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INTRADERMA

STUDY REPORT

2023-3638/23 23 00063

NOVASIN

European Standard Test Method
EN 1500:2013

Chemical disinfectants and antiseptics - Hygienic handrub -
Test method and requirements (Phase 2/Step 2)

MARCH 2023

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STUDY REPORT 2023-3638/23 23 00063

EUROPEAN STANDARD EN 1500:2013

Chemical disinfectants and antiseptics Hygienic handrub (Phase 2/Step 2)

TEST PRODUCT IDENTIFICATION

PRODUCT NAME	:	NOVASIN (Sodium hypochlorite 500ppm-pH neutral)
SUBSTANCES AND THEIR CONCENTRATIONS	:	Sodium Hypochlorite-NaOCl 20%, Hypochlorite acid-HOCl 80%
APPEARANCE OF THE PRODUCT	:	Liquid
STORAGE CONDITIONS	:	Room Temperature, Darkness
LOT	:	TBD
METHOD	:	EN 1500:2013
COMPANY NAME	:	INTRADERMA
STUDY SPONSOR	:	INTRADERMA
PRODUCT MANUFACTURER	:	INTRADERMA
RECEIPT DATE	:	22/02/2023
STUDY PERIOD	:	21/03/2023-23/03/2023
LAB ID	:	2023-3638/23 23 00063
APPLICATION CONDITIONS-REFERENCE	:	2 x 30sec; 2 x 3 ml (60% v/v propan-2-ol)
APPLICATION CONDITIONS-PRODUCT	:	4 x 15sec; 4 x 15 ml (total rubbing time 60sec, total application quantity 60ml)
No of VOLUNTEERS	:	20

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No of VOLUNTEERS	:	20

Test Method	:	European Standard EN 1500:2013
Test Procedures	:	Full details of all the test and control procedures used are given in the Test Method
Test Organism	:	Escherichia coli K12 NCTC 10538
Culture Media and Reagents	:	Tryptone Soya Agar, Tryptic Soya Selective Agar, Tryptone Soy Broth
Incubation	:	Plates were incubated at 37 ºC for 24 - 48 h

TEST METHOD

EN 1500:2013 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 NCTC 10538) 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 neutralizers to terminate the effect of any residual disinfectant. Propan-2-ol 60% (V/V) is used as reference. The organisms are enumerated, counts transposed to the Log system and the difference between the numbers recovered from the test or reference, and baseline counts is established and statistically analyzed 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 and test product after, or for the product first and the reference after. For the test product to conform to the standard, EN1500:2013, the mean log reduction factor obtained shall be at least not inferior to that achieved by the specified reference hygienic handrub (60% volume concentration of propan-2-ol).

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REQUIREMENTS

When tested in accordance with EN 1500:2013, the mean reduction of the release of the test organism *Escherichia coli K12* achieved by the hygienic handrub with the product under test shall be at least not inferior to that achieved by a specified reference hygienic handrub (60 % vol. concentration of propan-2-ol).

SUBJECTS

The test was performed on 20 persons (requirement of the Standard 18-22 subjects) who have 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).

Composition of the neutraliser

Lecithin	3.0g
Sodium thiosulphate	5.0g
Tryptic digest of casein	1.0g
Sodium chloride	8.5g
Disodium hydrogen phosphate	8.0g
Potassium dihydrogen phosphate	1.5g
L-histidine HCL	1.0g
Polysorbate 80	30g

METHOD OF APPLICATION

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).

The volunteers were randomly divided into two groups of approximately the same size. Group 1 used the reference hygienic handrub and Group 2 the product under test. The test was repeated on the same day with Group 1 using the handrub procedure with the test product and Group 2 using the reference handrub procedure.

Hands were immersed to the mid-metacarpals for 5 sec, fingers apart, in 2 l of cultured test organism, E. coli K12, containing 1.5-5.0 x 10^8 cfu/ml. 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, either for reference handrub procedure (R) or test product (P) as outlined below.

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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

Three (3) ml of Propan-2-ol 60% (V/V) 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 three (3) ml of Propan-2-ol 60% (V/V) to give a total rubbing time of 60 seconds.

TEST PRODUCT

Fifteen (15) ml of product under test was poured into the cupped dry hands and rubbed vigorously into the skin for 15 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 with product under test was repeated with 3 more doses of 15 ml each to give a total rubbing time of 60 seconds and total application quantity 60ml.

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 24-48h in order to detect slow-growing colonies.

