

**ENCLOSURE No. 1 TO REPORT OF ANALYSIS NO. 240517/20/JSHCR**

<b>A) IDENTIFICATION OF THE SAMPLE:</b>	
Name of the product	<b>HAND DESINFECTANT 1302/20 ANTISEPTIK ZA RUKE (HAND ANTISEPTIC), 1L</b>
The active substance	Ethanol ( 70mg/g)
<b>B) TEST METHOD :</b>	
Method	<b>EN 1500:2013 Chemical disinfectants and antiseptics - Hygienic handrub - Test method and requirements (phase 2, step 2)</b>
Neutralizer	Polysorbate 80 30 g/l, saponine 30g/l, histidine 1g/l, cysteine 1g/l
<b>C) EXPERIMENTAL CONDITIONS:</b>	
Product test concentrations (%V/V)	100%
Test temperature	20°C
Contact time	3ml of the preparation for 30s
Incubation temperature	36±1 °C
Test-organism	<i>E. coli</i> K12 NCTC 10538

Date: 29.06.2020

Authorized by: Agnieszka Erber, Cosmetics Microbiology Laboratory Manager

Approved by: Hanna Wachowska, Laboratory Director (*Approved with qualified electronic signature*)

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Table 1. PROCEDURE FOR REFERENCE HYGIENIC HANDRUB

PRODUCT: Standard 2-propanol 60% (V/V)

 TEST ORGANISM: *E. coli* K12 NCTC 10538

 NUMBER IN CONTAMINATION FLUID:  $1,7 \times 10^8$  cfu/g

volunteer		number of cfu per plate from dilution 10x							Reduction	
Nr	Hand left/right	prevalues			postvalues				log z	
		$\times 10^{-4}$	$\times 10^{-5}$	log x	$\times 10^0$	$\times 10^{-1}$	$\times 10^{-2}$	log y		
1	l	122	14	6,12	65	7	0	1,72	4,39	
	r	136	16		42	5	0			
2	l	145	13	6,22	18	2	0	1,18	5,03	
	r	187	21		12	2	0			
3	l	87	10	6,00	32	4	0	1,69	4,31	
	r	112	12		72	8	0			
4	l	196	20	6,31	69	7	0	1,61	4,70	
	r	215	22		24	3	0			
5	l	210	23	6,26	55	6	0	1,80	4,46	
	r	155	16		71	9	0			
6	l	139	14	6,11	82	10	1	1,88	4,24	
	r	112	20		66	8	0			
7	l	95	10	5,91	13	2	0	1,22	4,69	
	r	67	10		19	3	0			
8	l	156	17	6,17	47	5	0	1,52	4,65	
	r	141	15		23	3	0			
9	l	168	18	6,24	59	6	0	1,79	4,45	
	r	172	21		63	8	0			
10	l	188	19	6,30	87	10	1	1,91	4,40	
	r	215	22		73	8	0			
11	l	235	25	6,27	56	5	0	1,79	4,48	
	r	147	15		71	6	0			
12	l	136	14	6,12	59	7	0	1,61	4,51	
	r	125	16		28	3	0			
13	l	213	23	6,37	15	2	0	1,30	5,07	
	r	256	27		25	3	0			
14	l	86	10	5,90	47	5	0	1,81	4,09	
	r	71	8		86	9	0			
15	l	113	14	6,08	91	8	0	1,81	4,27	
	r	125	15		47	5	0			
16	l	146	16	6,15	33	4	0	1,60	4,54	
	r	132	14		48	5	0			
17	l	158	17	6,22	50	6	0	1,56	4,66	
	r	170	21		26	2	0			
18	l	169	22	6,16	43	6	0	1,70	4,46	
	r	122	10		57	5	0			
19	l	147	17	6,19	62	8	0	1,82	4,36	
	r	158	16		70	6	0			
20	l	169	18	6,28	14	2	0	1,19	5,09	
	r	208	23		16	2	0			
Xavg				6,17				1,63	4,54	
s				0,13				0,23	0,27	

log x-logarithm of the average value of the initial left and right hand

log y-logarithm of the average value of the final left and right hand

log z-logarithm reduction

x avg - overall average of log x, log y, log z

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Table 2. HYGIENIC HANDRUB PROCEDURE WITH THE PRODUCT

