Chemila, spol. s r.o., Za Dráhou 4386/3, 695 01 Hodonín, Phone.: +420 518 340 919, chemila@chemila.cz
Chemical and Microbiological Laboratory, Testing Laboratory No. 1273 certified by Czech Accreditation Institute according to CSN EN ISO/IEC 17025:2018.

Copy No.: 1
Issue No.: 1

Test report No.: S48/2022 - 1

DETERMINATION OF HYGIENIC HANDRUB (EN 1500:2013) ACTIVITY OF THE PRODUCT **CHEMISEPT GEL**

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Sample ID:	S48/2022	Page.: 1
Sample name:	**CHEMISEPT GEL**	From pages: 4
Client:	Chemi-Pharm AS, Tänassilma tee 11, Tänassilma küla, 76406 Saku vald, Estonia	
Manufacturer:	Chemi-Pharm AS, Tänassilma tee 11, Tänassilma küla, 76406 Saku vald, Estonia	
Sampling point:	Chemi-Pharm AS, Tänassilma tee 11, Tänassilma küla, 76406 Saku vald, Estonia	

Incoming date:
23.2.2022

Delivery date:
28.3.2022

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID:	S48/2022	Sampling date:	29.12.2021
Sample name:	**CHEMISEPT GEL**	Sample delivered:	23.2.2022
Sampled:	Client	Testing date:	16.3. - 17.3.2022
Sampling point:	Chemi-Pharm AS	Delivered amount:	2 x 500 ml
Client:	Chemi-Pharm AS	Page:	2

Subject of testing:
Hygienic handrub

Identification of the sample:

Name of the product:	**CHEMISEPT GEL**
Batch number (Lot):	198291221
Date of manufacture:	29.12.2021
Expiry date:	12/2024
Manufacturer:	Chemi-Pharm AS, Tänassilma tee 11, Tänassilma küla, 76406 Saku vald, Estonia
Incoming date:	23.2.2022
Storage conditions:	room temperature
Active ingredients:	CAS: 64-17-5 ethanol 72.5 CAS: 67-63-0 2-propanol 7.5

Experimental conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents on carriers**
SOP:	SOP-M-22-12 (EN 1500:2013)
Period of analysis:	16. 3. 2022 - 17.3.2022
Test method:	dilution neutralization method
Neutralization medium:	Dey-Engley Neutralizing Broth M 1062
Appearance of the product:	colourles gel
The test concentration:	100%
The volume of the product:	3 ml
The application time:	30 s
The soap:	liquid soap from linseed oil 200 g/l
Reference item:	2-propanol p.a., CAS 67-63-0, concentration: 60% (V/V) batch number: I1161134133, expiry date: 30.6.2026
The volume of the reference propan-2-ol used per person:	2 x 3 ml, according to reference handrub procedure, the total application volume is 6 ml
The application time:	2 x 30 s, according to reference handrub procedure, the total application volume is 1 min
Test organism:	*Escherichia coli K 12* NCTC 10538
Treatment procedure:	hygienic handrub disinfection in accordance with the standard handrub procedure also include the instructions to keep hands wet with the product for a given time

Preparation of the test:

1. Determination of the number of the microorganisms CFU/ml in the product
2. Preparation of the test suspension of *Escherichia coli*
3. Determination of the number of viable cells of *Escherichia coli*
4. Prevalue - number of cfu sampled after the contamination with *Escherichia coli*
5. Postvalue - number of cfu sampled after the treatment with the disinfectant
6. Reduction factor - ratio of prevalues and postvalues, generally expressed by decimal logarithms
7. Calculation (Hodges-Lehmann)

The standard:
EN 1500:2013 Chemical disinfectants and antiseptics – Hygienic handrub – Test method and requirements (phase 2/step 2), April 2013

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Sampled:	Client	Testing date:	16.3. - 17.3.2022
Sampling point:	Chemi-Pharm AS	Delivered amount:	2 x 500 ml
Client:	Chemi-Pharm AS	Page:	3

The Number of CFU in the tested product: 0 CFU/ml

Testing the efficacy of chemical disinfectant **CHEMISEPT GEL on** *Escherichia coli K 12* NCTC 10538

Test suspensions:

Dilutions	V1	V2	lgN	Weighted mean (ø)		
10^{-6}	>330	>330				
10^{-7}	33	47	8,6			
		$\Phi = 4{,}0x10^8$	$8{,}17 \leq lgN \leq 8{,}7$	for N	$5 \leq ø \leq 15$	-

