

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, Tännassilma tee 11, Tännassilma 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:	colourless 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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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 (σ)		
10^{-6}	>330	>330				
10^{-7}	33	47	8,6			
$\Phi = 3,98 \times 10^8$			$8,17 \leq \lg N \leq 8,7$	for N	$5 \leq \sigma \leq 15$	-

Verification of methodology

Validation of suspension N _{v0}		Validation of suspension N _{vb}		Neutralizer toxicity control (B)	
Vc1	96	Vc1	68	Vc1	112
Vc2	110	Vc2	129	Vc2	82
$30 \leq 103 \leq 160$		$30 \leq 98,5 \leq 160$		$97 \geq$	
$30 \leq \Phi_{Nv0} \leq 160$		$30 < \Phi_{Nvb}(Nvb/1000) < 160$		$\Phi_B \geq 0.0005 \Phi_{Nvb}$	

Method validation (C)

Testing conditions	Vc1	Vc2	σ C
80 %, 0,5 min, -, 20°C	84	116	$100 \geq 0,5 N_{v0}$

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 suspension, N_{v0} (C), N_{vb} (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 complete set of results from at least 18 volunteers shall be available. All complete sets of results shall be used for further evaluation.
- The overall means of the lg prevalences for RP and PP shall be both at least 5.00.
- Not more than three individual lg reductions less than 3.00 shall occur in RP.
- The absolute difference of mean differences between lg reductions of RP and PP of group RP → PP and group PP → RP shall be less than 2.00.
- All quotients of weighted mean counts between 5 and 15.

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Conclusion:

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 significance, 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 inferiority 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.

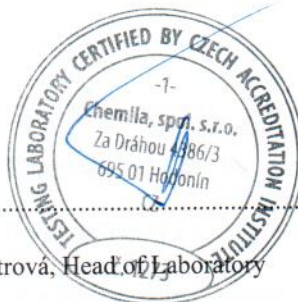
The tested product	CHEMISEPT GEL
Batch number:	198291221
Standard:	EN 1500:2013
Procedure:	handrub
Conditions:	
Application time:	30 s
Volume of the product:	3 ml
Concentration:	100%

The tested product is 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



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		
∅	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			
∅	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			
∅	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	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
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	0,07											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	-0,08										
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	0,03	-0,02	-0,11	-0,13												
15	-0,43	0,31	0,07	0,03	0,02	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; $\bar{\mu}$ = 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

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