

Test report No. hd0618

HYGIENIC HANDRUB TEST (EN 1500)

Name of the product: CHEMISEPT MED
Batch number: 196101017
Order number: 17029
Manufacturer: Chemi-Pharm Ltd.
Client, representative: Chemi-Pharm Ltd., Põllu 132, Tallinn, 10917, ESTONIA
Maris Millner, +372-51-77-090
Date of delivery: 10.01.2018
Test material conditions: No specific features, sample in the manufacturers tare
Storage conditions: In room temperature, dark
Active substance – conc.: Ethyl alcohol 72.5% wt, isopropyl alcohol 7.5% wt
Appearance of the product: Transparent liquid
Test concentration: Ready to use
Contact time: 30 s
Interfering substance: -
Rinsing liquid: -
Neutralizer: Polysorbate 80, 30 g/l; saponin, 30 g/l; lecithin, 3 g/l
Test organisms: *Escherichia coli* K12 NCTC 10538
Testing method based on: EVS-EN 1500:2013
Chemical disinfectants and antiseptics - Hygienic handrub - Test method and requirements (phase 2/step 2)
Testing date: 01.02.2018 - 02.02.2018
Results: look appendix 1 - 6



Diana Kaare, MSc
Head of laboratory, microbiologist

Date of test report: 06.02.2018

Appendix 1

Reference hygienic handrub – experimental results

Reference solution: [propan-2-ol alcohol 60% (v/v)]

Handrub procedure: 3 ml 30 sec. Repeat the procedure.

Date: 01.02.2018 - 02.02.2018

Test organism: *Escherichia coli* K12 NCTC 10538; Suspension: $2,19 \times 10^8$ cfu/ml

Volunteer			Number of CFU per plate from dilution 10^x					
No	Hand, left or right	Sequence	Prevalues			Postvalues		
			-3	-4	-5	0	-1	-2
1	l	PP>RP	>330	<u>104</u>	12	<u>31</u>	5	0
	r		>330	<u>92</u>	11	<u>36</u>	5	0
2	l	PP>RP	>330	<u>48</u>	7	<u>23</u>	<u>2</u>	0
	r		>330	<u>63</u>	10	<u>28</u>	5	0
3	l	PP>RP	>330	<u>114</u>	8	<u>52</u>	9	2
	r		>330	<u>86</u>	6	<u>43</u>	2	0
4	l	PP>RP	>330	<u>51</u>	<u>5</u>	<u>27</u>	6	2
	r		>330	<u>37</u>	5	<u>21</u>	4	0
5	l	PP>RP	<u>306</u>	35	5	<u>15</u>	1	0
	r		<u>281</u>	31	6	<u>18</u>	4	0
6	l	PP>RP	>330	<u>41</u>	7	<u>27</u>	5	0
	r		>330	<u>72</u>	13	<u>33</u>	6	2
7	l	PP>RP	>330	<u>91</u>	15	<u>62</u>	10	4
	r		>330	<u>74</u>	4	<u>49</u>	11	2
8	l	PP>RP	>330	<u>57</u>	9	<u>41</u>	8	0
	r		>330	<u>83</u>	14	<u>55</u>	4	0
9	l	RP>PP	>330	<u>66</u>	4	<u>29</u>	4	0
	r		>330	<u>81</u>	6	<u>25</u>	5	1
10	l	RP>PP	>330	<u>94</u>	16	<u>39</u>	<u>4</u>	0
	r		>330	<u>77</u>	13	<u>29</u>	1	0
11	l	RP>PP	>330	<u>42</u>	5	<u>22</u>	7	2
	r		>330	<u>59</u>	<u>6</u>	<u>31</u>	5	0
12	l	RP>PP	>330	<u>63</u>	13	<u>28</u>	2	0
	r		>330	<u>85</u>	14	<u>40</u>	9	4
13	l	RP>PP	>330	<u>91</u>	11	<u>71</u>	13	3
	r		>330	<u>54</u>	7	<u>58</u>	9	2
14	l	RP>PP	>330	<u>121</u>	20	<u>77</u>	15	3
	r		>330	<u>106</u>	15	<u>52</u>	10	2
15	l	RP>PP	>330	<u>198</u>	24	<u>93</u>	19	4
	r		>330	<u>175</u>	22	<u>78</u>	9	3
16	l	RP>PP	>330	<u>159</u>	23	<u>64</u>	5	1
	r		>330	<u>177</u>	19	<u>70</u>	10	3
17	l	RP>PP	>330	<u>314</u>	28	<u>103</u>	14	4
	r		>330	<u>286</u>	23	<u>92</u>	13	0
18	l	RP>PP	>330	<u>141</u>	19	<u>80</u>	11	0
	r		>330	<u>124</u>	17	<u>63</u>	4	1

