

Chemi-Pharm AS
Tänassilma tee 11, Tänassilma küla, Saku
EST – Harju Maakond 76406

Hamburg, 25 September 2023

Expert opinion

Suitability of **Chemisept Med** for hygienic handrub in the practice-like trial with test persons according to DIN EN 1500:2017 (Phase 2, Step 2)

The disinfectant **Chemisept Med** was tested and evaluated according to DIN EN 1500:2017 "Chemical disinfectants and antiseptics – Hygienic handrub – Test method and requirements (phase 2/step 2)".

According to the test report no. L23/00430.4 dated 25/09/2023 of Dr. Brill + Partner GmbH the preparation proved to be suitable for hygienic handrub in a practice-like trial with 19 test persons.

Chemisept Med complies with the requirements of DIN EN 1500:2017 (phase 2, step 2) with the following concentration-time relationship:

Use concentration:	100 %
Use quantity:	3.0 ml
Contact time:	30 seconds

Dr. Florian H. H. Brill

Test report no L23/00430.4

Efficacy of **Chemisept Med** in a practice-like trial with test persons
Hygienic Hand rub – Test method and requirements according to DIN EN 1500:2017*
(Phase 2, step 2)

In accordance with your order, we tested the preparation **Chemisept Med** for its activity in the practice-like trial with 19 test persons according to DIN EN 1500:2017* with an application rate of 3.0 ml.

1 General Information and Material

1.1 Client

Client: Chemi-Pharm AS, Mrs Kaal, Tännassilma tee 11, Tännassilma küla, Saku, EST – Harju Maakond 76406
Date of order: 17/08/2023
Confirmation no.: 232108

1.2 Identification of Test Laboratory

Location: Dr. Brill + Partner GmbH · Institute for Hygiene and Microbiology, Stiegstück 34, DE-22339 Hamburg, Germany
Study manager: Dipl.-Ing. Dr. rer. nat. Andreas Kampe
Scientific assistant: Dipl.-Biol. Dr. rer. nat. Jan-Hendrik Klock
Laboratory technicians: Martina Müller, Carmela Jänicke

1.3 Table of Contents

General Information and Material	1
Methods	3
Results	3
Tables	4
List of Abbreviations	12

1.4 Identification of Sample

Name of product: **Chemisept Med**
Batch no.: 196150723

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Internal product identifier:	23/00733
Manufacturer:	Chemi-Pharm AS, EST – Harju Maakond 76406
Date of delivery:	15/08/2023
Storage conditions:	room temperature
Appearance of product:	clear liquid
Odour:	characteristic
Product type:	Hand disinfectant
Recommended diluent:	Product is ready for use
Active agents (Manufacturer's data):	80 g ethanol per 100 g

1.5 Identification of standard product

Name of product:	2-Propanol (60 %)
Storage conditions:	room temperature and darkness
Appearance of concentrate:	water clear solution
Odour:	alcoholic
Contact time:	2 x 30 seconds
Application rate:	2 x 3.0 ml
concentration of standard product:	60 vol-% 2-propanol

1.6 Test Conditions

Test period:	07/09/ - 11/09/2023
Lab task no.:	L23/00430.4
Test persons:	19
Product test concentration:	100 %
Application rate:	3.0 ml
Contact time:	30 seconds
Test temperature:	20°C ± 1°C
Incubation temperature:	36°C ± 1°C
Neutraliser:	30 g/L polysorbate 80, 30 g/L saponine, 1 g/L cysteine, 1 g/L histidine, (TSHC)
Test organisms:	<i>Escherichia coli</i> K12 NCTC 10538

2 Methods

The tests were carried out according to DIN EN 1500:2017 "Chemical disinfectants and antiseptics - Hygienic hand disinfection - Test method and requirements (phase 2/step 2)". 20 test persons were chosen for testing and data of 19 was evaluable.

3 Results

The test results based on DIN EN 1500:2017 are summarised in tables 1.