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Test organism : *E. coli* K12 NCTC 10538.
Concentration of contamination fluid : 3.7×10^8 cfu/ml.
Application Conditions-Reference (RP) : 2 x 30sec / 2 x 3 ml (60% v/v propan-2-ol)
Date of experiment : 21/03/2023

Table 1 - Reference hygienic handrub – experimental results. Colony Counts per Plate

Volunteer	Hand	Prevalues		Postvalues		
No	left or right	10^{-4}	10^{-5}	10^{0}	10^{-1}	10^{-2}
1	l	201	25	>330	>330	54
	r	>330	93	>330	>330	128
2	l	>330	78	>330	>330	32
	r	>330	106	>330	>330	39
3	l	>330	82	>330	>330	72
	r	>330	58	>330	>330	63
4	l	>330	81	>330	>330	64
	r	>330	53	>330	>330	50
5	l	>330	41	>330	>330	36
	r	>330	37	>330	137	18
6	l	>330	120	>330	127	10
	r	>330	118	>330	>330	27
7	l	>330	84	>330	67	9
	r	>330	73	>330	149	21
8	l	>330	116	>330	82	11
	r	>330	140	>330	107	15
9	l	>330	72	>330	204	21
	r	>330	69	>330	181	16
10	l	>330	79	>330	195	17
	r	>330	128	>330	>330	26
11	l	>330	105	>330	>330	229
	r	>330	57	>330	>330	121
12	l	>330	163	>330	>330	52
	r	>330	147	>330	183	24
13	l	>330	102	>330	>330	35
	r	>330	158	>330	>330	40
14	l	>330	59	>330	>330	39
	r	>330	80	>330	>330	54
15	l	>330	46	>330	204	23
	r	>330	54	>330	232	27
16	l	>330	132	>330	>330	39
	r	>330	154	>330	>330	41
17	l	>330	89	>330	>330	85
	r	>330	103	>330	>330	74
18	l	>330	39	>330	176	19
	r	>330	42	>330	201	23
19	l	>330	121	>330	175	18
	r	>330	152	>330	136	14
20	l	>330	53	>330	>330	39
	r	>330	58	>330	>330	74

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Test organism : *Escherichia coli K12* NCTC 10538.
Concentration of contamination fluid : 3.7×10^8 cfu/ml.
Application Conditions-Product (PP): : 4 x 15sec; 4 x 15 ml (total rubbing time 60sec, total application quantity 60ml)
Date of experiment : 21/03/2023

Table 2-Hygienic handrub procedure with the product under test-experimental results. Colony Counts per Plate.

Volunteer	Hand	Prevalues		Postvalues		
No	left or right	10^{-4}	10^{-5}	10^{0}	10^{-1}	10^{-2}
1	l	>330	64	>330	>330	71
	r	>330	125	>330	>330	36
2	l	>330	117	>330	>330	88
	r	>330	109	>330	>330	30
3	l	>330	132	>330	>330	122
	r	>330	144	>330	>330	45
4	l	>330	69	>330	109	14
	r	>330	60	>330	>330	47
5	l	>330	46	>330	184	15
	r	>330	38	>330	172	21
6	l	>330	27	>330	205	17
	r	>330	32	>330	153	12
7	l	>330	45	>330	88	9
	r	>330	58	>330	206	17
8	l	>330	44	>330	161	17
	r	>330	46	>330	170	23
9	l	>330	108	>330	219	26
	r	>330	114	>330	103	8
10	l	>330	93	>330	>330	62
	r	>330	91	>330	>330	71
11	l	>330	77	>330	>330	28
	r	>330	59	>330	>330	53
12	l	>330	133	>330	251	22
	r	>330	100	>330	185	21
13	l	>330	131	>330	>330	42
	r	>330	99	>330	>330	36
14	l	>330	84	>330	>330	54
	r	>330	66	>330	>330	69
15	l	>330	110	>330	>330	50
	r	>330	102	>330	>330	38
16	l	>330	56	>330	136	17
	r	>330	41	>330	101	13
17	l	>330	39	>330	214	22
	r	>330	64	>330	231	30
18	l	>330	33	>330	98	12
	r	>330	42	>330	57	5
19	l	>330	54	>330	>330	54
	r	>330	50	>330	>330	36
20	l	>330	141	>330	>330	49
	r	>330	138	>330	>330	45

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Table 3 List of computed log_{10} values (mean of left and right hand) and log_{10} reduction