55= count used for computations; **55**= adjacent usage; >330 = not countable

Appendix 2

Hygienic handrub procedure with the product under test – experimental results

Product: CHEMISEPT MED

Handrub procedure: 1x3 ml 30 sec

Date: 01.02.2018 - 02.02.2018

Test organism: *Escherichia coli* K12 NCTC 10538; Suspension: $2,19 \times 10^8$ cfu/ml

Volunteer			Number of CFU per plate from dilution 10^x					
No	Hand, left or right	Sequence	Prevalues			Postvalues		
			-3	-4	-5	0	-1	-2
1	l	PP>RP	>330	<u>58</u>	11	<u>5</u>	0	0
	r		>330	<u>66</u>	4	<u>3</u>	0	0
2	l	PP>RP	>330	<u>81</u>	10	<u>11</u>	2	0
	r		>330	<u>119</u>	11	<u>14</u>	3	0
3	l	PP>RP	>330	<u>123</u>	15	<u>24</u>	5	1
	r		>330	<u>104</u>	8	<u>18</u>	2	0
4	l	PP>RP	>330	>330	<u>31</u>	<u>68</u>	10	2
	r		>330	<u>281</u>	30	<u>41</u>	7	2
5	l	PP>RP	>330	<u>94</u>	<u>10</u>	<u>23</u>	4	0
	r		>330	<u>73</u>	9	<u>28</u>	5	0
6	l	PP>RP	>330	>330	<u>47</u>	<u>51</u>	7	0
	r		>330	>330	<u>61</u>	<u>70</u>	9	2
7	l	PP>RP	>330	<u>101</u>	13	<u>9</u>	2	0
	r		>330	<u>85</u>	10	<u>14</u>	2	0
8	l	PP>RP	>330	<u>69</u>	5	<u>6</u>	0	0
	r		>330	<u>94</u>	12	<u>7</u>	0	0
9	l	RP>PP	>330	<u>121</u>	<u>12</u>	<u>17</u>	4	0
	r		>330	<u>139</u>	18	<u>12</u>	2	0
10	l	RP>PP	>330	<u>105</u>	17	<u>25</u>	4	0
	r		>330	<u>74</u>	11	<u>28</u>	5	0
11	l	RP>PP	<u>237</u>	19	3	<u>2</u>	0	0
	r		<u>281</u>	25	5	<u>6</u>	0	0
12	l	RP>PP	>330	<u>81</u>	13	<u>11</u>	2	0
	r		>330	<u>98</u>	<u>10</u>	<u>15</u>	5	1
13	l	RP>PP	>330	<u>57</u>	9	<u>17</u>	3	0
	r		>330	<u>73</u>	9	<u>23</u>	0	0
14	l	RP>PP	>330	<u>87</u>	11	<u>22</u>	4	0
	r		>330	<u>99</u>	15	<u>28</u>	5	1
15	l	RP>PP	>330	<u>224</u>	26	<u>48</u>	7	1
	r		>330	<u>191</u>	23	<u>34</u>	5	0
16	l	RP>PP	>330	<u>154</u>	18	<u>27</u>	<u>3</u>	1
	r		>330	<u>141</u>	17	<u>15</u>	3	0
17	l	RP>PP	>330	>330	52	<u>81</u>	10	3
	r		>330	>330	38	<u>70</u>	11	4
18	l	RP>PP	>330	<u>162</u>	14	<u>9</u>	2	0
	r		>330	<u>133</u>	10	<u>9</u>	3	0

