

EN 1500: Chemical disinfectants and Antiseptics— Hygienic handrub— Test method and requirements (phase 2/step 2)

TEST PRODUCT: Duomax Hand Sanitizer

PROJECT: BT-DUO-13

TESTED FOR: DUOTECH

SAMPLE RECEIVED: 31 August 2011

NEUTRALIZER: Lecithin 3g/l, Polysorbate 80 30g/l, sodium thiosulphate 5g/l, L-histidine 1g/l, phosphate buffer 0.0025mol/l in tryptone soya broth. Sterilized by autoclave.

TEST PERFORMED: 29 SEPTEMBER 2011

TESTING LABORATORY: BLUTEST LABORATORIES LTD
ROBERTSON Incubator (Level 4)
ROBERTSON BUILDING
56 DUMBARTON ROAD
GLASGOW, UK G11 6NU

INTRODUCTION

1.1 Requirements: When tested in accordance with clause 5, the mean reduction of the release of test organisms achieved by the hygienic handrub product shall not be significantly smaller than that achieved by a reference handrub (R) with propan-2-ol 60 % (V/V). Products under test shall at least have a bactericidal activity as specified in prEN 12054.

1.2 Principle: The number of test organisms released from the fingertips of artificially contaminated hands is assessed before and after the hygienic handrub. The ratio of the two resulting values is called the reduction factor. It represents a measure of the antimicrobial activity of the hygienic handrub product tested. The necessary precision is achieved by repeating the test on 12 to 15 subjects. To compensate for extraneous influences it is compared with the reduction factor obtained with a parallel reference handrub procedure (R) which is performed with the same subjects, on the same day and under comparable environmental conditions.

1.3 Experimental design: For testing one product at a time a cross-over design is used. The subjects are randomly divided into two groups of approximately the same size. The test is first performed with group 1 using the reference handrub procedure (see **1.6.4.2**) and group 2 using the handrub procedure with the test product (see **1.6.4.3**). The test is then repeated on the same day with group 1 using the handrub procedure with the test product and group 2 using the reference handrub procedure. Before every reference handrub procedure and every handrub procedure with the test product, the procedure described in 1.6.2 and 1.6.3 shall be carried out.

1.4 Subjects: The test shall be performed on 12 to 15 healthy persons who have hands with healthy skin, without cuts or abrasions, and with short and clean fingernails. Although, in general, age is not a limiting factor, subjects should be at least 18 years of age.

1.5 Test organism: *Escherichia coli* K12 NCTC 10538.

1.6 PROCEDURE FOR EN 1500

SUBJECTS SHOULD WEAR A PROTECTIVE DISPOSABLE SMOCK AND PROTECTIVE GOGGLES.



1.6.1 Preparation of the contamination fluid

Grow the E. coli in two tubes each containing 5 ml of TSB for 18 h to 24 h at $(36 \pm 1)^\circ\text{C}$. Inoculate these cultures into two bottles with 1 L TSB each and incubate for 18 h to 24 h at $(36 \pm 1)^\circ\text{C}$. Pool the resulting bacterial suspension. This contamination fluid shall contain between 2×10^8 cfu/ml and 2×10^9 cfu/ml which shall be confirmed by a standard quantitative culture technique in accordance with prEN 12054.

1.6.2 Application of the contamination fluid

Prepare the hands by washing for 1 min with soft soap to remove natural transients. Dry them thoroughly on paper towels. Pour the contamination fluid into the container and immerse the hands up to the mid-metacarpals for 5 s with fingers spread apart. Carefully allow surplus liquid to drain back into the container. Allow the hands to dry in the air for 3 min, holding them in a horizontal position with the fingers spread out and rotating them to and fro to avoid the formation of droplets. One batch of contamination fluid shall be used not longer than 3 h after the first subject's hands have been contaminated. Additionally, it shall be ensured that, in a test, all subject's hands shall be treated with the same batch of contamination fluid, even if various products are tested against the reference product.