The following reductions for the tests were determined:

	Application rate	Contact time	Reduction
2-Propanol (60 %)	2 x 3.0 ml	2 x 30 seconds	4.97
Chemisept Med	3.0 ml	30 seconds	4.74

As the mean reduction factor of the test procedure with 3.0 ml was lower compared to the reference procedure but there were test persons with greater as well as less reduction compared to standard, a statistical analysis according to Chapter F of the norm for inferiority was necessary. According to table E.5 the critical value for $n=19$ and a single sided level of significance of 0.025 is 46 and therefore $c=47$. If the pairwise differences are sorted downward the 53th value was 0.47.

Because this is below the inferiority limit of the Hodges-Lehmann test (one sided upper 97.5 % confidence limit) of 0.60 the difference between reductions of standard product and **Chemisept Med** was not significant.

Therefore **Chemisept Med** did show a sufficient efficacy in the practice-like test according to DIN EN 1500:2017 with *Escherichia coli* with 3.0 ml and a contact time of 30 seconds.

Hamburg, 25/09/2023

Dipl.-Ing. Dr. rer. nat. Andreas Kampe
Study Manager

Dipl.-Biol. Dr. rer. nat. Jan-Hendrik Klock
Deputy Head of Laboratory

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Table 1: Hygienic hand disinfection – Validation und Evaluation

Product name: **Chemisept Med** Batch: 196150723
Test germ / lg cfu/ml: *Escherichia coli* 8.59 Neutraliser: TSHC
Contact time: 30 seconds Application rate: 3.0 ml

Suspension for validation (N_{v0})			Suspension for validation B (N_{vB})			Control of neutraliser (B)			Validation of method (C) Product concentration: 10 %		
	microbial count	\bar{x}		microbial count	\bar{x}		microbial count	\bar{x}		microbial count	\bar{x}
V_{c1}	59	69.5	V_{c1}	68	74	V_{c1}	38	49.5	V_{c1}	46	51.5
V_{c2}	80		V_{c2}	80		V_{c2}	61		V_{c2}	57	
$30 \leq \bar{x} \text{ of } N_{v0} \leq 160$		Yes	$30 \leq \bar{x} \text{ of } N_{vB} \leq 160$		Yes	$\bar{x} \text{ if B is } \geq 0.5 \times \bar{x} \text{ of } N_{vB}?$		Yes	$\bar{x} \text{ of C (30'')} \text{ is } \geq 0.5 \times \bar{x} \text{ of } N_{v0}?$		Yes
Test suspension (N)	N	microbial counts of plates				V_{c1}	V_{c2}	$\bar{x}_{wm} / \lg N$	$8.17 \leq \lg N \leq 8.70 ?$		
	1,00E-06	>330		>330		>330	>330	3.85E+08	Yes		
	1,00E-07	40		37		40	37	8.59			

Validity of pre values

≥ 18 test persons? **Yes**
log-pre values > 5,00? **Yes**
RF more than 3 times < 3? **No**
Difference lgR RP-PP 0.23
Difference lgR PP-RP 0.22
Absolute difference of differences 0.01
Absolute difference of differences < 2 ? **Yes**

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Table 1.1: Hygienic hand disinfection – means and reduction factors

Product name: **Chemisept Med** Batch: 196150723
Test germ / lg cfu/ml: *Escherichia coli* 8.59 Neutraliser: TSHC
Contact time: 30 seconds Application rate: 3.0 ml

