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QUALITY ASSURANCE & CONTROL SYSTEMS

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Assessment of the Efficacy of a Wet Wipe Product Determined with Modified Protocol, based on the European Standard Test Method EN 1500:2013

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

STUDY SPONSOR	:	Saga ApS
MANUFACTURER	:	Saga ApS
RECEIPT DATE	:	21/09/2020
STUDY PERIOD	:	26/10/2020-28/10/2020
PRODUCT NAME	:	Hand/Wet Wipes Formula 73%
LAB ID	:	2020-9159/ 20 23 00917
LOT	:	Not provided
STORAGE CONDITIONS	:	Room Temperature, Darkness.
PRODUCT TYPE	:	Wet Wipes
DILUTION (AND IF APPLICABLE DILUENT)	:	Undiluted
APPLICATION CONDITIONS-PEFERENCE	:	1 wet wipe ; 60sec (wet wipes impregnated with 60% v/v propan-2-ol)
APPLICATION CONDITIONS-PRODUCT	:	1 wet wipe ; 30sec
No of VOLUNTEERS	:	20
ACTIVE SUBSTANCE	:	Ethanol 73% w/w

STUDY REPORT

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No of VOLUNTEERS	:	20
ACTIVE SUBSTANCE	:	Ethanol 73% w/w

Test Method	:	Modified Protocol, based on the 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 ºC for 24 - 48 h
Neutralizer	:	LPT Dilution Broth with tween 80 and saponin

TEST METHOD

EN 1500:2013 Chemical disinfectants and antiseptics – Wet Wipe Product – Modified Protocol, based on the European Standard EN 1500:2013 (phase 2/step 2).

This European Standard specifies a method of test simulating practical conditions for establishing whether a product for Wet Wipe Product 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 Wet Wipe Product (60% volume concentration of propan-2-ol).

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TEST METHOD MODIFICATION AND JUSTIFICATION

According to "Guidance on the BPR: Volume II Parts B+C, Version 3.0", issued on April 2018: "*Phase 2, step 2 tests for wet wipes hand disinfection (**modified EN 1500**) should be tests with the wipe applied on volunteers hands according to the intended use. The wipes should be used on full hands and not on the fingertips only.*"

Moreover, according to EN14885:2018 "§ 4.2.8 *Where in EN 14885 no standard exists that specifies the use conditions for a specific product activity in an area (e.g. activity at a temperature or contact time not specified in the obligatory or additional test conditions), a standard may be used with the relevant test condition modified for relevance to the area of application".*

In this study, the European Standard EN1500:2013 has been modified with relevance to the product's intended use. More specifically, the product supplier provided dry wipes along with the product under test. The dry wipes were of the same type, without impregnation liquid. The dry wipes were soaked with the same reference product Propan-2-ol 60% (v/v), with the same impregnation quantity, as product under test (5ml per wipe).

In this way, the reference product was modified as: one (1) wet wipe with the same impregnation factor as product under test, soaked with Propan-2-ol 60% (v/v), in order to be create directly comparable conditions for the reference product and the product under test.

REQUIREMENTS

When tested in accordance with EN 1500:2013, the mean reduction of the release of the test organism Escherichia coli K12 achieved by the hygienic handrub with the product under test shall be at least not inferior to that achieved by a specified reference hygienic handrub (60 % v/v 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

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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 Wet Wipe Product 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 x 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:

One (1) wet wipe, provided by product supplier, soaked with 5ml Propan-2-ol 60% (v/v) was used on dry hands and rubbed onto skin for **60 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.

TEST PRODUCT:

One (1) wet wipe, provided by product supplier, soaked with 5ml of the product under test was used on dry hands and rubbed onto 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.

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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.

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***E. coli* K12 NCTC 10538 concentration: 2.9 x 10^8 cfu/ml.**

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

No	Hand	Prevalues		Postvalues		
	left or right	10^{-4}	10^{-5}	10^{-1}	10^{-2}	10^{-3}
1	l	>330	184	>330	184	19
	r	>330	48	>330	91	6
2	l	>330	35	>330	>330	36
	r	>330	44	>330	45	6
3	l	>330	194	>330	204	25
	r	>330	156	>330	45	5
4	l	>330	48	>330	>330	33
	r	>330	84	>330	>330	41
5	l	>330	136	>330	84	8
	r	>330	201	>330	41	5
6	l	>330	89	>330	>330	33
	r	>330	45	>330	>330	48
7	l	>330	55	>330	188	19
	r	>330	68	>330	201	21
8	l	>330	91	>330	36	6
	r	>330	136	>330	59	5
9	l	>330	204	>330	45	6
	r	>330	25	>330	54	6
10	l	>330	34	>330	>330	84
	r	>330	55	>330	>330	74
11	l	>330	45	>330	>330	36
	r	>330	59	>330	>330	44
12	l	>330	136	>330	231	24
	r	>330	41	>330	204	28
13	l	>330	68	>330	45	6
	r	>330	196	>330	56	6
14	l	>330	133	>330	236	22
	r	>330	236	>330	84	6
15	l	>330	48	>330	45	7
	r	>330	41	>330	55	6
16	l	>330	124	>330	201	16
	r	>330	132	>330	177	19
17	l	>330	64	>330	44	6
	r	>330	59	>330	33	5
18	l	>330	130	>330	88	9
	r	>330	224	>330	202	18
19	l	>330	145	>330	45	6
	r	>330	129	>330	54	6
20	l	>330	51	>330	205	28
	r	>330	40	>330	54	6