NOTE During this procedure care should be taken to avoid contamination of the immediate work area. Additionally, clothing should be removed from the forearms and the subject protected by wearing a smock to protect the subjects clothes.

1.6.3 Pre-values

Immediately after drying, rub the fingertips (including that of the thumb) for 1 min on the base of a Petri dish containing 10 ml of TSB without neutralizer, in order to assess the release of test organisms before treatment of the hands (pre-values). Use a separate Petri dish for each hand. Prepare dilutions of these sampling fluids of 10^{-3} and 10^{-4} in TSB. For each dilution, spread 0.1 ml over the surface of a TSA plate using glass spreaders. The interval between sampling and plating shall not exceed 30 min.

1.6.4 Hygienic handrub procedure

1.6.4.1 General

Immediately after sampling for the pre-values and without re-contaminating the hands, the groups shall perform the handrub in accordance with either **1.6.4.2** or **1.6.4.3**, as applicable.

1.6.4.2 Reference handrub procedure R

A 3 ml volume of propan-2-ol 60 % (V/V) is poured into the cupped dry hands and rubbed vigorously for 30 s onto the skin up to the wrists, in accordance with the standard handrub procedure shown Figure A.1, to ensure total coverage of the hands. This comprises 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. Repeat with a further 3 ml propan-2-ol, to give a total rubbing time of 60 s. The reference procedure is completed by a 5 s rinse of the fingers under running tap water; excess water is shaken off.

1.6.4.3 Handrub procedure with test product (P)

This procedure is always performed according to information provided by the manufacturer, which shall include volume of product and frequency of application. The total rubbing time is limited to either 30 s or 60 s. The procedure with the test product is completed by a 5 s rinse of the fingers under running tap water; excess water is shaken off.

1.6.5 Post-values

Immediately afterwards, rub the fingertips and thumbtips on the base of a Petri dish containing 10 ml TSB containing neutralizer for 1 min. Use a separate Petri dish for each hand.

PREVENTATIVE DECONTAMINATION OF THE SUBJECTS HANDS. The subjects hands are extensively washed with a liquid soap solution twice and extensively rinsed in tap water to remove residual bacterial contamination. The subject should be satisfied that their hands are thoroughly cleaned before leaving the test area.

Spread 1.0 ml and 0.1 ml samples of the undiluted sampling fluid and 0.1 ml of a 10^{-1} dilution in TSB containing neutralizer over the surface of TSA plates using glass spreaders. If necessary, repeat with 0.1 ml of a 10^{-2} dilution. The interval between sampling and plating shall not exceed 30 min.

1.6.6 Incubation

Incubate all plates aerobically at $(36 \pm 1) ^\circ\text{C}$ for 18 h to 24 h. Count the colony forming units and reincubate for a further 24 h to detect any slow growing colonies.

1.7 Calculation

Record the number of colony forming units (cfu) per plate for each dilution step. Calculate the dilution factor by multiplying the sample dilution and the sample volume (in millilitres). Calculate the number of cfu per millilitre of sampling fluid by multiplying the plate count (cfu) by the dilution factor.

NOTE 1 Whenever possible, the counts should be obtained from plates showing 15 to 300 colonies. With very efficient handrubs some counting plates for post-values can show fewer than 15 colonies or no growth at all even if inoculated with 1 ml of undiluted sampling fluid. These values can then be accepted. If suitable counts are obtained from two adjacent dilution steps, e.g. 299 colonies from 10^{-1}

dilution and 31 from 10^{-2} dilution, the weighted arithmetic mean of both is calculated as follows: = 3 000 cfu/ml undiluted sampling fluid

NOTE 2 If colony counts of different dilution steps are grossly disproportional, insufficient neutralization of the antimicrobial agent should be suspected. All viable counts per millilitre sampling fluid are transformed to decimal logarithms. For computational reasons values of 0 ($\log 0 = -Z$) have to be set 1 ($\log 1 = 0$).