Test person	Mean from right and left hand					
	Standard preparation			Test preparation		
	log pre value	log post value	RF	log pre value	log post value	RF
1 (PP-RP)	5,70	0,00	5,70	6,15	1,45	4,70
2 (PP-RP)	5,81	1,05	4,76	6,06	0,93	5,14
3 (PP-RP)	6,53	1,15	5,37	6,48	1,36	5,12
4 (PP-RP)	6,37	0,00	6,37	6,29	0,15	6,14
5 (PP-RP)	5,35	2,06	3,29	5,63	1,16	4,47
6 (PP-RP)	6,13	1,83	4,30	6,13	2,52	3,62
7 (PP-RP)	6,38	2,39	3,99	6,22	2,96	3,25
8 (PP-RP)	6,49	2,00	4,49	6,60	2,22	4,38
9 (PP-RP)	6,49	0,48	6,01	6,46	0,98	5,48
10 (PP-RP)	6,09	0,54	5,55	6,43	1,21	5,22
11 (RP-PP)	5,97	1,23	4,74	5,76	0,90	4,86
12 (RP-PP)	6,59	1,57	5,02	6,43	1,14	5,29
13 (RP-PP)	5,08	0,24	4,84	5,69	0,69	5,00
14 (RP-PP)	6,97	1,39	5,59	6,63	1,72	4,91
15 (RP-PP)	6,38	2,06	4,32	6,35	2,19	4,16
16 (RP-PP)	6,44	0,00	6,44	6,54	0,80	5,74
17 (RP-PP)	6,68	0,80	5,88	6,45	1,46	4,99
18 (RP-PP)	6,64	1,55	5,09	6,55	1,41	5,14
19 (RP-PP)	5,12	2,45	2,67	5,34	2,83	2,51
Mean all:	6,17	1,20	4,97	6,22	1,48	4,74
Standarddev.:	0,52	0,80	0,97	0,36	0,73	0,85
Count of test persons:	19			20		
Mean R-P:	6,13	1,15	4,98	6,25	1,49	4,75
Standarddev.:	0,40	0,88	0,98	0,28	0,84	0,86
count of test persons:	10			10		
Mean P-R:	6,21	1,25	4,95	6,19	1,46	4,73
Standarddev.:	0,68	0,80	1,07	0,47	0,70	0,93
Count of test persons:	9			9		

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Table 1.2: Hygienic hand disinfection – Activity of standard preparation

Product name: 2-Propanol (60 %) Batch: -
Test germ / lg cfu/ml: *Escherichia coli* 8.59 Neutraliser: TSHC
Contact time: 2 x 30 seconds Application rate: 2 x 3.0 ml

Test person	Hand	pre value			post value				RF
		CFU/ml		log	CFU/ml			log	
		1,00E-04	1,00E-05		1,00E+00	1,00E-01	1,00E-02		
1	left	53	1	5,72	0	0	0	0,00	5,72
	right	47	6	5,67	0	0	0	0,00	5,67
2	left	67	2	5,83	8	0	0	0,90	4,92
	right	62	5	5,79	16	1	0	1,20	4,59
3	left	>330	53	6,72	12	2	0	1,08	5,65
	right	214	19	6,33	17	2	0	1,23	5,10
4	left	235	25	6,37	0	0	0	0,00	6,37
	right	236	17	6,36	0	0	0	0,00	6,36
5	left	22	3	5,34	119	15	0	2,09	3,26
	right	23	1	5,36	109	9	2	2,04	3,32
6	left	154	10	6,19	74	6	0	1,87	4,32
	right	118	9	6,07	62	3	0	1,79	4,28
7	left	224	21	6,35	186	20	4	2,27	4,08
	right	250	28	6,40	>330	32	1	2,51	3,90
8	left	326	22	6,50	96	8	0	1,98	4,52
	right	292	37	6,48	104	11	0	2,02	4,46
9	left	308	30	6,49	9	0	0	0,95	5,53
	right	304	12	6,48	1	0	0	0,00	6,48
10	left	112	7	6,05	2	0	0	0,30	5,75
	right	134	13	6,13	6	0	0	0,78	5,35

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Table 1.3: Hygienic hand disinfection – Activity of standard preparation

Product name: 2-Propanol (60 %) Batch: -
Test germ / lg cfu/ml: *Escherichia coli* 8.59 Neutraliser: TSHC
Contact time: 2 x 30 seconds Application rate: 2 x 3.0 ml

Test person	Hand	pre value			post value				RF
		CFU/ml		log	CFU/ml			log	
		1,00E-04	1,00E-05		1,00E+00	1,00E-01	1,00E-02		
11	left	137	20	6,15	16	2	0	1,20	4,95
	right	62	7	5,79	18	3	0	1,26	4,54
12	left	218	22	6,34	9	2	0	0,95	5,38
	right	>330	70	6,85	150	17	1	2,18	4,66
13	left	36	3	5,56	3	0	0	0,48	5,08
	right	4	0	4,60	0	0	0	0,00	4,60
14	left	>330	103	7,01	9	0	0	0,95	6,06
	right	>330	86	6,93	66	2	0	1,82	5,11
15	left	158	16	6,20	146	17	1	2,17	4,03
	right	>330	36	6,56	90	3	2	1,95	4,60
16	left	268	29	6,43	1	0	0	0,00	6,43
	right	278	30	6,45	0	0	0	0,00	6,45
17	left	>330	47	6,67	5	0	0	0,70	5,97
	right	>330	49	6,69	8	1	0	0,90	5,79
18	left	>330	49	6,69	40	1	1	1,60	5,09
	right	>330	39	6,59	32	1	0	1,51	5,09
19	left	19	3	5,28	>330	60	8	2,78	2,50
	right	9	0	4,95	133	4	1	2,12	2,83

