

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

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RECEIPT DATE	:	06/05/2016
STUDY PERIOD	:	14/05/16-17/05/16
PRODUCT NAME	:	ALCOMEDSEPT
LAB ID	:	2016-2956/16 23 00052
LOT	:	L153712
EXPIRY DATE	:	12/2018
STORAGE CONDITIONS	:	Room Temperature, Darkness.
DILUTION (AND IF APPLICABLE DILUENT)	:	As is
ACTIVE SUBSTANCE	:	Ethanol
CONTACT TIME (TEST PRODUCT)	:	60sec (2X30sec)
TEST DOSE	:	6ml (2X3ml)

STUDY REPORT

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ACTIVE SUBSTANCE	:	Ethanol
CONTACT TIME (TEST PRODUCT)	:	60sec (2X30sec)
TEST DOSE	:	6ml (2X3ml)
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
Neutraliser	:	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, EN1500:1999, 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) who have hands with healthy skin, without cuts or abrasions and with short and clean fingernails. Subject age was at least 18 years of age.

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 s, fingers apart, in 2 l of cultured test organism, E. coli K12, containing 4.1×10^8 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 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 three (3) ml** of product under test for **30 seconds** to **give a total rubbing time of 60 seconds**. The excess of the product (cream) that was not absorbed by skin, was rinsed of by a 5-10 sec rinse of the fingers under running tap water. Excess water was shaken off, and sampling commenced immediately.

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 24h in order to detect slow-growing colonies.

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

No	Hand	Prevalues		Postvalues		
		10 ⁻⁴	10 ⁻⁵	10 ⁰	10 ⁻¹	10 ⁻²
1	l	112	9	39	3	1
	r	165	17	>330	58	4
2	l	>330	30	>330	45	4
	r	201	22	150	17	3
3	l	123	19	>330	>330	119
	r	103	8	>330	>330	31
4	l	220	29	>330	69	7
	r	>330	41	>330	76	11
5	l	120	13	84	11	2
	r	159	12	>330	32	3
6	l	>330	32	162	26	2
	r	>330	27	>330	42	7
7	l	>330	35	>330	29	2
	r	175	23	>330	131	11
8	l	76	9	>330	52	6
	r	65	10	>330	57	3
9	l	105	8	28	4	1
	r	148	21	205	36	3
10	l	>330	28	>330	41	4
	r	195	20	145	13	2
11	l	164	13	>330	25	4
	r	>330	35	174	29	3
12	l	>330	27	>330	45	6
	r	>330	31	>330	21	3
13	l	210	23	>330	60	7
	r	187	18	>330	42	3
14	l	139	15	186	19	2
	r	209	19	126	14	2
15	l	254	28	>330	44	5
	r	278	30	>330	50	7
16	l	>330	46	>330	72	6
	r	163	15	>330	98	12
17	l	164	13	>330	116	13
	r	250	28	>330	58	6
18	l	>330	50	>330	84	8
	r	>330	69	>330	43	4
19	l	95	11	227	26	5
	r	87	8	>330	61	7
20	l	132	15	296	28	3
	r	124	16	>330	74	9

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

No	Hand	Prevalues		Postvalues		
		10 ⁻⁴	10 ⁻⁵	10 ⁰	10 ⁻¹	10 ⁻²
1	l	107	15	20	3	1
	r	>330	23	>330	32	4
2	l	123	14	>330	43	4
	r	96	12	139	14	2
3	l	127	21	>330	66	7
	r	129	12	>330	>330	80
4	l	260	28	>330	56	9
	r	>330	33	>330	108	11
5	l	175	24	94	14	1
	r	185	21	>330	85	9
6	l	>330	35	>330	37	5
	r	>330	34	>330	58	4
7	l	>330	60	>330	43	4
	r	>330	59	>330	>330	28
8	l	>330	38	>330	>330	64
	r	174	18	>330	>330	69
9	l	104	14	18	2	1
	r	>330	21	>330	29	2
10	l	117	13	>330	39	3
	r	91	10	125	12	2
11	l	184	30	85	10	1
	r	205	29	>330	57	4
12	l	188	21	>330	31	3
	r	105	10	105	11	1
13	l	>330	56	>330	65	7
	r	>330	48	>330	78	9
14	l	244	26	235	29	5
	r	263	24	>330	46	5
15	l	158	16	>330	60	8
	r	134	14	236	24	3
16	l	140	13	271	30	4
	r	98	10	156	19	2
17	l	206	26	145	15	3
	r	218	22	>330	93	8
18	l	258	30	>330	111	13
	r	263	28	>330	183	13
19	l	>330	34	>330	90	12
	r	>330	32	>330	117	13
20	l	147	13	291	36	5
	r	185	19	281	29	4

