at room temperature under clean conditions (0.3 g/l BSA) as interfering substance 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 Modified vaccinia virus Ankara enable to claim an efficacy against all enveloped virus. Moreover, Coronaviruses being included in the group of enveloped virus as described in Annex A of the EN 14476:2019-10, it can be concluded that Desinfektions-Handgel is effective against Coronavirus SARS-CoV-2.

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Das Prüfprodukt **Desinfektions-Handgel** wurde auf seine virusinakti¬vierenden Eigenschaften gegenüber dem Modifizierten Vacciniavirus Ankara untersucht. Die Prüfung erfolgte gemäß der Europäischen Norm DIN EN 14476:2019-10. Die Wirksamkeit des Desinfektionsmittels wurde unter geringer Belastung (0,3 g/l BSA) evaluiert. Das Produkt wurde in einer 20%igen, 40%igen und 80%igen Produktprüfkonzentration eingesetzt. Folgende Einwirkzeiten wurden geprüft: 15, 30 und 60 Sekunden.

Zusammenfassend kann gesagt werden, dass Desinfektions-Handgel in einer 80,0%igen Produktprüfkonzentration unter geringer Belastung (0,3 g/l BSA) nach 15 Sekunden das Modifizierte Vacciniavirus Ankara inaktiviert.

Gemäß der Guidance on the Biocidal Products Regulation, Volume II, Parts B & C vom April 2018, entspricht eine Wirksamkeit gegen das Modifizierten 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 Desinfektions-Handgel gegen Coronavirus SARS-CoV-2 wirksam ist.

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Efficacy of Desinfektions-Handgel against the Modified vaccinia virus Ankara in the virucidal quantitative suspension test for chemical disinfectants and antiseptics.

Test report

Desinfektions-Handgel 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 log reduction in the titre of the test strain. In the amendment of this standard, Modified vaccinia virus Ankara is the new test virus for the claim “virucidal efficacy against enveloped viruses for hygienic hand rub and hand wash products”. **Desinfektions-Handgel** was examined as a 20.0%, 40.0% and 80.0% concentration under clean conditions (0.3 g/l BSA). The contact times were 15, 30 and 60 seconds.

Laboratory

Labor Prof. Dr. G. Enders MVZ GbR
Rosenbergstr. 85
70193 Stuttgart
Germany

Identification of the sample

Laboratory project identification number	LI-020-225
Name of the product	**Desinfektions-Handgel**
Batch number	L-071420GGAC
Manufacturer	NEMADIA GmbH
Appearance of the undiluted product	clear, strongly viscous, colourless
Date of manufacture	2020-06-16
Expiry date	2022-06-15
Date of delivery	2020-06-22
Opened on	2020-06-26
Storage conditions	20.0°C, dark
pH value undiluted	pH 7.17
Active compounds	67.5g Ethanol / 100 g gel

Experimental conditions

Test period	2020-06-26 – 2020-07-03
Test temperature	20.0°C ± 1.0°C
Product test concentrations, pH value	20.0% (pH 6.39), 40.0% (pH 6.47), 80.0% (pH 6.97)
Contact times	15, 30 and 60 seconds
Interfering substance	clean conditions (0.3 g/l BSA)
Diluent used for product test solution	A. dest.
Appearance of product dilutions	20.0% and 40.0%: strongly turbid
Stability and appearance of the mixture during procedure	20.0% and 40.0%: slightly turbid 80.0%: slightly turbid, colour change to orange
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	300520
Virus, number of passage	n+4
Cell line	BHK-21 cells
Cell line, source	cell bank of the Friedrich-Loeffler Institut
Cell line, number of passage	87 / 16

Test strain virus and cell culture line

Modified vaccinia virus Ankara from the Institute of Animal Hygiene and Veterinary Public Health of the University Leipzig 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 cells of the cell bank of the Friedrich-Loeffler-Institut were cultivated at 37.0°C in a humid atmosphere under 5.0% CO_2. The cells were fed with Dulbeccos Minimum Essential Medium (D-MEM) supplemented with heat-inactivated foetal 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 at 2500 rpm for 10 minutes. 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 product test solution under clean conditions (0.3 g/l BSA) as interfering substance. The test assays were mixed in the following way:

Inactivity test

1 part	**(0.1 ml)**	virus suspension
1 part	**(0.1 ml)**	0.3 g/100 ml BSA (0.3 g/l BSA in the test mixture; clean conditions)
8 parts	**(0.8 ml)**	undiluted (80.0% in the test mixture) 25.0% dilution of the test product (20.0% in the test mixture) 50.0% dilution of the test product (40.0% in the test mixture)

virus control

1 part	**(0.1 ml)**	virus suspension
1 part	**(0.1 ml)**	0.3 g/100 ml BSA (0.3 g/l BSA in the test mixture; clean conditions)
8 parts	**(0.8 ml)**	A. dest.

cytotoxicity test

1 part	**(0.1 ml)**	A. dest.
1 part	**(0.1 ml)**	0.3 g/100 ml BSA (0.3 g/l BSA in the test mixture; clean conditions)
8 parts	**(0.8 ml)**	undiluted (80.0% in the test mixture) 25.0% dilution of the test product (20.0% in the test mixture) 50.0% dilution of the test product (40.0% in the test mixture)

Determination of infectivity

The test product **Desinfektions-Handgel** was examined as a 20.0%, 40.0% and 80.0% concentration. After the specified contact time, the virucidal activity was immediately suppressed by dilution with nine volumes of ice-cold medium (MEM + 2.0% FCS) and without delay the assay was serially diluted 10-fold. Due to the immediate titration, no after-effect of the product could occur. 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% CO_2.

After 7 days the cell cultures were stained with 50 µl crystal violet per well. The cells were examined microscopically 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 degree of the cell damage. The viral titre was calculated using the Spearman-Kärber-method (Br. J. Psychol. 2 (1908): 227-42, Arch. exp. Path. Pharmak. 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 (Δ $\log_{10}$ $TCID_{50}$/ml).

Cytotoxic effect

To check for possible morphological alterations of BHK-21 cells caused by the test product **Desinfektions-Handgel**, eight parts of the test product were diluted with one part of the interfering substance and one part of A.dest. 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 hour with 100 µl of a 10^{-2} dilution of the 80.0% concentration of **Desinfektions-Handgel**. Based on the results of the cytotoxicity test, only the lowest apparently non-cytotoxic dilution of the test mixture could be used. 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 **Desinfektions-Handgel** resulted in less than a log difference.

Control of efficiency of suppression of product's activity

100 µl of the undiluted product **Desinfektions-Handgel** and 850 µl of ice-cold medium with 2.0% FCS were mixed. Afterwards 50 µl of the virus inoculum were added and the mixture is incubated in an ice-bath for 30 min ± 10 s. A serial dilution in $\log_{10}$ steps was prepared and cell cultures were inoculated with the dilutions. The titre was determined. The difference between the titre of the suppression of virucidal activity (SVA) control and the titre of virus control should not exceed 0.50 $\log_{10}$.

Reference virus inactivation assay

As a validity control of the test system, formaldehyde was selected for inactivation of the reference virus. Contact times were 5, 15, 30 and 60 minutes. 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 for the cytotoxicity of the formaldehyde test solution, 1 ml of 1.4% formaldehyde was added to 0.8 ml of PBS. The infectivity of the virus control was determined at 0 min and 60 min. The formaldehyde solution was substituted by water. Immediately following the contact time, 0.2 ml of the test mixture was pipetted into a tube containing 1.8 ml ice-cold MEM + 2.0% FCS, which was then serially diluted ten-fold.

Results

The product **Desinfektions-Handgel** was tested as a 20.0%, 40.0% and 80.0% concentration under clean conditions (0.3 g/l BSA) as interfering substance and following exposure times of 15, 30 and 60 seconds.