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Table 1.4: Hygienic hand disinfection – Activity of test preparation

Product name: **Chemisept Med** Batch: 196150723
Test germ / lg cfu/ml: *Escherichia coli* 8.59 Neutraliser: TSHC
Contact time: 30 seconds Application rate: 3.0 ml

Test person	Hand	pre value			post value				RF
		CFU/ml		log	CFU/ml			log	
		1,00E-04	1,00E-05		1,00E+00	1,00E-01	1,00E-02		
1	left	156	15	6,19	60	9	0	1,78	4,41
	right	127	5	6,10	13	1	0	1,11	4,99
2	left	116	14	6,06	3	0	0	0,48	5,59
	right	116	13	6,06	24	0	0	1,38	4,68
3	left	>330	39	6,59	26	4	1	1,41	5,18
	right	236	13	6,37	20	4	1	1,30	5,07
4	left	278	28	6,44	2	0	0	0,30	6,14
	right	136	9	6,13	1	0	0	0,00	6,13
5	left	47	10	5,67	7	0	0	0,85	4,83
	right	39	4	5,59	30	2	0	1,48	4,11
6	left	140	13	6,15	>330	39	4	2,59	3,56
	right	133	14	6,12	>330	28	3	2,45	3,68
7	left	112	16	6,07	>330	76	6	2,88	3,19
	right	234	21	6,37	>330	111	10	3,05	3,32
8	left	>330	38	6,58	>330	38	3	2,58	4,00
	right	>330	42	6,62	74	7	0	1,87	4,75
9	left	284	37	6,47	7	1	0	0,85	5,62
	right	284	30	6,46	13	0	0	1,11	5,34
10	left	242	13	6,38	29	4	0	1,46	4,92
	right	288	37	6,47	9	1	0	0,95	5,52

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Table 1.5: Hygienic hand disinfection – Activity of test preparation

Product name: **Chemisept Med** Batch: 196150723
Test germ / lg cfu/ml: *Escherichia coli* 8.59 Neutraliser: TSHC
Contact time: 30 seconds Application rate: 3.0 ml

Test person	Hand	pre value			post value				RF
		CFU/ml		log	CFU/ml			log	
		1,00E-04	1,00E-05		1,00E+00	1,00E-01	1,00E-02		
11	left	65	4	5,81	16	1	0	1,20	4,61
	right	52	10	5,72	4	0	0	0,60	5,11
12	left	174	13	6,24	24	3	0	1,38	4,86
	right	>330	41	6,61	8	0	0	0,90	5,71
13	left	33	2	5,52	24	1	0	1,38	4,14
	right	72	5	5,86	0	0	0	0,00	5,86
14	left	>330	45	6,65	36	1	0	1,56	5,10
	right	>330	41	6,61	77	8	1	1,89	4,73
15	left	200	14	6,30	154	9	1	2,19	4,11
	right	>330	25	6,40	153	15	1	2,18	4,21
16	left	298	35	6,48	5	0	0	0,70	5,78
	right	>330	40	6,60	8	0	0	0,90	5,70
17	left	>330	18	6,26	44	0	0	1,64	4,61
	right	>330	44	6,64	19	2	0	1,28	5,36
18	left	>330	42	6,62	5	0	0	0,70	5,92
	right	288	40	6,47	132	10	2	2,12	4,35
19	left	26	2	5,41	>330	68	10	2,83	2,58
	right	18	3	5,26	>330	67	6	2,83	2,43

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Table 1.6: Hygienic hand disinfection – Evaluation and rank sums

