



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 20360
30 November 2015

Assessment of the Efficacy of a Hygienic Handrub Product (Purell Advanced), 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: Purell Advanced Hygienic Hand Rub

Active Substance of Disinfectant: Ethyl Alcohol 70%(v/v)

Interval between Lotion Application and Sanitizer Application: 60sec

Application of Disinfectant: 1 X 3mL/30sec

Storage Conditions for Products: Room Temperature, Darkness

Test Method: Modified test method based on European Standard EN 1500:2013

Test Procedures: As in Test Method

Test Organism: *Escherichia coli* 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

The objective of the study is to establish that client's hand lotion does not suppress the activity of hygienic handrub. Therefore a modified test method based on European Standard EN 1500:2013 (phase 2/step 2) was employed to assess the efficacy of a hygienic handrub product (Purell Advanced), when its application is combined with Gloves in a Bottle Shielding Lotion.

This modified test 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 of EN 1500:2013, when previously a moisturizing lotion (Gloves in a bottle Shielding Lotion) is applied.

The method involves applying live test microorganisms (*Escherichia coli* K12 NCTC 10538) to the hands of volunteers, then recovering the test organism 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 broth containing neutralizers to terminate the effect of any residual disinfectant. Purell Advanced Hygienic Hand Rub is used as reference in the test.

Afterwards, live test microorganisms (*Escherichia coli* K12 NCTC 10538) are applied to the hands of volunteers, again. The hygienic handrub product (Purell Advanced Hygienic Hand Rub) is then applied, when previously a moisturizing cream (Gloves in a bottle Shielding Lotion) is applied (2.5ml for both hands/60sec for 60sec for absorbance of cream). Again recovery of any surviving test organisms is performed by submerging the hands in broth containing neutralizers to terminate the effect of any residual disinfectant.

The organisms are enumerated, counts transposed to the Log system and the difference between the numbers recovered from the test, and baseline counts is 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 repeats the procedure for the reference first (without application of lotion) and 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 combine application is at least, not inferior to that achieved by the sanitizer application only.

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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 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). The composition of the neutraliser is listed below:

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 (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).

Hands were immersed to the mid-metacarpals for 5 sec, fingers apart, in cultured test microorganisms containing 3.0×10^8 cfu/ml of *E. coli* K12, The same container with the contamination fluid were 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.



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PRE-VALUES

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 Purell Advanced 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 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

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 1 minute. Subsequently, 3ml of Purell Advanced 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 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 completed by a 5 sec rinse of the fingers under running tap water. Excess water was shaken off, and sampling commenced immediately.



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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 24h in order to detect slow-growing colonies.

RESULTS

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Registered in England No. 1408264 Registered Office: Academy Place 1-9 Brook Street, Brentwood, Essex, CM14 5NQ



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

No	Hand	Pre-values		Post-values		
		10 ⁻⁴	10 ⁻⁵	10 ⁰	10 ⁻¹	10 ⁻²
1	Left (L) or Right (R)					
	L	81	14	>330	78	9
	R	57	6	>330	62	5
2	L	270	42	>330	124	15
	R	>330	92	>330	123	9
3	L	98	10	51	3	0
	R	129	23	>330	63	5
4	L	184	26	>330	>330	82
	R	166	27	>330	>330	41
5	L	138	22	>330	56	6
	R	139	12	180	25	1
6	L	187	22	>330	26	3
	R	210	25	>330	198	26
7	L	>330	39	>330	>330	52
	R	>330	47	>330	>330	19
8	L	>330	49	>330	53	7
	R	>330	91	>330	190	32
9	L	>330	29	>330	69	3
	R	>330	43	>330	76	17
10	L	>330	50	>330	34	3
	R	>330	55	>330	84	6
11	L	>330	46	>330	34	3
	R	>330	42	>330	>330	55
12	L	>330	50	>330	25	3
	R	>330	35	>330	196	33
13	L	199	31	>330	>330	21
	R	219	22	>330	59	7
14	L	>330	74	>330	68	10
	R	>330	61	>330	23	4
15	L	>330	38	>330	49	5
	R	>330	33	>330	78	9
16	L	>330	49	>330	182	19
	R	>330	41	>330	151	12
17	L	>330	50	>330	103	11
	R	>330	29	>330	76	5
18	L	>330	35	>330	91	9
	R	195	21	>330	60	8
19	L	257	23	>330	53	4
	R	236	23	>330	39	5
20	L	205	39	>330	30	3
	R	190	75	>330	24	4

