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INTRADERMA

STUDY REPORT

2023-20893/23 23 00368

NOVASIN GEL

European Standard Test Method
EN 1500:2013

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

DECEMBER 2023

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EUROPEAN STANDARD EN 1500:2013

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

TEST PRODUCT IDENTIFICATION

PRODUCT NAME	:	Novasin Gel
ACTIVE SUBSTANCES	:	Hypochlorite acid-HOCl 80%, Sodium Hypochlorite-NaOCl 20%
APPEARANCE OF THE PRODUCT	:	Gel
STORAGE CONDITIONS	:	Cool dry place, protected from light
LOT	:	Not listed
METHOD	:	EN 1500:2013
COMPANY NAME	:	INTRADERMA
STUDY SPONSOR	:	INTRADERMA
PRODUCT MANUFACTURER	:	INTRADERMA
RECEIPT DATE	:	13/12/2023
LAB ID	:	2023-19543/23 23 00358

EXPERIMENTAL CONDITIONS

STUDY PERIOD	:	14/12/2023-16/12/2023
PRODUCT TEST CONCENTRATION	:	Undiluted (100%)
REFERENCE PRODUCT (RP)	:	60.0 % v/v Propan-2-ol
APPLICATION CONDITIONS-REFERENCE (RP)	:	2 x 30sec; 2 x 3 ml (60% v/v propan-2-ol)
APPLICATION CONDITIONS-PRODUCT (PP)	:	4 x 15sec; 4 x 5 ml (total rubbing time 60sec, total application quantity 20ml)
No of VOLUNTEERS	:	20
INCUBATION	:	37°C ± 1°C for 24 - 48 h
TESTING METHOD	:	Dilution-neutralization
NEUTRALIZER	:	LPT Dilution Broth containing 3% polysorbate 80
CULTURE MEDIA	:	Tryptone Soya Agar, Tryptic Soya Selective Agar, Tryptone Soy Broth
SOFT SOAP	:	Linseed oil 50 parts by weight, Potassium hydroxide 9.5 parts by weight Ethanol 7 parts by weight, Water as needed

TEST MICROORGANISMS

Escherichia coli K12	NCTC 10538

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).

TEST METHOD

This European Standard specifies a test method simulating practical conditions for establishing whether a product for hygienic handrub reduces the release of transient microbial flora on hands when rubbed onto the 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)

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.

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.

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

Five (5) 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 5 ml each to give a total rubbing time of 60 seconds and total application quantity 20ml.

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.

ACCEPTANCE CRITERIA FOR TEST RESULTS

Only if the results of the test procedure fulfil the following requirements, shall they be accepted for further evaluation, otherwise the test shall be repeated.

- A complete set of results from at least 18 volunteers shall be available (here:20).
- The overall means of the lg prevalues for RP and PP shall be both at least 5.00
- No more than three individual lg reductions less than 3.00 shall occur in RP
- The absolute difference of mean differences between lg reductions of RP and PP of groups RP -> PP and PP -> RP shall be less than 2.00

BASIC LIMITS AND EXPLANATIONS

- Test Suspension (N) is between 1.5 to 5.0 X 10^8 CFU per mL ($8.17 \leq \log N \leq 8.70$).
- Validation Suspension=Nv is between 3.0 x 10^2 and 1.6 x 10^3.
- Nvo (Nv/10) is between 30 and 160.
- N_{VB} is between 3.0 x 10^4 and 1.6 x 10^5.
- Average recovery values for the Neutralizer control (B) is equal to or greater than 0,000 5 x N_{VB} (half of one-thousandth).
- Method Validation control (C) is equal to or greater than 0.5 x N_{V0}.
- Control of weighted mean counts: quotient is not lower than 5 and not higher than 15.

STATISTICAL EVALUATION (SIGNIFICANCE TESTING)

If the quality of the data (§ BASIC LIMITS & ACCEPTANCE CRITERIA) has been found to be acceptable, they shall be used for the evaluation of the product under test by applying the following pass criterion:

For testing the performance of PP against that of RP, a test for non-inferiority shall be applied to the lg Rs in each evaluation.
The statistical method Hodges-Lehmann shall be used or other methods.
The level of significance is set at p = 0,025. The test is to be used one-sided.
The margin of inferiority is 0,6 lg units.

