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Chemical and Microbiological Laboratory, Testing Laboratory No. 1273 certified by Czech Accreditation Institute according to ČSN EN ISO/IEC 17025:2018.

Copy No.: 1
Issue No.: 1

Test report No.: S47/2022 - 1

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

Digitally Signed by Moraru Grigore
Date: 2023.11.08 13:22:56 EET
Reason: MoldSign Signature
Location: Moldova

Sample ID:	S47/2022	Page.: 1
Sample name:	**CHEMISEPT MED**	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

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

Sample ID:	S47/2022	Sampling date:	1.2.2022
Sample name:	**CHEMISEPT MED**	Sample delivered:	23.2.2022
Sampled:	Client	Testing date:	3.3. - 4.3.2022
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 MED**
Batch number (Lot):	196010222
Date of manufacture:	1.2.2022
Expiry date:	02/2025
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 g 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:	3. 3. 2022 - 4.3.2022
Test temperature:	20°C ± 1°C
Test method:	dilution neutralization method
Neutralization medium:	Dey-Engley Neutralizing Broth M 1062
Appearance of the product:	colourless liquid
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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Sample ID:	S47/2022	Sampling date:	1.2.2022
Sample name:	**CHEMISEPT MED**	Sample delivered:	23.2.2022
Sampled:	Client	Testing date:	3.3. - 4.3.2022
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 MED on** *Escherichia coli K 12* NCTC 10538

Test suspensions:

Dilutions	V1	V2	lgN	Weighted mean (ø)		
10^{-6}	188	240				
10^{-7}	22	20	8,33			
$\Phi = 2{,}14 \times 10^{8}$			$8{,}17 \leq \lg N \leq 8{,}7$	for N	$5 \leq ø \leq 15$	10,19

Verification of methodology

Validation of suspension N_{V0}		Validation of suspenzion N_{Vb}		Neutralizer toxicity control (B)	
Vc1	49	Vc1	60	Vc1	49
Vc2	60	Vc2	35	Vc2	50
30 $\leq 54{,}5 \leq$ *160*		*30* $\leq$ 47,5 $\leq$ *160*		49,5 $\geq$	
$30 \leq \Phi_{Nvo} \leq 160$		$30 < \Phi_{NVB}(N_{VB}/1000) < 160$		$\Phi_B \geq 0.0005\ \Phi_{NVB}$	

Method validation (C)

Testing conditions	Vc1	Vc2	ø C
80 %, 30 s, -, 20°C	47	56	51,5 $\geq$ 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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Sample name:	**CHEMISEPT MED**	Sample delivered:	23.2.2022
Sampled:	Client	Testing date:	3.3. - 4.3.2022
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 0,33. Hence the Hodges-Lehmann upper one-sided 97,5% confidence limit for the difference in lg Rs between RP and PP is 0.33, 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 MED**
Batch number:	196010222
Standard:	EN 1500:2013
Procedure:	handrub
Conditions:	
Application time:	30 s
Volume of the product:	3 ml
Concentration:	100%

The tested product is deemed suitable to be used as medical hygienic handrub according to the standard EN 1500:2013.

Approved by:Ing. Barbora Stoklásková, Leader of Study

Hodonín, 28.3.2022

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Ing. Jana Šlitrová, Head of Laboratory

