



AB 815

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

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

A. Laboratory:

LAB – TEST LABORATORIUM S.C. 40-868 KATOWICE, Poland

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**.

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 $3,0 \cdot 10^4 \leq N_{vB} \leq 1,6 \cdot 10^5$ - metC $\geq 0,5 N_{v_0}$ - metB $\geq 0,05 N_{vB}$ - met

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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

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

Nr	subject 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	<u>49</u>	5	<u>8</u>	1	0
	R	>330	<u>78</u>	6	<u>10</u>	1	0
2	L	>330	<u>54</u>	7	<u>13</u>	2	0
	R	<u>144</u>	<u>18</u>	3	<u>7</u>	1	0
3	L	>330	<u>71</u>	10	<u>6</u>	0	0
	R	>330	<u>113</u>	9	<u>11</u>	1	0
4	L	<u>277</u>	<u>35</u>	4	<u>4</u>	0	0
	R	>330	<u>48</u>	5	<u>9</u>	1	0
5	L	>330	<u>104</u>	12	<u>28</u>	3	0
	R	>330	<u>80</u>	6	<u>39</u>	4	0
6	L	>330	<u>97</u>	11	<u>31</u>	2	0
	R	>330	<u>45</u>	6	<u>16</u>	2	0
7	L	>330	<u>96</u>	12	<u>25</u>	3	0
	R	>330	<u>74</u>	7	<u>14</u>	2	0
8	L	<u>256</u>	<u>33</u>	4	<u>12</u>	1	0
	R	>330	<u>47</u>	6	<u>17</u>	2	0
9	L	<u>207</u>	<u>26</u>	4	<u>6</u>	1	0
	R	<u>125</u>	<u>15</u>	1	<u>1</u>	0	0
10	L	>330	<u>81</u>	9	<u>22</u>	3	0
	R	>330	<u>106</u>	11	<u>5</u>	1	0
11	L	<u>69</u>	<u>9</u>	1	<u>2</u>	0	0
	R	<u>143</u>	<u>17</u>	2	<u>3</u>	0	0
12	L	>330	<u>70</u>	8	<u>19</u>	2	0
	R	<u>284</u>	<u>36</u>	4	<u>11</u>	2	0
13	L	>330	<u>51</u>	7	<u>7</u>	1	0
	R	>330	<u>79</u>	9	<u>13</u>	3	0
14	L	<u>326</u>	<u>41</u>	5	<u>4</u>	0	0
	R	>330	<u>82</u>	10	<u>8</u>	1	0
15	L	>330	<u>145</u>	<u>15</u>	<u>21</u>	2	0
	R	>330	<u>117</u>	13	<u>73</u>	9	1
16	L	<u>282</u>	<u>37</u>	2	<u>5</u>	1	0
	R	<u>223</u>	<u>28</u>	3	<u>3</u>	0	0
17	L	>330	<u>83</u>	10	<u>20</u>	2	0
	R	>330	<u>49</u>	6	<u>2</u>	0	0
18	L	>330	<u>108</u>	11	<u>27</u>	3	0
	R	>330	<u>139</u>	<u>16</u>	<u>55</u>	6	0
19	L	<u>204</u>	<u>21</u>	3	<u>10</u>	1	0
	R	<u>316</u>	<u>32</u>	3	<u>6</u>	1	0
20	L	<u>130</u>	<u>15</u>	2	<u>4</u>	0	0
	R	>330	<u>46</u>	5	<u>11</u>	1	0

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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 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				S = standard deviation X(m) = mean N = number of subjects in the test			

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

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TABLE 5

Computation of individual differences of IgR 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.....