NEUTRILIZATION

A suitable neutralizer was chosen and validated before the test procedure (LPT dilution broth with tween 80 30g/l).

Composition of the neutraliser g/L

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

CONTROL AND VALIDATION OF NEUTRALIZATION

Product was tested at concentration	:	Undiluted
Contact time	:	60 seconds
Date of test	:	14/12/2023

VALIDATION AND CONTROLS

Escherichia coli K12

Validation suspension (Nvo)			Validation suspension (NVB)			Neutralizer control (B)			Method validation (C) Product		
Vc 1	88	x mean	Vc 1	94	x mean	Vc 1	79	x mean	Vc 1	67	x mean
Vc 2	91	89.5	Vc 2	90	92	Vc 2	80	79.5	Vc 2	71	69
30<x mean of Nvo < 160? **Yes**			30<x mean of N_{VB}/1000 < 160? **Yes**			x mean of B is > 0,5*x mean of Nvo or N_{VB}/1000? **Yes**			x mean of C is > 0,5*x mean of Nvo? **Yes**		

1. Validation Suspension = Nv is between 3.0 x 10^2 and 1.6 x 10^3.
2. N_{V0} (N_V/10) is between 30 and 160
3. N_{VB} is between 3.0 x 10^4 and 1.6 x 10^5.
4. Neutralizer control (B): To verify the absence of toxicity of the neutralizer. Average recovery values for the Neutralizer control (B) should be equal to or greater than 0,000 5 x N_{VB}.
5. Method Validation control (C): To validate the neutralization method. Average recovery values for the Method Validation control (C) should be equal to or greater than 0.5 x N_{V0}.
6. Prior to testing, all reagents (product test solutions, propan-2-ol, diluted soft soap, test suspension, validation suspension, TSB, and neutralizer are stabilized to the test temperature of 20 °C.

Test organism : *Escherichia coli* K12 NCTC 10538.
Concentration of contamination fluid : 3.61 x 10^8 cfu/ml.
Application Conditions-Reference (RP) : 2 x 30sec / 2 x 3 ml (60% v/v propan-2-ol)
Date of experiment : 14/12/2023

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	>330	84	>330	>330	82
	r	>330	135	>330	>330	133
2	l	>330	157	>330	126	12
	r	>330	42	>330	58	6
3	l	>330	75	>330	>330	33
	r	>330	103	>330	129	11
4	l	>330	121	>330	>330	56
	r	>330	93	>330	>330	113
5	l	>330	134	>330	>330	175
	r	>330	126	>330	>330	96
6	l	>330	66	>330	>330	33
	r	>330	99	>330	>330	32
7	l	>330	104	>330	>330	28
	r	>330	102	>330	281	23
8	l	97	10	168	19	2
	r	93	11	107	12	1
9	l	>330	40	>330	115	13
	r	>330	70	>330	178	19
10	l	>330	45	>330	>330	33
	r	>330	52	>330	212	22
11	l	207	26	>330	86	8
	r	>330	74	>330	45	4
12	l	>330	59	>330	>330	36
	r	>330	53	>330	>330	40
13	l	>330	103	>330	102	11
	r	>330	98	>330	137	16
14	l	>330	45	>330	>330	38
	r	>330	36	>330	>330	42
15	l	>330	84	>330	>330	50
	r	>330	48	>330	>330	56
16	l	304	33	>330	128	16
	r	296	28	>330	101	13
17	l	>330	102	>330	>330	36
	r	>330	115	>330	>330	45
18	l	>330	45	>330	>330	58
	r	>330	31	>330	>330	50
19	l	>330	84	>330	86	9
	r	>330	58	>330	59	7
20	l	165	19	102	13	2
	r	204	26	97	10	1

Test organism : *Escherichia coli* K12 NCTC 10538.
Concentration of contamination fluid : 3.61 x 10^8 cfu/ml.
Application Conditions-Product (PP): : 4 x 15sec; 4 x 5 ml
ate of experiment : 14/12/2023