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***E. coli* K12 NCTC 10538 concentration: 2.9 x 10^8 cfu/ml.**

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

No	Hand	Prevalues		Postvalues		
	left or right	10^{-4}	10^{-5}	10^{0}	10^{-1}	10^{-2}
1	l	>330	195	>330	204	29
	r	>330	84	>330	185	22
2	l	>330	47	>330	>330	34
	r	89	6	>330	201	22
3	l	>330	198	>330	>330	45
	r	>330	154	>330	>330	36
4	l	>330	161	>330	184	19
	r	>330	208	>330	145	16
5	l	>330	154	>330	204	26
	r	>330	75	>330	54	5
6	l	>330	40	>330	48	6
	r	>330	99	>330	>330	35
7	l	>330	45	>330	>330	54
	r	>330	84	>330	>330	39
8	l	>330	48	>330	188	21
	r	>330	65	>330	169	21
9	l	>330	44	>330	204	22
	r	>330	69	>330	184	21
10	l	>330	45	>330	>330	41
	r	>330	84	>330	>330	33
11	l	>330	209	>330	56	6
	r	>330	123	>330	54	6
12	l	>330	45	>330	>330	45
	r	>330	59	>330	>330	30
13	l	96	9	>330	204	23
	r	154	19	>330	156	19
14	l	>330	64	>330	204	22
	r	>330	84	>330	66	8
15	l	>330	264	>330	>330	45
	r	>330	199	>330	201	29
16	l	>330	154	>330	>330	33
	r	>330	68	>330	>330	42
17	l	>330	45	>330	>330	29
	r	>330	96	>330	>330	31
18	l	>330	204	>330	169	15
	r	>330	128	>330	84	9
19	l	>330	164	>330	245	26
	r	>330	294	>330	154	16
20	l	>330	68	>330	201	29
	r	>330	49	>330	>330	33

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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	6.93	4.11	2.83	7.07	4.30	2.77
2	PP -> RP	6.55	4.09	2.46	6.28	4.40	1.89
3	PR -> PP	7.20	3.99	3.21	7.20	4.56	2.64
4	PP -> RP	6.76	4.52	2.24	7.22	4.22	3.01
5	PR -> PP	7.18	3.77	3.41	6.99	4.02	2.97
6	PP -> RP	6.76	4.56	2.20	6.76	4.10	2.66
7	PR -> PP	6.75	4.29	2.46	6.75	4.62	2.13
8	PP -> RP	7.00	3.67	3.33	6.71	4.26	2.45
9	PR -> PP	6.81	3.70	3.11	6.70	4.29	2.41
10	PP -> RP	6.59	4.86	**1.74**	6.75	4.52	2.22
11	PR -> PP	6.67	4.56	2.11	7.16	3.74	**3.42**
12	PP -> RP	6.83	4.34	2.49	6.67	4.52	2.15
13	PR -> PP	7.02	3.71	3.31	6.09	4.26	1.83
14	PP -> RP	7.21	4.14	3.07	6.82	4.07	2.75
15	PR -> PP	6.61	3.71	2.90	7.32	4.47	2.85
16	PP -> RP	7.07	4.27	2.79	6.97	4.53	2.44
17	PR -> PP	6.75	3.60	3.15	6.78	4.44	2.34
18	PP -> RP	7.19	4.12	3.07	7.17	4.08	3.09
19	PR -> PP	7.09	3.70	3.39	7.30	4.29	3.01
20	PP -> RP	6.61	4.03	2.58	6.72	4.40	2.32
X	Overall	6.88	4.09	**2.79**	6.87	4.30	**2.57**
s		0.23	0.36	0.48	0.32	0.22	0.42
NN		20	20	20	20	20	20
X	PR -> PP	6.90	3.91	2.99	6.94	4.30	2.64
s		0.21	0.32	0.42	0.37	0.26	0.47
NN		10	10	10	10	10	10
X	PP -> RP	6.86	4.26	2.60	6.81	4.31	2.50
s		0.24	0.33	0.48	0.27	0.19	0.38
NN		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):	0.35	
Difference of mean Rs (PR -> PP):	0.10	
Absolute difference of differences:	0.25	(<2.00)

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CHECK OF ACCEPTANCE CRITERIA

