



ITS Testing Services (UK) Limited
Centre Court, Meridian Business Park, Leicester
LE19 1WD, United Kingdom

Tel: +44 (0) 116 263 0330
Fax: +44 (0) 116 263 0311/12
Web: www.intertek.com/consumergoods

ITA REPORT NUMBER:-LECH000: ITA 20623
11 April 2016

Assessment of the Efficacy of a Hygienic Handrub Product (3M Avagard Surgical and Healthcare Personnel with moisturizers), when its Application is Combined with Moisturizing Lotion (Gloves in a Bottle Shielding Lotion).

Client:
Gloves In A Bottle Inc
3720 Park Place
Montrose
California
CA91021

UK Distributor:
Abbliss Ltd
41, NN11 0TF

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TEST PRODUCT IDENTIFICATION AND TEST CONDITIONS

Product Name	: Gloves in a bottle Shielding Lotion
Application of Lotion	: 2.5ml for both hands/60sec
Disinfectant Used	: 3M Avagard Surgical & Healthcare Personnel hand antiseptic with moisturizers
Active Substance of Disinfectant	: Chlorhexidine Gluconate 1% solution and Ethyl Alcohol 61%(w/w)
Interval between Lotion Application & Sanitizer Application	: Until hands are dry (approx. 2 minutes)
Application of Disinfectant	: 2X2ml/60sec
Storage Conditions for Products	: Room Temperature, Darkness
Test Method	: Modified test method based on European Standard EN 1500:2013
Test Procedures	: As described in Test Method
Test Organism	: <i>Escherichia coli</i> K12 NCTC 10538
Culture Media	: Tryptone Soya Agar, Tryptic Soya Selective Agar, Tryptone Soy Broth
Incubation	: Plates were incubated at 37 °C for 24-48 hours
Neutraliser	: LPT Dilution Broth with tween 80 and saponin

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PRINCIPLES AND METHODS

A modified test method based on European Standard EN 1500:2013 (phase 2/step 2) was employed for the test. This modified test method was used to simulate practical conditions required to establish whether a product for hygienic hand rub reduces the release of transient flora according to the requirements of EN 1500:2013, when its application is combined with moisturizing lotion (Gloves in a bottle Shielding Lotion). The method involves applying viable test organisms (*Escherichia coli* K12 NCTC 10538) to the hands of volunteers, then recovering the test organisms in order to obtain a baseline count. The reference disinfectant product is then applied to the hands, followed by recovering any surviving test organisms using a broth containing neutralizers to terminate the effect of any residual disinfectant. The disinfectant 3M Avagard Surgical & Healthcare Personnel hand antiseptic with moisturizers was used as a reference. Afterwards, viable test organisms (*Escherichia coli* K12 NCTC 10538) were applied to the hands of volunteers, again. The hygienic hand rub product (3M Avagard Surgical & Healthcare Personnel hand antiseptic with moisturizers Hygienic Hand Rub) was then applied, when previously a moisturizing cream (Gloves in a bottle Shielding Lotion) had been applied (2.5ml for both hands/60sec and 2 minutes for absorbance of cream or until hands are dry). No towel was used for Drying. Again recovery of any surviving test organisms was performed by submerging the hands in broth containing neutralizers to terminate the effect of any residual disinfectant. The organisms were enumerated, counts transposed to the Log system and the difference between the numbers recovered from the test, and baseline counts was established and statistically analysed 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 carried out the procedure for the reference first (without application of lotion) and for the test product after (application of lotion before use of sanitizer). The lotion does not interfere with sanitizer, if the mean log reduction factor obtained with combined application is at least, not inferior to that achieved by the sanitizer application only.

SUBJECTS

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



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NEUTRILIZATION

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

Ingredient	Amount (g)
Lecithin	3
Sodium thiosulphate	5
Tryptic digest of casein	1
Sodium chloride	8.5
Disodium hydrogen phosphate	8
Potassium dihydrogen phosphate	1.5
L-histidine HCL	1
Polysorbate 80	30
Saponin	30

TEST PROCEDURES

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. Hands were immersed to the mid-metacarpals for 5 sec, fingers apart, in 2 L of cultured test organism containing 5.0×10^8 cfu/ml of *E. coli* K12. 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 for both reference hand rub procedure (R) and 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

Two (2) ml of "3M Avagard Surgical & Healthcare Personnel hand antiseptic with moisturizers" Hygienic Hand Rub (previously passed EN 1500, according to its label claim) 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 hand rub procedure shown in Figure 1. This ensured total coverage of the hands. The technique comprises of five

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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 is repeated with another dose of 2ml for further 30 seconds. Total quantity: 2X2ml for 60 sec total rubbing time.

