

## Assessment of the Efficacy of Hygienic Handrub Determined using the European Standard Test Method EN 1500:2013

STUDY SPONSOR	:	K G Puntervold AS
MANUFACTURER	:	K G Puntervold AS
RECEIPT DATE	:	30/03/2020
STUDY PERIOD	:	31/03/2020-02/04/2020
PRODUCT NAME	:	Norsk Handsprit
LAB ID	:	2020-3134/20 23 00125
LOT	:	Sample Number: N00725437
EXPIRY DATE	:	Not listed
STORAGE CONDITIONS	:	Room Temperature, Darkness.
DILUTION (AND IF APPLICABLE DILUENT)	:	Undiluted – Neat
APPLICATION CONDITIONS-PREFERENCE	:	2 X 30sec; 2 X 3 ml (60% v/v propan-2-ol)
APPLICATION CONDITIONS-PRODUCT	:	2 X 30sec; 2 X 3 ml. 6ml total quantity for 60sec total rubbing time
No of VOLUNTEERS	:	20
ACTIVE SUBSTANCE	:	Ethanol 56.8% w/w Isopropyl alcohol 10.35% w/w

## STUDY REPORT

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Tests Carried Out By: QACS Ltd, 1 Antigonis str, 144-51 Metamorfosis, Greece,  
 Tel: +30-210-2934745, fax: +30-210-2934606, email: [info@qacs.gr](mailto:info@qacs.gr) website: [www.qacs.gr](http://www.qacs.gr)

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ACTIVE SUBSTANCE	:	Ethanol 56.8% w/w Isopropyl alcohol 10.35% w/w
Test Method	:	European Standard EN 1500:2013
Test Procedures	:	Full details of all the test and control procedures used are given in the Test Method
Test Organism	:	Escherichia coli K12 NCTC 10538
Culture Media and Reagents	:	Tryptone Soya Agar, Tryptic Soya Selective Agar, Tryptone Soy Broth
Incubation	:	Plates were incubated at 37 °C for 24 - 48 h
Neutralizer	:	LPT Dilution Broth with tween 80 and saponin

## TEST METHOD

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

This European Standard specifies a method of test simulating practical conditions for establishing whether a product for hygienic handrub reduces the release of transient flora according to the requirements when rubbed onto artificially contaminated hands of volunteers.

The method involves applying live test organisms (Escherichia coli K12 NCTC 10538) to the hands, then recovering the test organism in order to obtain a baseline count. The test or reference disinfectant product is then applied to the hands before once again recovering any surviving test organisms in sampling broth containing neutralizers to terminate the effect of any residual disinfectant. Propan-2-ol 60% (V/V) is used as reference. The organisms are enumerated, counts transposed to the Log system and the difference between the numbers recovered from the test or reference, and baseline counts is established and statistically analyzed for significance (WILCOXON'S matched-pairs, Hodges-Lehman). The larger the difference between the two counts, the less effective the product. Each of the volunteers repeats the procedure for the reference first and test product after, or for the product first and the reference after. For the test product to conform to the standard, EN1500:2013, the mean log reduction factor obtained shall be at least not inferior to that achieved by the specified reference hygienic handrub (60% volume concentration of propan-2-ol).

## SUBJECTS

The test was performed on 20 persons (requirement of the Standard 18-22 subjects) with healthy skin, without cuts or skin abrasions and with short and clean fingernails. All subjects, were at least 18 years old.

## NEUTRILIZATION

A suitable neutralizer was chosen and validated before the test procedure (LPT dilution broth with tween 80 30g/l and saponin 30g/l).

### Composition of the neutraliser

Lecithin	3.0g
Sodium thiosulphate	5.0g
Tryptic digest of casein	1.0g
Sodium chloride	8.5g
Disodium hydrogen phosphate	8.0g
Potassium dihydrogen phosphate	1.5g
L-histidine HCL	1.0g
Polysorbate 80	30g
Saponin	30g

## METHOD OF APPLICATION:

Application of the test organism: Hands were prepared by washing for 1 minute with 5ml soft soap to remove transients and dried thoroughly on paper towels (Soft soap, 200 g l-1: Linseed oil 50 parts (by weight); Potassium hydroxide 9.5 parts; Ethanol 7 parts in distilled water -as needed-, autoclave to sterilize, pH between 10-11).

