LAB – TEST LABORATORIUM S.C.
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AB 815

TEST REPORT NR DZ/44/08/20 CONTAINS 6 NUMBERED PAGES

Evaluation of activity according to PN-EN 1500:2013-07 'Hygienic handrub'

A. Laboratory:

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B. Identification of the sample:

1. Product tested..... **Zoono ® Hand Sanitiser and Protectant**
2. Batch.....364464
3. Test sponsor.....Zoono Holdings Ltd
4. Date of delivery.....30.07.2020
5. Storage conditions.....ambient
6. Active substances:

   alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC/BKC(C12-16)), 0,4% w/w (4 g/kg)
7. Product diluent recommended by the manufacturer.....product to be used as is

C.1. Test method.....handrub according to PN-EN 1500:2013-07

D. Test conditions:

1. Test period..... 30.07.2020 – 17.08.2020
2. Number of persons / hands in the test..... 20 / 20x2
3. Test strain..... *Escherichia coli* K12 NCTC 10538
4. Neutralizer:

   Tween 80, 100 g/l; lecithin 30 g/l; L-histidine 1 g/l; sodium thiosulphate 5 g/l; phosphate buffer 10 ml/l in TSB
5. Soft soap..... prepared according to PN-EN 1500:2013-07
6. Volume of product for single rub..... 3,0 ml
7. Single handrub time.....30 s
8. Number of handrubs..... 2
9. Handrub procedure..... according to annex A to PN-EN 1500:2013-07
10. Incubation..... 37°C ± 1°C; 48 h

E. Test results..... tables 1 – 4

F. Conclusion:

Product: **Zoono ® Hand Sanitiser and Protectant**

applied 3,0 ml on dry hands, 30 s handrub, is not significantly worse than the reference procedure with the level of significance at least 0,025, and meets the requirements of **PN-EN 1500:2013-07.**

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TABLE 1

dilution – neutralization validation

Product: **Zoono ® Hand Sanitiser and Protectant**

test strain	validation test		
	bacterial suspensions	neutralizer control	dilution-neutralization control
Escherichia coli K12 NCTC 10538	Vc: 10^{-3}: 104;119 Nv_0: $1,1 \cdot 10^2$ Nv_B: $1,1 \cdot 10^5$	Vc: 10^{-2}: 98;110 B: $1,0 \cdot 10^4$	Vc: 76;79 C: $7,8 \cdot 10^1$

Nv - cfu/ml in the bacterial suspension in the dilution-neutralization control test, $Nv_0 = Nv/1000 = N_B/1000$
Nv_B - cfu/ml in the bacterial suspension in the neutralizer toxicity control test,

B - cfu/ml in neutralizer control test
C - cfu/ml in the dilution - neutralization control test

Validation criteria verification:

$3,0 \cdot 10^2 \leq N_v \leq 1,6 \cdot 10^3$	-	met	$C \geq 0,5\ Nv_0$	-	met
$3,0 \cdot 10^4 \leq Nv_B \leq 1,6 \cdot 10^5$	-	met	$B \geq 0,05\ Nv_B$	-	met

TABLE 2 Reference handrub procedure – experimental results

Reference product: 2-propanol 60% v/v
Application: 3 ml on dry hands; 30 s rub; repeated
Test date: 16.08.2020
Test strain: *Escherichia coli* K12 NCTC 10538
Microbial suspension: $4{,}2 \cdot 10^8$ cfu/ml

