Assessment of the Efficacy of GAMA Healthcare Hand Rub (Clinell) – Determined using the European Standard Test Method EN 1500:1997

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Tests Carried Out By:	University of Huddersfield**.** School of Applied Sciences, Queensgate, Huddersfield HD1 3DH
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Microbiological Tests	
Test Method	European Standard EN 1500:1997
Test Procedures	Full details of all the test and control procedures used are given in the Test Method
Handrub	GAMA Healthcare Ltd. (Clinell)
Batch Tested	17012005
Temperature	20 ºC (± 1 ºC)
Contact Time Tested	1 minute
Test Organism	*Escherichia coli* K12 10083 (NCIMB), National Collections of Industrial, Food and Marine Bacteria, Aberdeen.
Culture Medium	Tryptone Soya Agar, LabM
Incubation	Plates were incubated at 37 ºC for 24 - 48 h
Counting procedure	Pour plate
Diluent	¼ Strength Ringer's (LabM)
Neutralisers	GAMA Healthcare hand rub (Clinell) a neutraliser containing polysorbate 80, L-histidine, saponin and cysteine in diluent. Propan-2-ol 60% (v/v) reference hand rub a neutraliser containing polysorbate, lecithin, and L-histidine in diluent. Tests were carried out to verify that these neutralisers were satisfactory. (See Annex 1)

Ethics committee approval was sought and granted for the application of the following test method.

Test Method and Validation

EN 1500:1997 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 10083 NCIMB) 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 neutralisers to terminate the effect of any residual disinfectant. The organisms are enumerated, counts transposed to the Log_{10} system and the difference between the numbers recovered from the test or reference, and baseline counts is established and statistically analysed for significance. The larger the difference between the two counts, the more effective the product. Each of the volunteers repeats the procedure for the reference and test product.

For the test product to conform to the standard, EN1500:1997, the mean log reduction should not be significantly smaller (at 90 % significance level) than that obtained when using the reference product.

Method of Application:

Application of the test organism: Hands were prepared by washing for 1 minute with 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, autoclave to sterilize). Hands were immersed to the mid-metacarpals for 5 s, fingers apart, in 2 l of cultured test organism, *E. coli* K12, containing between 2 x 10^8 and 2 x 10^9 cfu ml^{-1}. Hands were air dried for 3 minutes were upon the test procedure was commenced, either reference handrub procedure (R) or test product (P) as outlined below.

Reference Product (R): 60% (w/v) Propan-2-ol

Reference Handrub Procedure (R): Three mls of the reference product (propan-2-ol) 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 3 ml of the reference product, to give a total rubbing time of 60 seconds. The reference procedure was completed by a 5 second rinse of the fingers under running tap water. Excess water was shaken off. Sampling commenced immediately.

Test Product (P): GAMA Healthcare Handrub (Clinell), applied as 3 ml aliquots for 30 seconds repeated once

The test product was applied using the same rubbing technique as described for the reference product (R). The procedure was completed by a 5 second rinse of the fingers under running tap water. Excess water was shaken off, and sampling commenced immediately.

Results

Table 1 Reference handrub procedure propan-2-ol 60% (v/v)

Preparation		propan-2-ol 60% (v/v)									
Application		rub-in 3ml/30 s, repeat once									
Date of experiment		17-Mar-05									
Test organism		*E.coli* K12 NCIMB 10083									
Suspension		2.14 x 10^{9} cfu ml^{-1}									
Subject		**Number of CFU per plate from dilution 10^{x}**									
No.	**Hand**	**Prevalues**				**Postvalues**					
	left or right	10^{-4}	10^{-4}	10^{-5}	10^{-5}	10^{0}	10^{0}	10^{-1}	10^{-1}	10^{-2}	10^{-2}
1	l			61	50	48	64				
	r	212	200	19	22	106	92				
2	l	63	58							110	121
	r	151	165	15	15					31	41
3	l			367	397					92	94
	r			193	168					88	126
4	l			83	64			204	176		
	r			116	111					42	36
5	l			290	307			238	260		
	r			724	762					44	42
6	l			125	119			99	90		
	r			293	281			129	131		
7	l			95	88			188	224		
	r			73	66			97	90		
8	l			207	185					136	140
	r			97	85					121	115
9	l			138	131					78	79
	r			94	92					85	85
10	l			149	150					115	117
	r			113	102						
11	l			160	159					112	126
	r			175	163					58	60
12	l			128	124					233	240
	r			147	156					215	207
13	l			358	332					46	41
	r			412	346					105	100
14	l			23	21	160	170				
	r			21	30			260	252		
15	l			322	266			148	122		
	r			510	482			214	260		