The volunteers were randomly divided into two groups of approximately the same size. Group 1 used the reference hygienic handrub and Group 2 the product under test. The test was repeated on the same day with Group 1 using the handrub procedure with the test product and Group 2 using the reference handrub procedure.

Hands were immersed to the mid-metacarpals for 5 sec, fingers apart, in 2 l of cultured test organism, E. coli K12, containing  $1.5-5.0 \times 10^8$  cfu/ml. The same container with the contamination fluid was used for all volunteers. Hands were air dried for 3 minutes in horizontal position with the fingers spread out and rotating to avoid the formation of droplets, either for reference handrub procedure (R) or test product (P) as outlined below.

## PREVALUES

Immediately after treatment, the fingertips were immersed (including the thumb) for 1 min on the base of a petri dish containing 10ml of TSB as sampling fluid in order to assess the release of test micro-organisms before treatment of the hands. A separate petri dish was used for each hand.

#### REFERENCE PRODUCT:

**Three ml of Propan-2-ol 60% (V/V)** was poured into the cupped dry hands and rubbed vigorously into the skin for **30 seconds** up to the wrists in accordance with the standard handrub procedure shown in Figure 1. This ensured total coverage of the hands. The technique comprises of five strokes backwards and forwards, palm to palm, right palm over left dorsum and left palm over right dorsum, palm to palm with fingers interlaced, back of fingers to opposing palms with fingers interlocked, rotational rubbing of right thumb clasped in left palm and left thumb clasped in right palm, rotational rubbing with clasped fingers, of right hand in palm of left hand and clasped fingers of left hand in palm of right hand. **The procedure was repeated** with a further **three (3) ml** of Propan-2-ol 60% (V/V) to give a **total rubbing time of 60 seconds**.

#### TEST PRODUCT:

**Three (3) ml of product** under test was poured into the cupped dry hands and rubbed vigorously into the skin for **30 seconds** up to the wrists in accordance with the standard handrub procedure shown in Figure 1. This ensured total coverage of the hands. The technique comprises of five strokes backwards and forwards, palm to palm, right palm over left dorsum and left palm over right dorsum, palm to palm with fingers interlaced, back of fingers to opposing palms with fingers interlocked, rotational rubbing of right thumb clasped in left palm and left thumb clasped in right palm, rotational rubbing with clasped fingers, of right hand in palm of left hand and clasped fingers of left hand in palm of right hand. **The procedure was repeated** with a further dose of **three (3) ml** of product under test **for 30sec**, in order to give a **total rubbing time of 60 seconds**.

#### POST VALUES

Immediately after treatment, the fingertips were immersed (including the thumb) for 1 min on the base of a petri dish containing 10ml of neutralizer.

The interval between sampling and planting did not exceed 30 min.

#### INCUBATION

All plates were incubated aerobically at 37°C + 1°C for 20h to 24h; then, the colonies were counted and the plates re-incubated for a further 24-48h in order to detect slow-growing colonies.

***E. coli* K12 NCTC 10538 concentration: 2.3 x 10<sup>8</sup> cfu/ml.**

**Table 1 Handrub reference procedure Propan-2-ol 60% (V/V). Colony Counts per Plate**

No	Hand left or right	Prevalues		Postvalues		
		10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>0</sup>	10 <sup>-1</sup>	10 <sup>-2</sup>
1	l	>330	82	>330	>330	47
	r	>330	71	>330	>330	51
2	l	>330	171	>330	>330	140
	r	>330	174	>330	>330	168
3	l	>330	82	>330	>330	108
	r	>330	86	>330	>330	191
4	l	>330	110	>330	42	4
	r	>330	126	>330	29	5
5	l	91	7	75	9	1
	r	103	12	>330	106	11
6	l	>330	202	>330	>330	100
	r	>330	262	>330	>330	76
7	l	>330	90	>330	27	2
	r	>330	99	>330	53	6
8	l	>330	168	>330	14	1
	r	>330	192	>330	217	25
9	l	>330	175	>330	>330	29
	r	>330	207	>330	27	2
10	l	>330	63	>330	22	2
	r	>330	129	>330	101	7
11	l	>330	97	86	9	1
	r	>330	175	159	17	2
12	l	>330	166	>330	16	2
	r	>330	189	>330	25	5
13	l	>330	129	>330	220	28
	r	>330	155	>330	>330	33
14	l	>330	76	>330	138	14
	r	>330	111	>330	86	9
15	l	>330	66	>330	226	30
	r	>330	99	>330	257	25
16	l	>330	212	>330	188	19
	r	>330	156	>330	144	16
17	l	>330	71	>330	89	7
	r	>330	64	>330	51	6
18	l	>330	136	>330	99	10
	r	>330	170	>330	43	5
19	l	>330	89	>330	75	12
	r	>330	55	>330	103	11
20	l	>330	47	>330	68	5
	r	>330	101	>330	125	15