NOTE 3 Since 0-values should be found only among post-values and should occur only with the most active products, this adjustment can, at the worst, introduce a conservative bias of underestimating the antimicrobial efficiency of a product. For both reference and test procedure the log counts from right and left hands of each subject shall be averaged separately for pre-values and post-values.

NOTE 4 This double weighting increases the precision of the measurement. From the difference between this individual combined log pre-value and the log post-value a log reduction factor is established for each subject. Then, the two arithmetic means of all individual log reduction factors are calculated for both the reference and the test procedure. Compare the results for both procedures, P and R. This comprises 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. Repeat with a further 3 ml propan-2-ol, to give a total rubbing time of 60 s. The reference procedure is completed by a 5 s rinse of the fingers under running tap water; excess water is shaken off.

1.8 Test validation

The results of a test shall be accepted for further evaluation if they conform to the following criteria, otherwise the test shall be repeated.

Requirements for acceptance of test results are:

- a) All results from at least 12 subjects shall be available.
- b) The overall mean of the log pre-values for reference and test procedure(s) shall be at least 5.00.
- c) In each procedure, R and P, not more than three individual log reduction factors fewer than 3.00 shall occur.

1.9 Evaluation of P

If the quality of the data has been found acceptable (see 1.8), they shall be used for the evaluation of the antimicrobial efficacy of the product(s) under test by applying the following pass criterion:

- For any product, the mean log reduction factor obtained shall not be significantly smaller than that obtained for the reference propan-2-ol;
- If the mean log reduction factor of a test product is smaller than that of the reference propan-2-ol, the difference shall be tested for statistical significance;
- If the mean log reduction factor is significantly smaller than that obtained with the reference propan-2-ol, the test product does not conform to this standard.

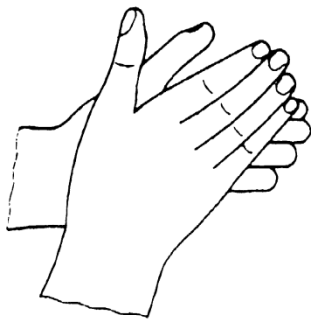
1.10 Significance testing

For testing the mean log RF of P against that of R, the Wilcoxon matched-pairs signed-ranks test shall be Used. For testing the data obtained in a Latin-square design experiment, the (k-l) sign test by Rhyne and Steel shall be used, comparing means of more than one test procedure with that of the reference in a pairwise manner. Because of the confirmative nature of the test in this application, the level of significance is set at $p = 0.1$. The test is to be used one-sided. The discrimination efficiency of the test

procedure described has been set to detect a difference between the two mean log reduction factors of approximately 0,6 log at a power of 95 %.

Standard handwash procedure

Pour an appropriate volume of handwash product, R or P, into the pre-wetted cupped hands and wash hands in accordance with the standard handwash shown below to ensure total coverage of the hands. The action in each step is repeated 5 times before proceeding to the next step. After concluding step 6, recommence the series of steps, as appropriate, to complete the washing time as specified in clauses 1.6.4.2 and 1.6.4.3.



Step 1
Palm to palm



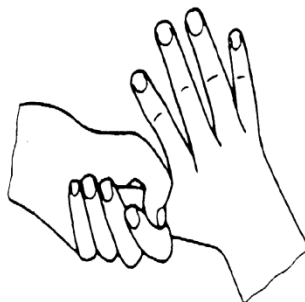
Step 2
Right palm over left
dorsum and left palm
over right dorsum



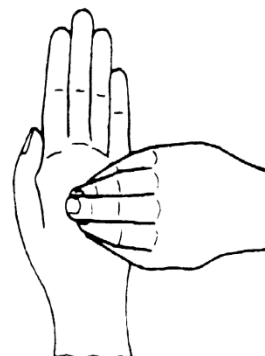
Step 3
Palm to palm with
fingers interlaced



