BluTest
GLOBAL MICROBIOLOGY EXPERTISE

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

TEST PRODUCT: **SANISCRUB E**

BATCH NUMBER: **12009RD10**

PROJECT: BT-SIR-01-02

TESTED FOR: **SIRMAXO CHEMICALS PVT LTD**

SAMPLE RECEIVED: 7 September 2012

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: 7 November 2012

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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 ± 1) °C. Inoculate these cultures into two bottles with 1 L TSB each and incubate for 18 h to 24 h at (36 ± 1) °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 subject's 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 ± 1) °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.

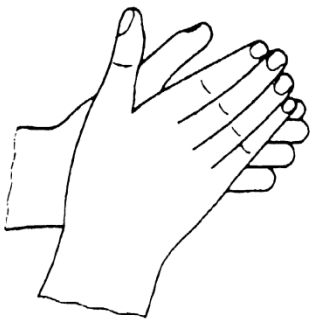
1.10 Significance testing

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

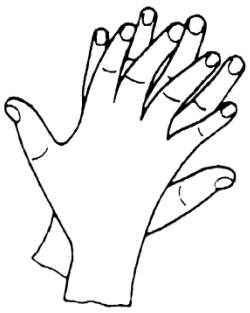
procedure described has been set to detect a difference between the two mean log reduction factors of approximately 0,6 log at a power of 95 %.

Standard handrub procedure

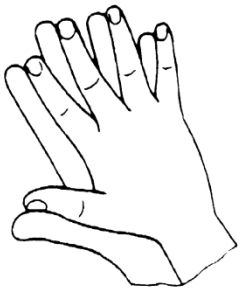
Pour an appropriate volume of handrub product, R or P, into the pre-wetted cupped hands and wash hands in accordance with the standard handrub shown below to ensure total coverage of the hands. The action in each step is repeated 5 times before proceeding to the next step. After concluding step 6, recommence 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

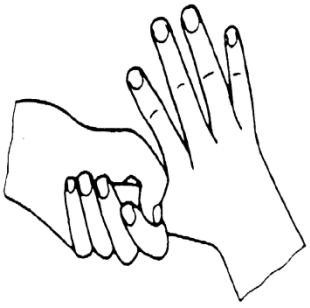


Step 2
Right palm over left dorsum and left palm over right dorsum

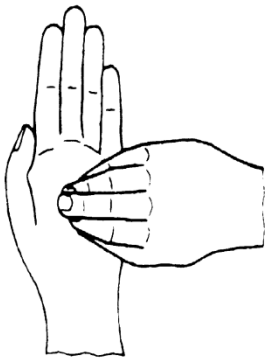


Step 3
Palm to palm with fingers interlaced

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

Validation of the efficacy of the product against E. coli K12 in a contact time of 60 seconds and validation of neutralization according to Control C

EN1276 Results for the efficacy of SANISCRUB E from SIRMAXO CHEMICALS PVT LTD under CLEAN CONDITIONS

Test organisms	Validation test				Bacterial test suspension (N)	Exposure (mins)	Test procedure at concentration % (V/V)			
	Bacterial Suspension (Nv)	Experimental conditions (A)	Neutralizer toxicity Control or filtration control (B)	Dilution-neutralization control or filtration test control (C)				1	50	80
Escherichia coli K12	*V* c: 84 ; 75	Vc: 66 ; 58	Vc: 76 ; 69	Vc: 61 ; 49	10^{-6}: 269 ; 274	1	Vc	0 ; 0	0 ; 0	0 ; 0
					10^{-7}: 31 ; 25		Na	<1.40E+02	<1.40E+02	<1.40E+02
					N: 2.72E+08		R	>10(5)	>10(5)	>10(5)
	N v: 7.95E+02	A: 6.20E+01	B: 7.25E+01	C: 5.50E+01						
Validation	30 ≤ Nv_0 ≤ 160 ?	A ≥ 0.5 x Nv_0 ?	B ≥ 0.5 x Nv_0 ?	C ≥ 0.5 x Nv_0?	7.17≤ log N_0 ≤7.70?		Test is valid			
	yes	yes	yes	yes	yes					

Please note: the upper limit for counting bacterial plates is 330 cfu. Enter as >330

V c = viable count
N = number of cfu/ml of the bacterial test suspension
N v = number of cfu/ml in the bacterial suspension
R = reduction in viability
N a = number of cfu/ml in the test mixture
A = number of cfu/ml of the experimental conditions validation
B = number of cfu/ml of the neutralizer toxicity validation or of the filtration validation
C = the number of cfu/ml of the dilution-neutralization validation or the membrane filtration test validation

Conclusion: According to EN 1276, the product demonstrates efficacy against *E. coli* K 12 (NCTC 10538) and the product can be effectively neutralised (Control C) by the neutraliser to be employed in EN 1500.

