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17489 GREIFSWALD
DEUTSCHLAND - GERMANY

DAkkS Deutsche Akkreditierungsstelle
D-PL-18411-01-01
D-PL-18411-01-02

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HYGIENE NORD GMBH, C/O BIOTECHNIKUM, W.-RATHENAU-STR. 49 A, D-17489 GREIFSWALD

NEMADIA GmbH

Landwehrstraße 55

64293 Darmstadt

Germany / Deutschland

CUSTOMER NUMBER	DATE
1881	July 31, 2020

REPORT 201407.V1

SEIFREI DESINFEKTIONS HAND-GEL

BACTERICIDAL AND YEASTICIDAL EFFICACY

(VAH 2015/2019)

Purpose

The bactericidal and yeasticidal efficacy of the product **SEIFREI Desinfektions Hand-Gel** (NEMADIA GmbH, Darmstadt, Germany) as a hygienic handrub product should be evaluated by in vitro tests in accordance with the "Requirements and methods for VAH-certification of chemical disinfection processes" **(VAH, 2015/2019)**.

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Test description

Order number:	A 20-0595
Manufacturer:	NEMADIA GmbH, Darmstadt, Germany
Product:	**SEIFREI Desinfektions Hand-Gel**
Batch number:	L-071420GGAC
Manufacture date:	not provided
Best before:	June 15, 2020
Sample number:	P 203647
Date of order:	May 28, 2020
Date of delivery:	June 22, 2020
Test date:	July 24, 2020 – July 31, 2020
Basis:	Requirements and methods for VAH-certification of chemical disinfection processes (VAH, 2015/2019)
Test organisms:	*Staphylococcus aureus* ATCC 6538 *Enterococcus hirae* ATCC 10541 *Pseudomonas aeruginosa* ATCC 15442 *Escherichia coli* K12 NCTC 10538 *Proteus mirabilis* ATCC 14153 *Candida albicans* ATCC 10231
Test solution:	80 %, 50 %, 10 %
Active ingredients in 100 g[1]:	67.5 g ethanol
Odour:	product specific, flowery
Appearance:	clear, colourless gel
Appearance of dilution:	clear, colourless viscous liquid
pH – value (pH-Meter):	100 %: 7.00 80 %: 6.69 50 %: 6.53 10 %: 6.34 WFI: 5.81
pH – value (pH-stripes):	100 %: 6
Neutralizer:	4 % Tween 80 + 3 % Saponin + 0.4 % Lecithin + 0.25 % SDS (Neutralizer XXIV)
Interfering substance:	0.03 % albumin (clean conditions)
Test temperature:	20 ± 1 °C
Incubation temperature:	36 ± 1 °C (*C. albicans*: 30 ± 1°C)

Test Method

MIC / Selection of neutralizer

The test is performed in accordance with the "Requirements and methods for VAH-certification of chemical disinfection processes". Accordingly, the following test organisms were used:

S. aureus	ATCC 6538	*P. aeruginosa*	ATCC 15442
E. coli K12	NCTC 10538	*E. hirae*	ATCC 10541
P. mirabilis	ATCC 14153	*C. albicans*	ATCC 10231

The test is performed using the following growth medium:

- Tryptone Soya Broth (TSB)
- Malt Extract Broth (MEB) for *C.albicans*

The product is diluted water for injection; tests are performed at room temperature (20 ± 1 °C). The test organisms are incubated at 36 ± 1 °C (*C. albicans*: 30 ± 1 °C). Detailed results presented in table 1.

Quantitative suspension test under dirty conditions

A test suspension of bacteria including an interfering substance (0.03 % albumin – clean conditions) is added to a sample of **SEIFREI Desinfektions Hand-Gel** (diluted with water for injection, if necessary). The mixture is maintained at 20 ± 1°C for the required contact time. At the end of the contact time, an aliquot is taken; the microbicidal activity in this portion is immediately neutralized. The number of surviving test organisms in each sample is determined by plating aliquots of the neutralized test suspensions and its dilutions. The reduction is calculated in relation to a sample containing water instead of the test product (water control, WFI control).