Volunteers	Chronological Sequence	Reference handrub			Handrub with product		
		log prevalues	log postvalues	log R	log prevalues	log postvalues	log R
1	PR -> PP	6.64	3.92	2.72	6.95	3.70	3.25
2	PP -> RP	6.96	3.55	3.41	7.05	3.71	3.34
3	PR -> PP	6.84	3.83	3.01	7.14	3.87	3.27
4	PP -> RP	6.82	3.75	3.06	6.81	3.36	3.45
5	PR -> PP	6.59	3.35	3.24	6.62	3.25	3.37
6	PP -> RP	7.08	3.26	3.81	6.47	3.24	3.23
7	PR -> PP	6.89	3.01	3.88	6.71	3.13	3.58
8	PP -> RP	7.11	2.99	4.12	6.65	3.23	3.43
9	PR -> PP	6.85	3.28	3.57	7.05	3.18	3.87
10	PP -> RP	7.00	3.35	3.65	6.96	3.82	3.14
11	PR -> PP	6.89	4.22	2.67	6.83	3.59	3.24
12	PP -> RP	7.19	3.50	3.69	7.06	3.33	3.73
13	PR -> PP	7.10	3.57	3.53	7.06	3.59	3.47
14	PP -> RP	6.84	3.66	3.18	6.87	3.79	3.09
15	PR -> PP	6.70	3.34	3.35	7.02	3.64	3.39
16	PP -> RP	7.15	3.60	3.55	6.68	3.08	3.60
17	PR -> PP	6.98	3.90	3.08	6.70	3.35	3.35
18	PP -> RP	6.61	3.28	3.33	6.57	2.88	3.70
19	PR -> PP	7.13	3.19	3.94	6.72	3.64	3.07
20	PP -> RP	6.74	3.73	3.01	7.14	3.67	3.47
X	Overall	6.91	3.51	3.39	6.85	3.45	3.40
s		0.19	0.32	0.40	0.20	0.28	0.21
NN		20	20	20	20	20	20
X	PR -> PP	6.86	3.56	3.30	6.88	3.49	3.39
s		0.18	0.39	0.44	0.19	0.25	0.22
NN		10	10	10	10	10	10
X	PP -> RP	6.95	3.47	3.48	6.83	3.41	3.42
s		0.19	0.24	0.35	0.23	0.32	0.22
NN		10	10	10	10	10	10

logR	: decimal log reduction	X	: Mean
PR -> PP	: Sequence: first RP, second PP	s	: standard deviation
PP -> PR	: Sequence: first PP, second RP	NN	: Number of values

Difference of mean Rs (PR -> PP):	-0.09
Difference of mean Rs (PR -> PP):	0.07
Absolute difference of differences:	0.15 (<2.00)

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CHECK OF ACCEPTANCE CRITERIA

- Complete set of 20 volunteers available (hence, more than the minimum of 18)
- Mean of log prevalues for RP=6.91 and for PP=6.85 (hence both greater than 5.00)
- Individual log reductions less than 3.00 for the Reference Product (RP)=2.
  (hence not more than three individual log reduction factors for RP, fewer than 3,00 log).
- Absolute difference of mean differences=0.15 (hence less than 2.00)
- All quotients of weighted mean counts between 5 and 15 (in Tables 1 and 2 and in validation of neutralizer)

All acceptance criteria are fulfilled

Table 4 Computation of individual differences of lg Rs of RP-PP

Volunteers	log reduction		Difference RP-PP
	Reference procedure (RP)	Product procedure (PP)	
1	2.72	3.25	-0.53
2	3.41	3.34	0.07
3	3.01	3.27	-0.26
4	3.06	3.45	-0.38
5	3.24	3.37	-0.13
6	3.81	3.23	0.58
7	3.88	3.58	0.30
8	4.12	3.43	0.69
9	3.57	3.87	-0.30
10	3.65	3.14	0.51
11	2.67	3.24	-0.58
12	3.69	3.73	-0.03
13	3.53	3.47	0.06
14	3.18	3.09	0.09
15	3.35	3.39	-0.03
16	3.55	3.60	-0.05
17	3.08	3.35	-0.26
18	3.33	3.70	-0.37
19	3.94	3.07	0.87
20	3.01	3.47	-0.46

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Table 5 Sorting of individual differences and computation for Hodges-Lehmann 97.5% upper confidence limits

Sorted differences		Mean pairwise differences (di+dii)/2										
		1	2	3	4	5	6	7	8	9	10	11
		0.872	0.693	0.584	0.511	0.297	0.089	0.069	0.064	-0.031	-0.034	-0.049
1	0.872	0.872										
2	0.693	0.782	0.693									
3	0.584	0.728	0.639	0.584								
4	0.511	0.691	0.602	0.547	0.511							
5	0.297	0.584	0.495	0.441	0.404	0.297						
6	0.089	0.48	0.391	0.337	0.3	0.193	0.089					
7	0.069	0.47	0.381	0.327	0.29	0.183	0.079	0.069				
8	0.064	0.468	0.378	0.324	0.287	0.181	0.076	0.066	0.064			
9	-0.031	0.42	0.331	0.277	0.24	0.133	0.029	0.019	0.016	-0.031		
10	-0.034	0.419	0.329	0.275	0.238	0.132	0.028	0.017	0.015	-0.033	-0.034	
11	-0.049	0.411	0.322	0.268	0.231	0.124	0.02	0.01	0.007	-0.04		
12	-0.133	0.369	0.28	0.226	0.189	0.082	-0.022	-0.032	-0.034	-0.082		
13	-0.259	0.306	0.217	0.163	0.126	0.019	-0.085	-0.095	-0.098	-0.145		
14	-0.263	0.304	0.215	0.161	0.124	0.017	-0.087					
15	-0.303	0.284	0.195	0.141	0.104	-0.003	-0.107					
16	-0.367	0.252	0.163	0.109	0.072							
17	-0.384	0.244	0.154	0.1								
18	-0.459	0.206	0.117	0.063								
19	-0.527	0.172										
20	-0.576											