subject Nr	hand L left/ R right	Number of cfu / 1,0 ml TSB on plate from dilutions					
		pre-count			post-count		
		10^{-3}	10^{-4}	10^{-5}	10^{0}	10^{-1}	10^{-2}
1	L	>330	158	19	63	6	1
	R	>330	114	13	70	9	1
2	L	>330	92	10	14	2	0
	R	>330	78	8	29	4	0
3	L	>330	67	8	15	1	0
	R	>330	42	5	12	1	0
4	L	>330	37	4	5	1	0
	R	>330	86	9	18	2	0
5	L	>330	51	6	30	3	0
	R	>330	110	12	33	3	0
6	L	>330	49	6	10	1	0
	R	>330	75	9	6	0	0
7	L	>330	127	14	28	3	0
	R	>330	72	8	34	3	0
8	L	310	38	4	19	2	0
	R	>330	59	4	4	0	0
9	L	174	21	1	11	1	0
	R	255	35	4	5	0	0
10	L	>330	103	11	43	4	0
	R	>330	80	9	31	4	0
11	L	156	19	3	6	0	0
	R	275	26	2	3	0	0
12	L	>330	85	10	52	5	0
	R	>330	61	5	48	6	1
13	L	>330	108	12	87	9	1
	R	230	29	3	7	1	0
14	L	>330	89	11	15	2	0
	R	>330	129	14	65	6	1
15	L	>330	116	12	57	7	1
	R	>330	58	7	5	1	0
16	L	288	37	4	8	1	0
	R	>330	74	6	21	2	0
17	L	>330	82	9	54	6	1
	R	>330	128	16	32	3	0
18	L	>330	79	8	25	3	0
	R	231	32	2	9	1	0
19	L	>330	43	5	6	1	0
	R	298	38	4	14	2	0
20	L	177	23	3	10	1	0
	R	286	35	4	17	2	0

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TABLE 3 Handrub test procedure – experimental results

Reference product: **Zoono ® Hand Sanitiser and Protectant**
Application: 3 ml on dry hands; 30 s rub, repeated
Test date: 16.08.2020
Test strain: *Escherichia coli* K12 NCTC 10538
Microbial suspension: $4{,}2 \cdot 10^8$ cfu/ml

subject Nr	hand L left/ R right	Number of cfu / 1,0 ml TSB on plate from dilutions					
		pre-count			post-count		
		10^{-3}	10^{-4}	10^{-5}	10^{0}	10^{-1}	10^{-2}
1	L	>330	49	5	8	1	0
	R	>330	78	6	10	1	0
2	L	>330	54	7	13	2	0
	R	144	18	3	7	1	0
3	L	>330	71	10	6	0	0
	R	>330	113	9	11	1	0
4	L	277	35	4	4	0	0
	R	>330	48	5	9	1	0
5	L	>330	104	12	28	3	0
	R	>330	80	6	39	4	0
6	L	>330	97	11	31	2	0
	R	>330	45	6	16	2	0
7	L	>330	96	12	25	3	0
	R	>330	74	7	14	2	0
8	L	256	33	4	12	1	0
	R	>330	47	6	17	2	0
9	L	207	26	4	6	1	0
	R	125	15	1	1	0	0
10	L	>330	81	9	22	3	0
	R	>330	106	11	5	1	0
11	L	69	9	1	2	0	0
	R	143	17	2	3	0	0
12	L	>330	70	8	19	2	0
	R	284	36	4	11	2	0
13	L	>330	51	7	7	1	0
	R	>330	79	9	13	3	0
14	L	326	41	5	4	0	0
	R	>330	82	10	8	1	0
15	L	>330	145	15	21	2	0
	R	>330	117	13	73	9	1
16	L	282	37	2	5	1	0
	R	223	28	3	3	0	0
17	L	>330	83	10	20	2	0
	R	>330	49	6	2	0	0
18	L	>330	108	11	27	3	0
	R	>330	139	16	55	6	0
19	L	204	21	3	10	1	0
	R	316	32	3	6	1	0
20	L	130	15	2	4	0	0
	R	>330	46	5	11	1	0

TABLE 4

Calculated logs (means for two hands) and reduction factors according to tables 2,3