Product name: **Chemisept Med** Batch: 196150723
Test germ / lg cfu/ml: *Escherichia coli* 8.59 Neutraliser: TSHC
Contact time: 30 seconds Application rate: 3.0 ml

Test person	log-Reduction			Rank of numbers	
	Standard	Test preparation	Difference	w/o prefix	with prefix
1	5,70	4,70	+ 1,00	18,0	18,0
2	4,76	5,14	- 0,38	11,0	-11,0
3	5,37	5,12	+ 0,25	8,0	8,0
4	6,37	6,14	+ 0,23	7,0	7,0
5	3,29	4,47	- 1,18	19,0	-19,0
6	4,30	3,62	+ 0,68	14,0	14,0
7	3,99	3,25	+ 0,73	16,0	16,0
8	4,49	4,38	+ 0,11	2,0	2,0
9	6,01	5,48	+ 0,53	12,0	12,0
10	5,55	5,22	+ 0,33	10,0	10,0
11	4,74	4,86	- 0,12	3,0	-3,0
12	5,02	5,29	- 0,26	9,0	-9,0
13	4,84	5,00	- 0,16	5,0	-5,0
14	5,59	4,91	+ 0,68	13,0	13,0
15	4,32	4,16	+ 0,15	4,0	4,0
16	6,44	5,74	+ 0,70	15,0	15,0
17	5,88	4,99	+ 0,89	17,0	17,0
18	5,09	5,14	- 0,05	1,0	-1,0
19	2,67	2,51	+ 0,16	6,0	6,0
Mean:	4.97	4.74	Rank sums:	+ 142	- 48

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Table 1.7: Hygienic hand disinfection – Significance test after Hodges-Lehmann

Product name: **Chemisept Med** Batch: 196150723
Test germ / lg cfu/ml: *Escherichia coli* 8.59 Neutraliser: TSHC
Contact time: 30 seconds Application rate: 3.0 ml

No.	Sorted diff. from SP-TP (downward)	mean of differences of data pairs ($d_i + d_{ji}$) / 2										
		1,00	0,89	0,73	0,70	0,68	0,68	0,53	0,33	0,25	0,23	
1	1,00	1,00										
2	0,89	0,94	0,89									
3	0,73	0,87	0,81	0,73								
4	0,70	0,85	0,80	0,72	0,70							
5	0,68	0,84	0,79	0,71	0,69	0,68						
6	0,68	0,84	0,78	0,70	0,69	0,68	0,68					
7	0,53	0,76	0,71	0,63	0,61	0,61	0,60	0,53				
8	0,33	0,66	0,61	0,53	0,51	0,51	0,50	0,43	0,33			
9	0,25	0,62	0,57	0,49	0,47	0,46	0,46	0,39	0,29	0,25		
10	0,23	0,61	0,56	0,48	0,46	0,46	0,45	0,38	0,28	0,24	0,23	
11	0,16	0,58	0,53	0,45	0,43	0,42	0,42	0,34	0,24			
12	0,15	0,57	0,52	0,44	0,43	0,42	0,41	0,34	0,24			
13	0,11	0,55	0,50	0,42	0,41	0,40	0,39	0,32				
14	-0,05	0,47	0,42	0,34	0,32	0,32	0,31	0,24				
15	-0,12	0,44	0,39	0,31	0,29	0,28	0,28					
16	-0,16	0,42	0,37	0,29	0,27	0,26	0,26					
17	-0,26	0,37	0,32	0,24								
18	-0,38	0,31	0,26									
19	-1,18											

The Median of the differences of SP and TP is 0.23.

The mean pair differences not exceeding the median of 0.23 were calculated. The critical value for the Wilcoxon rank sum test for pair differences for $n=19$ at a single sided level of significance is 46. For this follows $c=46+1=47$ marking the 47th Value of the downward sorted pair differences to be chosen for evaluation being **0.47**. Therefore the one sided upper 97.5% interval of confidence is below the agreed level for inferiority of **0.60** and the reduction proved not to be statistically inferior to the standard product.

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4 List of Abbreviations

CFU	=	Colony forming units
RF	=	reduction in organisms in log ₁₀ steps

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