*E. coli* K12 NCTC 10538 concentration:  $4.3 \times 10^8$  cfu/ml.

Table 2 Handrub procedure with the test product. Colony Counts per Plate.

No	Hand left or right	Prevalues		Postvalues		
		$10^{-4}$	$10^{-5}$	$10^0$	$10^{-1}$	$10^{-2}$
1	l	>330	135	>330	185	19
	r	>330	129	>330	259	23
2	l	>330	117	>330	>330	170
	r	>330	86	>330	>330	234
3	l	>330	145	>330	35	7
	r	>330	189	>330	49	5
4	l	>330	86	185	22	4
	r	>330	83	221	27	2
5	l	>330	94	>330	39	5
	r	>330	76	>330	61	7
6	l	>330	143	>330	86	9
	r	>330	132	>330	>330	45
7	l	>330	153	>330	35	3
	r	>330	105	>330	61	8
8	l	>330	151	>330	91	6
	r	>330	140	>330	>330	77
9	l	>330	143	>330	>330	74
	r	>330	185	>330	>330	8
10	l	>330	137	>330	>330	4
	r	>330	120	>330	>330	75
11	l	>330	201	123	19	2
	r	>330	153	202	17	1
12	l	>330	112	>330	37	3
	r	>330	130	195	18	3
13	l	>330	249	>330	>330	90
	r	>330	261	>330	>330	108
14	l	>330	152	>330	49	5
	r	>330	229	>330	36	5
15	l	>330	89	>330	204	25
	r	>330	113	>330	130	17
16	l	>330	221	>330	289	35
	r	>330	265	>330	>330	45
17	l	>330	76	>330	82	11
	r	>330	54	>330	53	6
18	l	>330	253	>330	235	30
	r	>330	230	>330	118	9
19	l	>330	156	>330	>330	48
	r	>330	144	>330	>330	52
20	l	>330	93	>330	90	7
	r	>330	157	>330	104	13

**Table 3 List of computed log<sub>10</sub> values (mean of left and right hand) and log<sub>10</sub> reduction**

Volunteers	Chronological Sequence	Reference handrub			Handrub with product		
		log prevalues	log postvalues	log R	log prevalues	log postvalues	log R
1	PR -> PP	6.84	3.65	3.19	7.08	3.34	3.74
2	PP -> RP	7.20	4.14	3.05	6.96	4.26	<b>2.70</b>
3	PR -> PP	6.88	4.12	<b>2.77</b>	7.18	2.64	4.54
4	PP -> RP	7.03	2.56	4.47	6.89	2.31	4.57
5	PR -> PP	5.98	2.49	3.49	6.89	2.70	4.19
6	PP -> RP	7.32	3.90	3.42	7.10	3.27	3.82
7	PR -> PP	6.93	2.58	4.36	7.06	2.67	4.39
8	PP -> RP	7.21	2.74	4.47	7.12	3.40	3.73
9	PR -> PP	7.24	2.92	4.32	7.17	3.34	3.83
10	PP -> RP	6.91	2.67	4.25	7.07	3.20	3.87
11	PR -> PP	7.07	2.10	4.98	7.20	2.20	5.00
12	PP -> RP	7.21	2.32	4.88	7.04	2.42	4.62
13	PR -> PP	7.11	3.42	3.69	7.37	3.95	3.41
14	PP -> RP	6.92	3.04	3.88	7.23	2.63	4.60
15	PR -> PP	6.87	3.39	3.48	6.96	3.22	3.74
16	PP -> RP	7.22	3.22	4.00	7.34	3.54	3.80
17	PR -> PP	6.79	2.83	3.96	6.77	2.83	3.94
18	PP -> RP	7.14	2.82	4.32	7.34	3.22	4.12
19	PR -> PP	6.80	2.96	3.85	7.13	3.66	3.48
20	PP -> RP	6.80	2.96	3.83	7.04	2.99	4.05
X s NN	Overall	6.97	3.04	<b>3.93</b>	7.10	3.09	<b>4.01</b>
		0.29	0.57	0.59	0.16	0.54	0.52
		20	20	20	20	20	20
X s NN	PR -> PP	6.85	3.04	3.81	7.08	3.05	4.03
		0.34	0.60	0.64	0.17	0.54	0.50
		10	10	10	10	10	10
X s NN	PP -> RP	7.10	3.04	4.06	7.11	3.12	3.99
		0.17	0.58	0.54	0.15	0.58	0.57
		10	10	10	10	10	10
logR : decimal log reduction				X : Mean			
PR -> PP : Sequence: first RP, second PP				s : standard deviation			
PP -> PR : Sequence: first PP, second RP				NN : Number of values			