Step 4
Backs of fingers
to opposing palms
with fingers interlocked



Step 5
Rotational rubbing of
right thumb clasped
in left palm and vice versa



Step 6
Rotational rubbing,
backwards and forwards with
clasped fingers of right hand
in left palm and vice versa

RESULTS

TABLE 1
Product Results

Handwash procedure BS EN 1500 : 1997							
Preparation :		DUOMAX HAND FOAM					
Application :		3g X 2					
Date of Experiment :		29-Sep-11					
Test Organism :		E.COLI K12					
Suspension :		8.7 X 10 ⁸					
Subject Number	Hand	Prevalues			Post Values		
		10 ⁻⁴	10 ⁻⁵	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³
1	L	148	19	TNTC	TNTC	36	3
	R	TNTC	41	TNTC	TNTC	41	5
2	L	TNTC	58	TNTC	202	33	4
	R	TNTC	51	TNTC	TNTC	68	16
3	L	TNTC	164	TNTC	119	46	8
	R	TNTC	128	TNTC	234	20	7
4	L	TNTC	0	TNTC	182	17	1
	R	TNTC	76	TNTC	TNTC	58	6
5	L	TNTC	55	TNTC	TNTC	46	7
	R	TNTC	73	TNTC	TNTC	66	6
6	L	99	11	TNTC	132	8	1
	R	101	8	TNTC	TNTC	54	28
7	L	39	5	TNTC	83	7	1
	R	65	9	TNTC	99	20	1
8	L	354	45	TNTC	TNTC	131	14
	R	TNTC	44	TNTC	TNTC	79	11
9	L	TNTC	43	TNTC	TNTC	50	2
	R	TNTC	43	TNTC	279	30	5
10	L	46	6	TNTC	TNTC	68	11
	R	56	11	TNTC	TNTC	83	11
11	L	TNTC	53	TNTC	285	46	5
	R	TNTC	53	TNTC	241	21	1
12	L	222	23	TNTC	TNTC	26	3
	R	282	27	TNTC	TNTC	68	8
13	L	TNTC	58	TNTC	TNTC	239	27
	R	298	28	TNTC	TNTC	TNTC	48
14	L	TNTC	37	TNTC	TNTC	97	36
	R	TNTC	50	TNTC	TNTC	47	5
15	L	TNTC	89	TNTC	127	39	3
	R	TNTC	81	TNTC	50	14	0

TABLE 2
Control Results

Handwash procedure BS EN 1500 : 1997							
Preparation :		60% IPA					
Application :		3mls X 2					
Date of Experiment :		29-Sep-11					
Test Organism :		E.COLI K12					
Suspension :		8.7 X 10 ⁸					
Subject Number	Hand	Prevalues			Post Values		
		10 ⁻⁴	10 ⁻⁵	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³
1	L	238	37	TNTC	TNTC	62	2
	R	223	30	TNTC	TNTC	57	5
2	L	TNTC	65	TNTC	TNTC	78	7
	R	TNTC	58	TNTC	TNTC	137	8
3	L	TNTC	193	TNTC	6	1	0
	R	TNTC	140	TNTC	81	4	1
4	L	TNTC	37	TNTC	TNTC	106	17
	R			TNTC	TNTC	260	20
5	L	306	32	TNTC	TNTC	113	8
	R	231	19	TNTC	TNTC	241	33
6	L	235	37	TNTC	55	5	0
	R	276	39	TNTC	TNTC	265	21
7	L	79	8	TNTC	106	26	3
	R	23	2	TNTC	35	0	0
8	L	145	16	TNTC	TNTC	7	19
	R	242	36	TNTC	175	26	4
9	L	TNTC	40	TNTC	166	23	1
	R	298	42	TNTC	82	2	2
10	L	86	11	TNTC	TNTC	49	1
	R	80	5	TNTC	284	28	5
11	L	TNTC	89	TNTC	158	15	3
	R	TNTC	95	TNTC	TNTC	113	1
12	L	TNTC	108	TNTC	59	2	0
	R	TNTC	88	TNTC	89	12	0
13	L	TNTC	53	TNTC	TNTC	108	10
	R	293	37	TNTC	TNTC	32	3
14	L	122	21	TNTC	TNTC	TNTC	107
	R	269	42	TNTC	TNTC	256	44
15	L	TNTC	79	TNTC	117	14	0
	R	TNTC	76	TNTC	46	8	0