PRODUCT P

 TEST ORGANISM: *E. coli* K12 NCTC 10538

 NUMBER IN CONTAMINATION FLUID:  $1,7 \times 10^8$  cfu/g

volunteer		number of cfu per plate from dilution 10x							Reduction	
Nr	Hand left/right	prevalues			postvalues				log z	
		$\times 10^{-4}$	$\times 10^{-5}$	log x	$\times 10^0$	$\times 10^{-1}$	$\times 10^{-2}$	log y		
1	l	100	12		16	1	0			
	r	188	21	6,14	21	2	0	1,11	5,03	
2	l	128	14		25	3	0			
	r	140	16	6,13	18	1	0	1,20	4,93	
3	l	104	11		32	4	0			
	r	180	20	6,14	27	2	0	1,41	4,73	
4	l	182	22		41	5	0			
	r	160	18	6,24	52	5	0	1,66	4,58	
5	l	192	21		64	6	0			
	r	136	14	6,21	35	4	0	1,65	4,56	
6	l	172	19		45	4	0			
	r	146	11	6,20	54	4	0	1,56	4,64	
7	l	176	20		63	7	0			
	r	128	11	6,18	45	3	0	1,62	4,56	
8	l	195	23		55	6	0			
	r	193	25	6,30	42	3	0	1,59	4,71	
9	l	181	13		38	4	0			
	r	152	19	6,22	41	5	0	1,61	4,61	
10	l	168	21		46	5	0			
	r	156	14	6,21	42	4	0	1,61	4,60	
11	l	171	18		37	4	0			
	r	136	12	6,18	41	5	0	1,61	4,57	
12	l	145	11		36	4	0			
	r	138	10	6,14	52	6	0	1,65	4,49	
13	l	165	15		47	5	0			
	r	110	8	6,12	62	3	0	1,55	4,58	
14	l	147	16		68	7	0			
	r	189	22	6,23	53	4	0	1,68	4,54	
15	l	192	23		45	5	0			
	r	123	14	6,19	53	6	0	1,70	4,50	
16	l	136	11		32	4	0			
	r	155	16	6,16	28	2	0	1,41	4,75	
17	l	181	19		17	2	0			
	r	144	21	6,22	31	2	0	1,26	4,96	
18	l	170	18		36	4	0			
	r	165	17	6,23	42	5	0	1,61	4,62	
19	l	193	22		51	5	0			
	r	123	14	6,19	48	5	0	1,66	4,54	
20	l	112	13		42	5	0			
	r	130	12	6,08	31	4	0	1,61	4,47	
X <sub>avg</sub>					6,19				1,54	4,65
s					0,05				0,17	0,16

log x - logarithm of the average value of the initial left and right hand

log y - logarithm of the average value of the final left and right hand

log z - logarithm reduction

x avg - overall average of log x, log y, log z

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Table 3. LIST OF COMPUTED IG VALUES AND IG REDUCTIONS

volunteer		R 2-propanol 60% (V/V)			P		
Nr		log x	log y	log z	log x	log y	log z
1	R-P	6,12	1,72	4,39	6,14	1,11	5,03
2	R-P	6,22	1,18	5,03	6,13	1,20	4,93
3	R-P	6,00	1,69	4,31	6,14	1,41	4,73
4	R-P	6,31	1,61	4,70	6,24	1,66	4,58
5	R-P	6,26	1,80	4,46	6,21	1,65	4,56
6	P-R	6,11	1,88	4,24	6,20	1,56	4,64
7	P-R	5,91	1,22	4,69	6,18	1,62	4,56
8	P-R	6,17	1,52	4,65	6,30	1,59	4,71
9	P-R	6,24	1,79	4,45	6,22	1,61	4,61
10	P-R	6,30	1,91	4,40	6,21	1,61	4,60
11	R-P	6,27	1,79	4,48	6,18	1,61	4,57
12	R-P	6,12	1,61	4,51	6,14	1,65	4,49
13	R-P	6,37	1,30	5,07	6,12	1,55	4,58
14	R-P	5,90	1,81	4,09	6,23	1,68	4,54
15	R-P	6,08	1,81	4,27	6,19	1,70	4,50
16	P-R	6,15	1,60	4,54	6,16	1,41	4,75
17	P-R	6,22	1,56	4,66	6,22	1,26	4,96
18	P-R	6,16	1,70	4,46	6,23	1,61	4,62
19	P-R	6,19	1,82	4,36	6,19	1,66	4,54
20	P-R	6,28	1,19	5,09	6,08	1,61	4,47
X <sub>20</sub>		<b>6,17</b>	1,63	4,54	<b>6,19</b>	1,54	4,65
X10(R-P)		6,16	1,63	<b>4,53</b>	6,17	1,52	<b>4,65</b>
X10 (P-R)		6,17	1,62	<b>4,55</b>	6,20	1,55	<b>4,64</b>