Difference of mean Rs (PR -> PP): -0.22  
 Difference of mean Rs (PR -> PP): 0.07  
 Absolute difference of differences: 0.29 (<2.00)

## CHECK OF ACCEPTANCE CRITERIA

- Complete set of 20 volunteers available (hence, more than the minimum of 18)
- Mean of log prevalues for RP=6.97 and for PP=7.10 (hence both greater than 5.00)
- Individual log reductions less than 3.00: with Reference Product (RP)=1, with Test Product (PP)=1 (hence not more than three individual log reduction factors for each, fewer than 3,00 log).
- Absolute difference of mean differences=0.29 (hence less than 2.00)
- All quotients of weighted mean counts between 5 and 15 (in Tables 1 and 2 and in validation of neutralizer)

**All acceptance criteria are fulfilled**

**Table 4 Computation of individual differences of lg Rs of RP-PP**

Volunteers	log reduction		Difference RP-PP
	Reference procedure (RP)	Product procedure (PP)	
1	3.19	3.74	-0.55
2	3.05	2.70	0.35
3	2.77	4.54	-1.77
4	4.47	4.57	-0.10
5	3.49	4.19	-0.70
6	3.42	3.82	-0.40
7	4.36	4.39	-0.04
8	4.47	3.73	0.75
9	4.32	3.83	0.49
10	4.25	3.87	0.38
11	4.98	5.00	-0.02
12	4.88	4.62	0.27
13	3.69	3.41	0.28
14	3.88	4.60	-0.71
15	3.48	3.74	-0.26
16	4.00	3.80	0.20
17	3.96	3.94	0.02
18	4.32	4.12	0.20
19	3.85	3.48	0.37
20	3.83	4.05	-0.22



**Table 5 Sorting of individual differences and computation for Hedges-Lehman 97.5% upper confidence limits**

Sorted differences		Mean pairwise differences (di+dii)/2										
		1	2	3	4	5	6	7	8	9	10	11
		0.75	0.49	0.38	0.37	0.35	0.28	0.27	0.20	0.20	0.02	-0.02
1	0.75	0.748										
2	0.49	0.62	0.492									
3	0.38	0.564	0.435	0.379								
4	0.37	0.559	0.431	0.374	0.37							
5	0.35	0.549	0.421	0.364	0.36	0.35						
6	0.28	0.515	0.387	0.33	0.325	0.315	0.281					
7	0.27	0.507	0.379	0.322	0.318	0.308	0.274	0.266				
8	0.20	0.476	0.348	0.291	0.287	0.277	0.243	0.235	0.204			
9	0.20	0.473	0.345	0.288	0.284	0.274	0.24	0.232	0.201	0.198		
10	0.02	0.386	0.257	0.201	0.196	0.186	0.152	0.144	0.113	0.11	0.023	
11	-0.02	0.363	0.235	0.178	0.174	0.164	0.13	0.122	0.091	0.088		
12	-0.04	0.357	0.228	0.172	0.167	0.157	0.123	0.115	0.084	0.081		
13	-0.10	0.325	0.197	0.14	0.136	0.126	0.092	0.084	0.053	0.05		
14	-0.22	0.264	0.135	0.079	0.074	0.064	0.03					
15	-0.26	0.245	0.116	0.06	0.055	0.045	0.011					
16	-0.40	0.174	0.045	-0.011	-0.016							
17	-0.55	0.1	-0.028	-0.085								
18	-0.70	0.026	-0.103	-0.159								
19	-0.71	0.017										
20	-1.77											

The differences of the individual logR of RP – PP from Table 4 are sorted in the second column and in the headline according to their size in descending order.