Annex
raw data

EN 1500:2013

Chemisept MED,
sample S47/2022

Period of analysis: 3.3.-4.3.2022

Prepared by: Mgr. Alena Holíková

Volunteer	Hand	2-Propanol batch I1161134133, expiry date 2026/06/30, 60%, 2x3 ml, 2x30 s						Product Chemisept MED, sample S47/2022, 100%, 3 ml, 30 s					
	left or right	-3	-4	-5	0	-1	-2	-3	-4	-5	0	-1	-2
1	l	>330	117	<14	>330	89	<14	>330	101	<14	>330	39	<14
	r	>330	124	<14	>330	73	<14	>330	77	<14	>330	45	<14
2	l	>330	164	18	>330	81	<14	>330	103	<14	>330	56	<14
	r	>330	147	14	>330	65	<14	>330	89	<14	>330	48	<14
3	l	>330	148	15	>330	244	28	>330	>330	41	>330	>330	85
	r	>330	173	19	>330	252	20	>330	>330	33	>330	>330	74
4	l	>330	203	22	>330	47	<14	>330	188	24	96	<14	<14
	r	>330	162	17	>330	32	<14	>330	169	20	112	<14	<14
5	l	>330	171	17	>330	87	<14	>330	82	<14	>330	85	<14
	r	>330	156	16	>330	82	<14	>330	73	<14	>330	79	<14
6	l	>330	138	<14	>330	52	<14	>330	44	<14	>330	57	<14
	r	>330	124	<14	>330	46	<14	>330	66	<14	>330	49	<14
7	l	>330	200	20	>330	43	<14	>330	246	25	>330	113	<14
	r	>330	218	18	>330	36	<14	>330	225	24	>330	99	<14
8	l	>330	111	<14	>330	36	<14	>330	34	<14	>330	50	<14
	r	>330	149	<14	>330	42	<14	>330	45	<14	>330	46	<14
9	l	>330	142	14	137	<14	<14	>330	287	32	133	14	<14
	r	>330	117	<14	122	<14	<14	>330	280	26	147	15	<14
10	l	>330	131	14	>330	93	<14	>330	244	26	>330	75	<14
	r	>330	150	15	>330	88	<14	>330	233	17	>330	66	<14
11	l	116	<14	<14	80	<14	<14	72	<14	<14	40	<14	<14
	r	112	<14	<14	71	<14	<14	57	<14	<14	48	<14	<14
12	l	>330	134	<14	248	29	<14	>330	101	<14	>330	46	<14
	r	>330	115	<14	240	22	<14	>330	75	<14	>330	35	<14
13	l	>330	264	21	284	27	<14	>330	303	30	>330	61	<14
	r	>330	277	33	291	34	<14	>330	278	25	>330	48	<14
14	l	>330	126	<14	162	15	<14	>330	>330	111	>330	40	<14
	r	>330	90	<14	155	14	<14	>330	>330	73	>330	42	<14
15	l	>330	159	15	>330	80	<14	>330	72	<14	>330	67	<14
	r	>330	143	14	>330	75	<14	>330	60	<14	>330	59	<14
16	l	>330	112	<14	>330	47	<14	>330	59	<14	>330	55	<14
	r	>330	91	<14	>330	58	<14	>330	70	<14	>330	40	<14
17	l	>330	252	22	>330	33	<14	>330	229	27	>330	90	<14
	r	>330	263	33	>330	45	<14	>330	220	18	>330	83	<14
18	l	>330	>330	57	>330	36	<14	>330	75	<14	>330	36	<14
	r	>330	>330	75	>330	60	<14	>330	100	<14	>330	48	<14
19	l	>330	112	<14	>330	116	<14	>330	192	18	>330	>330	43
	r	>330	146	<14	>330	105	<14	>330	171	20	>330	>330	54
20	l	>330	251	25	>330	82	<14	>330	219	17	>330	62	<14
	r	>330	193	22	>330	66	<14	>330	244	25	>330	46	<14