- Complete set of 20 volunteers available (hence, more than the minimum of 18)
- Mean of log prevalues for RP=6.88 and for PP=6.87 (hence both greater than 5.00)

  The criterion requires that not more than three individual log reduction factors for each procedure is fewer than 3,00 log. This criterion cannot by applied in this Study, due to Modification of the method.
- Absolute difference of mean differences=0.25 (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	2.83	2.77	0.06
2	2.46	1.89	0.58
3	3.21	2.64	0.57
4	2.24	3.01	-0.77
5	3.41	2.97	0.44
6	2.20	2.66	-0.46
7	2.46	2.13	0.33
8	3.33	2.45	0.88
9	3.11	2.41	0.70
10	1.74	2.22	-0.48
11	2.11	3.42	-1.31
12	2.49	2.15	0.34
13	3.31	1.83	1.48
14	3.07	2.75	0.31
15	2.90	2.85	0.04
16	2.79	2.44	0.35
17	3.15	2.34	0.81
18	3.07	3.09	-0.02
19	3.39	3.01	0.38
20	2.58	2.32	0.26

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

Sorted dufferences		Mean pairwise differences (di+dii)/2										
		1	2	3	4	5	6	7	8	9	10	11
		1.48	0.88	0.81	0.70	0.58	0.57	0.44	0.38	0.35	0.34	0.33
1	1.48	1.482										
2	0.88	1.183	0.884									
3	0.81	1.145	0.846	0.808								
4	0.70	1.093	0.793	0.755	0.703							
5	0.58	1.03	0.73	0.693	0.64	0.577						
6	0.57	1.028	0.729	0.691	0.638	0.575	0.574					
7	0.44	0.961	0.662	0.624	0.571	0.508	0.507	0.44				
8	0.38	0.933	0.634	0.596	0.543	0.48	0.478	0.412	0.383			
9	0.35	0.918	0.619	0.581	0.528	0.465	0.464	0.397	0.368	0.354		
10	0.34	0.911	0.612	0.574	0.521	0.459	0.457	0.39	0.362	0.347	0.34	
11	0.33	0.905	0.606	0.568	0.515	0.453	0.451	0.384	0.356	0.341		
12	0.31	0.897	0.598	0.56	0.507	0.444	0.443	0.376	0.348	0.333		
13	0.26	0.872	0.572	0.535	0.482	0.419	0.417	0.35	0.322	0.307		
14	0.06	0.771	0.472	0.434	0.382	0.319	0.317					
15	0.04	0.763	0.464	0.426	0.373	0.31	0.309					
16	-0.02	0.729	0.43	0.392	0.339							
17	-0.46	0.512	0.212	0.175								
18	-0.48	0.499	0.2	0.162								
19	-0.77	0.357										
20	-1.31											

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: [(0.354) + (0.340)]/2 = 0.334. The small exponents represent the ranks.

The mean pairwise differences that do not exceed the median (here: 0.334) 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: 0.507**

Hence the Hodges-Lehmann upper one-sided 97,5 % confidence limit for the difference in log Rs between RP and PP is 0.507, 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 product under test PP is not inferior to reference product RP.**

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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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PRODUCT TYPE	:	Wet Wipes
DILUTION (AND IF APPLICABLE DILUENT)	:	Undiluted
APPLICATION CONDITIONS-PEFERENCE	:	1 wet wipe ; 60sec (wet wipes impregnated with 60% v/v propan-2-ol)
APPLICATION CONDITIONS-PRODUCT	:	1 wet wipe ; 30sec
No of VOLUNTEERS	:	20
ACTIVE SUBSTANCE	:	Ethanol 73% w/w

CONCLUSION

The product under test: “Hand/Wet Wipes Formula 73%”, when applied as: one (1) wet wipe applied on full hands for total rubbing time of 30sec, is not inferior to the reference product RP, which was modified as: one (1) wet wipe of the same type, with the same impregnation factor as product under test, soaked with Propan-2-ol 60% (v/v). The product under test was tested in accordance with the standard **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:

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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

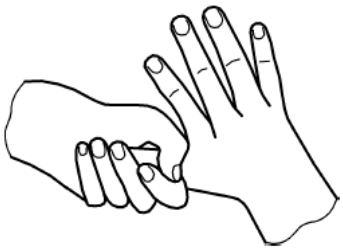
Right palm over left dorsum and left palm over right dorsum (five times)

Step 3

Palm to palm with fingers interlaced (five times)

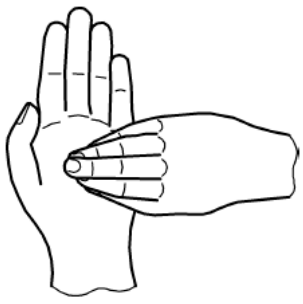
Step 4

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 – Wet Wipe Product - Test method and requirements (phase 2/step2)

End of Study Report