Nordic Tersus
Laboratory

Test report No. hd1518

HYGIENIC HANDRUB TEST (EN 1500)

Name of the product:	CHEMISEPT GEL
Batch number:	198251017
Order number:	17030
Manufacturer:	Chemi-Pharm Ltd.
Client, representative:	Chemi-Pharm Ltd., Põllu 132, Tallinn, 10917, ESTONIA
Date of delivery:	15.01.2018
Test material conditions:	No specific features, sample in the manufacturers tare
Storage conditions:	In room temperature, dark
Active substance – conc.:	Ethyl alcohol 72.5% wt, isopropyl alcohol 7.5% wt
Appearance of the product:	Transparent liquid
Test concentration:	Ready to use
Contact time:	30 s
Interfering substance:	-
Rinsing liquid:	-
Neutralizer:	Polysorbate 80, 30 g/l; saponin, 30 g/l; lecithin, 3 g/l
Test organisms:	*Escherichia coli* K12 NCTC 10538
Testing method based on:	EVS-EN 1500:2013 Chemical disinfectants and antiseptics - Hygienic handrub - Test method and requirements (phase 2/step 2)
Testing date:	15.02.2018 – 16.02.2018
Results:	look appendix 1 - 6

Date of test report: 22.02.2018

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Appendix 1

Reference hygienic handrub – experimental results

Reference solution: [propan-2-ol alcohol 60% (v/v)]
Handrub procedure: 3 ml 30 sec. Repeat the procedure.
Date: 15.02.2018 – 16.02.2018
Test organism: *Escherichia coli* K12 NCTC 10538; Suspension: 1,54 x 10^8 cfu/ml

Volunteer			Number of CFU per plate from dilution 10^x					
			Prevalues			Postvalues		
No	Hand, left or right	Sequence	-3	-4	-5	0	-1	-2
1	l	PP>RP	>330	108	18	11	4	1
	r		>330	91	14	16	2	0
2	l	PP>RP	>330	64	10	8	2	0
	r		>330	85	11	3	0	0
3	l	PP>RP	>330	42	8	7	2	0
	r		>330	66	8	6	0	0
4	l	PP>RP	>330	55	7	11	3	0
	r		>330	73	7	9	3	0
5	l	PP>RP	>330	128	15	16	4	0
	r		>330	153	20	14	2	0
6	l	PP>RP	>330	92	14	28	5	1
	r		>330	109	12	32	6	1
7	l	PP>RP	>330	117	16	36	5	0
	r		>330	142	21	25	3	0
8	l	PP>RP	>330	85	7	16	1	0
	r		>330	64	6	11	0	0
9	l	RP>PP	>330	164	19	22	2	0
	r		>330	125	10	30	3	1
10	l	RP>PP	>330	153	21	33	4	0
	r		>330	121	17	38	6	1
11	l	RP>PP	>330	85	4	11	0	0
	r		>330	99	8	19	4	0
12	l	RP>PP	>330	116	10	21	1	0
	r		>330	132	15	26	1	0
13	l	RP>PP	>330	75	9	30	5	1
	r		>330	94	16	14	2	0
14	l	RP>PP	>330	83	6	14	2	0
	r		>330	62	4	8	0	0
15	l	RP>PP	>330	108	14	23	3	0
	r		>330	117	19	34	3	1
16	l	RP>PP	>330	124	16	36	5	0
	r		>330	151	11	42	2	0
17	l	RP>PP	>330	67	10	19	3	0
	r		>330	84	12	26	5	0
18	l	RP>PP	303	28	5	14	3	0
	r		284	24	7	18	1	0

55= count used for computations; 55= adjacent usage; >330 = not countabable

Appendix 2

Hygienic handrub procedure with the product under test – experimental results

Product: CHEMISEPT GEL
Handrub procedure: 1x3 ml 30 sec
Date: 15.02.2018 – 16.02.2018
Test organism: *Escherichia coli* K12 NCTC 10538; Suspension: 1,54 x 10^8 cfu/ml

Volunteer			Number of CFU per plate from dilution 10^x					
			Prevalues			Postvalues		
No	Hand, left or right	Sequence	-3	-4	-5	0	-1	-2
1	l	PP>RP	>330	99	14	23	3	0
	r		>330	62	8	22	2	0
2	l	PP>RP	>330	103	16	36	5	0
	r		>330	85	10	41	5	1
3	l	PP>RP	294	31	5	12	2	0
	r		263	23	1	10	2	0
4	l	PP>RP	>330	62	8	5	1	0
	r		>330	49	7	14	3	0
5	l	PP>RP	>330	143	18	36	6	1
	r		>330	159	22	27	2	0
6	l	PP>RP	>330	118	14	28	2	0
	r		>330	132	11	20	1	0
7	l	PP>RP	>330	108	6	18	4	0
	r		>330	116	8	26	2	0
8	l	PP>RP	>330	75	8	19	5	0
	r		>330	42	3	13	5	1
9	l	RP>PP	>330	138	17	37	2	0
	r		>330	134	18	33	2	0
10	l	RP>PP	>330	162	20	41	6	0
	r		>330	107	13	35	2	0
11	l	RP>PP	>330	74	5	16	3	0
	r		>330	53	9	21	3	0
12	l	RP>PP	>330	107	11	27	2	0
	r		>330	125	18	34	5	1
13	l	RP>PP	>330	82	5	19	1	0
	r		>330	71	4	25	4	1
14	l	RP>PP	>330	106	16	16	1	0
	r		>330	91	11	29	3	0
15	l	RP>PP	>330	93	14	24	3	0
	r		>330	108	15	15	3	0
16	l	RP>PP	>330	122	9	23	4	0
	r		>330	113	16	34	5	0
17	l	RP>PP	>330	37	6	15	3	0
	r		>330	49	8	19	1	0
18	l	RP>PP	>330	43	2	20	3	0
	r		>330	59	8	13	2	1

55 = count used for computations; 55 = adjacent usage; >330 = not countable

Appendix 3

List of computed lg values (means of left and right hands) and lg reductions

Volunteer	Sequence	Reference handrub (RP) (Propan-2-ol 60% V/V)			Handrub with product under test (pp)		
		lg prevalues	lg postvalues	lg R	lg prevalues	lg postvalues	lg R
1	PP>RP	5.89	2.35	3.54	6.00	2.13	3.87
2	PP>RP	5.97	2.58	3.39	5.87	1.69	4.18
3	PP>RP	5.43	2.04	3.39	5.72	1.81	3.91
4	PP>RP	5.74	1.92	3.82	5.80	2.00	3.80
5	PP>RP	6.18	2.49	3.68	6.15	2.18	3.97
6	PP>RP	6.10	2.37	3.72	6.00	2.48	3.52
7	PP>RP	6.05	2.34	3.71	6.11	2.48	3.63
8	PP>RP	5.75	2.20	3.55	5.87	2.12	3.74
9	PP>RP	6.13	2.54	3.59	6.16	2.41	3.75
10	RP>PP	6.12	2.58	3.54	6.13	2.55	3.58
11	RP>PP	5.80	2.26	3.53	5.96	2.16	3.80
12	RP>PP	6.06	2.48	3.58	6.09	2.37	3.72
13	RP>PP	5.88	2.34	3.54	5.92	2.31	3.61
14	RP>PP	5.99	2.33	3.66	5.86	2.02	3.83
15	RP>PP	6.00	2.28	3.72	6.05	2.44	3.61
16	RP>PP	6.07	2.45	3.62	6.14	2.59	3.55
17	RP>PP	5.63	2.23	3.40	5.88	2.35	3.53
18	RP>PP	5.70	2.21	3.49	5.41	2.20	3.21
X	Overall	5.92	2.33	3.58	5.95	2.24	3.71
s		0.20	0.18	0.12	0.19	0.25	0.21
NN		18	18	18	18	18	18
X	PP>RP	5.92	2.32	3.60	5.96	2.14	3.82
s		0.24	0.23	0.15	0.16	0.28	0.19
NN		9	9	9	9	9	9
X	RP>PP	5.92	2.35	3.57	5.94	2.33	3.61
s		0.17	0.13	0.09	0.22	0.18	0.18
NN		9	9	9	9	9	9

Difference of mean Rs (RP>PP): 3.57 – 3.61 = (-0.04);
Difference of mean Rs (PP>RP): 3.60 – 3.82 = (-0.22);
Absolute difference of differences: Abs [(-0.22) - (-0.04)] = 0.18

Check of acceptance criteria:

1) Complete set of results from 18 volunteers available (minimum 18)
2) Mean of lg prevalues for RP = 5.92 and for PP = 5.95 (hence both greater than 5.00)
3) Individual lg reductions less than 3.00: with RP = 0 and with PP = 0
4) For group with sequence RP>PP difference of lg R: 3.57 – 3.61 = (-0.04); for group with sequence PP>RP difference of lg R: 3.60 – 3.82 = (-0.22); absolute difference of mean differences: Abs [(-0.22) - (-0.04)] = 0.18 (hence less than 2.00)
5) All quotients of weighted mean counts between 5 and 15 (results which were used for weighted mean counts in appendix 1 and appendix 2)

Acceptance criteria are fulfilled.

Appendix 4

Computation of individual differences of lg Rs of RP - PP

Volunteer	lg reduction (R)		Difference RP-PP
	Reference procedure (RP)	Product procedure (PP)	
1	3.54	3.87	-0.32
2	3.39	4.18	-0.79
3	3.39	3.91	-0.52
4	3.82	3.80	0.02
5	3.68	3.97	-0.29
6	3.72	3.52	0.20
7	3.71	3.63	0.08
8	3.55	3.74	-0.19
9	3.59	3.75	-0.16
10	3.54	3.58	-0.04
11	3.53	3.80	-0.27
12	3.58	3.72	-0.14
13	3.54	3.61	-0.07
14	3.66	3.83	-0.17
15	3.72	3.61	0.12
16	3.62	3.55	0.08
17	3.40	3.53	-0.13
18	3.49	3.21	0.28

Appendix 5

Sorting of individual differences and computation for Hodges-Lehmann 97,5% upper confidence limits

No	Sorted differences	Mean pairwise differences (di+dii)/2							
		0.28	0.2	0.12	0.08	0.08	0.02	-0.04	-0.07
1	0.28	0.28/1							
2	0.20	0.24/2	0.20/4						
3	0.12	0.20/3	0.16/7	0.12/12					
4	0.08	0.18/5	0.14/9	0.10/15	0.08/19				
5	0.08	0.18/6	0.14/10	0.10/16	0.08/20	0.08/21			
6	0.02	0.15/8	0.11/14	0.07/24	0.05/28	0.05/29	0.02/37		
7	-0.04	0.12/11	0.08/18	0.04/31	0.02/35	0.02/36	-0.01	-0.04	
8	-0.07	0.11/13	0.07/23	0.03/33	0.01/41	0.01	-0.02	-0.05	-0.01
9	-0.13	0.08/17	0.04/30	0	-0.02	-0.02	-0.05	-0.08	-0.04
10	-0.14	0.07/22	0.03/32	-0.01	-0.03	-0.03	-0.06	-0.09	-0.05
11	-0.16	0.06/25	0.02/34	-0.02	-0.04	-0.04	-0.07	-0.10	-0.05
12	-0.17	0.05/26	0.01/39	-0.03	-0.05	-0.05	-0.08	-0.11	
13	-0.19	0.05/27	0.01/40	-0.03	-0.05	-0.05	-0.08		
14	-0.27	0.01/38	-0.03	-0.07	-0.09	-0.09			
15	-0.29	0	-0.04	-0.08	-0.10				
16	-0.32	-0.02	-0.06	-0.10					
17	-0.52	-0.12	-0.16						
18	-0.79	-0.26							

The differences of the individual lg R's of RP-PP from table at the Appendix 4 are sorted in the second column and in the headline according to their size in descending order.

The median is between 9th and 10th value: [-0.13+(-0.14)]/2 = (-0.135). The numbers behind the fracture line represent the ranks.

The mean pairwise differences that not exceed the median are computed. From the table E.5 (EVS-EN 1500:2013) of critical values for Wilcoxon's matched-pairs signed-ranks test the entry for n=18 and for one-sided 0,025 level of significance, the critical value of 40 is found. Hence c = 40+1 = 41. The pairwise differences are sorted in descending order. The 41st value is 0.01. Hence the Hodges-Lehmann upper one-sided 97,5% confidence limit for the difference in lg Rs between RP and PP is 0.01, 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.