Verification of methodology

Validation of suspension N_{V0}		Validation of suspenzion N_{Vb}		Neutralizer toxicity control (B)	
Vc1	96	Vc1	68	Vc1	112
Vc2	110	Vc2	129	Vc2	82
30	$\leq 103 \leq$ *160*	*30* $\leq$	98,5 $\leq$ *160*	97 $\geq$	
$30 \leq \Phi_{Nvo} \leq 160$		$30 < \Phi_{NVB}(N_{VB}/1000) < 160$		$\Phi_B \geq 0.0005\ \Phi_{NVB}$	

Method validation (C)

Testing conditions	Vc1	Vc2	ø C
80 %, 0,5 min, -, 20°C	84	116	100 $\geq$ 0,5 Nvo

Note: Vc = value is the number of cfu per ml, Φ = average Vc1 a Vc2 (1. + 2. duplicate Vc values), N = the number of cfu/ml of the bacterial test suspenzion, Nvo (C), Nvb (B) = the number of cfu/ml of the bacterial test suspensions for validation in the test mixture B, C at the beginning of the contact time = 0, B, C, = the number of surviving bacteria per ml in control tests (B - neutralizer toxicity validation, C - method validation)

Acceptance criteria for test results:

Only if the results of the test procedure fulfil the following requirements, they shall be accepted for further evaluation, otherwise the test shall be repeated:

a) A complete set of results from at least 18 volunteers shall be available. All complete sets of results shall be used for further evaluation.

b) The overall means of the lg prevalues for RP and PP shall be both at least 5.00.

c) Not more than three individual lg reductions less than 3.00 shall occur in RP.

d) The absolute diference of mean differences between lg reductions of RP and PP of group RP →PP and group PP→RP shall be less than 2.00.

e) All quotients of weighted mean counts between 5 and 15.

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

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Sampled:	Client	Testing date:	16.3. - 17.3.2022
Sampling point:	Chemi-Pharm AS	Delivered amount:	2 x 500 ml
Client:	Chemi-Pharm AS	Page:	4

Conslusion:

The acceptance criteria for the test results were met.

From table (see Table E.5 in EN 1500) of critical values for Wilcoxon´s matched-pairs signed-ranks test the entry for n = 20 and a one-sided 0.025 level of significante, the critical value of 52 is found. Hence c = 52 + 1 = 53. The pairwise differences are sorted in descending order. The 53rd value is 0,17. Hence the Hodges-Lehmann upper one-sided 97,5% confidence limit for the difference in lg Rs between RP and PP is 0,17, which is less than the agreed inheriority margin of 0,6. Therefore the hypothesis of inferiority of PP is rejected and it can be concluded the test preparation PP is non-inferior to RP.

The tested product	**CHEMISEPT GEL**
Batch number:	198291221
Standard:	EN 1500:2013
Procedure:	handrub
Conditions:	
Application time:	30 s
Volume of the product:	3 ml
Concentration:	100%

The tested product is seemed suitable to be used as medical hygienic handrub.

Approved by: Ing. Barbora Stoklásková, Leader of Study

Hodonín, 28.3.2022

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Ing. Jana Slitrová, Head of Laboratory