Criteria:

$$R_s (R-P) = 4,53 - 4,65 = - 0,12$$

$$R_s (P-R) = 4,55 - 4,64 = - 0,09$$

$$Abs = - 0,12 - (- 0,09) = - 0,03 < 2$$

$$\log x(R) = 6,17 > 5$$

$$\log x(P) = 6,19 > 5$$

$$\log z (P), \log z (R) > 3$$

Validation conditions of neutralizer and methods have been satisfied

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Table 4. COMPUTATION OF INDIVIDUAL DIFFERENCES OF lg R-P

volunteer	log RF		difference R-P	difference high to low	Range +/-
	R	P			
1	4,39	5,03	-0,64	0,61	1
2	5,03	4,93	0,10	0,50	2
3	4,31	4,73	-0,42	0,14	3
4	4,70	4,58	0,12	0,12	4
5	4,46	4,56	-0,11	0,10	5
6	4,24	4,64	-0,40	0,02	6
7	4,69	4,56	0,14	-0,06	-7
8	4,65	4,71	-0,06	-0,10	-8
9	4,45	4,61	-0,16	-0,11	-9
10	4,40	4,60	-0,21	-0,16	-10
11	4,48	4,57	-0,10	-0,16	-11
12	4,51	4,49	0,02	-0,17	-12
13	5,07	4,58	0,50	-0,21	-13
14	4,09	4,54	-0,45	-0,21	-14
15	4,27	4,50	-0,23	-0,23	-15
16	4,54	4,75	-0,21	-0,29	-16
17	4,66	4,96	-0,29	-0,40	-17
18	4,46	4,62	-0,16	-0,42	-18
19	4,36	4,54	-0,17	-0,45	-19
20	5,09	4,47	0,61	-0,64	-20
sum range (+): 21					
sum range (-): 189					

Table 5. SORTING OF INDIVIDUAL DIFFERENCES AND COMPUTATION FOR HODGES-LEHMANN 97,5% UPPER CONFIDENCE LIMITS FOR THE DIFFERENCE IN lg BETWEEN R-P

	0,61	0,50	0,14	0,12	0,10	0,02	-0,06	-0,10	-0,11
1	0,61	0,61							
2	0,50	0,56	0,50						
3	0,14	0,38	0,32	0,14					
4	0,12	0,37	0,31	0,13	0,12				
5	0,10	0,36	0,30	0,12	0,11	0,10			
6	0,02	0,32	0,26	0,08	0,07	0,06	0,02		
7	-0,06	0,28	0,22	0,04	0,03	<b>0,02</b>	-0,02	0,06	
8	-0,10	0,26	0,20	0,02	0,01	0,00	-0,04	0,08	0,10
9	-0,11	0,25	0,20	0,02	0,01	-0,01	-0,05	0,09	0,11
10	-0,16	0,23	0,17	-0,01	-0,02	-0,03	-0,07	0,11	0,13
11	-0,16	0,23	0,17	-0,01	-0,02	-0,03	-0,07	0,11	0,13
12	-0,17	0,22	0,17	-0,02	-0,03	-0,04	-0,08	0,12	0,14
13	-0,21	0,20	0,15	-0,04	-0,05	-0,06	-0,10	0,14	
14	-0,21	0,20	0,15	-0,04	-0,05	-0,06	-0,10		
15	-0,23	0,19	0,14	-0,05	-0,06	-0,07			
16	-0,29	0,16	0,11	-0,08	-0,09				
17	-0,40	0,11	0,05	-0,13					
18	-0,42	0,10	0,04						
19	-0,45	0,08							
20	-0,64								

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Table 6. WILCOXON'S TMATCHED PAIRS SIGNED-RANKS TEST:  
CRITICAL VALUES LESS WITH RANG SUM (+) OR (-) AT DIFFERENT LEVELS OF SIGNIFICANCE

n	one-sided level of significance		
	0,05	<b>0,025</b>	0,01
18	47	<b>40</b>	32
19	53	<b>46</b>	27
20	60	<b>52</b>	43
21	68	<b>59</b>	49
22	75	<b>66</b>	56

For the designated level of significance 0,025 for n=20 the value read from the table 6 is 52.

Hence  $c = 52+1 = 53$ .

For the distribution of 53 Table 5 assigns a value of 0,02 which is less than the agreed inferiority margin of 0,6.

Therefore, the hypothesis of inferiority of PP compared to the reference RP is rejected.

The test preparation (PP) is non-inferior to RP.

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