Appendix 6

Interpretation

In the case of the reference substance the mean of the log of the pre-values (lg prevalues) is 5.92, which overrides the validation criterion ≥ 5. Tested product CHEMISEPT GEL (Batch no. 198251017) shows the mean of the log of the pre-values (lg prevalues) 5.95, which is higher than the validation rate ≥ 5 as well.

There were not any of the lg reductions less than 3,00. The validation criterion is not more than three individual lg reductions less than 3,00 in RP.

Conclusion

Therefore, it can be claimed that by the validation criteria, test results and following counting: lg values of reduction, Wilcoxon test and Hodges-Lehmann system: the tested product – CHEMISEPT GEL - is accepted for usage in hygienic handrub disinfection procedures on the following application: rub 3 ml of the product onto the hands within 30 seconds.

Nordic Tersus
Laboratory

Test report No. shd1718

SURGICAL HAND DISINFECTION (EN 12791)

Name of the product:	Chemisept GEL
Batch number:	198251017
Order number:	17030
Manufacturer:	Chemi-Pharm Ltd.
Client, representative:	Chemi-Pharm Ltd., Põllu 132, Tallinn, 10917, ESTONIA
Date of delivery:	15.01.2018
Test material conditions:	No specific features, sample in the manufacturers tare
Storage conditions:	In room temperature, dark
Active substance – conc.:	Ethyl alcolol 72,5% wt, isopropyl alcohol 7.5% wt
Appearance of the product:	Transparent liquid
Test concentration:	Ready to use
Contact time:	90 sec
Interfering substance:	-
Neutralizer:	Polysorbate 80 30g/l; saponin 30 g/l, lecithin 3 g/l
Rinsing liquid:	-
Test organisms:	Normal skin flora
Testing method:	EVS-EN 12791:2016+A1:2017 Chemical disinfectants and antiseptics – Surgical hand disinfection – Test method and requirements (phase 2, step2)
Testing date:	12.02.2018-20.02.2018
Results:	look appendix 1-10

Date of test report: 23.02.2018

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Appendix 1

Validations and controls

Neutralizer validation:

The selected neutralizer is tested according to EN 13727:2012 + A2:2015 for *P.aeruginosa* ATCC 15442, *S. aureus* ATCC 6538, *E. hirae* ATCC 10541, *E. coli* K12, NCTC 10538 and according to EN 13624:20123 for *C. albicans* ATCC 10231. The neutralizer fulfilled all the criteria and passed the controls, therefore, the neutralizer is suitable for test.

P. aeruginosa ATCC 15442:

Validation suspension N_{vo}			Experimental conditions control (A)			Neutralizer control (B)			Method validation (C)		
V_{C1}	54	$\bar{x}$ = 52.5	V_{C1}	37	$\bar{x}$ = 40.5	V_{C1}	28	$\bar{x}$ = 32	V_{C1}	50	$\bar{x}$ = 47.5
V_{C2}	51		V_{C2}	44		V_{C2}	36		V_{C2}	45	
30 ≤ $\bar{x}$ N_{vo} ≤160 ? yes X; no □			$\bar{x}$ A is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no □			$\bar{x}$ B is ≥ 0,5 $\bar{x}$ N_{vo}? yesX; no □			$\bar{x}$ C is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no □		

Test suspension and test

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 2.31 x 10^9; log$N$ = 9.36
N and N_0	10^{-7}	223	241	N_0 = N/100; logN_0 = 7.36
	10^{-8}	24	21	7,17≤ logN_0≤7,70; yesX; no □

S. aureus ATCC 6538

Validation suspension N_{vo}			Experimental conditions control (A)			Neutralizer control (B)			Method validation (C)		
V_{C1}	47	$\bar{x}$ = 43.5	V_{C1}	36	$\bar{x}$ = 33.5	V_{C1}	28	$\bar{x}$ = 31	V_{C1}	36	$\bar{x}$ = 33
V_{C2}	40		V_{C2}	31		V_{C2}	34		V_{C2}	30	
30 ≤ $\bar{x}$ N_{vo} ≤160 ? yes X; no □			$\bar{x}$ A is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no □			$\bar{x}$ B is ≥ 0,5 $\bar{x}$ N_{vo}? yesX; no □			$\bar{x}$ C is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no □		

Test suspension and test

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 2.03 x 10^9; log$N$ = 9.31
N and N_0	10^{-7}	195	207	N_0 = N/100; logN_0 = 7.31
	10^{-8}	20	24	7,17≤ logN_0≤7,70; yesX; no □

E. hirae ATCC 10541

Validation suspension N_{vo}			Experimental conditions control (A)			Neutralizer control (B)			Method validation (C)		
V_{C1}	49	$\bar{x}$ = 41	V_{C1}	42	$\bar{x}$ = 42	V_{C1}	38	$\bar{x}$ = 31.5	V_{C1}	34	$\bar{x}$ = 36.5
V_{C2}	33		V_{C2}	42		V_{C2}	25		V_{C2}	39	
30 ≤ $\bar{x}$ N_{vo} ≤160? yes X;no □			$\bar{x}$ A is ≥ 0,5 $\bar{x}$ N_{vo}?yes X;no□			$\bar{x}$ B is ≥ 0,5$\bar{x}$ N_{vo}? yesX; no □			$\bar{x}$ C is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no □		

Test suspension and test

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 1.87 x 10^9; log$N$ = 9.28
	10^{-7}	180	199	N_0 = N/100; logN_0 = 7.28
N and N_0	10^{-8}	16	20	7,17≤ logN_0≤7,70; yesX; no □

E. coli K12, NCTC 10538

Validation suspension N_{vo}			Experimental conditions control (A)			Neutralizer control (B)			Method validation (C)		
V_{C1}	37	$\bar{x}$ = 34	V_{C1}	26	$\bar{x}$ = 23.5	V_{C1}	22	$\bar{x}$ = 23.5	V_{C1}	32	$\bar{x}$ = 29.5
V_{C2}	31		V_{C2}	21		V_{C2}	25		V_{C2}	27	
30 ≤ $\bar{x}$ N_{vo} ≤160 yes X; no □			$\bar{x}$ A is ≥ 0,5 $\bar{x}$ N_{vo}? yesX;no□			$\bar{x}$ B is ≥ 0,5 $\bar{x}$ N_{vo}? yesX;no □			$\bar{x}$ C is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no □		

Test suspension and test

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 1.60 x 10^9; log$N$ = 9.21
	10^{-7}	152	166	N_0 = N/100; logN_0 = 7.21
N and N_0	10^{-8}	17	18	7,17≤ logN_0≤7,70; yesX; no □

C. albicans ATCC 10231

Validation suspension N_{vo}			Experimental conditions control (A)			Neutralizer control (B)			Method validation (C)		
V_{C1}	62	$\bar{x}$ = 59.5	V_{C1}	49	$\bar{x}$ = 45.5	V_{C1}	38	$\bar{x}$ = 41.5	V_{C1}	48	$\bar{x}$ = 52.5
V_{C2}	57		V_{C2}	42		V_{C2}	45		V_{C2}	57	
30 ≤ $\bar{x}$ N_{vo} ≤160? yes X;no □			$\bar{x}$ A is ≥ 0,5 $\bar{x}$ N_{vo}?yes X;no□			$\bar{x}$ B is ≥ 0,5 $\bar{x}$ N_{vo}? yesX;no □			$\bar{x}$ C is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no □		

Test suspension and test

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 2.28 x 10^8; log$N$ = 8.36
N and N_0	10^{-6}	217	239	$N_0 = N/100$; logN_0 = 6.36
	10^{-7}	25	20	6,17≤ logN_0≤6,70; yesX; no □

Quality control on soft soap:
The soft soap met the quality criteria:

1. Identity: when sulphuric acid (10%) was added to an undiluted soft soap solution the free fatty acids separated out as a dense white precipitate which, when gently heated, melted into oily droplets and collected on the surface of the liquid.
2. Purity: 1g of soft soap dissolved in 2.0ml of warm water into clear liquid.
3. Alcohol-insoluble substances: 2.5g of soft soap contained 3 mg of alcohol-insoluble substances which meets the criterion (must be <5mg)
4. Loss on drying: 39,0% which is less than the maximum of 45% and therefore, passed the test.
5. Determination of content: after following the procedures described in EN12791 standard, the residue of soft soap weighed 1.173g, which meets the quality criterion (shall weight 1.125g to 1.25g, corresponding to a content of 45.0% to 50,0% of fatty acids)

Quality control of surgical gloves

Quality control of surgical gloves was carried out on the instructions given in EN12791. The tested gloves did not show any microbial growth, meaning the gloves were sterile and free from any microbial activity.

Appendix 2

Reference surgical hand disinfection procedure – Experimental results

Preparation: "RP" Reference [propan-1-ol 60% (v/v)]
Date of test: 12.02.2018 - 20.02.2018
Application: Rubbing hands during 3 min

Volunteer			Number of cfu per plate from dilution 10^x								
RP		Hand	Prevalues			Immediate postvalues			3h Postvalues		
No	Sequence	left or right	-1	-2	-3	0	-1	-2	0	-1	-2
1	PP>RP	l	>330	>330	81	105	18	4			
		r	>330	>330	76				92	15	3
2	PP>RP	l	>330	>330	100				103	10	3
		r	>330	>330	91	128	16	1			
3	PP>RP	l	>330	>330	87	119	8	2			
		r	>330	>330	104				99	13	2
4	PP>RP	l	>330	>330	76				93	11	0
		r	>330	>330	61	82	8	0			
5	PP>RP	l	>330	>330	116	68	19	4			
		r	>330	>330	90				54	8	0
6	PP>RP	l	>330	>330	84				89	12	2
		r	>330	>330	97	100	14	2			
7	PP>RP	l	>330	>330	42	74	10	1			
		r	>330	>330	36				55	8	1
8	PP>RP	l	>330	>330	72				62	6	0
		r	>330	>330	60	49	7	0			
9	PP>RP	l	>330	>330	46	106	14	2			
		r	>330	>330	37				84	10	2
10	PP>RP	l	>330	>330	92				91	11	0
		r	>330	>330	51	99	6	0			
11	PP>RP	l	>330	>330	64	84	5	0			
		r	>330	>330	85				71	14	3
12	PP>RP	l	>330	>330	101				41	6	0
		r	>330	>330	75	58	9	3			
13	RP >PP	l	>330	138	16	73	5	0			
		r	>330	116	16				59	8	1
14	RP >PP	l	>330	>330	64				79	15	4
		r	>330	>330	87	82	14	4			
15	RP >PP	l	>330	>330	106	94	15	3			
		r	>330	>330	113				104	22	5
16	RP >PP	l	>330	291	32				54	8	2
		r	>330	>330	37	70	10	2			
17	RP >PP	l	>330	>330	54	113	17	5			
		r	>330	>330	85				92	14	3
18	RP >PP	l	>330	>330	88				28	5	0
		r	>330	>330	50	33	2	0			
19	RP >PP	l	>330	>330	42	81	8	2			
		r	>330	>330	51				68	10	2
20	RP >PP	l	>330	>330	49				61	4	0
		r	>330	>330	71	75	11	2			
21	RP >PP	l	>330	174	19	107	16	5			
		r	>330	205	28				83	6	0
22	RP >PP	l	>330	>330	126				51	9	2
		r	>330	>330	120	77	6	0			
23	RP >PP	l	>330	>330	79	42	3	0			
		r	>330	>330	109				30	5	0
24	RP >PP	l	>330	>330	115				53	7	0
		r	>330	>330	101	85	9	3			

Underlined = count used for further computation
Highlighted = indicates adjacent dilutions used for computation

Appendix 3

Surgical hand rub procedure with test product – Experimental results
Preparation: "PP" Chemisept GEL
Date of test: 12.02.2018 - 20.02.2018
Application: 2 x 3 ml, 90 s