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	8,10E+02	6,08	2,91	3,17	8,90E+05	4,20E+02	5,95	2,62	3,33	-0,15
2	RP	1,56E+06	7,30E+02	6,19	2,86	3,33	9,60E+05	5,20E+02	5,98	2,72	3,26	0,07
3	RP	1,61E+06	2,47E+03	6,21	3,39	2,82	3,70E+06	8,00E+03	6,57	3,90	2,67	0,15
4	RP	1,84E+06	4,00E+02	6,26	2,60	3,66	1,82E+06	1,04E+02	6,26	2,02	4,24	-0,58
5	RP	1,64E+06	8,50E+02	6,21	2,93	3,28	7,80E+05	8,20E+02	5,89	2,91	2,98	0,30
6	RP	1,31E+06	4,90E+02	6,12	2,69	3,43	5,50E+05	5,30E+02	5,74	2,72	3,02	0,41
7	RP	2,07E+06	4,00E+02	6,32	2,60	3,72	2,36E+06	1,06E+03	6,37	3,03	3,34	0,38
8	RP	1,30E+06	3,90E+02	6,11	2,59	3,52	4,00E+05	4,80E+02	5,60	2,68	2,92	0,60
9	RP	1,30E+06	1,30E+02	6,11	2,11	4,00	2,84E+06	1,40E+02	6,45	2,15	4,30	-0,30
10	RP	1,41E+06	9,10E+02	6,15	2,96	3,19	2,36E+06	7,10E+02	6,37	2,85	3,52	-0,33
11	PP	1,14E+05	7,60E+01	5,06	1,88	3,18	6,50E+04	4,40E+01	4,81	1,64	3,17	0,01
12	PP	1,25E+06	2,45E+02	6,10	2,39	3,71	8,80E+05	4,10E+02	5,94	2,61	3,33	0,38
13	PP	2,70E+06	2,89E+02	6,43	2,46	3,97	2,89E+06	5,50E+02	6,46	2,74	3,72	0,25
14	PP	1,08E+06	1,57E+02	6,03	2,20	3,83	9,20E+06	4,10E+02	6,96	2,61	4,35	-0,52
15	PP	1,50E+06	7,80E+02	6,18	2,89	3,29	6,60E+05	6,30E+02	5,82	2,80	3,02	0,27
16	PP	1,02E+06	5,30E+02	6,01	2,72	3,29	6,50E+05	4,80E+02	5,81	2,68	3,13	0,16
17	PP	2,59E+06	3,90E+02	6,41	2,59	3,82	2,25E+06	8,70E+02	6,35	2,94	3,41	0,41
18	PP	6,60E+06	4,80E+02	6,82	2,68	4,14	8,80E+05	4,20E+02	5,94	2,62	3,32	0,82
19	PP	1,29E+06	1,11E+03	6,11	3,05	3,07	1,82E+06	4,90E+03	6,26	3,69	2,57	0,50
20	PP	2,23E+06	7,40E+02	6,35	2,87	3,48	2,30E+06	5,40E+02	6,36	2,73	3,63	-0,15
Ø	Overall	1,78E+06	6,19E+02	6,16	2,67	3,49	1,91E+06	1,10E+03	6,10	2,73	3,36	
s		1,27E+06	5,20E+02	0,32	0,35	0,35	1,99E+06	1,91E+03	0,45	0,49	0,50	
n				20	20	20			20	20	20	
Ø	RP → PP			6,18	2,77	3,41			6,12	2,76	3,36	0,05
s				0,08	0,33	0,33			0,33	0,51	0,54	
n				10	10	10			10	10	10	
Ø	PP → RP			6,15	2,57	3,58			6,07	2,71	3,37	0,21
s				0,46	0,35	0,37			0,57	0,49	0,47	
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	0,82	0,82										
2	0,60	0,71	0,60									
3	0,50	0,66	0,55	0,50								
4	0,41	0,62	0,51	0,46	0,41							
5	0,41	0,62	0,51	0,46	0,41	0,41						
6	0,38	0,60	0,49	0,44	0,40	0,40	0,38					
7	0,38	0,60	0,49	0,44	0,40	0,40	0,38	0,38				
8	0,30	0,56	0,45	0,40	0,36	0,36	0,34	0,34	0,30			
9	0,27	0,55	0,44	0,39	0,34	0,34	0,33	0,33	0,29	0,27		
10	0,25	0,54	0,43	0,38	0,33	0,33	0,32	0,32	0,28	0,26	0,25	
11	0,16	0,49	0,38	0,33	0,29	0,29	0,27	0,27	0,23	0,22	0,21	
12	0,15	0,49	0,38	0,33	0,28	0,28	0,27	0,27	0,23	0,21		
13	0,07	0,45	0,34	0,29	0,24	0,24	0,23	0,23	0,19			
14	0,01	0,42	0,31	0,26	0,21	0,21	0,20	0,20				
15	-0,15	0,34	0,23	0,18	0,13	0,13	0,12					
16	-0,16	0,33	0,22	0,17	0,13	0,13						
17	-0,30	0,26	0,15	0,10	0,06							
18	-0,33	0,25	0,14	0,09								
19	-0,52	0,15	0,04									
20	-0,58	0,12										

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): 3,41 - 3,36 = 0,05; Difference of mean Rs (PP→RP): 3,58 - 3,37 = 0,21; Absolute difference of differences: |0,05 - 0,21| = 0,16

The median is between the 10th and 11th value: [0,25+0,16]/2 = 0,205. The mean pairwise differences that do not exceed the median (here: 0,205) 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 0,33.** Hence the Hodges-Lehmann upper one sided 97,5% confidence limit for the difference in lg Rs between RP and PP is 0,33, 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.