TEST PRODUCT

2.5ml liquid cream under test was poured into the cupped dry hands and rubbed vigorously into the skin for 60sec and allowed to soak in for about 2 minute or until hands were visibly dry. No towel was used. Subsequently, 2ml of "3M Avagard Surgical & Healthcare Personnel hand antiseptic with moisturizers" Hygienic Hand Rub, 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 hand rub 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 another dose of 2ml for further 30 seconds. Total quantity: 2X2ml for 60 sec total rubbing time. The procedure was completed by a 5 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.

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RESULTS

Table 1 Colony Counts per Plate: Handrub Reference Procedure Propan-2-ol 60% (V/V).

No	Hand Left (L) or Right (R)	Pre-values		Post-values		
		10^{-4}	10^{-5}	10^0	10^{-1}	10^{-2}
1	L	>300	50	>300	216	30
	R	>300	55	>300	212	17
2	L	>300	67	>300	>300	53
	R	>300	89	>300	275	33
3	L	>300	30	>300	117	19
	R	168	21	>300	135	23
4	L	>300	43	>300	205	17
	R	>300	64	>300	288	32
5	L	227	18	>300	153	18
	R	238	25	>300	84	10
6	L	>300	34	>300	185	20
	R	>300	60	>300	108	10
7	L	>300	30	>300	105	21
	R	>300	33	>300	>300	38
8	L	>300	53	>300	222	24
	R	>300	70	>300	>300	39
9	L	83	11	>300	64	8
	R	144	14	>300	53	8
10	L	175	14	>300	108	10
	R	189	19	>300	186	26
11	L	>300	32	>300	>300	39
	R	>300	52	>300	>300	30
12	L	>300	66	>300	>300	56
	R	>300	80	>300	>300	42
13	L	>300	115	>300	68	9
	R	>300	85	>300	79	11
14	L	214	23	>300	36	5
	R	153	18	205	22	3
15	L	>300	63	>300	107	9
	R	>300	38	>300	201	26
16	L	147	11	>300	34	3
	R	196	19	>300	39	5
17	L	>300	58	>300	>300	26
	R	>300	36	>300	251	20
18	L	>300	72	>300	98	10
	R	>300	40	>300	57	8
19	L	114	19	>300	33	4
	R	68	8	>300	55	9
20	L	264	26	>300	136	14
	R	237	28	>300	130	12

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Table 2 Colony Counts per Plate: Handrub Procedure with the Test Product.

No	Hand Left (L) or Right (R)	Pre-values		Post-values		
		10^{-4}	10^{-5}	10^0	10^{-1}	10^{-2}
1	L	>300	34	>300	>300	65
	R	>300	50	>300	156	31
2	L	>300	48	>300	66	11
	R	>300	38	>300	>300	42
3	L	206	18	>300	140	28
	R	153	19	>300	152	13
4	L	>300	41	>300	>300	33
	R	>300	39	>300	85	9
5	L	263	26	>300	189	20
	R	239	27	>300	108	8
6	L	184	23	296	85	9
	R	167	18	>300	123	11
7	L	>300	41	>300	155	26
	R	>300	34	>300	124	16
8	L	>300	27	>300	246	27
	R	>300	26	>300	279	31
9	L	120	14	190	17	2
	R	103	15	208	26	4
10	L	245	20	163	12	2
	R	209	24	>300	29	4
11	L	>300	41	>300	231	18
	R	>300	26	>300	186	20
12	L	>300	77	>300	>300	96
	R	>300	61	>300	250	20
13	L	205	28	>300	72	10
	R	188	19	>300	205	17
14	L	>300	33	>300	99	11
	R	>300	50	>300	45	4
15	L	257	31	296	33	5
	R	293	36	216	22	4
16	L	113	19	>300	63	8
	R	136	10	>300	139	15
17	L	>300	63	>300	>300	26
	R	>300	52	>300	260	23
18	L	157	19	>300	223	19
	R	213	22	>300	89	10
19	L	238	29	>300	78	8
	R	196	18	>300	115	15
20	L	150	16	>300	129	11
	R	123	14	>300	176	12

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Table 3 List of Computed Log₁₀ Values (mean of left and right hand) and Log₁₀ Reduction