Volunteer			Number of cfu per plate from dilution 10^x								
PP		Hand	Prevalues			Immediate postvalues			3h Postvalues		
No	Sequence	left or right	-1	-2	-3	0	-1	-2	0	-1	-2
1	RP >PP	l	>330	>330	93	87	12	2			
		r	>330	>330	85				74	5	0
2	RP >PP	l	>330	>330	122				42	8	2
		r	>330	>330	154	61	7	3			
3	RP >PP	l	>330	>330	63	101	18	3			
		r	>330	>330	49				92	9	2
4	RP >PP	l	>330	>330	38				104	15	1
		r	>330	>330	51	122	13	5			
5	RP >PP	l	>330	>330	106	39	5	0			
		r	>330	>330	84				44	13	2
6	RP >PP	l	>330	>330	117				75	4	1
		r	>330	>330	136	88	14	2			
7	RP >PP	l	>330	>330	59	61	8	0			
		r	>330	>330	41				73	11	2
8	RP >PP	l	>330	>330	75				22	4	0
		r	>330	>330	84	34	5	0			
9	RP >PP	l	>330	>330	44	133	16	3			
		r	>330	>330	67				107	14	2
10	RP >PP	l	>330	>330	106				99	6	0
		r	>330	>330	81	116	20	4			
11	RP >PP	l	>330	>330	124	73	11	3			
		r	>330	>330	165				26	2	0
12	RP >PP	l	>330	>330	120				22	2	1
		r	>330	>330	103	25	3	1			
13	PP>RP	l	>330	184	22	49	8	3			
		r	>330	142	16				41	7	2
14	PP>RP	l	>330	>330	59				88	13	3
		r	>330	>330	74	109	16	5			
15	PP>RP	l	>330	>330	104	53	8	0			
		r	>330	>330	119				37	5	0
16	PP>RP	l	>330	254	28				76	8	2
		r	>330	233	20	85	11	0			
17	PP>RP	l	>330	>330	99	69	10	4			
		r	>330	>330	72				52	8	0
18	PP>RP	l	>330	>330	61				36	2	0
		r	>330	>330	84	47	2	0			
19	PP>RP	l	>330	163	20	91	15	4			
		r	>330	>330	34				108	18	4
20	PP>RP	l	>330	>330	68				75	12	3
		r	>330	>330	90	66	9	2			
21	PP>RP	l	>330	>330	42	129	15	4			
		r	>330	>330	58				85	21	5
22	PP>RP	l	>330	>330	142				48	5	0
		r	>330	>330	116	61	8	0			
23	PP>RP	l	>330	>330	84	28	4	0			
		r	>330	>330	91				39	5	0
24	PP>RP	l	>330	>330	138				65	3	0
		r	>330	>330	106	71	14	2			

Underlined = count used for further computation
Highlighted = indicates adjacent dilutions used for computation

Appendix 4

List of computed lg values and lg reductions
Preparation: "RP" Reference [propan-1-ol 60% (v/v)]

Voluntee	Sequence	Immediate effect			3h effect		
No		lg Prevalues	lg Postvalues	lg Reduction	lg Prevalues	lg Postvalues	lg Reduction
1	RP >PP	4.91	3.02	1.89	4.88	2.96	1.92
2	RP >PP	4.96	3.11	1.85	5.00	3.01	1.99
3	RP >PP	5.02	3.08	1.86	5.02	3.00	2.02
4	RP >PP	4.88	2.91	1.87	4.88	2.97	1.91
5	RP >PP	4.95	2.83	2.23	4.95	2.73	2.22
6	RP >PP	4.92	3.00	1.99	4.92	2.95	1.97
7	RP >PP	4.56	2.87	1.75	4.56	2.74	1.82
8	RP >PP	4.86	2.69	2.09	4.86	2.79	2.07
9	RP >PP	4.57	3.03	1.64	4.57	2.92	1.64
10	RP >PP	4.96	3.00	1.71	4.96	2.96	2.00
11	RP >PP	4.93	2.92	1.88	4.93	2.85	2.08
12	RP >PP	5.00	2.76	2.11	5.00	2.61	2.39
13	PP>RP	4.06	2.86	1.28	4.06	2.77	1.29
14	PP>RP	4.81	2.91	2.03	4.81	2.90	1.91
15	PP>RP	5.05	2.97	2.05	5.05	3.02	2.04
16	PP>RP	4.46	2.85	1.72	4.46	2.73	1.73
17	PP>RP	4.93	3.05	1.68	4.93	2.96	1.97
18	PP>RP	4.94	2.52	2.18	4.94	2.45	2.50
19	PP>RP	4.71	2.91	1.72	4.71	2.83	1.88
20	PP>RP	4.69	2.88	1.98	4.69	2.79	1.90
21	PP>RP	4.31	3.03	1.21	4.31	2.92	1.39
22	PP>RP	5.10	2.89	2.19	5.10	2.71	2.39
23	PP>RP	5.04	2.62	2.27	5.04	2.48	2.56
24	PP>RP	5.06	2.93	2.07	5.06	2.72	2.34
Mean		4.82	2.90	1.89	4.82	2.82	2.00
Standard deviation		0.26	0.14	0.27	0.26	0.16	0.31
N		24	24	24	24	24	24

Appendix 5

List of computed lg values and lg reductions

Preparation: "PP" Chemisept GEL

Volunteer	Sequence	Immediate effect			3h effect		
No		lg Prevalues	lg Postvalues	lg Reduction	lg Prevalues	lg Postvalues	lg Reduction
1	PP>RP	4.97	2.94	2.03	4.93	2.87	2.06
2	PP>RP	5.19	2.79	2.40	5.09	2.62	2.46
3	PP>RP	4.80	3.00	1.80	4.69	2.96	1.73
4	PP>RP	4.71	3.09	1.62	4.58	3.02	1.56
5	PP>RP	5.03	2.59	2.43	4.92	2.64	2.28
6	PP>RP	5.13	2.94	2.19	5.07	2.88	2.19
7	PP>RP	4.77	2.79	1.99	4.61	2.86	1.75
8	PP>RP	4.92	2.53	2.39	4.88	2.34	2.53
9	PP>RP	4.64	3.12	1.52	4.83	3.03	1.80
10	PP>RP	4.91	3.06	1.84	5.03	3.00	2.03
11	PP>RP	5.09	2.86	2.23	5.22	2.41	2.80
12	PP>RP	5.01	2.41	2.61	5.08	2.34	2.74
13	RP >PP	4.26	2.69	1.57	4.15	2.61	1.54
14	RP >PP	4.87	3.04	1.83	4.77	2.94	1.83
15	RP >PP	5.02	2.72	2.29	5.08	2.57	2.51
16	RP >PP	4.37	2.93	1.44	4.40	2.88	1.52
17	RP >PP	5.00	2.84	2.16	4.86	2.72	2.14
18	RP >PP	4.92	2.67	2.25	4.79	2.56	2.23
19	RP >PP	4.21	2.96	1.25	4.53	3.03	1.50
20	RP >PP	4.95	2.82	2.13	4.83	2.88	1.96
21	RP >PP	4.62	3.11	1.51	4.76	2.93	1.83
22	RP >PP	5.06	2.79	2.28	5.15	2.68	2.47
23	RP >PP	4.92	2.45	2.48	4.96	2.59	2.37
24	RP >PP	5.03	2.85	2.17	5.14	2.81	2.33
Mean		4.85	2.83	2.02	4.85	2.76	2.09
Standard deviation		0.26	0.20	0.38	0.26	0.22	0.39
N		24	24	24	24	24	24

Appendix 6

Test for sequence effects

Test of sequence of lgR "Immediate effect"

	Sequence		Absolute difference
Procedure	RP>PP	PP>RP	[RP>PP]-[PP>RP]
	Mean s.d.N	Mean s.d.N	
RP (Propan-1-ol 60% v/v)	1.91 0.17 12	1.87 0.35 12	
PP	1.95 0.41 12	2.09 0.34 12	
Difference of Means			
RP - PP	0.04	0.22	0.18
s.d. standard deviation			

Test of sequence of lg R "3-hours effect"

	Sequence		Absolute difference
Procedure	RP>PP	PP>RP	[RP>PP]-[PP>RP]
	Mean s.d.N	Mean s.d.N	
RP (Propan-1-ol 60% v/v)	2.00 0.19 12	1.99 0.41 12	
PP	2.02 0.37 12	2.16 0.41 12	
Difference of Means			
RP - PP	0.02	0.17	0.15
s.d. standard deviation			

"RP>PP" means: RP tested before PP and
"PP>RP" means: PP tested before RP

Appendix 7

Individual differences of lgRs between RP and PP for immediate and 3 h effects

	Immediate effect			3h effect		
Volunteer	RP	PP	Difference RP-PP	RP	PP	Difference RP-PP
1	1.89	2.03	-0.14	1.92	2.06	-0.14
2	1.85	2.40	-0.55	1.99	2.46	-0.47
3	1.86	1.80	0.06	2.02	1.73	0.29
4	1.87	1.62	0.25	1.91	1.56	0.35
5	2.23	2.43	-0.20	2.22	2.28	-0.06
6	1.99	2.19	-0.20	1.97	2.19	-0.22
7	1.75	1.99	-0.24	1.82	1.75	0.07
8	2.09	2.39	-0.30	2.07	2.53	-0.46
9	1.64	1.52	0.12	1.64	1.80	-0.16
10	1.71	1.84	-0.13	2.00	2.03	-0.03
11	1.88	2.23	-0.35	2.08	2.80	-0.72
12	2.11	2.61	-0.50	2.39	2.74	-0.35
13	1.28	1.57	-0.29	1.29	1.54	-0.25
14	2.03	1.83	0.20	1.91	1.83	0.08
15	2.05	2.29	-0.24	2.04	2.51	-0.47
16	1.72	1.44	0.28	1.73	1.52	0.21
17	1.68	2.16	-0.48	1.97	2.14	-0.17
18	2.18	2.25	-0.07	2.50	2.23	0.27
19	1.72	1.25	0.47	1.88	1.50	0.38
20	1.98	2.13	-0.15	1.90	1.96	-0.06
21	1.21	1.51	-0.30	1.39	1.83	-0.44
22	2.19	2.28	-0.09	2.39	2.47	-0.08
23	2.27	2.48	-0.21	2.56	2.37	0.19
24	2.07	2.17	-0.10	2.34	2.33	0.01

Acceptance criteria for test results

a) number of complete test results: 24

b) overall mean

of individual lg prevalues RP (immediate/3 h effect): 4.82/4.82 (req. min 3.5/min 3.5)

of individual lg prevalues PP (immediate/3 h effect): 4.85/4.85 (req.min 3.5/min 3.5)

c) absolute differences of mean between RP and PP

c1) between groups RP>PP and PP<RP, immediate effect: 0.18 (<2.0)

c2) between groups PP>RP and RP<PP, immediate effect: 0.15 (<2.0)

d) all quotients of two adjacent dilutions used for computation i.e. counts highlighted in tables appendix 2 and appendix 3 are between 5 and 15

All acceptance criteria are fulfilled.

Appendix 8

Computation of the Hodges – Lehmann 97.5 upper confidence limit

Computation of the Hodges Lehmann 97.5 upper confidence limit for immediate effect

Sorted differences of RP-PP (descending order)	Mean pairwise differences $(d_i + d_{jj})/2$										
	0.47	0.28	0.25	0.20	0.12	0.06	-0.07	-0.09	-0.10	-0.13	-0.14
0.47	0.47/1										
0.28	0.38/2	0.28/6									
0.25	0.36/3	0.27/7	0.25/9								
0.2	0.34/4	0.24/10	0.23/11	0.20/14							
0.12	0.30/5	0.20/13	0.19/17	0.16/23	0.12/28						
0.06	0.27/8	0.17/20	0.16/22	0.13/27	0.09/37	0.08/38					
-0.07	0.20/12	0.11/31	0.09/36	0.07/45	0.03/55	0.01/65	-0.07				
-0.09	0.19/15	0.10/32	0.08/39	0.06/46	0.02/64	0/71	-0.08	-0.09			
-0.1	0.19/16	0.09/35	0.08/40	0.05/41	0.01/67	-0.01/79	-0.09	-0.1	-0.1		
-0.13	0.17/18	0.08/41	0.06/47	0.03/56	-0.01/77	-0.02	-0.1	-0.11	-0.12	-0.13	
-0.14	0.17/19	0.07/43	0.06/48	0.03/57	-0.01/78	-0.03	-0.11	-0.12	-0.12	-0.14	-0.14
-0.15	0.16/21	0.07/44	0.05/50	0.03/58	-0.02	-0.03	-0.11	-0.12	-0.13	-0.14	-0.15
-0.2	0.14/24	0.04/52	0.03/59	0/68	-0.04	-0.06	-0.14	-0.15	-0.15	-0.17	-0.17
-0.2	0.14/24	0.04/53	0.02/62	0/69	-0.04	-0.06	-0.14	-0.15	-0.15	-0.17	-0.17
-0.21	0.13/26	0.04/54	0.02/63	0/70	-0.05	-0.06	-0.14	-0.15	-0.16	-0.17	
-0.24	0.12/29	0.02/60	0.01/66	-0.02/82	-0.06	-0.08	-0.16	-0.17	-0.17		
-0.24	0.12/30	0.02/61	0/72	-0.02	-0.06	-0.08	-0.16	-0.17			
-0.29	0.09/33	-0.01/74	-0.02/81	-0.05	-0.09	-0.1	-0.18				
-0.3	0.09/34	-0.01/75	-0.03	-0.05	-0.09	-0.11					
-0.3	0.08/42	-0.01/76	-0.03	-0.05	-0.09						
-0.35	0.06/49	-0.04	-0.05	-0.08							
-0.48	-0.01/73	-0.1	-0.12								
-0.5	-0.02/80	-0.02									
-0.55	-0.04										

The median of the difference between RP-PP is between 12th and 13th value: [(-0.15) + (-0.2)] /2 = = (-0.175) The numbers after the values represent ranks.