Test person/testing order		Reference procedure (R) 2 – propanol 60%			**Test procedure (P) Zoono ® Hand Sanitiser and Protectant**		
		Log x	Log y	Log z	Log x	Log y	Log z
1	PP/RP	7,13	2,82	4,31	6,79	1,95	4,84
2		6,93	2,30	4,63	6,45	1,98	4,47
3		6,72	2,13	4,59	6,95	1,91	5,04
4		6,75	1,98	4,77	6,57	1,78	4,79
5		6,87	2,50	4,37	6,96	2,53	4,43
6		6,78	1,89	4,89	6,82	2,35	4,47
7		6,98	2,49	4,49	6,93	2,27	4,66
8		6,64	1,94	4,70	6,55	2,15	4,40
9		6,33	1,87	4,46	6,22	1,39	4,83
10		6,96	2,56	4,40	6,97	2,02	4,95
11	RP/PP	6,32	1,63	4,69	6,00	1,39	4,61
12		6,86	2,70	4,16	6,65	2,16	4,49
13		6,70	2,39	4,31	6,80	1,98	4,82
14		7,03	2,49	4,54	6,72	1,75	4,97
15		6,91	2,23	4,68	7,12	2,59	4,53
16		6,67	2,11	4,56	6,41	1,59	4,82
17		7,02	2,62	4,40	6,81	1,80	5,01
18		6,64	2,18	4,46	7,09	2,59	4,50
19		6,56	1,96	4,60	6,41	1,89	4,52
20		6,36	2,12	4,24	6,39	1,82	4,57
Total							
X(m)		6,76	2,25	4,51	6,68	1,99	4,69
S		0,24	0,32	0,19	0,30	0,35	0,21
N		20	20	20	20	20	20
Testing order: PP / RP							
X(m)		6,81	2,25	4,56	6,72	2,03	4,69
S		0,22	0,33	0,19	0,26	0,32	0,23
N		10	10	10	10	10	10
Testing order: RP / PP							
X(m)		6,71	2,24	4,46	6,64	1,96	4,68
S		0,25	0,32	0,18	0,35	0,39	0,20
N		10	10	10	10	10	10

Log x = log of pre-count value
Log y = log of post-count value
Log z = log of reduction factor
S = standard deviation
X(m) = mean
N = number of subjects in the test

Difference of mean reductions (RP->PP) = 4,46-4,68 = - 0,22; (PP->RP) = 4,56-4,69 = - 0,13
| RP->PP-PP->RP | = 0,09 < 2,0

Mean reduction for the product (4,69) is higher than the mean reduction for the reference procedure (4,51).

TABLE 5

Computation of individual differences of lgR for:
RP (reference procedure) and PP (test procedure for: Zoono ® Hand Sanitiser and Protectant)

person	Log RF for:		Difference
	RP	PP	RP-PP
1	4,31	4,84	-0,53
2	4,63	4,47	0,16
3	4,59	5,04	-0,45
4	4,77	4,79	-0,02
5	4,37	4,43	-0,06
6	4,89	4,47	0,42
7	4,49	4,66	-0,17
8	4,70	4,40	0,30
9	4,46	4,83	-0,37
10	4,40	4,95	-0,55
11	4,69	4,61	0,08
12	4,16	4,49	-0,33
13	4,31	4,82	-0,51
14	4,54	4,97	-0,43
15	4,68	4,53	0,15
16	4,56	4,82	-0,26
17	4,40	5,01	-0,61
18	4,46	4,50	-0,04
19	4,60	4,52	0,08
20	4,24	4,57	-0,33

Statistical comparison – sorting of individual differences and computation for Hodges-Lehmann 97,5% upper confidence limit.

The agreed inferiority margin is 0,6 at the level of significance 97,5%.

Because none of the mean-pair differences is larger than 0,6, for the critical value of 52 such differences for 20 paired data different than 0, the hypothesis of inferiority of **PP (test procedure for: Zoono ® Hand Sanitiser and Protectant)** to **RP (reference procedure)** is rejected with the level of significance of at least 97,5%.

Authorized by: Laboratory Manager.....date.....25.08.2020

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