The experimental conditions, the non-toxicity of the neutralizer and the dilution-neutralization method are validated according to the VAH standard methods:

- Co 1 = Water control (WFI)
- Co 2 = Method validation (Dilution-neutralization method)
- Co 3 = Non-toxicity of the neutralizer

The tests were performed under dirty conditions (0.3 % albumin + 0.3 % sheep erythrocytes) using *S. aureus*, *E. hirae, P. aeruginosa*, *E. coli, P. mirabilis* and *C. albicans* as test-organisms.

Results are presented in tables 2 – 7.

Results [2]

Selection of neutralizer / Minimal inhibitory concentration (MIC)

In accordance with the test results presented in table 1, neutralizer XXIV (4 % Tween 80 + 3 % Saponin + 0.4 % Lecithin + 0.25 % SDS) was selected as a suitable neutralizer for the test bacteria and *C. albicans*. A product concentration of **10 %** was determined as the minimal inhibitory concentration at the methodically determined contact time of 48 h (72 for *C. albicans*).

Quantitative suspension test

According to the "Requirements and methods for VAH-certification of chemical disinfection processes" (2015/2019), the test product **SEIFREI Desinfektions Hand-Gel**, when applied at the concentration/contact time relation of at least **80 % / 15 s**, **possesses** bactericidal and yeasticidal efficacy ($\log_{10}$ RF ≥ 5 or $\log_{10}$ RF ≥ 4, respectively) at 20 °C under dirty conditions (0.3 % albumin + 0.3 % sheep erythrocytes) for reference strains *S. aureus*, *E. hirae, P. aeruginosa, E. coli, P. mirabilis and C. albicans* (Tab. 2 – 7).

Results are considered validated in accordance with VAH (2015/2019) requirements.

Greifswald, July 31, 2020

Dr. rer. med. (Dipl. Biol.) T. Koburger-Janssen
- General Manager -

Prof. Dr. med. A. Kramer
- MD for Hygiene and Environmental Medicine -

Table 1: Identification of a suitable neutralizer (according to VAH methods, 2015/2019)

Date:	July 31, 2020	**Order number:**	A 20-0595
Product:	**SEIFREI Desinfektions Hand-Gel**	**Sample number:**	P 203647
Test organism:	see table 1	**Batch-number:**	L-071420GGAC
Incubation temperature:	36 ± 1 °C (*C. albicans*: 30 ± 1°C)	**Test suspension:**	see table 1
Incubation time:	48 h		

concentration (%)	*S. aureus* 2.73*10^7 cfu /ml (7.44 log)				*E. hirae* 2.27*10^7 cfu/ml (7.36 log)				*E. coli* 1.66*10^7 cfu/ml (7.22 log)				*P. mirabilis* 2.32*10^7 cfu/ml (7.37 log)				*P. aeruginosa* 3.45*10^7 cfu/ml (7.54 log)				*C. albicans* 7.45*10^7 cfu/ml (7.87 log)			
	0	II	III	XXIV	0	II	III	XXIV	0	II	III	XXIV	0	II	III	XXIV	0	II	III	XXIV	0	II	III	XXIV
50	(+)	-	-	(+)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	+	+	(+)	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	(+)	(+)	-	-	-	-
10	+	+	+	+	(+)	+	+	+	-	-	(+)	-	+	-	+	-	-	(+)	(+)	(+)	-	-	-	-
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
control*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

0 = TSB / (*C. albicans* MEB)
II = 3 % Tween 80 + 3 % Saponin + 0.1 % L-Histidine + 0.1 % Cysteine
III = 3 % Tween 80 + 0.1 % Histidine + 0.3 % Lecithin+ 0.5 % Sodium-Thiosulfate
XXIV = 4 % Tween 80 + 3 % saponin + 0.4 % lecithin + 0.25 % SDS

*control = WFI
- = no growth
+ = growth
(+) = minimal growth

Table 2: Results of the quantitative suspension test (according to VAH methods, 2015/2019)