The median is between the 10th and 11th value:  $[(0.02) + (-0.02)]/2 = 0.00$ . The small exponents represent the ranks.

The mean pairwise differences that do not exceed the median (here: 0.00) are computed. From Table 6 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: 0,201**. Hence the Hodges-Lehmann upper one-sided 97,5 % confidence limit for the difference in log Rs between RP and PP is 0.201, 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 not inferior to RP**.

**Table 6 WILCOXON'S matched-pairs signed - ranks test:**

**One-sided level of significance (directional test)**

<b>No (Number of pairs)</b>	0,05	0,025	0,01
18	47	<b>40</b>	32
19	53	<b>46</b>	37
20	60	<b>52</b>	43
21	68	<b>59</b>	49
22	75	<b>66</b>	56

## Assessment of the Efficacy of Hygienic Handrub Determined using the European Standard Test Method EN 1500:2013

### STUDY SUMMARY

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APPLICATION CONDITIONS-PRODUCT	:	2 X 30sec; 2 X 3 ml. 6ml total quantity for 60sec total rubbing time
No of VOLUNTEERS	:	20
ACTIVE SUBSTANCE	:	Ethanol 56.8% w/w Isopropyl alcohol 10.35% w/w

### CONCLUSION

The test product: “Norsk Handsprit” tested at concentration: Undiluted-neat, when applied for total rubbing time of 60sec (2X30sec), using total quantity of: 6 ml of product (2 doses of 3ml per 30sec), conforms to the requirements of EN 1500:2013.

Results refer to the sample as received and analyzed in the period specified above.

The test report shall not be reproduced except in full, without written approval of the laboratory.

The samples will be stored by the laboratory during 2 months from the end test date.

The study report and raw data will be stored by the laboratory during 2 years.

### RESULTS AUTHENTICITY

The study concerned by this report was carried out under my responsibility, according to the experimental protocol and the quality plan of the QACS Ltd laboratory.

Study Manager:

  
1 Antigonis str 14451 Metamorfosis Greece  
VAT no EL-999703411, email: [info@qacs.gr](mailto:info@qacs.gr)  
Tel +30-2102934745 Fax +30-210-2934606  
[www.qacs.gr](http://www.qacs.gr)

Agronomist – Food Scientist MSc  
Pharmaceutical Biologist PgCert  
Technical Manager of Microbiology Dpt.  
Date: 03/04/2020

### Figure 1. Standard handrub procedure

Pour appropriate volume of handrub product into the cupped dry hands and rub hands 30 s – 60 s in accordance with the standard handrub shown below to ensure total coverage of the hands. The action in each step is repeated five times before proceeding to the next step. After concluding step 6, recommence the series of steps as appropriate to complete the washing time.



Step 1  
Palm to palm



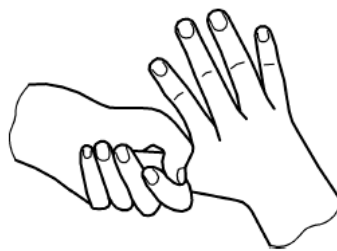
Step 2  
Right palm over left dorsum and left palm over right dorsum (five times)



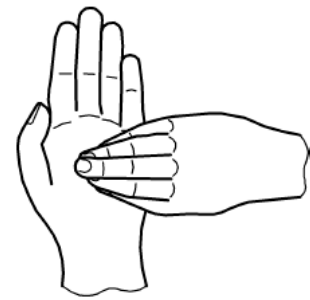
Step 3  
Palm to palm with fingers interlaced (five times)



Step 4  
Backs of fingers to opposing palms with fingers interlocked (five times)



Step 5  
Rotational rubbing of right thumb clasped in left palm and vice versa (five times)



Step 6  
Rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa (five times)

Adapted from EN 1500:2013 Chemical disinfectants and antiseptics – hygienic handrub - Test method and requirements (phase 2/step2)

End of Study Report