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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	228	31	>330	159	20
	R	186	23	>330	142	13
2	L	>330	69	>330	155	22
	R	>330	50	>330	114	17
3	L	170	23	>330	22	2
	R	168	27	204	55	9
4	L	102	11	>330	>330	76
	R	113	11	>330	>330	39
5	L	153	20	>330	31	5
	R	>330	31	>330	142	16
6	L	125	13	>330	134	16
	R	180	23	>330	>330	74
7	L	>330	131	>330	>330	123
	R	>330	77	>330	>330	64
8	L	208	26	>330	110	9
	R	235	19	>330	290	32
9	L	>330	35	120	22	1
	R	97	7	>330	51	3
10	L	>330	42	>330	43	2
	R	226	24	>330	60	6
11	L	>330	51	>330	131	16
	R	>330	65	>330	115	21
12	L	>330	63	>330	>330	27
	R	>330	51	>330	116	20
13	L	>330	39	>330	289	24
	R	>330	64	>330	280	35
14	L	>330	125	>330	90	5
	R	>330	132	>330	139	18
15	L	>330	31	>330	55	4
	R	>330	25	>330	63	8
16	L	239	26	>330	93	14
	R	>330	69	>330	99	10
17	L	>330	70	>330	144	10
	R	>330	36	>330	90	8
18	L	>330	31	>330	50	4
	R	>330	49	>330	69	4
19	L	>330	35	>330	201	23
	R	125	15	>330	179	15
20	L	186	13	>330	44	4
	R	208	24	>330	78	11

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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	5.85	2.84	3.01	6.33	3.18	3.15
2	PR -> PP	6.69	3.09	3.60	6.73	3.14	3.59
3	PR -> PP	6.07	2.11	3.95	6.25	2.36	3.89
4	PR -> PP	6.26	3.72	2.54	6.03	3.69	2.34
5	PR -> PP	6.15	2.56	3.59	6.32	2.84	3.49
6	PR -> PP	6.30	2.86	3.44	6.18	3.48	2.70
7	PR -> PP	6.59	3.46	3.13	6.96	3.91	3.05
8	PR -> PP	6.78	3.02	3.76	6.35	3.25	3.10
9	PR -> PP	6.51	2.87	3.64	6.24	2.40	3.84
10	PR -> PP	6.68	2.72	3.96	6.47	2.69	3.77
11	PR -> PP	6.60	3.11	3.49	6.72	3.11	3.61
12	PR -> PP	6.58	2.86	3.72	6.66	3.24	3.42
13	PR -> PP	6.33	3.03	3.30	6.66	3.46	3.20
14	PR -> PP	6.79	2.62	4.17	7.07	3.05	4.02
15	PR -> PP	6.51	2.79	3.71	6.40	2.77	3.63
16	PR -> PP	6.61	3.22	3.39	6.59	2.99	3.60
17	PR -> PP	6.54	2.94	3.60	6.66	3.05	3.61
18	PR -> PP	6.40	2.87	3.52	6.55	2.76	3.79
19	PR -> PP	6.39	2.66	3.73	6.30	3.28	3.03
20	PR -> PP	6.36	2.44	3.92	6.29	2.77	3.52
X		6.45	2.89	3.56	6.49	3.07	3.42
s		0.24	0.35	0.37	0.27	0.40	0.42
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=6.45 and for PP=6.49 (hence both greater than 5.00).
- iii. Individual log reductions less than 3.00: with Reference Product (RP)=3, with Test Product (PP)=3 (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	3.01	3.15	-0.14
2	3.60	3.59	0.01
3	3.95	3.89	0.06
4	2.54	2.34	0.20
5	3.59	3.49	0.10
6	3.44	2.70	0.74
7	3.13	3.05	0.08
8	3.76	3.10	0.67
9	3.64	3.84	-0.20
10	3.96	3.77	0.18
11	3.49	3.61	-0.12
12	3.72	3.42	0.30
13	3.30	3.20	0.10
14	4.17	4.02	0.14
15	3.71	3.63	0.08
16	3.39	3.60	-0.20
17	3.60	3.61	-0.01
18	3.52	3.79	-0.27
19	3.73	3.03	0.70
20	3.92	3.52	0.41

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Table 5 Sorting of Individual Differences and Computation for Hodges-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
		0.86	0.84	0.73	0.73	0.64	0.57	0.47	0.47	0.46	0.45	0.36
1	0.86	0.861										
2	0.84	0.852	0.843									
3	0.73	0.795	0.786	0.73								
4	0.73	0.794	0.785	0.728	0.727							
5	0.64	0.75	0.741	0.684	0.683	0.639						
6	0.57	0.716	0.707	0.65	0.649	0.605	0.571					
7	0.47	0.666	0.657	0.601	0.599	0.555	0.521	0.472				
8	0.47	0.666	0.657	0.6	0.599	0.555	0.521	0.471	0.471			
9	0.46	0.663	0.654	0.597	0.596	0.552	0.518	0.468	0.468	0.465		
10	0.45	0.657	0.648	0.592	0.59	0.546	0.512	0.463	0.462	0.459	0.454	
11	0.36	0.608	0.599	0.543	0.541	0.497	0.463	0.414	0.413	0.41	0.405	
12	0.35	0.605	0.596	0.539	0.538	0.494	0.46	0.41	0.41	0.407		
13	0.34	0.598	0.589	0.533	0.531	0.487	0.453	0.404	0.403	0.4		
14	0.33	0.595	0.586	0.529	0.527	0.483	0.449					
15	0.31	0.588	0.579	0.522	0.52	0.476	0.442					
16	0.22	0.538	0.529	0.473	0.471							
17	0.19	0.523	0.514	0.457								
18	0.13	0.496	0.487	0.43								
19	0.12	0.491										
20	-0.04											

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: $[0.10 + 0.08]/2 = 0.09$. 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: 0.27. Hence the Hodges-Lehmann upper one-sided 97,5 % confidence limit for the difference in log Rs between RP and PP is 0.27, which is less than the agreed

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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 handrub product).