Annex
raw data

EN 1500:2013

Chemisept Gel,
sample S48/2022

Period of analysis: 16.3.-17.3.2022

Prepared by: Mgr. Alena Holíková

Volunteer	Hand	2-Propanol batch I1161134133, expiry date 2026/06/30, 60%, 2x3 ml, 2x30 s						Product Chemisept Gel, sample S48/2022, 100%, 3 ml, 30 s					
	left or right	-3	-4	-5	0	-1	-2	-3	-4	-5	0	-1	-2
1	l	>330	126	<14	>330	58	<14	>330	>330	50	>330	142	14
	r	>330	116	<14	>330	70	<14	>330	>330	32	>330	145	16
2	l	>330	117	<14	>330	93	<14	>330	>330	38	>330	79	<14
	r	>330	134	<14	>330	101	<14	>330	>330	51	>330	96	<14
3	l	>330	>330	49	>330	105	<14	>330	>330	85	>330	70	<14
	r	>330	>330	38	>330	96	<14	>330	>330	67	>330	59	<14
4	l	>330	209	20	>330	41	<14	>330	127	17	300	21	<14
	r	>330	166	19	>330	33	<14	>330	152	15	311	32	<14
5	l	>330	139	<14	>330	43	<14	>330	250	24	>330	37	<14
	r	>330	114	<14	>330	57	<14	>330	232	25	>330	38	<14
6	l	135	<14	<14	132	<14	<14	136	<14	<14	184	16	<14
	r	129	<14	<14	124	<14	<14	115	<14	<14	192	24	<14
7	l	>330	>330	45	>330	82	<14	>330	219	29	>330	>330	57
	r	>330	>330	36	>330	101	<14	>330	204	15	>330	>330	51
8	l	>330	106	<14	>330	142	14	>330	122	<14	>330	45	<14
	r	>330	123	<14	>330	127	<14	>330	108	<14	>330	39	<14
9	l	>330	207	23	>330	168	17	>330	131	20	>330	144	17
	r	>330	191	20	>330	173	21	>330	145	15	>330	137	14
10	l	>330	>330	77	>330	96	<14	>330	>330	51	>330	82	<14
	r	>330	>330	59	>330	90	<14	>330	>330	37	>330	88	<14
11	l	>330	91	<14	>330	65	<14	>330	>330	44	>330	220	22
	r	>330	79	<14	>330	77	<14	>330	>330	33	>330	231	20
12	l	>330	148	14	>330	101	<14	>330	>330	32	>330	90	<14
	r	>330	153	20	>330	124	<14	>330	>330	59	>330	96	<14
13	l	>330	>330	43	>330	85	<14	>330	>330	70	130	<14	<14
	r	>330	>330	33	>330	81	<14	>330	>330	52	126	<14	<14
14	l	>330	182	19	>330	73	<14	>330	301	30	>330	64	<14
	r	>330	160	16	>330	90	<14	>330	273	32	>330	88	<14
15	l	>330	71	<14	>330	48	<14	>330	225	24	>330	35	<14
	r	>330	55	<14	>330	70	<14	>330	195	20	>330	41	<14
16	l	>330	130	<14	>330	132	14	>330	116	<14	>330	>330	34
	r	>330	111	<14	>330	153	16	>330	110	<14	>330	>330	46
17	l	>330	297	34	>330	184	16	>330	200	15	>330	>330	45
	r	>330	288	23	>330	193	25	>330	218	20	>330	>330	37
18	l	>330	153	18	>330	33	<14	>330	53	<14	>330	40	<14
	r	>330	211	21	>330	45	<14	>330	44	<14	>330	36	<14
19	l	>330	>330	46	>330	266	27	>330	179	18	>330	139	<14
	r	>330	>330	62	>330	277	26	>330	234	25	>330	133	<14
20	l	>330	>330	70	>330	>330	81	>330	275	31	>330	>330	79
	r	>330	>330	42	>330	>330	73	>330	268	25	>330	>330	97

Volunteer	Chronological Sequence	Reference hand disinfection procedure RP					Reference handwash procedure with product PP					Difference RP - PP
		N prevalues	N postvalues	lg prevalues	lg postvalues	lg R	N prevalues	N postvalues	lg prevalues	lg postvalues	lg R	
1	RP	1,21E+06	6,40E+02	6,08	2,81	3,27	4,10E+06	1,44E+03	6,61	3,16	3,45	-0,18
2	RP	1,26E+06	9,70E+02	6,10	2,99	3,11	4,50E+06	8,80E+02	6,65	2,94	3,71	-0,60
3	RP	4,40E+06	1,01E+03	6,64	3,00	3,64	7,60E+06	6,45E+02	6,88	2,81	4,07	-0,43
4	RP	1,88E+06	3,70E+02	6,27	2,57	3,70	1,41E+06	3,02E+02	6,15	2,48	3,67	0,03
5	RP	1,27E+06	5,00E+02	6,10	2,70	3,40	2,41E+06	3,75E+02	6,38	2,57	3,81	-0,40
6	RP	1,32E+05	1,28E+02	5,12	2,11	3,01	1,26E+05	1,89E+02	5,10	2,28	2,82	0,19
7	RP	4,10E+06	9,20E+02	6,61	2,96	3,65	2,12E+06	5,40E+03	6,33	3,73	2,60	1,05
8	RP	1,15E+06	1,35E+03	6,06	3,13	2,93	1,15E+06	4,20E+02	6,06	2,62	3,44	-0,51
9	RP	2,00E+06	1,72E+03	6,30	3,24	3,06	1,41E+06	1,42E+03	6,15	3,15	3,00	0,06
10	RP	6,80E+06	9,30E+02	6,83	2,97	3,86	4,40E+06	8,50E+02	6,64	2,93	3,71	0,15
11	PP	8,50E+05	7,10E+02	5,93	2,85	3,08	3,90E+06	2,24E+03	6,59	3,35	3,24	-0,16
12	PP	1,52E+06	1,13E+03	6,18	3,05	3,13	4,60E+06	9,30E+02	6,66	2,97	3,69	-0,56
13	PP	3,80E+06	8,30E+02	6,58	2,92	3,66	6,10E+06	1,28E+02	6,79	2,11	4,68	-1,02
14	PP	1,71E+06	8,20E+02	6,23	2,91	3,32	2,89E+06	7,60E+02	6,46	2,88	3,58	-0,26
15	PP	6,30E+05	5,90E+02	5,80	2,77	3,03	2,11E+06	3,80E+02	6,32	2,58	3,74	-0,71
16	PP	1,21E+06	1,43E+03	6,08	3,16	2,92	1,13E+06	4,00E+03	6,05	3,60	2,45	0,47
17	PP	2,92E+06	1,90E+03	6,47	3,28	3,19	2,06E+06	4,10E+03	6,31	3,61	2,70	0,49
18	PP	1,83E+06	3,90E+02	6,26	2,59	3,67	4,90E+05	3,80E+02	5,69	2,58	3,11	0,56
19	PP	5,40E+06	2,71E+03	6,73	3,43	3,30	2,07E+06	1,36E+03	6,32	3,13	3,19	0,11
20	PP	5,60E+06	7,70E+03	6,75	3,89	2,86	2,72E+06	8,80E+03	6,43	3,94	2,49	0,37
Ø	Overall	2,48E+06	1,34E+03	6,26	2,97	3,29	2,86E+06	1,75E+03	6,33	2,97	3,36	
s		1,88E+06	1,61E+03	0,40	0,36	0,31	1,91E+06	2,22E+03	0,41	0,50	0,57	
n				20	20	20			20	20	20	
Ø	RP → PP			6,21	2,85	3,36			6,30	2,87	3,43	-0,07
s				0,47	0,33	0,33			0,50	0,42	0,47	
n				10	10	10			10	10	10	
Ø	PP → RP			6,30	3,09	3,22			6,36	3,08	3,29	-0,07
s				0,32	0,37	0,28			0,31	0,57	0,68	
n				10	10	10			10	10	10	