Date:	July 27, 2020	**Order number:**	A 20-0595
Product:	**SEIFREI Desinfektions Hand-Gel**	**Batch-number:**	L-071420GGAC
Test organism:	*S. aureus*	**Sample number:**	P 203647
Interfering substance:	0.03 % albumin		
Incubation time:	24 h – 48 h	**Neutralizer:**	XXIV
Test suspension :	$3.65*10^8$ cfu/1 ml (8.56 log)	**Incubation temperature:**	36 ± 1 °C
Method:	Dilution-neutralization-method	**Reaction temperature:**	20 ± 1 °C

concentration	dilution	time: 15 s			time: 30 s			time: 60 s		
		cfu / plate	log_{10} (x)	RF	cfu / plate	log_{10} (x)	RF	cfu / plate	log_{10} (x)	RF
80 %	1 ml 10^0	0	0.00	**≥ 6.61**	0	0.00	**≥ 6.66**	0	0.00	**≥ 6.51**
	0.1 ml 10^0	0			0			0		
	0.1 ml 10^{-1}	0			0			0		
	0.1 ml 10^{-2}	0			0			0		
50 %	1 ml 10^0	10	1.00	**5.61**	104	2.02	**4.65**	26	1.41	**5.09**
	0.1 ml 10^0	1			11			2		
	0.1 ml 10^{-1}	0			0			0		
	0.1 ml 10^{-2}	0			0			0		
10 %	1 ml 10^0	> 330			> 330			> 330		
	0.1 ml 10^0	> 330			> 330			> 330		
	0.1 ml 10^{-1}	> 330			> 330			> 330		
	0.1 ml 10^{-2}	> 330	> 5.52	**< 1.09**	> 330	5.52	**1.14**	> 330	> 5.52	**< 0.99**
WFI (co 1)	0.1 ml 10^{-1}	> 330			> 330			> 330		
	0.1 ml 10^{-2}	> 330			> 330			> 330		
	0.1 ml 10^3	> 330			> 330			> 330		
	0.1 ml 10^{-4}	41	**6.61**		46	**6.66**		32	**6.51**	

Controls and Validation:

		number of colonies [cfu/plate]	cfu /ml (with regard to the validation suspension)	log_{10} (x)
control 2 (co 2)	0.1 ml 10^0	> 330		
	0.1 ml 10^{-1}	55	5.50E+03	3.74
control 3 (co 3)	0.1 ml 10^0	> 330		
	0.1 ml 10^{-1}	38	3.80E+03	3.58
Validation suspension:			3.65E+03	3.56
		80 % - 60 s	controls o.k.?	**Yes**

Table 3: Results of the quantitative suspension test (according to VAH methods, 2015/2019)

Date:	July 27, 2020	**Order number:**	A 20-0595
Product:	**SEIFREI Desinfektions Hand-Gel**	**Batch-number:**	L-071420GGAC
Test organism:	*E. hirae*	**Sample number:**	P 203647
Interfering substance:	0.03 % albumin		
Incubation time:	24 h – 48 h	**Neutralizer:**	XXIV
Test suspension:	$1.66*10^8$ cfu/1 ml (8.22 log)	**Incubation temperature:**	36 ± 1 °C
Method:	Dilution-neutralization-method	**Reaction temperature:**	20 ± 1 °C

concentration	dilution	time: 15 s			time: 30 s			time: 60 s		
		cfu / plate	log_{10} (x)	RF	cfu / plate	log_{10} (x)	RF	cfu / plate	log_{10} (x)	RF
80 %	1 ml 10^0	0	0.00	**≥ 6.15**	0	0.00	**≥ 6.07**	0	0.00	**≥ 6.21**
	0.1 ml 10^0	0			0			0		
	0.1 ml 10^{-1}	0			0			0		
	0.1 ml 10^{-2}	0			0			0		
50 %	1 ml 10^0	32	1.51	**4.65**	4	0.60	**5.47**	0	0.00	**≥ 6.21**
	0.1 ml 10^0	2			0			0		
	0.1 ml 10^{-1}	0			0			0		
	0.1 ml 10^{-2}	0			0			0		
10 %	1 ml 10^0	> 330			> 330			> 330		
	0.1 ml 10^0	> 330			> 330			> 330		
	0.1 ml 10^{-1}	> 330			> 330			> 330		
	0.1 ml 10^{-2}	> 330	> 5.52	**< 0.64**	> 330	> 5.52	**< 0.55**	> 330	> 5.52	**< 0.69**
WFI (co 1)	0.1 ml 10^{-1}	> 330			> 330			> 330		
	0.1 ml 10^{-2}	> 330			> 330			> 330		
	0.1 ml 10^3	140	**6.15**		113	**6.07**		160	**6.21**	
	0.1 ml 10^{-4}	17			16			19		