The mean pairwise differences that do not exceed the median (here -0.175) are computed. The critical values for Wilcoxin's matched-pair signed-ranks test the entry for n=24 and a one-sided P=0.025 level of significance the critical value is 81. Hence c = 81 + 1 = 82. The pairwise differences are sorted in descending order. The 82nd value is -0.02. Hence, the Hodges – Lehmann upper one-sided 97.5% confidence limit for the difference in lgRs between RP and PP is -0.02, which is below the agreed inferiority margin of 0.75.

Therefore, the hypothesis of inferiority of the immediate effect of PP versus RP can be rejected.

Appendix 9

Computation of the Hodges Lehmann 97.5% upper confidence limit for the 3 h effect

Sorted differences of RP-PP (descending order)	Mean pairwise differences $(d_i + d_j)/2$										
	0.38	0.35	0.29	0.27	0.21	0.19	0.08	0.07	0.01	-0.03	-0.06
0.38	0.38/1										
0.35	0.37/2	0.35/3									
0.29	0.34/4	0.32/6	0.29/10								
0.27	0.33/5	0.31/7	0.28/11	0.27/14							
0.21	0.30/8	0.28/12	0.25/15	0.24/17	0.21/23						
0.19	0.29/9	0.27/13	0.24/16	0.23/20	0.20/24	0.19/27					
0.08	0.23/18	0.22/21	0.19/26	0.18/31	0.15/40	0.14/41	0.08/63				
0.07	0.23/19	0.21/22	0.18/30	0.17/32	0.14/42	0.13/46	0.08/64	0.07/75			
0.01	0.20/25	0.18/27	0.15/39	0.14/43	0.11/58	0.10/59	0.04	0.04	0.01		
-0.03	0.18/28	0.16/35	0.13/45	0.12/47	0.09/62	0.08/65	0.03	0.02	-0.01	-0.03	
-0.06	0.16/33	0.15/36	0.12/48	0.11/56	0.08/66	0.07/73	0.01	0.01	-0.02	-0.04	-0.06
-0.06	0.16/34	0.15/37	0.12/49	0.11/57	0.08/67	0.07/74	0.01	0	-0.03	-0.05	-0.06
-0.08	0.15/38	0.14/44	0.11/54	0.10/58	0.07/72	0.06/79	0	-0.01	-0.04	-0.06	-0.07
-0.14	0.12/50	0.11/53	0.07/71	0.06/78	0.03	0.02	-0.03	-0.04	-0.07	-0.09	-0.1
-0.16	0.11/51	0.09/60	0.06/76	0.05/80	0.02	0.01	-0.04	-0.05	-0.08	-0.1	
-0.17	0.11/52	0.09/61	0.06/77	0.05/81	0.02	0.01	-0.05	-0.05	-0.08		
-0.22	0.08/68	0.07/70	0.04	0.03	0	-0.02	-0.07	-0.08			
-0.25	0.07/69	0.05/82	0.02	0.01	-0.02	-0.03	-0.09				
-0.35	0.02	0	-0.03	-0.04	-0.07	-0.08					
-0.44	-0.03	-0.05	-0.08	-0.09	-0.12						
-0.46	-0.04	-0.06	-0.09	-0.1							
-0.47	-0.04	-0.06	-0.09								
-0.47	-0.05	-0.06									
-0.72	-0.17										

The median of the difference between RP-PP is between 12th and 13th value: [(-0.06) + (-0.08)] /2 = = (-0.07) The numbers after the values represent ranks.

The mean pairwise differences that do not exceed the median (here -0.07) are computed. The critical values for Wilcoxin's matched-pair signed-ranks test the entry for n=24 and a one-sided P=0.025 level of significance the critical value is 81. Hence c = 81 + 1 = 82. The pairwise differences are sorted in descending order. The 82nd value is 0.05. Hence, the Hodges – Lehmann upper one-sided 97.5% confidence limit for the difference in IgRs between RP and PP is 0.05, which is below the agreed inferiority margin of 0.85.

Therefore, the hypothesis of inferiority of the immediate effect of PP versus RP can be rejected.

Conclusion: as both the immediate and 3 h effects of RP are significantly inferior to those of PP the product fulfilled the requirements of EN 12791.

Appendix 10

Interpretation:

Both, the reference substance and tested product (Chemisept GEL, batch no. 198251017) fulfilled all acceptance criteria in case of number of complete test results, overall means of individual lg pre-values and absolute differences of mean between RP and PP (see appendix 7).

Conclusion:

According to the EVS-EN 12791:2016+A1:2017, the fulfilled validation criteria, test results, lg values and Hodges Lehmann system show that the tested product – Chemisept GEL – is accepted in surgical hand disinfection procedures on the following application: rub 2x3 ml of the product onto the hands within 90 seconds.

Nordic Tersus
Laboratory

Test report No. shd0318

EVALUATION OF FUNGICIDAL OR YEASTICIDAL ACTIVITY IN THE MEDICAL AREA (EN 13624)

Name of the product:	Chemisept GEL
Batch number:	198251017
Order number:	17030
Manufacturer:	Chemi-Pharm Ltd.
Client, representative:	Chemi-Pharm Ltd., Põllu 132, Tallinn, 10917, ESTONIA
Date of delivery:	15.01.2018
Test material conditions:	No specific features, sample in the manufacturers tare
Storage conditions:	In room temperature, dark
Active substance – conc.:	Ethyl alcohol 72.5% wt, isopropyl alcohol 7.5% wt
Appearance of the product:	Transparent liquid
Test concentration:	Ready to use
Contact time:	15 sec
Interfering substance:	15 g/l bovine albumin + 15 ml/l sheep blood erythrocytes = Dirty conditions; 1.5 g/l bovine albumin = clean conditions
Rinsing liquid:	Tryptone 1 g/l + NaCl 9 g/l
Neutralizer:	-
Test organisms:	*Candida albicans* ATCC 10231
Testing method:	EVS-EN 13624:2013 Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity in the medical area.
Testing date:	23.01.2018 – 25.01.2018
Results:	look appendix 1-2

Date of test report: 26.01.2018

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TEST RESULTS (yeasticidal suspension test)

EVS-EN 13624:2013; Phase 2, step 1;
Membrane filtration method;
Rinsing liquid: Tryptone 1 g/l + NaCl 9 g/l;
Test organism: *Candida albicans* ATCC 10231;
Test temperature: +20° C; Incubation temperature: +30° C
Interfering substance: 15 g/l bovine albumin + 15 ml/l sheep blood erythrocytes = Dirty conditions;
1.5g/l bovine albumin = clean conditions;
Nordic Tersus Laboratory LLC.; Date of test: 23.01.2018 – 25.01.2018.
Responsible person: I

Validation and controls

Dirty and clean conditions

Validation suspension N_{vo}			Experimental conditions control (A)			Filtration control (B)			Method validation (C)		
V_{C1}	45	$\bar{x}$ = 43.5	V_{C1}	38	$\bar{x}$ = 35	V_{C1}	41	$\bar{x}$ = 37	V_{C1}	39	$\bar{x}$ = 41.5
V_{C2}	42		V_{C2}	32		V_{C2}	33		V_{C2}	44	
30 ≤ $\bar{x}$ N_{vo} ≤160?yes X; no □			$\bar{x}$ A is ≥ 0.5 $\bar{x}$ N_{vo}?yes X; no □			$\bar{x}$ B is ≥ 0.5 $\bar{x}$ N_{vo}?yesX; no□			$\bar{x}$ C is ≥ 0.5 $\bar{x}$ N_{vo}? yesX; no□		

Test suspension and test

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 1.71 x 10^8; log$N$ = 8.23
N and N_0	10^{-6}	172	164	N_0 = N/100; logN_0 = 6.23
	10^{-7}	19	22	6.17≤ logN_0≤6.70; yesX; no □

Experimental results

Concentration of the product	Dilution step	V_{C1}	V_{C2}	Na (=$\bar{x}$*10)	log Na	logR	Contact time	Conditions
Ready to use	-	<14	<14	<140	<2.15	>4.08	15 s	dirty
Ready to use	-	<14	<14	<140	<2.15	>4.08	15 s	clean

Explanations:

V_C = count per ml (one plate or more)

$\bar{x}$ = average of V_{C1} and V_{C2} (1. + 2. duplicate)

N = cfu/ml microbes in testsuspension

N_0 = cfu/ml at the start of the contact time (t=0)

N_{vo} = cfu/ml in the validation suspension (t=0)

Na = surviving microbes after the test

R = reduction factor (R= N_0/ Na; LogR=LogN_0 - Log Na)

Interpretation:

The ready to use product for surgical hand disinfection **CHEMISEPT GEL** (batch no. 198251017) was tested according to the test method EVS-EN 13624:2013. The test was performed at 20 °C ± 1 °C, under dirty and clean conditions with the contact time of 15 s. The membrane filtration method was used for testing the produts effectiveness against the reference strain: *Candida albicans* ATCC 10231. Under dirty and clean conditions the tested product was effective against the reference strain within 15 s of contact time.

Conclusion:

The surviving count of the reference strains showed at least 4 lg reduction meaning that **under dirty and clean conditions the ready to use product CHEMISEPT GEL has a yeasticidal effect in case of surgical hand disinfection within 15 sec.**

Nordic Tersus
Laboratory

Test report No. shd1318

EVALUATION OF MYCOBACTERICIDAL ACTIVITY (EN 14348)

Name of the product:	Chemisept GEL
Batch number:	198251017
Order number:	17030
Manufacturer:	Chemi-Pharm Ltd.
Client, representative:	Chemi-Pharm Ltd., Põllu 132, Tallinn, 10917, Estonia,
Date of delivery:	15.01.2018
Test material conditions:	No specific features, sample in the manufacturers tare
Storage conditions:	In room temperature, dark;
Active substance – conc.:	Ethyl alcohol 72.5% wt, isopropyl alcohol 7.5% wt
Appearance of the product:	Transparent liquid
Test concentration:	Ready to use
Test conditions:	Clean conditions
Contact time:	30 sec, 60 min(obligatory)
Interfering substance:	0,3 g/l bovine albumin = clean conditions
Test neutralizer:	-
Rinsing liquid:	Distilled water
Test organisms:	*Mycobacterium terrae* ATCC 15755; *Mycobacterium avium* ATCC 15769
Testing method base:	EVS-EN 14348:2005 – Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants in the medical area including instrument disinfectants - Test methods and requirements (phase 2, step 1)
Testing date:	29.01.2018 – 19.02.2018
Results:	look appendix 1-3

Date of test report: 26.02.2018

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Appendix 1

TEST RESULTS (mycobactericidal suspension test)

EVS-EN 14348:2005; Phase 2, step 1;
Membrane filtration method; Spread plate;
Rinsing liquid: Distilled water;
Test organism: *Mycobacterium terrae* ATCC 15755;
Test temperature: +20° C; Incubation temperature: +37° C
Solvents: diluent, water;
Interfering substance: 0,3 g/l bovine albumin = clean conditions
Nordic Tersus Laboratory LLC.;
Date of test: 29.01.2018 – 19.02.2018
Responsible person:

Validation and controls

Clean conditions

Validation suspension N_{vo}			Experimental conditions (A)			Filtration control (B)			Method validation (C)		
V_{C1}	80	$\bar{x}$ = 76	V_{C1}	49	$\bar{x}$ = 49,5	V_{C1}	62	$\bar{x}$ = 53,5	V_{C1}	74	$\bar{x}$ = 72,5
V_{C2}	72		V_{C2}	50		V_{C2}	45		V_{C2}	71	
$30 \leq \bar{x}\ N_{vo} \leq 160$? yes X; no □			$\bar{x}$ A is $\geq 0,5\ \bar{x}\ N_{vo}$? yes X; no □			$\bar{x}$ B is $\geq 0,5\ \bar{x}\ N_{vo}$? yes X; no □			$\bar{x}$ C is $\geq 0,5\ \bar{x}\ N_{vo}$? yes X; no □		

Test suspension and test

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm} = 2,56 \times 10^9$; $\log N = 9,41$
	10^{-7}	262	249	$N_0 = N/10$; $\log N_0 = 8,41$
N and N_0	10^{-8}	30	23	$8,17 \leq \log N_0 \leq 8,7$; yes X; no □

Experimental results

Concentration of the product.%	Dilution step	V_{C1}	V_{C2}	Na (=x̄*10)	log Na	logR	Contact time	Conditions
RTU	10^0	<14	<14	<140	<2,15	>6,26	30 sec	clean
	10^{-1}	<14	<14					
	10^{-2}	<14	<14					
	10^{-3}	<14	<14					
RTU	10^0	<14	<14	<140	<2,15	>6,26	60 min	clean
	10^{-1}	<14	<14					
	10^{-2}	<14	<14					
	10^{-3}	<14	<14					

Explanations:

V_C = count per ml (one plate or more)

x̄ = average of V_{C1} and V_{C2} (1. + 2. duplicate)

N = cfu/ml microbes in testsuspension

N_0 = cfu/ml at the start of the contact time (t=0)

N_{vo} = cfu/ml in the validation suspension (t=0)

Na = surviving microbes after the test

R = reduction factor (R= N_0/ Na; LogR=LogN_0 - Log Na)

Appendix 2

TEST RESULTS (mycobactericidal suspension test)

EVS-EN 14348:2005; Phase 2, step 1;
Membrane filtration method; Spread plate;
Rinsing liquid: Distilled water;
Test organism: *Mycobacterium avium* ATCC 15769;
Test temperature: +20° C; Incubation temperature: +37° C
Solvents: diluent, water;
Interfering substance: 0,3 g/l bovine albumin = clean conditions
Nordic Tersus Laboratory LLC.;
Date of test: 29.01.2018 – 19.02.2018
Responsible person:

Validation and controls

Clean conditions

Validation suspension N_{vo}			Experimental conditions (A)			Filtration control (B)			Method validation (C)		
V_{C1}	94	$\bar{x}$ = 93	V_{C1}	80	$\bar{x}$ = 74,5	V_{C1}	65	$\bar{x}$ = 62	V_{C1}	93	$\bar{x}$ = 94,5
V_{C2}	92		V_{C2}	69		V_{C2}	59		V_{C2}	96	
30 ≤ $\bar{x}$ N_{vo} ≤160 ? yes X; no □			$\bar{x}$ A is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no □			$\bar{x}$ B is ≥ 0,5 $\bar{x}$ N_{vo}? yesX; no □			$\bar{x}$ C is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no □		

Test suspension and test

Test suspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 2,86 x 10^9; log$N$ = 9,46
N *and* N_0	10^{-7}	283	291	$N_0 = N/10$; logN_0 = 8,46
	10^{-8}	31	25	8,17≤ logN_0≤8,7; yes X; no □

Experimental results

Concent-ration of the product.%	Dilu-tion step	V_{C1}	V_{C2}	Na (=x̄*10)	log Na	logR	Contact time	Condi-tions
RTU	10^0	<14	<14	<140	<2,15	>6,31	30 sec	clean
	10^{-1}	<14	<14					
	10^{-2}	<14	<14					
	10^{-3}	<14	<14					
RTU	10^0	<14	<14	<140	<2,15	>6,31	60 min	clean
	10^{-1}	<14	<14					
	10^{-2}	<14	<14					
	10^{-3}	<14	<14					

Explanations:

V_C = count per ml (one plate or more)

x̄ = average of V_{C1} and V_{C2} (1. + 2. duplicate)

N = cfu/ml microbes in testsuspension

N_0 = cfu/ml at the start of the contact time (t=0)

N_{vo} = cfu/ml in the validation suspension (t=0)

Na = surviving microbes after the test

R = reduction factor (R= N_0/ Na; LogR=LogN_0 - Log Na)

Appendix 3

Interpretation

Using the EN 14348 standard, there was tested ready to use product – **Chemisept GEL** (Batch No. 198251017) at 20 °C ± 1 °C, with the contact time 30 sec under clean conditions. The membrane filtration method was used for testing products' effectiveness against the reference strains: *Mycobacterium terrae* ATCC 15755, *Mycobacterium avium* ATCC 15769. Under clean conditions the tested product was active against all the testorganisms at contact time tested.

Conclusion

By the test results can be concluded that as treated by the product the surviving microorganisms count was decreasing at least four grades that under clean conditions the ready to use product **Chemisept GEL** is mycobactericidal in case of surgical hand disinfection, during conctact time of 30 sec.

DR. BRILL + DR. STEINMANN
INSTITUTE FOR HYGIENE AND MICROBIOLOGY

DAkkS Deutsche Akkreditierungsstelle D-PL-13412-01-01 D-PL-13412-01-02
Anerkannt durch/Recognized by Zentralstelle der Länder für Gesundheitsschutz bei Arzneimitteln und Medizinprodukten
ZLG-AP-216.11.02

28/03/2018

Test report L17/0666dM.1

Evaluation of the effectiveness of

Chemisept GEL

Test virus: murine norovirus (as surrogate of human norovirus)

Method: EN 14476:2013+A1:2015 (clean conditions)

quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in human medicine

Sponsor:
Chemi-Pharm AS
Pollu 132
EST – TALLINN 10917

TEST REPORT

Norderoog 2, DE - 28259 Bremen
Tel.: +49 40-557631-0, Fax: +49 40-557631-11
info@brillhygiene.com, http://www.brillhygiene.com

1. Identification of test laboratory

Dr. Brill + Partner GmbH Institute for Hygiene and Microbiology, Norderoog 2, DE - 28259 Bremen

2. Identification of sample

Manufacturer	Chemi-Pharm AS
Name of product	Chemisept GEL
Confirmation no.	203905
Product diluent recommended by the manufacturer	-
Batch number	198251017
Application	hand disinfection
Production date	25/10/2017
Expiry date	25/10/2020
Active compound (s) (100 g)	72.5 g ethanol 7.5 g IPA
Appearance, odour	clear, colorless liquid product specific
pH-values	undiluted: 7.16 (20 °C)
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	01/11/2017

3. Materials

3.1 Culture medium and reagents

- Dulbecco's Modified Eagle's Medium (DMEM, Biozym Scientific GmbH, catalogue no. 880006)
- Fetal calf serum (Thermo Fisher, article no. CH30160.02)
- 1.4 % formaldehyde solution (dilution of Roti®-Histofix 4 %, Carl Roth GmbH)
- Aqua bidest. (SG ultrapure water system, type Ultra Clear; serial no. 86996-1)
- PBS (Invitrogen, article no. 18912-014)
- BSA (Sigma-Aldrich-Chemie GmbH, article no. CA-2153).

3.2 Virus and cells

Murine norovirus (MNV) was obtained from PD. Dr. E. Schreier, Head of FG15 Molecular Epidemiology of Viral Pathogens at the Robert Koch-Institute (RKI) in Berlin. Prior to inactivation, MNV was passaged three times in *RAW 264.7 cells* (a macrophage-like, Abelson leukemia virus transformed cell line derived from BALB/c mice, ATCC TIB-71). *RAW 264.7 cells* were cultured with Dulbecco's Modified Eagle's Medium with 4.5 g/l glucose and fetal calf serum with low endotoxin. Furthermore, cells (passage 25) were inspected regularly for morphological alterations and for contamination by mycoplasmas. No morphological alterations of cells and no contamination by mycoplasmas could be detected.

3.3 Apparatus, glassware and small items of equipment

- CO_2 incubator, Nunc GmbH & Co. KG, model QWJ 350
- Agitator (Vortex Genie Mixer, type G 560E)
- pH measurement 315i (WTW, article no. 2A10-100)
- Centrifuge (Sigma-Aldrich-Chemie GmbH, type 113)
- Microscope (Olympus, type CK 30)
- Centrifuge 5804 R (Eppendorf AG)
- Water bath (JULABO, Julabo U 3)
- Adjustable and fixed-volume pipettes (Eppendorf AG)
- Polysterol 96-well microtitre plate (Nunc GmbH & Co. KG, Wiesbaden)
- Cell culture flask (Nunc GmbH & Co. KG, Wiesbaden)
- Sealed test tubes (Sarstedt AG & Co., Nümbrecht).

4. Experimental conditions

Test temperature	20 °C ± 1.0 °C
Concentration of test product	undiluted (80.0 %) and as 50.0 % and 10.0 % (demonstration of non-active range) solutions
Appearance of product dilutions	no precipitation
Contact times	30 and 60 seconds and 30 minutes
Interfering substance	0.3 g/l bovine serum albumin (clean conditions, EN 14476)
Procedure to stop action of disinfectant	immediate dilution
Diluent	water
Stability of product in the mix with virus and interfering substance (80.0 % solution)	medium clouding, no precipitation
Virus strain	murine norovirus (Berlin 06 / 06 / DE Isolate S99)
Date of testing	29/12/2017 – 28/03/2018
End of testing	28/03/2018

5. Methods

5.1 Preparation of test virus suspension

To prepare the test virus suspension, *RAW 264.7 cells* which have been cultured with Dulbecco's Modified Eagle's Medium with 4.5 g/l glucose and 10 % fetal calf serum with low endotoxin were inoculated with MNV (stock virus solution) in a 175 cm^2 cell culture flask. Once a cytopathic effect had been induced (approx. 1-3 days), freezing and thawing was carried out two times. The cell debris was removed by low speed centrifugation and the supernatant was recovered as test viral suspension, aliquoted and stored at -80 °C.

5.2 Preparation of disinfectant (dilutions)

The test product was tested undiluted. Due to the addition of interfering substance and test virus suspension an 80.0 % solution resulted. Furthermore, the product was evaluated as 50.0 % and 10.0 % solutions (demonstrating of non-active range). This solution was prepared with water immediately before the inactivation tests.

5.3 Infectivity assay

Infectivity was determined as endpoint titration according to EN 5.5 transferring 0.1 ml of each dilution into eight wells of a microtitre plate to 0.1 ml of *RAW 264.7 cells* (10-15 x 10^3 cells per well) freshly prepared by scraping, beginning with the highest dilution. Microtitre plates were incubated at 37 °C in a 5 % CO_2-atmosphere. The cytopathic effect was read by using an inverted microscope after five days. Calculation of the infective dose $TCID_{50}$/ml was calculated with the method of Spearman (2) and Kärber (3) with the following formula:

$$- \log_{10} TCID_{50} = X_0 - 0.5 + \sum r/n$$

meaning

X_0 = $\log_{10}$ of the lowest dilution with 100 % positive reaction

r = number of pos. determinations of lowest dilution step with 100 % positive and all higher positive dilution steps

n = number of determinations for each dilution step.

5.4 Calculation and verification of virucidal activity

The virucidal activity of the test disinfectant was evaluated by calculating the decrease in titre in comparison with the control titration without disinfectant. The difference is given as reduction factor (RF).

According to the EN 14476, a disinfectant or a disinfectant solution at a particular concentration is having virus-inactivating efficacy if the titre is reduced at least by four $\log_{10}$ steps within the recommended exposure period. This corresponds to an inactivation of ≥ 99.99 %.

5.5 Inactivation assay

Determination of virucidal activity has been carried out in accordance to EN 5.5. The test product was examined undiluted (80.0 %) and as 50.0 % and 10.0 % (demonstration of non-active range) solutions in water at 20 °C according to EN 14476. 30 and 60 seconds and 30 minutes were chosen as contact times.

Immediately at the end of a chosen contact time, activity of the disinfectant was stopped by dilution to 10^{-8}.

Titrations of the virus control were performed at the beginning of the test and after the longest exposure time (EN 5.5.7). One part by volume of test virus suspension was mixed with one part interfering substance and eight parts by volume of WSH or Aqua bidest. (RTU products).