Volunteer	Chronological Sequence	Reference handrub			Handrub with product		
		Log Pre-values	Log Post-values	Log R	Log Pre-values	Log Post-values	Log R
1	PR -> PP	6,68	3,33	3,34	6,57	3,50	3,07
2	PR -> PP	6,85	3,57	3,28	6,59	3,21	3,38
3	PR -> PP	6,34	3,12	3,21	6,25	3,18	3,07
4	PR -> PP	6,68	3,38	3,29	6,56	3,20	3,36
5	PR -> PP	6,36	3,06	3,30	6,40	3,15	3,25
6	PR -> PP	6,61	3,15	3,46	6,25	2,81	3,44
7	PR -> PP	6,46	3,30	3,16	6,53	3,16	3,37
8	PR -> PP	6,74	3,45	3,29	6,38	3,42	2,96
9	PR -> PP	6,04	2,78	3,26	6,06	2,30	3,76
10	PR -> PP	6,26	3,16	3,10	6,35	2,34	4,01
11	PR -> PP	6,57	3,49	3,08	6,47	3,31	3,16
12	PR -> PP	6,82	3,64	3,18	6,79	3,67	3,13
13	PR -> PP	6,95	2,88	4,07	6,30	3,09	3,21
14	PR -> PP	6,26	2,46	3,80	6,57	2,82	3,74
15	PR -> PP	6,65	3,17	3,48	6,45	2,41	4,04
16	PR -> PP	6,22	2,56	3,66	6,10	2,98	3,12
17	PR -> PP	6,62	3,38	3,24	6,72	3,39	3,32
18	PR -> PP	6,69	2,88	3,81	6,27	3,15	3,12
19	PR -> PP	5,96	2,65	3,32	6,34	2,98	3,35
20	PR -> PP	6,40	3,12	3,28	6,14	3,17	2,97
X		6,51	3,13	3,38	6,40	3,06	3,34
s		0,27	0,34	0,26	0,20	0,37	0,32
NN		20	20	20	20	20	20

Key: Log R: Decimal log reduction
 s : standard deviation
 X : Mean value
 NN : Number of subjects
 PR -> PP: Sequence: first Reference Product, second Product under test

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ACCEPTANCE CRITERIA

- i. Complete set of 20 volunteers available (hence, more than the minimum of 18).
- ii. Mean of log prevalues for RP=6.51 and for PP=6.40 (hence both greater than 5.00).
- iii. Individual log reductions less than 3.00: with Reference Product (RP) =0, with Test Product (PP) =2 (hence not more than three individual log reduction factors for each, fewer than 3,00 log).
- iv. Absolute difference of mean differences less than 2.00. This criterion is not applied here, as the reference was always applied first and the cream was always applied second.
- v. All quotients of weighted mean counts between 5 and 15 (criterion met)

Acceptance Criteria conclusion: Test fulfilled the acceptance criteria.

Table 4 Computation of Individual Differences of Log Reductions of RP-PP

Volunteers	log reduction		Difference RP-PP
	Reference procedure (RP)	Product procedure (PP)	
1	3,34	3,07	0,27
2	3,28	3,38	-0,09
3	3,21	3,07	0,14
4	3,29	3,36	-0,06
5	3,30	3,25	0,05
6	3,46	3,44	0,03
7	3,16	3,37	-0,21
8	3,29	2,96	0,33
9	3,26	3,76	-0,49
10	3,10	4,01	-0,92
11	3,08	3,16	-0,08
12	3,18	3,13	0,05
13	4,07	3,21	0,86
14	3,80	3,74	0,06
15	3,48	4,04	-0,56
16	3,66	3,12	0,54
17	3,24	3,32	-0,09
18	3,81	3,12	0,69
19	3,32	3,35	-0,04
20	3,28	2,97	0,31

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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
		0,92	0,56	0,49	0,21	0,09	0,09	0,08	0,06	0,04	-0,03	-0,05
1	0,92	0,916										
2	0,56	0,739	0,562									
3	0,49	0,704	0,527	0,492								
4	0,21	0,564	0,387	0,352	0,212							
5	0,09	0,505	0,328	0,293	0,153	0,094						
6	0,09	0,502	0,325	0,29	0,15	0,091	0,088					
7	0,08	0,499	0,322	0,287	0,147	0,088	0,085	0,082				
8	0,06	0,489	0,312	0,277	0,137	0,078	0,075	0,072	0,062			
9	0,04	0,478	0,301	0,266	0,126	0,067	0,064	0,061	0,051	0,039		
10	-0,03	0,445	0,268	0,233	0,094	0,034	0,031	0,028	0,018	0,007	-0,025	
11	-0,05	0,435	0,258	0,223	0,083	0,024	0,021	0,018	0,008	-0,004	-0,036	
12	-0,05	0,433	0,256	0,221	0,081	0,022	0,019	0,016	0,006	-0,006		
13	-0,06	0,43	0,253	0,218	0,078	0,019	0,016	0,013	0,003	-0,008		
14	-0,14	0,389	0,211	0,177	0,037	-0,022	-0,025					
15	-0,27	0,322	0,145	0,11	-0,029	-0,089	-0,092					
16	-0,31	0,303	0,126	0,091	-0,049							
17	-0,33	0,291	0,114	0,079								
18	-0,54	0,19	0,013	-0,022								
19	-0,69	0,114										
20	-0,86											

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: $[-0.03 + (-0.05)]/2 = -0.04$. The small exponents represent the ranks.