Controls and Validation:

		number of colonies [cfu/plate]	cfu /ml (with regard to the validation suspension)	log_{10} (x)
control 2 (co 2)	0.1 ml 10^0	235	2.35E+03	3.37
	0.1 ml 10^{-1}	23		
control 3 (co 3)	0.1 ml 10^0	189	1.89E+03	3.28
	0.1 ml 10^{-1}	12		
Validation suspension:			1.66E+03	3.22
		80 % - 60 s	controls o.k.?	**Yes**

Table 4: Results of the quantitative suspension test (according to VAH methods, 2015/2019)

Date:	July 27, 2020	**Order number:**	A 20-0595
Product:	**SEIFREI Desinfektions Hand-Gel**	**Batch-number:**	L-071420GGAC
Test organism:	*E. coli*	**Sample number:**	P 203647
Interfering substance:	0.03 % albumin		
Incubation time:	24 h – 48 h	**Neutralizer:**	XXIV
Test suspension:	$1.50*10^8$ cfu/1 ml (8.18 log)	**Incubation temperature:**	36 ± 1 °C
Method:	Dilution-neutralization-method	**Reaction temperature:**	20 ± 1 °C

concentration	dilution	time: 15 s			time: 30 s			time: 60 s		
		cfu / plate	$\log_{10}$ (x)	RF	cfu / plate	$\log_{10}$ (x)	RF	cfu / plate	$\log_{10}$ (x)	RF
80 %	1 ml 10^0	0	0.00	**≥ 6.21**	0	0.00	**≥ 6.14**	0	0.00	**≥ 6.19**
	0.1 ml 10^0	0			0			0		
	0.1 ml 10^{-1}	0			0			0		
	0.1 ml 10^{-2}	0			0			0		
50 %	1 ml 10^0	0	0.00	**≥ 6.21**	0	0.00	**≥ 6.14**	0	0.00	**≥ 6.19**
	0.1 ml 10^0	0			0			0		
	0.1 ml 10^{-1}	0			0			0		
	0.1 ml 10^{-2}	0			0			0		
10 %	1 ml 10^0	> 330			> 330			> 330		
	0.1 ml 10^0	> 330			> 330			> 330		
	0.1 ml 10^{-1}	> 330			> 330			> 330		
	0.1 ml 10^{-2}	> 330	> 5.52	**< 0.70**	> 330	> 5.52	**< 0.62**	> 330	> 5.52	**< 0.67**
WFI (co 1)	0.1 ml 10^{-1}	> 330			> 330			> 330		
	0.1 ml 10^{-2}	> 330			> 330			> 330		
	0.1 ml 10^3	163	**6.21**		136	**6.14**		154	**6.19**	
	0.1 ml 10^{-4}	17			15			15		

Controls and Validation:

		number of colonies [cfu/plate]	cfu /ml (with regard to the validation suspension)	$\log_{10}$ (x)
control 2 (co 2)	0.1 ml 10^0	178	1.75E+03	3.24
	0.1 ml 10^{-1}	15		
control 3 (co 3)	0.1 ml 10^0	155	1.61E+03	3.21
	0.1 ml 10^{-1}	22		
Validation suspension:			1.50E+03	3.18
		80 % - 60 s	controls o.k.?	**Yes**

Table 5: Results of the quantitative suspension test (according to VAH methods, 2015/2019)