Furthermore, a cell control (only addition of medium) was incorporated.

Inactivation tests were carried out in sealed test tubes in a water bath at 20 °C ± 1.0 °C. Aliquots were retained after appropriate exposure times and residual infectivity was determined.

5.6 Determination of cytotoxicity

Determination of cytotoxicity was performed according to EN 5.5.4.1.

5.7 Cell sensitivity to virus

For the control of cell sensitivity to virus two parts by volume of water were mixed with eight parts by volume of the lowest apparently non-cytotoxic dilution of the product. This mixture or PBS as control was added to a volume of double concentrated cell suspension. After 1 h at 37 °C the cells were centrifuged and re-suspended in cell culture medium (EN 5.5.4.2b).

Finally, a comparative titration of the test virus suspension was performed on the pre-treated (disinfectant) and non-pre-treated (PBS) cells as described above.

5.8 Control of efficacy for suppression of disinfectant's activity

Furthermore, a control of efficiency for suppression of disinfectant's activity was included (EN 5.5.5.1).

5.9 Reference virus inactivation test

As reference for test validation a 0.7 % formaldehyde solution according to EN 5.5.6 was included. 5, 15, 30 and 60 minutes were chosen as contact times. In addition, cytotoxicity of formaldehyde test solution was determined following EN 5.5.6.2 with dilutions up to 10^{-5}.

6. Verification of the methodology

The following criteria as mentioned in EN 5.7 were fulfilled:

a) The titre of the test virus suspension allowed the determination of a ≥ 4 $\log_{10}$ reduction (maximal virus reduction ≥ 5.88 ± 0.35, 80.0 % assay).

b) The test product (80.0 % solution) showed cytotoxicity in the 1:10 dilutions thus allowing the detection of a 4 $\log_{10}$ reduction of virus titre.

c) The comparative titration on pre-treated (disinfectant) and non-pre-treated (PBS) *RAW 264.7 cells* showed no significant difference (< 1 $\log_{10}$; EN 5.7) of virus titre: 8.38 ± 0.41 (PBS) versus 8.50 ± 0.35 (1:100 dilutions of disinfectant as 80.0 % solution) $\log_{10}$ $TCID_{50}$/ml.

d) The control of efficacy for suppression of disinfectant's activity (80.0 % solution) showed no decrease (≤ 0.5 $\log_{10}$; EN 5.5.5.1) in virus titre (8.00 ± 0.38 versus 8.25 ± 0.33 $\log_{10}$ $TCID_{50}$/ml).

e) One concentration demonstrated a 4 $\log_{10}$ reduction and (at least) one concentration demonstrated a $\log_{10}$ reduction of less than 4.

Since all criteria according EN 5.7 were fulfilled, examination with MNV according to EN 14476 is valid.

7. Results

Results of examination are shown in tables 1 to 8. Tables 1 to 7 demonstrate the raw data, whereas table 8 (a+b) gives a summary of results.

The undiluted test product (80.0 %) was able to inactivate MNV after 30 seconds under clean conditions in this quantitative suspension test (tables 1 and 2). The reduction factors were 4.63 ± 0.61 and ≥ 4.63 ± 0.45. The mean value was ≥ 4.63 ± 0.38. This corresponded to an inactivation of ≥ 99.99 %.

Tested as 50.0 % solution, the test product was not active within 30 seconds of exposure time (table 3).

Tested as 10.0 % solution, the test product was not active within 30 minutes of exposure time (table 4).

8. Conclusion

The hand disinfectant Chemisept GEL tested undiluted demonstrated effectiveness against MNV after an exposure time of 30 seconds under clean conditions.

Therefore, the hand disinfectant Chemisept GEL can be declared as active against MNV as follows:

undiluted **30 seconds** **clean conditions**

Bremen, 28/03/2018

9. Quality control

The Quality Assurance of the results was maintained by performing the determination of the virus-inactivating properties of the disinfectant in accordance with Good Laboratory Practice regulations:

1) Chemicals Act of Germany, Appendix 1, dating of 01.08 1994 (BGBI. I, 1994, page 1703). Appendix revised at 14. 05. 1997 (BGBI. I, 1997, page 1060).
2) OECD Principles of Good Laboratory Practice (revised 1997); OECD Environmental Health and Safety Publications; Series on Principles of Good Laboratory Practice and Compliance Monitoring – Number 1. Environment Directorate, Organization for Economic Co-operation and Development, Paris 1998.

The plausibility of the results was additionally confirmed by controls incorporated in the inactivation assays.

10. Records to be maintained

All testing data, protocol, protocol modifications, the final report, and correspondence between Dr. Brill + Partner GmbH and the sponsor will be stored in the archives at Dr. Brill + Partner GmbH.

11. Literature

1. EN 14476:2013+A1:2015: Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity of chemicals disinfectants and antiseptics in human medicine test - Test method and requirements (phase 2, step 1)

2. Spearman, C.: The method of `right or wrong cases` (constant stimuli) without Gauss's formulae. Brit J Psychol; 2 1908, 227-242

3. Kärber, G.: Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Arch Exp Path Pharmak; 162, 1931, 480-487

Appendix:

Legend to the Tables

Table 1:	Raw data for Chemisept GEL (80.0 %) tested against MNV (1st assay)
Table 2:	Raw data for Chemisept GEL (80.0 %) tested against MNV (2nd assay)
Table 3:	Raw data for Chemisept GEL (50.0 %) tested against MNV
Table 4:	Raw data for Chemisept GEL (10.0 %) tested against MNV
Table 5:	Raw data for formaldehyde solution (0.7 %) tested against MNV
Table 6:	Raw data for control of efficacy for suppression of disinfectant's activity (80.0 %)
Table 7:	Raw data (MNV) for cell sensitivity (80.0 %)
Table 8 (a+b):	Summary of results with Chemisept GEL and MNV

Legend to the Figures

Figure 1:	Virus-inactivating properties of Chemisept GEL (80.0 %)
Figure 2:	Virus-inactivating properties of formaldehyde (0.7 %)

Table 1: Raw data for Chemisept GEL (80.0 %) tested against MNV at 20 °C (quantal test; 8 wells) (#5355) (1st assay)

Product	Concentration	Interfering substance	Contact time	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	80.0 %	clean conditions	15 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			20 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30 s	tttt tttt	4444 4444	0000 3000	0200 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			60 s	tttt tttt	4004 0440	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
test product cytotoxicity	80.0 %	clean conditions	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	clean conditions	0 min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0444 4400	0000 0304	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 2: Raw data for Chemisept GEL (80.0 %) tested against MNV at 20 °C (quantal test; 8 wells) (#5378) (2nd assay)

Product	Concentration	Interfering substance	Contact time	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	80.0 %	clean conditions	15 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			20 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30 s	tttt tttt	4444 0444	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			30 min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	80.0 %	clean conditions	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	clean conditions	0 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0000 0000	0040 0000	0000 0000
			60 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0044 0440	0000 0000	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 3: Raw data for Chemisept GEL (50.0 %) tested against MNV at 20 °C (quantal test; 8 wells) (#5378)

Product	Concentration	Interfering substance	Contact time	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	50.0 %	clean conditions	15 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			20 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30 s	n.d.	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0400 0444	n.d.	n.d.
			30 min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	50.0 %	clean conditions	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	clean conditions	0 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0000 0000	0040 0000	0000 0000
			60 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0044 0440	0000 0000	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 4: Raw data for Chemisept GEL (10.0 %) tested against MNV at 20 °C (quantal test; 8 wells) (#5378)

Product	Concentration	Interfering substance	Contact time	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	10.0 %	clean conditions	15 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			20 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3400 0004	0000 0400	n.d.
test product cytotoxicity	10.0 %	clean conditions	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	clean conditions	0 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0000 0000	0040 0000	0000 0000
			60 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0044 0440	0000 0000	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 5: Raw data for formaldehyde solution (0.7 %) tested against MNV at 20 °C (quantal test; 8 wells) (#5378)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
formaldehyde	0.7 % (m/V)	PBS	5	tttt tttt	tttt tttt	tttt tttt	4444 4444	4444 4444	4444 4444	0400 4000	n.d.	n.d.
			15	tttt tttt	tttt tttt	tttt tttt	4444 4444	4444 4444	0440 4044	0040 0000	n.d.	n.d.
			30	tttt tttt	tttt tttt	tttt tttt	4444 4444	4444 4444	0030 0044	0000 0000	n.d.	n.d.
			60	tttt tttt	tttt tttt	tttt tttt	3443 4444	0000 3044	0000 0000	0000 0000	n.d.	n.d.
formaldehyde cytotoxicity	0.7 % (m/V)	PBS	n.a.	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	PBS	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4442 4444	4040 0400	0000 0000	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 6: Raw data for control of efficacy for suppression of disinfectant's activity (80.0 %) (#5451)

Product	Interfering substance	dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
test product	clean conditions	n.d.	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4004 4004	0000 0000	n.d.
corresponding virus control	clean conditions	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4403 0444	0000 0000	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 7: Raw data (MNV) for cell sensitivity (80.0 %) (#5451)

Product	Dilution	Dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
PBS	-	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4443 4004	4000 0000	n.d.
test product	1:100	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4433 0444	0004 0040	n.d.

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 8a: Summary of results with Chemisept GEL and MNV

Product*	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after …					> 4 log_{10} reduction after …
				15 s	20 s	30 s	60 s	30 min	
test product (1)	80.0 %	clean conditions	2.50	n.d.	n.d.	3.75±0.35	≤ 3.00±0.38	n.d.	30 s (RF = 4.63±0.61)
test product (2)	80.0 %	clean conditions	2.50	n.d.	n.d.	≤ 3.38±0.25	n.d.	n.d.	30 s (RF ≥ 4.63±0.45)
test product (2)	50.0 %	clean conditions	2.50	n.d.	n.d.	8.00±0.38	n.d.	n.d.	> 30 s (RF = 0.00±0.53)
test product (2)	10.0 %	clean conditions	1.50	n.d.	n.d.	n.d.	n.d.	8.00±0.44	> 30 min (RF = 0.00±0.58)

*The number in brackets gives the number of the corresponding virus control, see table 8b

n.a. = not applicable n.d. = not done

Table 8b: Summary of results with Chemisept GEL and MNV

Product	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after ... min
				0	5	15	30	60	
formaldehyde	0.7 % (w/v)	PBS	4.50	n.d.	7.75±0.33	7.25±0.44	6.88±0.37	5.88±0.37	> 60 (RF = 2.00±0.52)
virus control	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	7.88±0.57	n.a.
virus control (1)	n.a.	clean conditions	n.a.	n.d.	n.d.	n.d.	n.d.	8.38±0.49	n.a.
virus control (2)	n.a.	clean conditions	n.a.	7.63±0.25	n.d.	n.d.	n.d.	8.00±0.38	n.a.
virus control suppression	n.a.	clean conditions	n.a.	n.d.	n.d.	n.d.	n.d.	8.25±0.33	n.a.
suppression control	80.0 %	clean conditions	2.50	n.d.	n.d.	n.d.	8.00±0.38	n.d.	n.a.
sens.control PBS	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	8.38±0.41	n.a.
sens. control test product	80.0 %→ 1:100	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	8.50±0.35	n.a.

n.a. = not applicable n.d. = not done sens. = sensitivity

Figure 1: Virus-inactivating properties of Chemisept GEL (80.0 %)