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	54	>330	>330	56
	r	>330	109	>330	>330	82
2	l	>330	90	>330	41	5
	r	>330	32	78	9	0
3	l	>330	226	>330	>330	28
	r	>330	136	>330	103	12
4	l	>330	73	>330	>330	91
	r	>330	96	>330	>330	58
5	l	>330	67	>330	>330	297
	r	>330	37	>330	295	31
6	l	>330	35	>330	>330	38
	r	>330	58	>330	>330	41
7	l	>330	111	>330	226	26
	r	>330	116	>330	137	12
8	l	>330	46	>330	52	6
	r	>330	133	>330	75	8
9	l	>330	48	>330	37	3
	r	>330	145	>330	58	7
10	l	>330	86	>330	>330	42
	r	263	31	>330	>330	51
11	l	>330	34	>330	162	17
	r	>330	41	>330	190	21
12	l	135	16	>330	84	9
	r	204	22	>330	45	6
13	l	>330	49	>330	89	9
	r	>330	69	>330	45	6
14	l	>330	45	>330	102	13
	r	>330	36	>330	96	11
15	l	>330	101	>330	109	15
	r	>330	96	284	27	3
16	l	>330	45	>330	235	26
	r	>330	33	>330	196	18
17	l	>330	50	>330	>330	45
	r	>330	49	>330	>330	50
18	l	>330	62	>330	168	15
	r	>330	60	>330	166	20
19	l	>330	39	>330	45	6
	r	>330	45	>330	31	4
20	l	>330	102	>330	>330	204
	r	>330	86	>330	>330	131

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	7.03	4.02	3.01	6.88	3.83	3.05
2	PP -> RP	6.91	2.93	3.98	6.73	2.26	4.47
3	PR -> PP	6.94	3.31	3.63	7.24	3.23	4.01
4	PP -> RP	7.03	3.90	3.13	6.92	3.86	3.06
5	PR -> PP	7.11	4.11	3.00	6.70	3.97	2.72
6	PP -> RP	6.91	3.51	3.40	6.65	3.60	3.06
7	PR -> PP	7.01	3.44	3.57	7.05	3.25	3.81
8	PP -> RP	5.98	2.13	3.85	6.89	2.80	4.09
9	PR -> PP	6.72	3.16	3.56	6.92	2.67	4.26
10	PP -> RP	6.68	3.42	3.26	6.68	3.67	3.02
11	PR -> PP	6.60	2.79	3.81	6.57	3.25	3.32
12	PP -> RP	6.75	3.58	3.17	6.23	2.80	3.43
13	PR -> PP	7.00	3.08	3.92	6.76	2.81	3.96
14	PP -> RP	6.60	3.60	3.00	6.60	3.00	3.60
15	PR -> PP	6.80	3.72	3.08	6.99	2.75	4.24
16	PP -> RP	6.48	3.07	3.41	6.59	3.33	3.25
17	PR -> PP	7.03	3.60	3.43	6.69	3.68	3.02
18	PP -> RP	6.57	3.73	2.84	6.79	3.22	3.56
19	PR -> PP	6.84	2.86	3.99	6.62	2.58	4.04
20	PP -> RP	6.27	2.00	4.27	6.97	4.21	2.76
X	Overall	6.76	3.30	3.47	6.78	3.24	3.54
s		0.29	0.56	0.41	0.22	0.53	0.54
NN		20	20	20	20	20	20
X	PR -> PP	6.91	3.41	3.50	6.84	3.20	3.64
s		0.16	0.46	0.37	0.21	0.50	0.56
NN		10	10	10	10	10	10
X	PP -> RP	6.62	3.19	3.43	6.71	3.28	3.43
s		0.32	0.66	0.46	0.22	0.58	0.53
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): -0.14
Difference of mean Rs (PR -> PP): 0.00
Absolute difference of differences: 0.14 (<2.00)

CHECK OF ACCEPTANCE CRITERIA

- Complete set of 20 volunteers available (hence, more than the minimum of 18)
- Mean of log prevalues for RP=6.76 and for PP=6.78 (hence both greater than 5.00)
- Individual log reductions less than 3.00 with Reference Product (RP)=1.
  (hence not more than three individual log reduction factors for RP, fewer than 3,00 log).
- Absolute difference of mean differences=0.14 (hence less than 2.00)