Table 2 Handrub procedure with the test product													
Preparation		GAMA Healthcare											
Application		rub-in 3ml/30 s, repeat once											
Date of experiment		17-Mar-05											
Test organism		*E.coli* K12 NCIMB 10083											
Suspension		2.14 x 10^9 cfu ml^{-1}											
Subject		**Number of CFU per plate from dilution 10^x**											
No.	**Hand**	**Prevalues**				**Postvalues**							
	left or right	10^{-4}	10^{-4}	10^{-5}	10^{-5}	10^0	10^0	10^{-1}	10^{-1}	10^{-2}	10^{-2}		
1	l	180	197					318	304				
	r	103	128							45	35		
2	l			110	74			312	294				
	r			37	39					34	35		
3	l			166	171			334	298				
	r			77	78			306	308				
4	l	84	105					144	146				
	r			25	24			130	124				
5	l			270	264			316	314				
	r			222	226			240	282				
6	l			59	53			152	168				
	r			63	59			180	156				
7	l			44	35			192	182				
	r			36	41			66	90				
8	l			23	30					77	75		
	r			27	35			266	318				
9	l			63	91			154	138				
	r			27	25			188	192				
10	l			95	98					93	98		
	r			41	47					91	89		
11	l			88	91			102	104				
	r			37	46			200	208				
12	l			28	33			110	112				
	r			25	24			104	102				
13	l			304	292					63	64		
	r			342	318					52	52		
14	l			132	136			264	268				
	r			176	137			64	90				
15	l			240	248			74	90				
	r			276	290			118	130				

Table 3 Computed log_{10} values (mean of left and right hand) and log_{10} reduction factors of the experimental results (Table 1 and Table 2)						
Subject	**Reference**			**Product**		
	procedure propan-2-ol 60% v/v (R)			GAMA Healthcare test product (P)		
	log x	**log y**	**log z**	**log x**	**log y**	**log z**
1	6.580354661	1.889302	4.691053	6.181844	3.55206	2.629784
2	6.038421446	3.879239	2.1591822	6.812913	3.511215	3.301699
3	7.449092531	4.000868	3.4482248	7.089905	3.493458	3.596447
4	6.970811611	3.460522	3.5102896	6.22981	3.133539	3.096271
5	7.716629279	3.5302	4.1864296	7.390051	3.459392	3.930659
6	7.310693312	3.050186	4.260507	6.767156	3.214844	3.552312
7	6.90579588	3.175367	3.730429	6.591065	3.214844	3.376221
8	7.156851901	4.10738	3.0494723	6.458638	3.720159	2.738479
9	7.055951405	3.91169	3.1442612	6.711807	3.225309	3.486498
10	7.108903128	3.827369	3.2815339	6.846646	3.967548	2.879098
11	7.215505378	3.948413	3.2670924	6.816241	3.186108	3.630133
12	7.142232992	4.349763	2.7924699	6.439333	3.029384	3.409949
13	7.558708571	3.86362	3.6950884	7.49693	3.762491	3.734439
14	6.375663614	3.134337	3.2413271	7.162116	3.234264	3.927852
15	7.596597096	3.269513	4.3270842	7.420781	3.012837	4.407943
mean	7.078814187	3.559851	3.518963	6.827682	3.381163	3.446519
s	0.462385412	0.605528	0.6567008	0.41678	0.283976	0.476787
N	15	15	15	15	15	15

X – log_{10} pre values mean value from left and right hands
Y – log_{10} post values mean value from left and right hands
Z – X-Y log_{10} reduction mean value
S – Standard deviation
N – Sample size

Statistical Analysis

Table 4 Statistical comparison of values as obtained with R (control) and P (product)						
Subject	log RF derived from		Difference		Rank of difference	
	R	P	R-P		without sign	with sign
1	4.691053	2.629784	2.0612689		15	15
2	2.159182	3.301699	-1.142516		14	-14
3	3.448225	3.596447	-0.148222		3	-3
4	3.51029	3.096271	0.4140187		10	10
5	4.18643	3.930659	0.2557706		4	4
6	4.260507	3.552312	0.7081949		13	13
7	3.730429	3.376221	0.3542083		7	7
8	3.049472	2.738479	0.3109938		5	5
9	3.144261	3.486498	-0.342237		6	-6
10	3.281534	2.879098	0.4024355		9	9
11	3.267092	3.630133	-0.363041		8	-8
12	2.79247	3.409949	-0.617479		11	-11
13	3.695088	3.734439	-0.03935		1	-1
14	3.241327	3.927852	-0.686525		12	-12
15	4.327084	4.407943	-0.080859		2	-2
RF: reduction factor z						
Sum of ranks (+): 63						
Sum of ranks (-): 57						