Validity of the test

The test product dilutions caused cytotoxic effects as shown in Table 1. As shown 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 after-effect control, which measures the efficiency of suppression of product's activity, shall be ≤ 0.50 lg. As shown in Table 3, the control was 0.34 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 1:1000 dilution. The difference of the logarithmic titre of the virus control minus the test virus was ≥ 2.83 +/– 0.54 $\log_{10}$ $TCID_{50}$/ml after 60 min.

Test results

The data of the virucidal efficacy of the product **Desinfektions-Handgel** is presented in Table 5. The 20.0% and 40.0% concentration of **Desinfektions-Handgel** showed no virucidal activity. However, under clean conditions, the 80.0% concentration of **Desinfektions-Handgel** showed excellent virucidal activity against Modified vaccinia virus Ankara following a 15 seconds exposure time.

Table 1: Cytotoxic factor of Desinfektions-Handgel

Concentration	Interfering substance	Dilution (log_{10})						
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
20.0%	clean conditions	–	–	–	–	–	–	–
40.0%	clean conditions	**+**	–	–	–	–	–	–
80.0%	clean 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)
80.0%	clean conditions	7.33 +/– 0.61	7.50 +/– 0.60	**-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 after effect control titration (log_{10} $TCID_{50}$/ml)	Difference in virus titre (log_{10} $TCID_{50}$/ml)
80.0%	6.83 +/– 0.42	7.17 +/– 0.42	**-0.34**

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
4.50 +/– 0.00	7.50 +/– 0.60	7.33 +/– 0.54	5.33 +/– 0.33	≤ 4.50 +/– 0.00	≤ 4.50 +/– 0.00	≤ 4.50 +/– 0.00	**2.00 +/– 0.63**	**≥ 2.83 +/– 0.54**	**≥ 2.83 +/– 0.54**	**≥ 2.83 +/– 0.54**

Table 5: Virucidal activity of Desinfektions-Handgel 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 "residual 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**
20.0%	clean conditions	6.67 +/– 0.33	1.50	7.50 +/– 0.60	7.33 +/– 0.54	7.00 +/– 0.45	**-0.83 +/– 0.68**	**-0.67 +/– 0.63**	**-0.33 +/– 0.56**
40.0%	clean conditions	6.67 +/– 0.33	2.50	6.33 +/– 0.33	6.00 +/– 0.54	5.67 +/– 0.33	**0.33 +/– 0.47**	**0.67 +/– 0.63**	**1.00 +/– 0.47**
80.0%	clean conditions	6.67 +/– 0.33	2.50	≤ 2.50 +/– 0.00	≤ 2.50 +/– 0.00	≤ 2.50 +/– 0.00	**≥ 4.17 +/– 0.33**	**≥ 4.17 +/– 0.33**	**≥ 4.17 +/– 0.33**
80.0%	clean conditions	7.50 +/– 0.00	2.50	≤ 2.50 +/– 0.00	≤ 2.50 +/– 0.00	≤ 2.50 +/– 0.00	**≥ 5.00 +/– 0.00**	**≥ 5.00 +/– 0.00**	**≥ 5.00 +/– 0.00**
Mean reduction factor with 95.0% confidence interval against Modified vaccinia virus Ankara							**≥ 4.58 +/– 0.17**	**≥ 4.58 +/– 0.17**	**≥ 4.58 +/– 0.17**

n.d. not done

Conclusion

The 80.0% concentration of the test product **Desinfektions-Handgel** efficiently inactivates Modified vaccinia virus Ankara at room temperature under clean conditions (0.3 g/l BSA) within 15 seconds exposure time.