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	3.01	3.05	-0.05
2	3.98	4.47	-0.49
3	3.63	4.01	-0.38
4	3.13	3.06	0.06
5	3.00	2.72	0.28
6	3.40	3.06	0.34
7	3.57	3.81	-0.24
8	3.85	4.09	-0.24
9	3.56	4.26	-0.69
10	3.26	3.02	0.25
11	3.81	3.32	0.48
12	3.17	3.43	-0.26
13	3.92	3.96	-0.03
14	3.00	3.60	-0.60
15	3.08	4.24	-1.16
16	3.41	3.25	0.16
17	3.43	3.02	0.41
18	2.84	3.56	-0.72
19	3.99	4.04	-0.05
20	4.27	2.76	1.51

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
		1.510	0.482	0.411	0.338	0.276	0.246	0.158	0.063	-0.032	-0.045	-0.051
1	1.510	1.510										
2	0.482	0.996	0.482									
3	0.411	0.961	0.447	0.411								
4	0.338	0.924	0.410	0.375	0.338							
5	0.276	0.893	0.379	0.344	0.307	0.276						
6	0.246	0.878	0.364	0.329	0.292	0.261	0.246					
7	0.158	0.834	0.320	0.285	0.248	0.217	0.202	0.158				
8	0.063	0.787	0.273	0.237	0.201	0.170	0.155	0.111	0.063			
9	-0.032	0.739	0.225	0.190	0.153	0.122	0.107	0.063	0.016	-0.032		
10	-0.045	0.732	0.218	0.183	0.146	0.115	0.100	0.056	0.009	-0.039	-0.045	
11	-0.051	0.730	0.216	0.180	0.144	0.113	0.098	0.053	0.006	-0.042		
12	-0.240	0.635	0.121	0.085	0.049	0.018	0.003	-0.041	-0.089	-0.136		
13	-0.244	0.633	0.119	0.084	0.047	0.016	0.001	-0.043	-0.090	-0.138		
14	-0.260	0.625	0.111	0.076	0.039	0.008	-0.007					
15	-0.378	0.566	0.052	0.017	-0.020	-0.051	-0.066					
16	-0.492	0.509	-0.005	-0.040	-0.077							
17	-0.598	0.456	-0.058	-0.093								
18	-0.691	0.410	-0.104	-0.140								
19	-0.720	0.395										
20	-1.162											

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: [(-0.045) + (-0.051)]/2 = -0.048.

The mean pairwise differences that do not exceed the median (here: -0.048) 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: 0.146.** Hence the Hodges-Lehmann upper one-sided 97,5 % confidence limit for the difference in log Rs between RP and PP **is 0.146,** 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 non-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

CONCLUSION

The mean reduction of the release of the test organism *Escherichia coli* K12 achieved by the product under test, is non-inferior to that achieved by the reference (60.0 % v/v Propan-2-ol).

Therefore, the product under test: **"NOVASIN GEL",** when tested at concentration: Undiluted, by the following application: 4 doses of 5ml each per 15 sec rubbing time (total application quantity 20ml, total rubbing time 60sec), conforms to the requirements of EN 1500:2013 for hygienic handrub disinfection.

For the QACS Ltd Laboratory:

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Date: 19/12/2023

The method is accredited according to EN ISO/IEC 17025:2017.

Testing Cert. No 195

RESULTS AUTHENTICITY

The study concerned by this report was carried out according to the experimental protocol, under responsibility and quality plan of the QACS Ltd laboratory. All the observations and data recorded during this trial are reported in this study report.

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

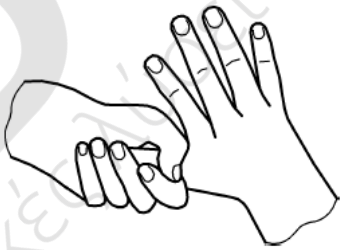
Right palm over left dorsum and left palm over right dorsum (five times)

Step 3

Palm to palm with fingers interlaced (five times)

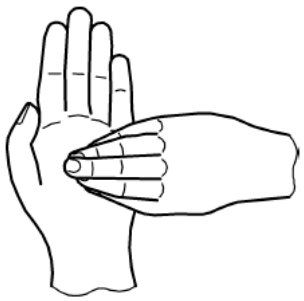
Step 4

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)

Results refer to the sample as received and analyzed in the period specified above.
The test report shall not be reproduced except in full, without written approval of the laboratory.
The samples will be stored by the laboratory for 2 months from the end of the test date.
The study report and raw data will be stored by the laboratory for 5 years.

End of Study Report