When the smaller sum of the ranks (here 57) is compared with the tabulated values from the Wilcoxon table (table 5) at a significance level of 0.1 i.e. 90% (value 36), the sum of ranks is not smaller than the tabulated value. The product i.e. 3 ml GAMA Healthcare hand rub (Clinell) rubbed in for 30 seconds (repeated once) is therefore not significantly different in efficacy (either more or less) than the reference product. The product, GAMA Healthcare hand rub (Clinell) meets the efficacy requirements of EN 1500:1997

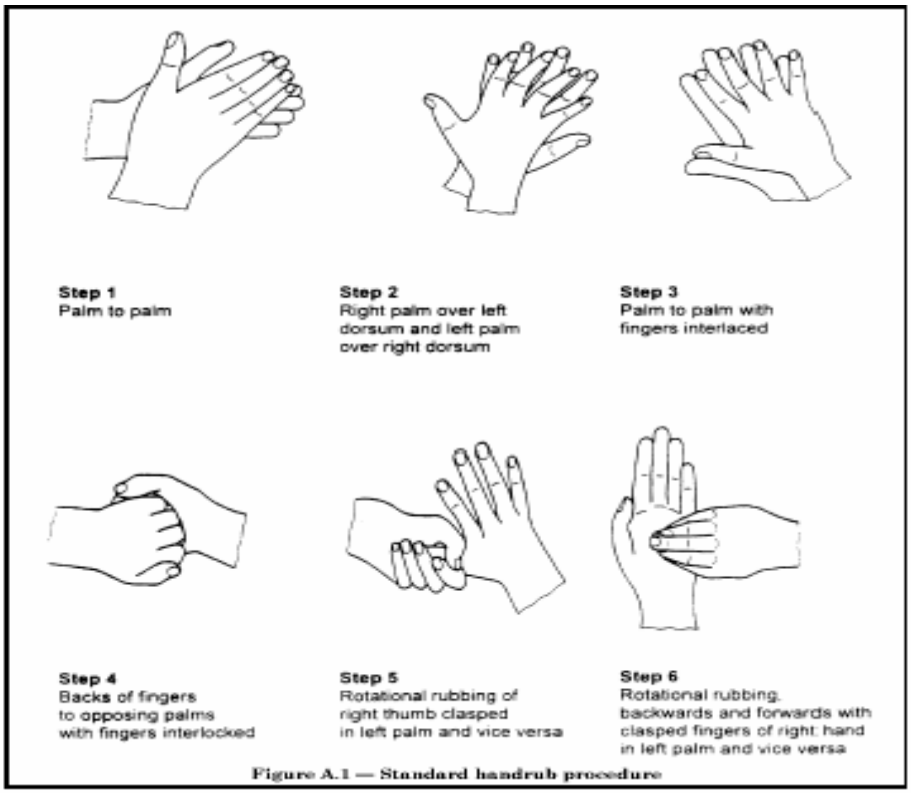
Table 5 Wilcoxons matched-pairs signed-rank test critical values of the lower of both sums of ranks with (+) or (-) sign at different significance levels			
Number of pairs with difference not equal to 0	Level of significance		
n	0.1 (90%)	0.05 (95%)	0.01 (99%)
12	12	17	9
13	26	21	12
14	31	25	15
15	36	30	19

Conclusion:

According to EN 1500:1997, the GAMA Healthcare Handrub (Clinell) when applied in two 3ml aliquots and rubbed into the hands for 60 seconds in total, is not significantly more or less effective at a 90 % confidence level than the reference product propan-2-ol 60% (w/v), which is applied in two 3 ml aliquots and rubbed into the hands for 60 seconds in total. The GAMA Healthcare handrub (Clinell) when applied in the manner described therefore meet the requirement stipulated in EN 1500:1997 i.e. the mean reduction of the release of test organisms achieved by the GAMA Healthcare handrub product (Clinell) is not significantly smaller than that achieved by the reference product (propan-2-ol 60% w/v) and therefore passes the European Standard EN 1500:1997 for a hygienic hand rub.

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.