The following concentration and exposure time is active against enveloped viruses in the quantitative suspension test:

clean conditions	**undiluted**	**15 seconds**

PD Dr. rer. nat. Maren Eggers

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 the product Desinfektions-Handgel tested against the Modified vaccinia virus Ankara under clean conditions (quantal test; 6 wells)

2020-06-26 – 2020-07-03

Product	Con-centra-tion	Interfering substance	Contact time	Dilution (log_{10})							
				1	2	3	4	5	6	7	8
Desinfektions-Handgel	20.0%	clean conditions	15 s	444 444	444 444	444 444	444 444	443 433	210 202	200 100	000 000
			30 s	444 444	444 444	444 444	444 444	444 444	024 420	002 000	000 000
			60 s	444 444	444 444	444 444	444 444	443 334	003 012	000 000	000 000
Virus control			60 s	444 444	444 444	444 444	444 444	344 323	000 020	000 000	000 000
Cytotoxicity				000 000	000 000	000 000	000 000	000 000	000 000	000 000	000 000
Desinfektions-Handgel	40.0%	clean conditions	15 s	xxx xxx	444 444	444 444	333 432	121 230	000 000	000 000	000 000
			30 s	xxx xxx	444 444	444 444	444 333	000 101	000 100	000 000	000 000
			60 s	xxx xxx	444 444	444 444	444 444	000 001	000 000	000 000	000 000
Virus control			60 s	444 444	444 444	444 444	444 444	344 323	000 020	000 000	000 000
Cytotoxicity				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*

Raw data for the product Desinfektions-Handgel tested against the Modified vaccinia virus Ankara under clean conditions (quantal test; 6 wells)

2020-06-26 – 2020-07-03

Product	Con-centra-tion	Interfering substance	Contact time	Dilution (log_{10})							
				1	2	3	4	5	6	7	8
Desinfektions-Handgel	80.0%	clean conditions	15 s	xxx xxx	000 000	000 000	000 000	000 000	000 000	000 000	000 000
			30 s	xxx xxx	000 000	000 000	000 000	000 000	000 000	000 000	000 000
			60 s	xxx xxx	000 000	000 000	000 000	000 000	000 000	000 000	000 000
Virus control			60 s	444 444	444 444	444 444	444 444	344 323	000 020	000 000	000 000
Cytotoxicity				xxx xxx	000 000	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	043 044	000 032	000 000
Cell sensitivity to virus after preincubation with PBS				444 444	444 444	444 444	444 444	444 444	001 302	001 002	000 000
After-effect control without product			30 min	444 444	444 444	444 444	444 444	344 443	020 020	000 000	000 000
After-effect control with product			30 min	xxx xxx	444 444	444 444	444 444	332 344	012 230	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 the product Desinfektions-Handgel tested against the Modified vaccinia virus Ankara under clean conditions (quantal test; 6 wells)

2020-06-25 – 2020-07-03

Product	Con-centra-tion	Interfering substance	Contact time	Dilution (log_{10})							
				1	2	3	4	5	6	7	8
Desinfektions-Handgel	80.0%	clean conditions	15 s	xxx xxx	000 000	000 000	000 000	000 000	000 000	000 000	000 000
			30 s	xxx xxx	000 000	000 000	000 000	000 000	000 000	000 000	000 000
			60 s	xxx xxx	000 000	000 000	000 000	000 000	000 000	000 000	000 000
Virus control			60 s	444 444	444 444	444 444	444 444	444 344	333 244	000 000	000 000
Cytotoxicity				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*

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

2020-06-26 – 2020-07-03

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	xxx xxx	112 120	000 000	000 000	000 000	000 000	000 000
			15 min	xxx xxx	xxx xxx	xxx xxx	000 000	000 000	000 000	000 000	000 000	000 000
			30 min	xxx xxx	xxx xxx	xxx xxx	000 000	000 000	000 000	000 000	000 000	000 000
			60 min	xxx xxx	xxx xxx	xxx xxx	000 000	000 000	000 000	000 000	000 000	000 000
Virus control			0 min	444 444	444 444	444 444	444 444	444 444	140 014	020 001	000 000	000 000
Virus control			60 min	444 444	444 444	444 444	444 444	443 444	420 031	000 001	000 000	000 000
Cytotoxicity				xxx xxx	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*