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	5

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 1 minute, does not inhibit the activity of the hygienic handrub product "Purell Advanced Hygienic Hand Rub". The hygienic handrub product "Purell Advanced Hygienic Hand Rub" conforms to the requirements of EN 1500:2013, according to its label claim.

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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.

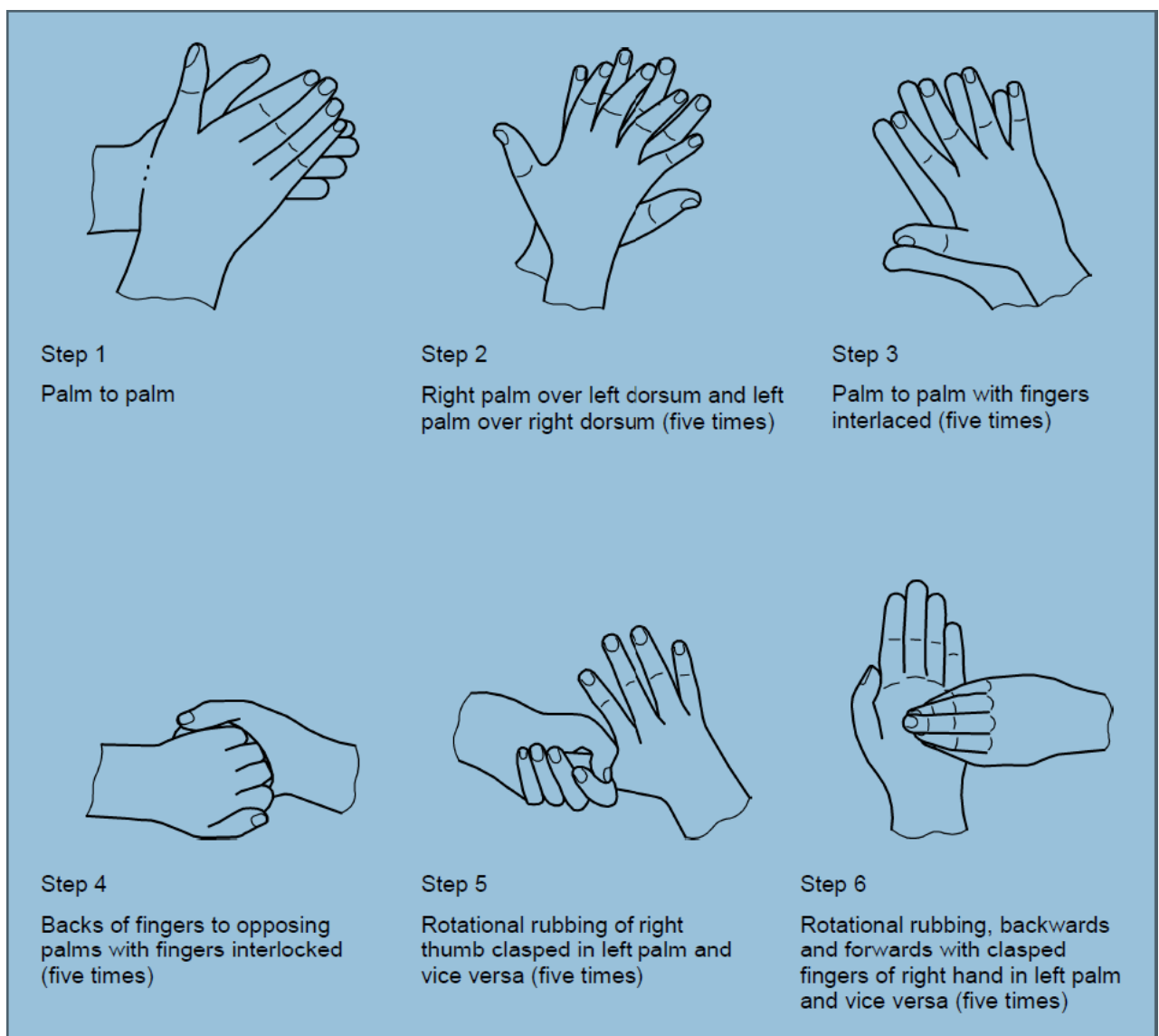


Figure 1 Standard Handrub Procedure: Adapted from EN 1500:2013.

END OF REPORT

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