Adapted from EN 1500:1997 Chemical disinfectants and antiseptics – hygienic handrub test method and requirements (phase 2/step2)

Annex 1

Method Validation

A1. Validation of non-toxicity of the neutralisation medium (control)
To 9 ml of neutralisation medium 1 ml of bacterial test suspension diluted to 1 x 10^3 to 3 x10^3 is added, mixed and left in the water bath at 20 ºC ± 1 ºC for a contact time of 1 min ± 5 s. At the end of the contact time 1 ml samples are taken in duplicate and pour plates prepared, after incubation at 37 ºC for 48 h. The average plate count N' of the neutralisation medium is determined.

A2. Validation of the inactivation by the dilution neutralisation method (test)
To 1 ml of the bacterial diluent 9 ml of the handrub product is added, mixed and left in a water bath at 20 ºC ± 1 ºC for 5 min ± 10 s. 1 ml of the mixture is then transferred to 8 ml of neutralising medium, maintained at 20 ºC ± 1 ºC and left for 5 min ± 10 s. 1 ml of bacterial test suspension diluted to 1 x 10^3 to 3 x10^3 is added, mixed and left for a contact time of 1 min ± 5 s. At the end of the contact time 1 ml samples are taken in duplicate and pour plates prepared, after incubation at 37 ºC for 48 h. The average plate count n' of the dilution neutralisation method is determined.

Table 2A Validation of dilution-neutralisation method

Neutraliser	**Bacterial test suspension (cfu ml^{-1})**	**Validation of neutralisation**	
	Escherichia coli	**A1. Control**	**A2. Test**
polysorbate 80, L-histidine, saponin and cysteine – GAMA Healthcare (P)	211, 205 (N=208)	200, 199 (N'=199.5)	193, 185 (n'= 189)
polysorbate, lecithin, and L-histidine – Propan-2-ol (R)	211, 205 (N=208)	203, 201 (N' = 202)	189, 180 (n' = 184.5)

Results
For the test organism *Escherichia coli* K12:

N and N' are between 100 and 300 cfu

N' is greater than 0.5 times N

n' is greater than 0.5 times N'

The neutralisation is validated with the neutralisation mediums tested.

User Opinion

Although European test methods such as EN1500:1997 exclude user acceptability studies it is widely acknowledged that user acceptability is essential to ensure hand washing /disinfection compliance. On conclusion of the test procedure the participants were invited to wash/disinfect their hands using Purell alcohol gel. The results of an opinion poll of the 15 participants are shown in table 6.

	Soft soap	Propan-2-ol	GAMA Healthcare	Purell
Smell Feel	-13 -3	-9 -5	13 13	5 -13
Overall preference	-16 (4)	-14 (3)	26 (1)	-10 (2)

Score: Good = +1 **No. of participants = 15**
No opinion = 0 **(1-4) = order of preference**
Poor = -1

What is clear are the commercial products, GAMA Healthcare handrub (Clinell) and Purell were all far more popular than the standard un-medicated soft soap or the reference control formulations, propan-2-ol 60 %. A favourable majority of the participants (13) found the feel and smell of the GAMA Healthcare product (Clinell) acceptable/good. What is interesting is that the majority of the participants (13) did not like the feel of the Purell product, reporting it as feeling sticky.

Summary

Cosmetic or user acceptability is an essential issue in the selection of hand disinfection and hand rubs. If the product is unpleasant it will not be used and compliance with national good practice hand disinfection guidelines will be unlikely.
User opinion was gained from all 15 participants in this study on the smell and feel of the reference and test products and a current in-use hand gel. The GAMA Healthcare handrub product (Clinell) was reasonably popular whereas the reference standards, propan-2-ol 60% and the un-medicated soft soap were not. From this study it is difficult to make a direct comparison between the Purell hand rub and the GAMA Healthcare hand rub (Clinell), however initial reaction would appear to favour the use of the GAMA Healthcare handrub (Clinell) over the Purell hand gel.

Opinion

During the research supporting these results we encountered some difficulty identifying an effective neutraliser for alcohol based products (active ingredients of 70%). The published literature suggests the use of universal neutralisers. However, we failed to find an effective chemical neutraliser, using the membrane filtration method where appropriate. This problem of neutralising a product which is effectively 70 % active does not seem to have been encountered or addressed within the published literature within this field of microbiology. Potentially this may lead to the questioning of results on high activity products, predominantly those that contain alcohol, where neutralisation of the product has been via a chemical neutraliser.