Table 3 List of computed \log_{10} values (mean of left and right hand) and \log_{10} reduction

Volunteers	Chronological Sequence	Reference handrub			Handrub with product		
		log prevalues	log postvalues	log R	log prevalues	log postvalues	log R
1	PR -> PP	6,13	2,16	3,97	6,18	1,92	4,27
2	PP -> RP	6,37	2,45	3,92	6,04	2,39	3,66
3	PR -> PP	6,06	3,74	2,32	6,12	3,34	2,78
4	PP -> RP	6,46	2,87	3,59	6,45	2,90	3,54
5	PR -> PP	6,14	2,29	3,85	6,26	2,46	3,80
6	PP -> RP	6,43	2,53	3,90	6,50	2,67	3,83
7	PR -> PP	6,38	2,78	3,60	6,73	3,02	3,71
8	PP -> RP	5,86	2,73	3,13	6,39	3,78	2,61
9	PR -> PP	6,10	2,10	4,00	6,16	1,85	4,30
10	PP -> RP	6,35	2,37	3,98	6,02	2,34	3,68
11	PR -> PP	6,35	2,44	3,91	6,31	2,34	3,97
12	PP -> RP	6,42	2,50	3,92	6,67	2,26	4,42
13	PR -> PP	6,30	2,70	3,60	6,67	2,86	3,82
14	PP -> RP	6,23	2,22	4,01	6,40	2,52	3,88
15	PR -> PP	6,43	2,68	3,75	6,16	2,58	3,58
16	PP -> RP	6,42	2,93	3,49	6,07	2,32	3,75
17	PR -> PP	6,30	2,92	3,39	6,33	2,56	3,77
18	PP -> RP	6,73	2,78	3,95	6,42	3,15	3,27
19	PR -> PP	5,96	2,62	3,34	6,48	3,02	3,46
20	PP -> RP	6,12	2,66	3,45	6,22	2,46	3,75
X s NN	Overall	6,28	2,62	3,65	6,33	2,64	3,69
		0,20	0,36	0,41	0,21	0,47	0,44
		20	20	20	20	20	20
X s NN	PR -> PP	6,21	2,64	3,57	6,34	2,60	3,75
		0,16	0,47	0,50	0,22	0,48	0,43
		10	10	10	10	10	10
X s NN	PP -> RP	6,34	2,60	3,73	6,32	2,68	3,64
		0,23	0,23	0,30	0,22	0,48	0,46
		10	10	10	10	10	10
logR : decimal log reduction				X : Mean			
PR -> PP : Sequence: first RP, second PP				s : standard deviation			
PP -> RP : Sequence: first PP, second RP				NN : Number of values			