Date:	July 27, 2020	**Order number:**	A 20-0595
Product:	**SEIFREI Desinfektions Hand-Gel**	**Batch-number:**	L-071420GGAC
Test organism:	*P. aeruginosa*	**Sample number:**	P 203647
Interfering substance:	0.03 % albumin		
Incubation time:	24 h – 48 h	**Neutralizer:**	XXIV
Test suspension:	$2.00*10^8$ cfu/1 ml (8.30 log)	**Incubation temperature:**	36 ± 1 °C
Method:	Dilution-neutralization-method	**Reaction temperature:**	20 ± 1 °C

concentration	dilution	time: 15 s			time: 30 s			time: 60 s		
		cfu / plate	log_{10} (x)	RF	cfu / plate	log_{10} (x)	RF	cfu / plate	log_{10} (x)	RF
80 %	1 ml 10^0	0	0.00	**≥ 6.34**	0	0.00	**≥ 6.32**	0	0.00	**≥ 6.30**
	0.1 ml 10^0	0			0			0		
	0.1 ml 10^{-1}	0			0			0		
	0.1 ml 10^{-2}	0			0			0		
50 %	1 ml 10^0	0	0.00	**≥ 6.34**	4	0.60	**5.72**	0	0.00	**≥ 6.30**
	0.1 ml 10^0	0			0			0		
	0.1 ml 10^{-1}	0			0			0		
	0.1 ml 10^{-2}	0			0			0		
10 %	1 ml 10^0	> 330			> 330			> 330		
	0.1 ml 10^0	> 330			> 330			> 330		
	0.1 ml 10^{-1}	> 330			> 330			> 330		
	0.1 ml 10^{-2}	> 330	> 5.52	**< 0.82**	> 330	> 5.52	**< 0.80**	> 330	> 5.52	**< 0.78**
WFI (co 1)	0.1 ml 10^{-1}	> 330			> 330			> 330		
	0.1 ml 10^{-2}	> 330			> 330			> 330		
	0.1 ml 10^3	219	**6.34**		210	**6.32**		190	**6.30**	
	0.1 ml 10^{-4}	20			21			29		

Controls and Validation:

		number of colonies [cfu/plate]	cfu /ml (with regard to the validation suspension)	log_{10} (x)
control 2 (co 2)	0.1 ml 10^0	190	1.92E+03	3.28
	0.1 ml 10^{-1}	21		
control 3 (co 3)	0.1 ml 10^0	155	1.59E+03	3.20
	0.1 ml 10^{-1}	20		
Validation suspension:			2.00E+03	3.30
		80 % - 60 s	controls o.k.?	**Yes**

Table 6: Results of the quantitative suspension test (according to VAH methods, 2015/2019)

Date:	July 27, 2020	**Order number:**	A 20-0595
Product:	**SEIFREI Desinfektions Hand-Gel**	**Batch-number:**	L-071420GGAC
Test organism:	*P. mirabilis*	**Sample number:**	P 203647
Interfering substance:	0.03 % albumin		
Incubation time:	24 h – 48 h	**Neutralizer:**	XXIV
Test suspension:	$2.17*10^8$ cfu/1 ml (8.34 log)	**Incubation temperature:**	36 ± 1 °C
Method:	Dilution-neutralization-method	**Reaction temperature:**	20 ± 1 °C

concentration	dilution	time: 15 s			time: 30 s			time: 60 s		
		cfu / plate	log_{10} (x)	RF	cfu / plate	log_{10} (x)	RF	cfu / plate	log_{10} (x)	RF
80 %	1 ml 10^0	0	0.00	**≥ 6.34**	0	0.00	**≥ 6.41**	0	0.00	**≥ 6.36**
	0.1 ml 10^0	0			0			0		
	0.1 ml 10^{-1}	0			0			1		
	0.1 ml 10^{-2}	1			0			0		
50 %	1 ml 10^0	0	0.00	**≥ 6.34**	0	0.00	**≥ 6.41**	0	0.00	**≥ 6.36**
	0.1 ml 10^0	0			0			0		
	0.1 ml 10^{-1}	0			0			0		
	0.1 ml 10^{-2}	0			0			0		
10 %	1 ml 10^0	> 330			> 330			> 330		
	0.1 ml 10^0	> 330			> 330			> 330		
	0.1 ml 10^{-1}	> 330			> 330			> 330		
	0.1 ml 10^{-2}	> 330	> 5.52	**< 0.83**	> 330	> 5.52	**< 0.89**	> 330	> 5.52	**< 0.84**
WFI (co 1)	0.1 ml 10^{-1}	> 330			> 330			> 330		
	0.1 ml 10^{-2}	> 330			> 330			> 330		
	0.1 ml 10^3	216	**6.34**		260	**6.41**		229	**6.36**	
	0.1 ml 10^{-4}	27			21			21		