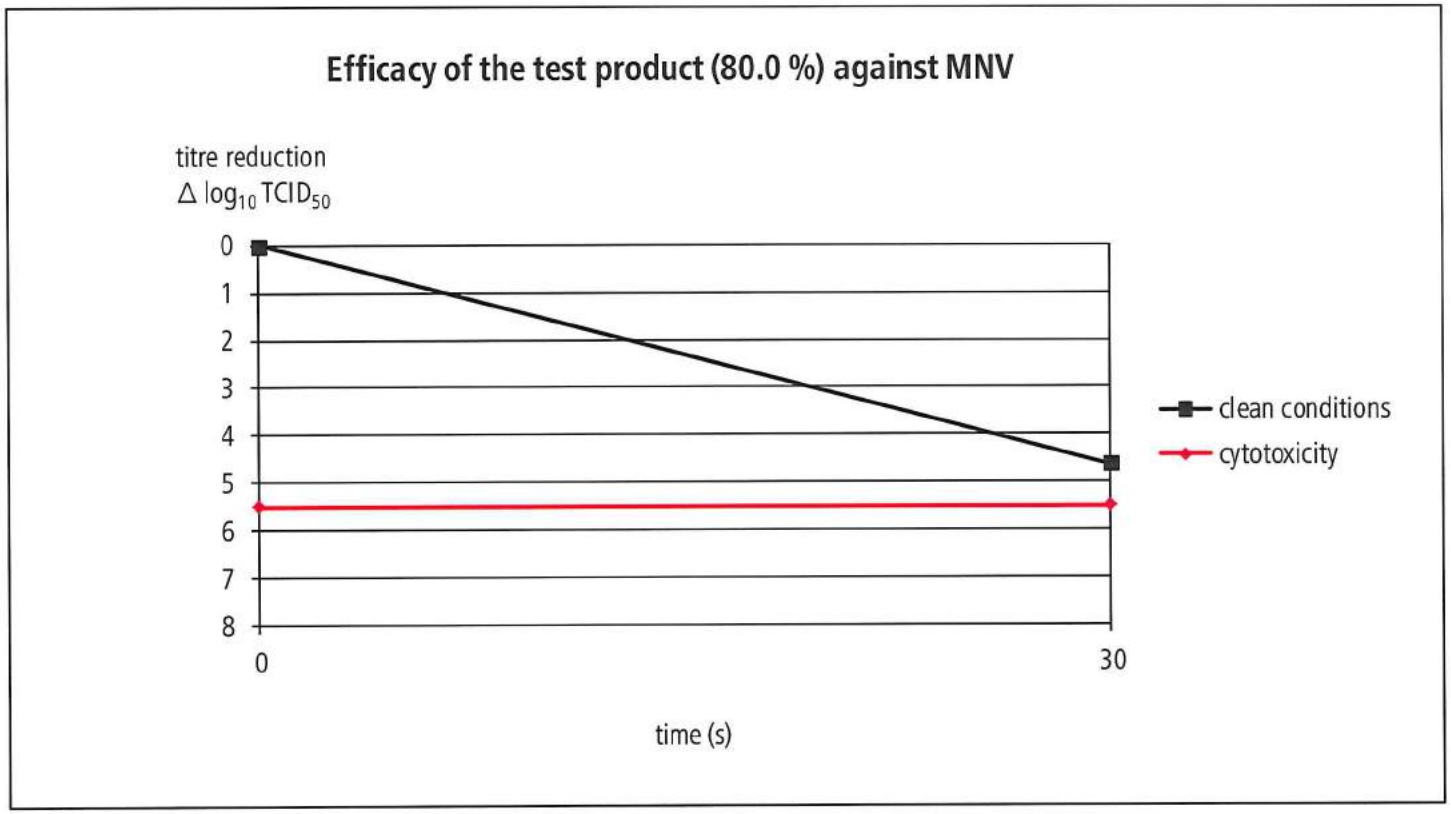
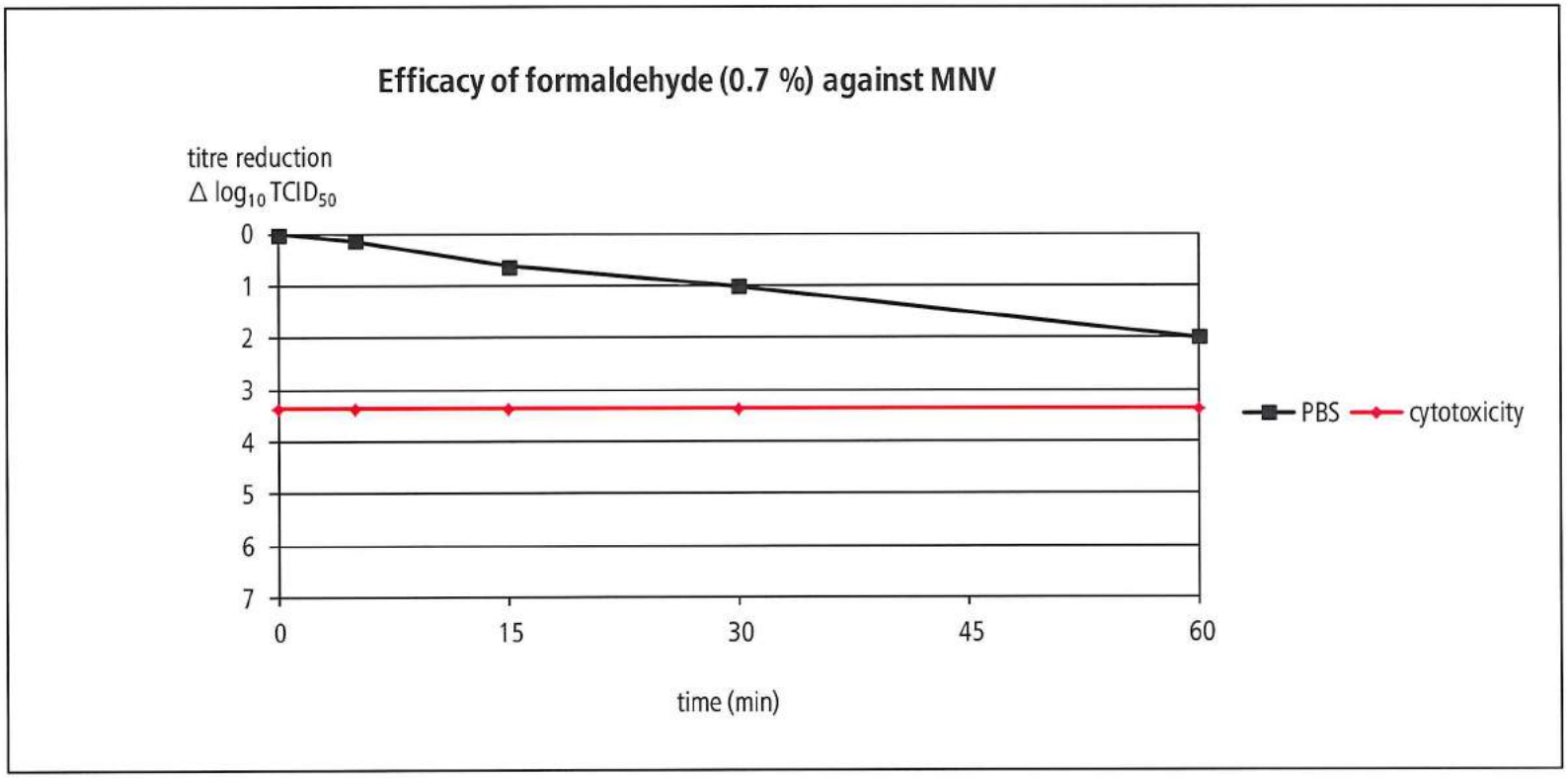


Figure 2: Virus-inactivating properties of formaldehyde (0.7 %)



Nordic Tersus
Laboratory

Test report No. shd1118

EVALUATION OF BACTERICIDAL ACTIVITIES OF A DISINFECTANTS AND ANTISEPTICS USED IN THE MEDICAL AREA (EN 13727)

Name of the product:	Chemisept GEL
Batch number:	198251017
Order number:	17030
Manufacturer:	Chemi-Pharm Ltd
Client, representative:	Chemi-Pharm Ltd., Põllu 132, Tallinn, 10917, ESTONIA
Date of delivery:	15.01.2018
Test material conditions:	No specific features, sample in the manufacturers tare
Storage conditions:	In room temperature, dark
Active substance – conc.:	Ethyl alcohol – 72.5 % wt; isopropyl alcohol – 7.5 % wt
Appearance of the product:	Transparent liquid
Test concentration:	Ready to use
Contact time:	15sec, 30 sec
Interfering substance:	15 g/l bovine albumin + 15 ml/l sheep blood erythrocytes = Dirty conditions; 1,5g/l bovine albumin = clean conditions
Neutralizer:	-
Rinsing liquid:	Tryptone 1 g/l + NaCl, 9 g/l
Test organisms:	*Pseudomonas aeruginosa* ATCC 15442 *Staphylococcus aureus* ATCC 6538 *Enterococcus hirae* ATCC 10541 *Escherichia coli* K12, NTCT 10538 *Staphylococcus aureus* MRSA ATCC 33592 *Enterococcus faecium* VRE ATCC 700221
Testing method:	EVS-EN 13727:2012+A2:2015 Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity in the medical area - Test method and requirements (phase 2, step 1)
Testing date:	17.02.2018 – 19.02.2018
Results:	look appendix 1-7

Date of test report: 21.02.2018

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Appendix 1

TEST RESULTS (bactericidal suspension test)

EVS-EN 13727:2012+A2:2015; Phase 2, step 1;
Membrane filtration method;
Rinsing liquid: tryptone, 1 g/l+ NaCl, 9 g/l;
Test organism: *Staphylococcus aureus* ATCC 6538;
Test temperature: +20° C; Incubation temperature: +37 °C
Interfering substance: 15 g/l bovine albumin + 15 ml/l sheep blood erythrocytes = Dirty conditions;
1,5g/l bovine albumin = clean conditions
Nordic Tersus Laboratory LLC.;
Date of test: 17.02.2018 – 19.02.2018
Responsible person:

Validation and controls

Dirty and clean conditions

Validation suspension N_{vo}			Experimental conditions control (A)			Filtration control (B)			Method validation (C)		
V_{C1}	83	$\bar{x}$ = 86.5	V_{C1}	72	$\bar{x}$ = 68.5	V_{C1}	68	$\bar{x}$ = 71.5	V_{C1}	81	$\bar{x}$ = 77
V_{C2}	90		V_{C2}	65		V_{C2}	75		V_{C2}	73	
30 ≤ $\bar{x}$ N_{vo} ≤160? yes X; no□			$\bar{x}$ A is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no□			$\bar{x}$ B is ≥ 0,5 $\bar{x}$ N_{vo}? yesX; no□			$\bar{x}$ C is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no□		

Test suspension and test

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 2.45 x 10^9; log$N$ = 9.39
N and N_0	10^{-7}	257	224	N_0 = N/100; logN_0 = 7,39
	10^{-8}	28	30	7,17≤ logN_0≤7,70; yesX; no □

Experimental results

Concentration of the product %	Dilution step	V_{C1}	V_{C2}	Na (=x̄*10)	log Na	logR	Contact time	Conditions
Ready to use	-	<14	<14	<140	<2.15	>5.24	15 sec	dirty
Ready to use	-	<14	<14	<140	<2.15	>5.24	15 sec	clean
Ready to use	-	<14	<14	<140	<2.15	>5.24	30 sec	dirty
Ready to use	-	<14	<14	<140	<2.15	>5.24	30 sec	clean

Explanations:

V_C = count per ml (one plate or more)

x̄ = average of V_{C1} and V_{C2} (1. + 2. duplicate)

N = cfu/ml microbes in testsuspension

N_0 = cfu/ml at the start of the contact time (t=0)

N_{v0} = cfu/ml in the validation suspension (t=0)

Na = surviving microbes after the test

R = reduction factor (R= N_0/ Na; LogR=LogN_0 - Log Na)

Appendix 2

TEST RESULTS (bactericidal suspension test)

EVS-EN 13727:2012+A2:2015; Phase 2, step 1;
Membrane filtration method;
Rinsing liquid: tryptone, 1 g/l+ NaCl, 9 g/l;
Test organism: *Enterococcus hirae* ATCC 10541;
Test temperature: +20° C; Incubation temperature: +37 °C
Interfering substance: 15 g/l bovine albumin + 15 ml/l sheep blood erythrocytes = Dirty conditions;
1,5g/l bovine albumin = clean conditions
Nordic Tersus Laboratory LLC.;
Date of test: 17.02.2018 – 19.02.2018
Responsible person:

Validation and controls

Dirty and clean conditions

Validation suspension N_{vo}			Experimental conditions control (A)			Filtration control (B)			Method validation (C)		
V_{C1}	62	$\bar{x}$ = 66	V_{C1}	47	$\bar{x}$ = 46	V_{C1}	52	$\bar{x}$ = 53.5	V_{C1}	59	$\bar{x}$ = 61
V_{C2}	70		V_{C2}	45		V_{C2}	55		V_{C2}	53	
30 ≤ $\bar{x}$ N_{vo} ≤160? yes X; no□			$\bar{x}$ A is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no□			$\bar{x}$ B is ≥ 0,5 $\bar{x}$ N_{vo}? yesX; no□			$\bar{x}$ C is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no□		