TABLE 3

List of computed log values (means of left and right hand) and log reduction factors of the experimental results (Tables 1 and 2)

Subject	Reference			Test			
	Log x	Log y	Log z	Log x	Log y	Log z	
1	6.38	3.77	2.61	6.31	3.59	2.72	
2	6.79	4.03	2.76	6.41	3.70	2.71	
3	7.22	2.64	4.58	6.81	3.28	3.53	
4				6.58	3.57	3.01	
5	6.43	4.25	2.18	6.81	3.75	3.06	
6	6.43	4.13	2.30	6.00	3.49	2.51	
7	5.71	2.85	2.86	5.73	2.96	2.77	
8	6.30	3.41	2.89	6.65	4.02	2.63	
9	6.61	3.09	3.52	6.63	3.60	3.03	
10	5.92	3.59	2.33	5.73	3.90	1.83	
11	6.96	3.81	3.15	6.72	3.43	3.29	
12	6.99	2.87	4.12	6.40	3.67	2.73	
13	6.65	3.85	2.80	6.63	4.57	2.06	
14	6.31	4.88	1.43	6.64	3.86	2.78	
15	6.89	2.91	3.98	6.93	3.02	3.91	
Mean	6.54	3.58	2.97	6.47	3.63	2.84	
S	0.42	0.65	0.85	0.38	0.40	0.52	
N	14	14	14	15	15	15	

TABLE 4

**Statistical comparison of values as obtained with R and P
(WILCOXON's matched-pairs signed-ranks test)**

Subject	Log RF derived from		Difference R - P	Rank of difference	
	R	P		Without sign	With sign
1	2.61	2.72	-0.11	4	(-)4
2	2.76	2.71	0.05	1	1
3	4.58	3.53	1.05	12	12
4					
5	2.18	3.06	-0.88	11	(-)11
6	2.30	2.51	-0.21	6	(-)6
7	2.86	2.77	0.09	3	3
8	2.89	2.63	0.26	7	7
9	3.52	3.03	0.49	8	8
10	2.33	1.83	0.50	9	9
11	3.15	3.29	-0.14	5	(-)5
12	4.12	2.73	1.39	14	14
13	2.80	2.06	0.74	10	10
14	1.43	2.78	-1.35	13	(-)13
15	3.98	3.91	0.07	2	2
RF : Reduction factor Sum of ranks (+) : 66 Sum of ranks (-) : 39					

CONCLUSION

The mean log reduction factor for the reference propan-2-ol is 2.97 and the mean log reduction factor for the product is 2.84; therefore there is a requirement for statistical analysis because the mean log reduction for the reference > product. Analysis with WILCOXON's matched-pairs signed-ranks test shows that the null hypothesis that R is significantly more effective than P is rejected at 99.5% confidence for N=14.

DUOMAX hand sanitizer, therefore DOES comply with EN 1500. Chemical disinfectants and Antiseptics - Hygienic handwash - Test method and requirements (phase 2/step 2), when 3.0g of foam is applied twice consecutively for 30 seconds in comparison with 3.0 mls of 60.0% V/V propan-2-ol applied twice consecutively for 30 seconds.

RESULT = PASS

Signed

A handwritten signature in blue ink, consisting of a series of loops and a long horizontal stroke at the end.

Dr Chris Woodall, Director
BluTest Laboratories Ltd
Glasgow, UK
17 October 2011