The mean pairwise differences that do not exceed the median (here: -0.04) 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 product (hand lotion), combined with (PP) is non-inferior to RP (Reference-Hygienic hand rub product).

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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

CONCLUSION

The test product: "Gloves in A Bottle Shielding Lotion", when applied as: 2.5ml on both clean, dry hands for 60sec total rubbing time and allowed to soak in for about 2 minutes, does not inhibit the activity of the hygienic hand rub product "3M Avagard Surgical & Healthcare Personnel hand antiseptic with moisturizers".

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ANNEX 1

Pour appropriate volume of handrub product into the cupped dry hands and rub hands 30s – 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.

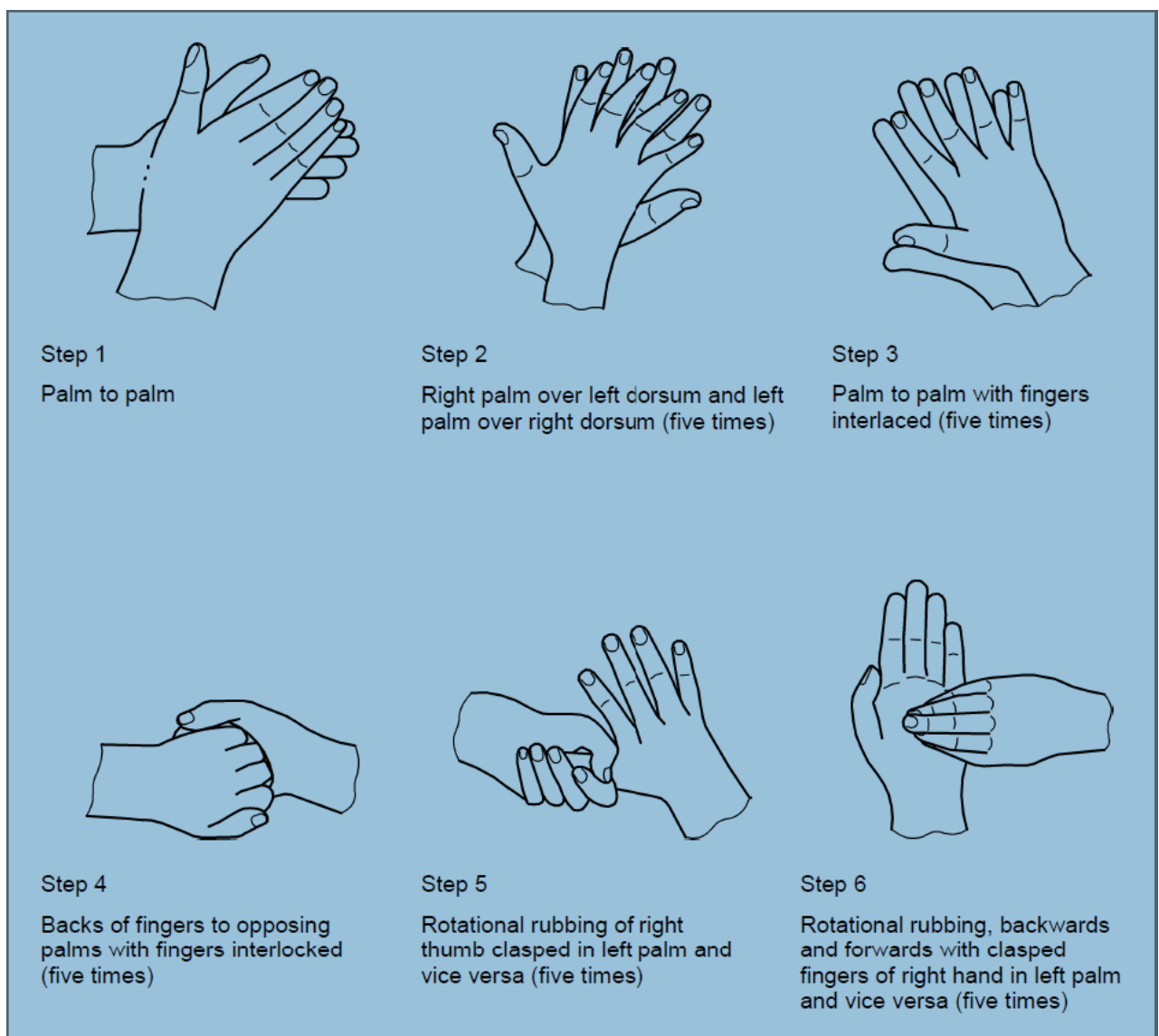


Figure 1 Standard Handrub Procedure: Adapted from EN 1500:2013.

END OF REPORT

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