The differences of the individual logR 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: [(-0.034) + (-0.049)]/2 = -0.041.

The mean pairwise differences that do not exceed the median (here: -0.041) 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: 0.189.** Hence the Hodges-Lehmann upper one-sided 97,5 % confidence limit for the difference in log Rs between RP and PP is **0.189,** 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 preparation PP is not inferior to RP.**

Table 6 WILCOXON'S matched-pairs signed - ranks test:

Np (Number of pairs)	One-sided level of significance (directional test)		
	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

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Assessment of the Efficacy of Hygienic Handrub Determined using the European Standard Test Method EN 1500:2013

TEST PRODUCT IDENTIFICATION

PRODUCT NAME	:	NOVASIN (Sodium hypochlorite 500ppm-pH neutral)
SUBSTANCES AND THEIR CONCENTRATIONS	:	Sodium Hypochlorite-NaOCl 20%, Hypochlorite acid-HOCl 80%
APPEARANCE OF THE PRODUCT	:	Liquid
STORAGE CONDITIONS	:	Room Temperature, Darkness
LOT	:	TBD
METHOD	:	EN 1500:2013
COMPANY NAME	:	INTRADERMA
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PRODUCT MANUFACTURER	:	INTRADERMA
RECEIPT DATE	:	22/02/2023
STUDY PERIOD	:	21/03/2023-23/03/2023
LAB ID	:	2023-3638/23 23 00063
APPLICATION CONDITIONS-REFERENCE	:	2 x 30sec; 2 x 3 ml (60% v/v propan-2-ol)
APPLICATION CONDITIONS-PRODUCT	:	4 x 15sec; 4 x 15 ml (total rubbing time 60sec, total application quantity 60ml)
No of VOLUNTEERS	:	20

CONCLUSION

The test product: "NOVASIN", when tested at concentration: Undiluted, by the following application: 4 doses of 15ml each per 15 sec rubbing time (total application quantity 60ml, total rubbing time 60sec), conforms to the requirements of EN 1500:2013.

The method is accredited according to EN ISO/IEC 17025:2017 (Cert. No 195).

For the QACS Ltd Laboratory

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ΕΣΥΔ
Testing
Cert. No 195

Lagiopoulos Giorgos
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Agronomist – Food Technologist, MSc
Pharmaceutical Microbiologist PgCert
Date: 27/03/2023

RESULTS AUTHENTICITY

The study concerned by this report was carried out under responsibility of QACS laboratory, according to the experimental protocol as stated in EN 1500:2013

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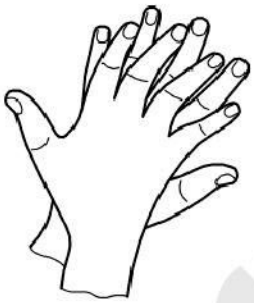
Figure 1. Standard handrub procedure

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.

Step 1

Palm to palm



Step 2

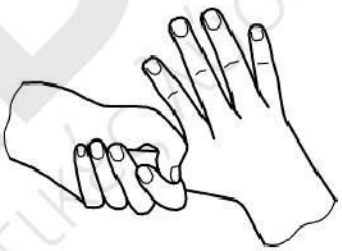
Right palm over left dorsum and left palm over right dorsum (five times)

Step 3

Palm to palm with fingers interlaced (five times)

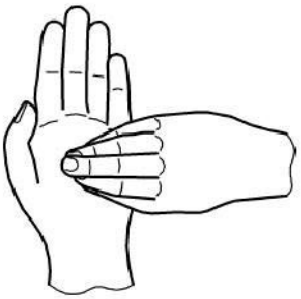
Step 4

Backs of fingers to opposing palms with fingers interlocked (five times)



Step 5

Rotational rubbing of right thumb clasped in left palm and vice versa (five times)



Step 6

Rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa (five times)

Adapted from EN 1500:2013 Chemical disinfectants and antiseptics – hygienic handrub - Test method and requirements (Phase 2/Step2)

End of Study Report