TABLE 1

Product Results

Handrub procedure BS EN 1500: 1997

Preparation : 3 mls

Application : 1 application for 30secs

Date of Experiment : 07-Nov-12

Test Organism: E.coli K12

Suspension : 10^{-7}: 90, 11 viable count

10^{-8}: 14, 5

Subject Number	Hand	Prevalues			Post Values		
		10^{-4}	10^{-5}	10^{-0}	10^{-1}	10^{-2}	10^{-3}
1	L	156	20	>330	>330	261	40
	R	117	12	>330	>330	>330	62
2	L	>330	102	>330	>330	>330	55
	R	>330	119	>330	>330	>330	23
3	L	43	4	>330	>330	135	18
	R	70	12	135	10	1	0
4	L	328	53	>330	>330	274	19
	R	>330	72	>330	>330	296	55
5	L	>330	103	>330	159	15	7
	R	>330	109	>330	>330	110	15
6	L	>330	62	185	18	3	0
	R	>330	71	127	7	0	0
7	L	55	7	>330	201	15	2
	R	156	16	>330	>330	58	7
8	L	>330	47	>330	156	25	2
	R	>330	108	>330	146	18	2
9	L	>330	49	177	9	1	0
	R	>330	48	180	6	0	0
10	L	>330	98	>330	115	9	0
	R	>330	91	>330	>330	100	14
11	L	212	35	>330	134	13	3
	R	256	30	>330	58	9	1
12	L	>330	86	>330	>330	239	22
	R	>330	112	>330	>330	36	13
13	L	>330	109	>330	35	7	0
	R	>330	96	>330	299	41	2
14	L	157	0	>330	35	3	3
	R	>330	125	>330	122	10	4
15	L	>330	86	>330	>330	60	2
	R	>330	153	>330	65	10	0

Red ink indicates data that was excluded. Yellow highlights are the data points used for each calculation.

TABLE 2

Control Results

Handwash procedure BS EN 1500 : 1997

Preparation : 60% IPA
Application : 3mls 2xapplications in 1 minute at 30sec intervals
Date of Experiment : 07-Nov-12
Test Organism: E.coli K12
Suspension : See Product

Subject Number	Hand	Prevalues			Post Values		
		10^{-4}	10^{-5}	10^{-0}	10^{-1}	10^{-2}	10^{-3}
1	L	0	0	>330	>330	>330	>330
	R	0	0	>330	>330	>330	>330
2	L	>330	103	>330	>330	255	28
	R	>330	112	>330	>330	63	5
3	L	124	19	>330	159	10	2
	R	165	17	9	1	0	0
4	L	50	49	>330	>330	>330	171
	R	>330	67	>330	>330	>330	188
5	L	>330	186	>330	234	29	2
	R	>330	102	>330	>330	152	8
6	L	>330	48	302	28	1	1
	R	>330	71	193	9	3	0
7	L	121	22	294	17	0	0
	R	>330	59	>330	49	0	0
8	L	65	2	>330	200	12	1
	R	146	17	309	16	5	1
9	L	86	8	62	1	0	1
	R	95	15	83	0	0	0
10	L	>330	89	>330	214	17	1
	R	>330	102	>330	>330	>330	44
11	L	>330	62	>330	194	34	0
	R	>330	78	>330	189	11	0
12	L	>330	56	>330	>330	246	20
	R	>330	74	>330	292	71	4
13	L	>330	88	>330	19	2	2
	R	>330	66	>330	22	7	1
14	L	>330	63	>330	45	11	0
	R	303	38	>330	66	8	2
15	L	>330	58	>330	113	12	3
	R	>330	108	>330	31	9	0

Red ink indicates data that was excluded. Yellow highlights are the data points used for each calculation.

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	ND	ND	ND	ND	ND	ND
2	7.05	4.22	2.83	7.06	4.59	2.47
3	6.17	2.90	3.27	ND	ND	ND
4	ND	ND	ND	ND	ND	ND
5	7.16	3.95	3.21	7.03	3.86	3.17
6	6.77	2.38	4.39	6.82	2.19	4.63
7	6.58	2.58	4.00	6.02	3.58	2.44
8	6.04	3.04	3.00	6.89	3.20	3.69
9	6.02	1.86	4.16	6.69	2.25	4.44
10	6.98	4.36	2.62	6.98	3.75	3.23
11	6.85	3.36	3.49	6.38	2.98	3.40
12	6.81	4.15	2.66	7.00	4.12	2.88
13	6.89	2.31	4.58	7.01	3.29	3.72
14	6.69	2.74	3.95	6.85	2.89	3.96
15	6.92	2.86	4.06	7.08	3.52	3.56
Mean	**6.69**	**3.13**	**3.56**	**6.82**	**3.35**	**3.47**
S	0.38	0.81	0.67	0.32	0.71	0.69
N	13	13	13	12	12	12

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	Rank of difference	
	R	P	R - P	Without sign	With sign
1	ND	ND	ND	ND	ND
2	2.83	2.47	0.36	7	7
3	3.27	ND	ND	ND	ND
4	ND	ND	ND	ND	ND
5	3.21	3.17	0.04	2	2
6	4.39	4.63	-0.24	5	(-)5
7	4	2.44	1.56	12	12
8	3	3.69	-0.69	10	(-)10
9	4.16	4.44	-0.28	6	(-)6
10	2.62	3.23	-0.61	9	(-)9
11	3.49	3.4	0.09	3	3
12	2.66	2.88	-0.22	4	(-)4
13	4.58	3.72	0.86	11	11
14	3.95	3.96	-0.01	1	(-)1
15	4.06	3.56	0.5	8	8
RF : Reduction factor Sum of ranks (+) : **32** Sum of ranks (-) : **35**					

CONCLUSION

The mean log reduction factor for the reference propan-2-ol is 3.56 and the mean log reduction factor for the product is 3.47; therefore there is a requirement for statistical analysis because the mean log reduction for the Product < Reference.

Applying Wilcoxons signed rank test. The sum of the positive ranks is 32 and the sum of the negative ranks is 35. For N = 12, the lowest sum of ranks has to be <12 for the populations to be significantly different at a confidence level of 0.1.

SANISCRUB E, batch number 12009RD10, therefore DOES comply with EN 1500: Chemical disinfectants and Antiseptics - Hygienic handrub - Test method and requirements (phase 2/step 2), when 3.0 ml is applied once for 30 seconds.

RESULT = PASS

Signed

Dr Chris Woodall, Director
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Glasgow, UK
14 November 2012