Sorting of individual differences and computation for Hodges-Lehmann 97,5% upper confidence limits												
	Sorted differences	Mean pairwise differences (di+dii)/2										
1	1,05	1,05										
2	0,56	0,81	0,56									
3	0,49	0,77	0,53	0,49								
4	0,47	0,76	0,52	0,48	0,47							
5	0,37	0,71	0,47	0,43	0,42	0,37						
6	0,19	0,62	0,38	0,34	0,33	0,28	0,19					
7	0,15	0,60	0,36	0,32	0,31	0,26	0,17	0,15				
8	0,11	0,58	0,34	0,30	0,29	0,24	0,15	0,13	0,11			
9	0,06	0,56	0,31	0,28	0,27	0,22	0,13	0,11	0,09	0,06		
10	0,03	0,54	0,30	0,26	0,25	0,20	0,11	0,09	0,07	0,05	0,03	
11	-0,16	0,45	0,20	0,17	0,16	0,11	0,02	-0,01	-0,03	-0,05	-0,07	
12	-0,18	0,44	0,19	0,16	0,15	0,10	0,01	-0,02	-0,04	-0,06		
13	-0,26	0,40	0,15	0,12	0,11	0,06	-0,04	-0,06	-0,08			
14	-0,41	0,32	0,08	0,04	0,03	-0,02	-0,11	-0,13				
15	-0,43	0,31	0,07	0,03	0,02	-0,03	-0,12					
16	-0,51	0,27	0,03	-0,01	-0,02	-0,07						
17	-0,56	0,25	0,00	-0,04	-0,05							
18	-0,60	0,23	-0,02	-0,06								
19	-0,71	0,17	-0,08									
20	-1,02	0,02										

log R = decimal log reduction; RP→PP sequence: first RP, second PP; PP→RP sequence: first PP, second RP; Ø = mean; s = standard deviation; n = number of values (volunteer)

Difference of mean Rs (RP→PP): 3,36 - 3,43 = -0,07; Difference of mean Rs (PP→RP): 3,22 - 3,29 = -0,07; Absolute difference of differences: |-0,07 - (-0,07)| = 0,00

The median is between the 10th and 11th value: [0,03+(-0,16)]/2 = -0,065. The mean pairwise differences that do not exceed the median (here: -0,065) are computed.

From table (see E.5 in EN 1500:2013) of critical values for Wilcoxon's matched-pairs signed-ranks test the entry for n = 20 and a one-sided 0,025 level of significance, the critical value of 52 is found. Hence c = 52 + 1 = 53. **The 53rd value is 0,17.** Hence the Hodges-Lehmann upper one sided 97,5% confidence limit for the difference in lg Rs between RP and PP is 0,17, which is less than the agreed inferiority margin of 0,6. Therefore the hypothesis of inferiority of PP is rejected and it can be concluded that the test preparation PP is non-inferior to RP.