Test suspension and test

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 2.15 x 10^9; log$N$ = 9.33
N and N_0	10^{-7}	203	224	N_0 = N/100; logN_0 = 7,33
	10^{-8}	22	25	7,17≤ logN_0≤7,70; yesX; no □

Experimental results

Concentration of the product %	Dilution step	V_{C1}	V_{C2}	Na (=x̄*10)	log Na	logR	Contact time	Conditions
Ready to use	-	<14	<14	<140	<2.15	>5.18	15 sec	dirty
Ready to use	-	<14	<14	<140	<2.15	>5.18	15 sec	clean
Ready to use	-	<14	<14	<140	<2.15	>5.18	30 sec	dirty
Ready to use	-	<14	<14	<140	<2.15	>5.18	30 sec	clean

Explanations:

V_C = count per ml (one plate or more)

x̄ = average of V_{C1} and V_{C2} (1. + 2. duplicate)

N = cfu/ml microbes in testsuspension

N_0 = cfu/ml at the start of the contact time (t=0)

N_{v0} = cfu/ml in the validation suspension (t=0)

Na = surviving microbes after the test

R = reduction factor (R= N_0/ Na; LogR=LogN_0 - Log Na)

Appendix 3

TEST RESULTS (bactericidal suspension test)
EVS-EN 13727:2012+A2:2015; Phase 2, step 1;
Membrane filtration method;
Rinsing liquid: tryptone, 1 g/l+ NaCl, 9 g/l;
Test organism: *Pseudomonas aeruginosa* ATCC 15442
Test temperature: +20° C; Incubation temperature: +37 °C
Interfering substance: 15 g/l bovine albumin + 15 ml/l sheep blood erythrocytes = Dirty conditions;
1,5g/l bovine albumin = clean conditions
Nordic Tersus Laboratory LLC.;
Date of test: 17.02.2018 – 19.02.2018
Responsible person: I

Validation and controls

Dirty and clean conditions

Validation suspension N_{vo}			Experimental conditions control (A)			Filtration control (B)			Method validation (C)		
V_{C1}	94	$\bar{x}$ = 87	V_{C1}	71	$\bar{x}$ = 72.5	V_{C1}	66	$\bar{x}$ = 68	V_{C1}	75	$\bar{x}$ = 78.5
V_{C2}	80		V_{C2}	74		V_{C2}	70		V_{C2}	82	
30 ≤ $\bar{x}$ N_{vo} ≤160? yes X; no□			$\bar{x}$ A is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no□			$\bar{x}$ B is ≥ 0,5 $\bar{x}$ N_{vo}? yesX; no□			$\bar{x}$ C is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no□		

Test suspension and test

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 2.74 x 10^9; log$N$ = 9.44
N and N_0	10^{-7}	284	266	$N_0 = N/100$; logN_0 = 7,44
	10^{-8}	24	29	7,17≤ logN_0≤7,70; yesX; no □

Experimental results

Concentration of the product %	Dilution step	V_{C1}	V_{C2}	Na (=x̄*10)	log Na	logR	Contact time	Conditions
Ready to use	-	<14	<14	<140	<2.15	>5.29	15 sec	dirty
Ready to use	-	<14	<14	<140	<2.15	>5.29	15 sec	clean
Ready to use	-	<14	<14	<140	<2.15	>5.29	30 sec	dirty
Ready to use	-	<14	<14	<140	<2.15	>5.29	30 sec	clean

Explanations:

V_C = count per ml (one plate or more)

x̄ = average of V_{C1} and V_{C2} (1. + 2. duplicate)

N = cfu/ml microbes in testsuspension

N_0 = cfu/ml at the start of the contact time (t=0)

N_{vo} = cfu/ml in the validation suspension (t=0)

Na = surviving microbes after the test

R = reduction factor (R= N_0/ Na; LogR=LogN_0 - Log Na)

Appendix 4

TEST RESULTS (bactericidal suspension test)
EVS-EN 13727:2012+A2:2015; Phase 2, step 1;
Membrane filtration method;
Rinsing liquid: tryptone, 1 g/l+ NaCl, 9 g/l;
Test organism: *Escherichia coli* K12, NTCT 10538
Test temperature: +20° C; Incubation temperature: +37 °C
Interfering substance: 15 g/l bovine albumin + 15 ml/l sheep blood erythrocytes = Dirty conditions;
1,5g/l bovine albumin = clean conditions
Nordic Tersus Laboratory LLC.;
Date of test: 17.02.2018 – 19.02.2018
Responsible person:

Validation and controls

Dirty and clean conditions

Validation suspension N_{vo}			Experimental conditions control (A)			Filtration control (B)			Method validation (C)		
V_{C1}	82	$\bar{x}$ = 78.5	V_{C1}	68	$\bar{x}$ = 60	V_{C1}	69	$\bar{x}$ = 69.5	V_{C1}	70	$\bar{x}$ = 68.5
V_{C2}	75		V_{C2}	52		V_{C2}	73		V_{C2}	67	
30 ≤ $\bar{x}$ N_{vo} ≤160? yes X; no□			$\bar{x}$ A is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no□			$\bar{x}$ B is ≥ 0,5 $\bar{x}$ N_{vo}? yesX; no□			$\bar{x}$ C is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no□		

Test suspension and test

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 2.48 x 10^9; log$N$ = 9.39
N and N_0	10^{-7}	241	257	$N_0 = N/100$; logN_0 = 7.39
	10^{-8}	22	26	7,17≤ logN_0≤7,70; yesX; no □

Experimental results

Concentration of the product %	Dilution step	V_{C1}	V_{C2}	Na (=x̄*10)	log Na	logR	Contact time	Conditions
Ready to use	-	<14	<14	<140	<2.15	>5.24	15 sec	dirty
Ready to use	-	<14	<14	<140	<2.15	>5.24	15 sec	clean
Ready to use	-	<14	<14	<140	<2.15	>5.24	30 sec	dirty
Ready to use	-	<14	<14	<140	<2.15	>5.24	30 sec	clean

Explanations:

V_C = count per ml (one plate or more)

x̄ = average of V_{C1} and V_{C2} (1. + 2. duplicate)

N = cfu/ml microbes in testsuspension

N_0 = cfu/ml at the start of the contact time (t=0)

N_{v0} = cfu/ml in the validation suspension (t=0)

Na = surviving microbes after the test

R = reduction factor (R= N_0/ Na; LogR=LogN_0 - Log Na)

Appendix5

TEST RESULTS (bactericidal suspension test)
EVS-EN 13727:2012+A2:2015; Phase 2, step 1;
Membrane filtration method;
Rinsing liquid: tryptone, 1 g/l+ NaCl, 9 g/l;
Test organism: *Staphylococcus aureus* MRSA ATCC 33592
Test temperature: +20° C; Incubation temperature: +37 °C
Interfering substance: 15 g/l bovine albumin + 15 ml/l sheep blood erythrocytes = Dirty conditions;
1,5g/l bovine albumin = clean conditions
Nordic Tersus Laboratory LLC.;
Date of test: 17.02.2018 – 19.02.2018
Responsible person:

Validation and controls

Dirty and clean conditions

Validation suspension N_{vo}			Experimental conditions control (A)			Filtration control (B)			Method validation (C)		
V_{C1}	66	$\bar{x}$ = 61.5	V_{C1}	42	$\bar{x}$ = 45.5	V_{C1}	49	$\bar{x}$ = 47.5	V_{C1}	52	$\bar{x}$ = 53
V_{C2}	57		V_{C2}	49		V_{C2}	46		V_{C2}	54	
30 ≤ $\bar{x}$ N_{vo} ≤160? yes X; no□			$\bar{x}$ A is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no□			$\bar{x}$ B is ≥ 0,5 $\bar{x}$ N_{vo}? yesX; no□			$\bar{x}$ C is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no□		

Test suspension and test

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 1.99 x 10^9; log$N$ = 9.30
	10^{-7}	204	191	N_0 = N/100; logN_0 = 7.30
N and N_0	10^{-8}	18	24	7,17≤ logN_0≤7,70; yesX; no □

Experimental results

Concentration of the product %	Dilution step	V_{C1}	V_{C2}	Na (=x̄*10)	log Na	logR	Contact time	Conditions
Ready to use	-	<14	<14	<140	<2.15	>5.15	15 sec	dirty
Ready to use	-	<14	<14	<140	<2.15	>5.15	15 sec	clean
Ready to use	-	<14	<14	<140	<2.15	>5.15	30 sec	dirty
Ready to use	-	<14	<14	<140	<2.15	>5.15	30 sec	clean

Explanations:

V_C = count per ml (one plate or more)

x̄ = average of V_{C1} and V_{C2} (1. + 2. duplicate)

N = cfu/ml microbes in testsuspension

N_0 = cfu/ml at the start of the contact time (t=0)

N_{v0} = cfu/ml in the validation suspension (t=0)

Na = surviving microbes after the test

R = reduction factor (R= N_0/ Na; LogR=LogN_0 - Log Na)

Appendix 6

TEST RESULTS (bactericidal suspension test)
EVS-EN 13727:2012+A2:2015; Phase 2, step 1;
Membrane filtration method;
Rinsing liquid: tryptone, 1 g/l+ NaCl, 9 g/l;
Test organism: *Enterococcus faecium* VRE ATCC 700221
Test temperature: +20° C; Incubation temperature: +37 °C
Interfering substance: 15 g/l bovine albumin + 15 ml/l sheep blood erythrocytes = Dirty conditions;
1,5g/l bovine albumin = clean conditions
Nordic Tersus Laboratory LLC.;
Date of test: 17.02.2018 – 19.02.2018
Responsible person:

Validation and controls

Dirty and clean conditions

Validation suspension N_{vo}			Experimental conditions control (A)			Filtration control (B)			Method validation (C)		
V_{C1}	62	$\bar{x}$ = 64	V_{C1}	52	$\bar{x}$ = 54.5	V_{C1}	43	$\bar{x}$ = 47	V_{C1}	58	$\bar{x}$ = 59
V_{C2}	66		V_{C2}	57		V_{C2}	51		V_{C2}	60	
30 ≤ $\bar{x}$ N_{vo} ≤160? yes X; no□			$\bar{x}$ A is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no□			$\bar{x}$ B is ≥ 0,5 $\bar{x}$ N_{vo}? yesX; no□			$\bar{x}$ C is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no□		

Test suspension and test

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 1.88 x 10^9; log$N$ = 9.27
	10^{-7}	181	195	$N_0 = N/100$; logN_0 = 7.27
N and N_0	10^{-8}	22	18	7,17≤ logN_0≤7,70; yesX; no □

Experimental results

Concentration of the product %	Dilution step	V_{C1}	V_{C2}	Na (=x̄*10)	log Na	logR	Contact time	Conditions
Ready to use	-	<14	<14	<140	<2.15	>5.12	15 sec	dirty
Ready to use	-	<14	<14	<140	<2.15	>5.12	15 sec	clean
Ready to use	-	<14	<14	<140	<2.15	>5.12	30 sec	dirty
Ready to use	-	<14	<14	<140	<2.15	>5.12	30 sec	clean

Explanations:

V_C = count per ml (one plate or more)

x̄ = average of V_{C1} and V_{C2} (1. + 2. duplicate)

N = cfu/ml microbes in testsuspension

N_0 = cfu/ml at the start of the contact time (t=0)

N_{vo} = cfu/ml in the validation suspension (t=0)

Na = surviving microbes after the test

R = reduction factor (R= N_0/ Na; LogR=LogN_0 - Log Na)

Appendix 7

Interpretation:

The product for surgical handrub **Chemisept GEL** (batch no. 198251017) was tested according to the test method EVS-EN 13727:2012+A2:2015. The test was performed at 20 °C ± 1 °C, under clean and dirty conditions with the contact times of 15 sec and 30 sec. The membrane filtration method was used for testing the products' effectiveness against the reference strains: *Pseudomonas aeruginosa* ATCC 15442, *Enterococcus hirae* ATCC 10541, *Staphylococcus aureus* ATCC 6538, Escherichia coli K12 NTCT 10538, Staphylococcus aureus MRSA ATCC 33592 and *Enterococcus faecium* VRE ATCC 700221. Under clean and dirty conditions the tested product was effective against all the reference strains within 15 sec and 30 sec of contact times.

Conclusion:

The surviving count of bacterial reference strains showed at least 5lg reduction meaning that **under clean and dirty conditions the ready to use product Chemisept GEL has a bactericidal effect in case of hand disinfection within 15 sec.**