Difference of mean Rs (PR -> PP): -0.17
 Difference of mean Rs (PP -> RP): 0.09
 Absolute difference of differences: 0.27 (<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=6.28 and for PP=6.33 (hence both greater than 5.00)
- Individual log reductions less than 3.00: with Reference Product (RP)=1, with Test Product (PP)=2 (hence not more than three individual log reduction factors for each, fewer than 3,00 log)
- Absolute difference of mean differences=0.27 (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	3,97	4,27	-0,29
2	3,92	3,66	0,26
3	2,32	2,78	-0,46
4	3,59	3,54	0,05
5	3,85	3,80	0,05
6	3,90	3,83	0,07
7	3,60	3,71	-0,12
8	3,13	2,61	0,52
9	4,00	4,30	-0,31
10	3,98	3,68	0,30
11	3,91	3,97	-0,06
12	3,92	4,42	-0,50
13	3,60	3,82	-0,22
14	4,01	3,88	0,13
15	3,75	3,58	0,16
16	3,49	3,75	-0,26
17	3,39	3,77	-0,38
18	3,95	3,27	0,68
19	3,34	3,46	-0,12
20	3,45	3,75	-0,30

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
		0,68	0,52	0,30	0,26	0,16	0,13	0,07	0,05	0,05	-0,06	-0,12
1	0,68	0,682										
2	0,52	0,602	0,522									
3	0,30	0,489	0,409	0,296								
4	0,26	0,471	0,391	0,278	0,26							
5	0,16	0,423	0,344	0,23	0,213	0,165						
6	0,13	0,406	0,326	0,213	0,195	0,147	0,13					
7	0,07	0,376	0,296	0,183	0,165	0,117	0,1	0,07				
8	0,05	0,366	0,286	0,173	0,155	0,107	0,09	0,06	0,05			
9	0,05	0,364	0,284	0,171	0,153	0,106	0,088	0,058	0,048	0,046		
10	-0,06	0,313	0,233	0,12	0,102	0,054	0,037	0,007	-0,003	-0,005	-0,056	
11	-0,12	0,283	0,203	0,09	0,072	0,024	0,007	-0,023	-0,033	-0,035	-0,086	
12	-0,12	0,282	0,202	0,089	0,071	0,024	0,006	-0,024	-0,034	-0,036		
13	-0,22	0,233	0,153	0,04	0,022	-0,026	-0,043	-0,073	-0,083	-0,085		
14	-0,26	0,212	0,132	0,019	0,001	-0,047	-0,064		-0,104			
15	-0,29	0,196	0,116	0,003	-0,015	-0,063	-0,081					
16	-0,30	0,19	0,11	-0,003	-0,021							
17	-0,31	0,188	0,109	-0,005								
18	-0,38	0,151	0,071	-0,043								
19	-0,46	0,11										
20	-0,50											

The differences of the individual log Rs 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: $[(-0.06) + (-0.12)]/2 = -0,09$. The small exponents represent the ranks.

The mean pairwise differences that do not exceed the median (here: -0.09) 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: 0,11**. Hence the Hodges-Lehmann upper one-sided 97,5 % confidence limit for the difference in log Rs between RP and PP is 0.11, 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**.

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

One-sided level of significance (directional test)

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

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STUDY SUMMARY

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TEST DOSE	:	6ml (2X3ml)

CONCLUSION

The test product: “**ALCOMEDSEPT**”, tested at concentration: **as is (100%)**, when applied for total rubbing time of **60sec (2X30sec)**, using **total quantity of: 6ml (2X3ml dose) of product, 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:

Lagiopoulos Giorgos

Agronomist – Food Technologist, MSc

Date: 13/06/16

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



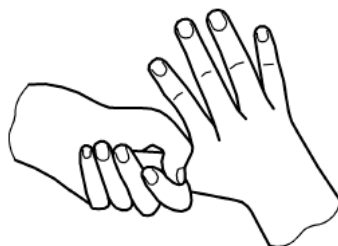
Step 2
Right palm over left dorsum and left palm over right dorsum (five times)



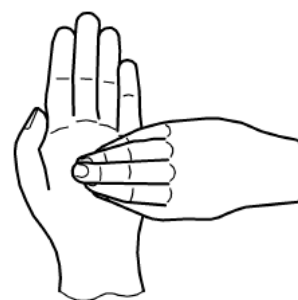
Step 3
Palm to palm with fingers interlaced (five times)



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