Controls and Validation:

		number of colonies [cfu/plate]	cfu /ml (with regard to the validation suspension)	log_{10} (x)
control 2 (co 2)	0.1 ml 10^0	253	2.49E+03	3.40
	0.1 ml 10^{-1}	21		
control 3 (co 3)	0.1 ml 10^0	218	2.17E+03	3.34
	0.1 ml 10^{-1}	21		
Validation suspension:			2.17E+03	3.34
		80 % - 60 s	controls o.k.?	**Yes**

Table 7: Results of the quantitative suspension test (according to VAH methods, 2015/2019)

Date:	July 27, 2020	**Order number:**	A 20-0595
Product:	**SEIFREI Desinfektions Hand-Gel**	**Batch-number:**	L-071420GGAC
Test organism:	*C. albicans*	**Sample number:**	P 203647
Interfering substance:	0.03 % albumin		
Incubation time:	48 h	**Neutralizer:**	XXIV
Test suspension:	$4.45*10^7$ cfu/1 ml (7.65 log)	**Incubation temperature:**	30 ± 1 °C
Method:	Dilution-neutralization-method	**Reaction temperature:**	20 ± 1 °C

concentration	dilution	time: 15 s			time: 30 s			time: 60 s		
		cfu / plate	log_{10} (x)	RF	cfu / plate	log_{10} (x)	RF	cfu / plate	log_{10} (x)	RF
80 %	1 ml 10^0	0	0.00	**≥ 5.70**	0	0.00	**≥ 5.68**	0	0.00	**≥ 5.63**
	0.1 ml 10^0	0			0			0		
	0.1 ml 10^{-1}	0			0			0		
	0.1 ml 10^{-2}	0			0			0		
50 %	1 ml 10^0	> 330			> 330			> 330		
	0.1 ml 10^0	> 330			> 330			> 330		
	0.1 ml 10^{-1}	> 330			> 330			> 330		
	0.1 ml 10^{-2}	105	5.02	**0.68**	103	5.01	**0.67**	45	4.65	**0.98**
10 %	1 ml 10^0	> 330			> 330			> 330		
	0.1 ml 10^0	> 330			> 330			> 330		
	0.1 ml 10^{-1}	> 330			> 330			> 330		
	0.1 ml 10^{-2}	> 330	> 5.52	**< 0.18**	> 330	> 5.52	**< 0.16**	312	5.49	**0.14**
WFI (co 1)	0.1 ml 10^0	> 330			> 330			> 330		
	0.1 ml 10^{-1}	> 330			> 330			> 330		
	0.1 ml 10^{-2}	> 330			> 330			> 330		
	0.1 ml 10^3	50	**5.70**		48	**5.68**		43	**5.63**	

Controls and Validation:

		number of colonies [cfu/plate]	cfu /ml (with regard to the validation suspension)	log_{10} (x)
control 2 (co 2)	0.1 ml 10^0	> 330		
	0.1 ml 10^{-1}	50	5.00E+04	4.70
control 3 (co 3)	0.1 ml 10^0	> 330		
	0.1 ml 10^{-1}	55	5.50E+04	4.74
Validation suspension:			4.45E+04	4.65
		80 % - 60 s	controls o.k.?	**Yes**

Legend:

[1]	=	as provided by the sponsor / manufacturer, unless stated otherwise
[2]	=	according to EN 17025. § 7.8.2.1 I. we are required to state that the results presented in this report relate to the item(s) tested only. That is quite obvious in the first place. anyway. And it is also ridiculous. of course. with regard to these tests and reports typically being used for a product's generalized efficacy evaluation and market authorization. Which. as such. is then fully acceptable by all other relevant authorizing and responsible parties. too. And which is why this disclaimer is only to be found at the very back end of this report.
MW	=	average value
x	=	average value
RF	=	reduction factor
> 330	=	not countable
n.d.	=	not determined
Co 1	=	Control 01
Co 2	=	Control 02
Co 3	=	Control 03
WFI	=	water for injection