55 = count used for computations; **55** = adjacent usage; >330 = not countable

List of computed lg values (means of left and right hands) and lg reductions

Volunteer	Sequence	Reference handrub (RP) (Propan-2-ol 60% V/V)			Handrub with product under test (pp)		
		lg prevalues	lg postvalues	lg R	lg prevalues	lg postvalues	lg R
1	PP>RP	5.99	2.52	3.47	5.79	1.59	4.20
2	PP>RP	5.74	2.40	3.34	5.99	2.09	3.90
3	PP>RP	6.00	2.67	3.32	6.05	2.32	3.74
4	PP>RP	5.64	2.38	3.26	6.47	2.72	3.75
5	PP>RP	5.47	2.22	3.25	5.92	2.40	3.52
6	PP>RP	5.74	2.47	3.26	6.73	2.78	3.95
7	PP>RP	5.91	2.74	3.17	5.97	2.05	3.92
8	PP>RP	5.84	2.68	3.16	5.91	1.81	4.09
9	PP>RP	5.86	2.43	3.43	6.11	2.15	3.96
10	RP>PP	5.93	2.53	3.40	5.95	2.42	3.52
11	RP>PP	5.70	2.42	3.28	5.41	1.54	3.87
12	RP>PP	5.86	2.52	3.34	5.95	2.11	3.84
13	RP>PP	5.85	2.81	3.04	5.81	2.30	3.51
14	RP>PP	6.05	2.80	3.25	5.97	2.39	3.57
15	RP>PP	6.27	2.93	3.34	6.32	2.61	3.71
16	RP>PP	6.22	2.83	3.40	6.17	2.31	3.86
17	RP>PP	6.48	2.99	3.49	6.65	2.88	3.77
18	RP>PP	6.12	2.85	3.27	6.17	1.95	4.21
X	Overall	5.93	2.62	3.30	6.07	2.25	3.83
s		0.24	0.22	0.11	0.32	0.38	0.22
NN		18	18	18	18	18	18
X	PP>RP	5.80	2.50	3.30	6.10	2.21	3.89
s		0.17	0.17	0.11	0.30	0.39	0.20
NN		9	9	9	9	9	9
X	RP>PP	6.05	2.74	3.31	6.04	2.28	3.76
s		0.25	0.20	0.13	0.34	0.38	0.22
NN		9	9	9	9	9	9
lg R = decimal log reduction				X = Mean			
RP>PP Sequence: first RP, second PP				s = Standard deviation			
PP>RP Sequence: first PP, second RP				NN = Number on values (=volunteers)			

Difference of mean Rs (RP>PP): $3.31 - 3.76 = (-0.45)$;
Difference of mean Rs (PP>RP): $3.30 - 3.89 = (-0.59)$;
Absolute difference of differences: $\text{Abs} [(-0.59) - (-0.45)] = 0.14$

Check of acceptance criteria:

- 1) Complete set of results from 18 volunteers available (minimum 18)
- 2) Mean of lg prevalues for RP = 5.93 and for PP = 6.07 (hence both greater than 5.00)
- 3) Individual lg reductions less than 3.00: with RP = 0 and with PP = 0
- 4) For group with sequence RP>PP difference of lg R: $3.31 - 3.76 = (-0.45)$; for group with sequence PP>RP difference of lg R: $3.30 - 3.89 = (-0.59)$; absolute difference of mean differences: $\text{Abs} [(-0.59) - (-0.45)] = 0.14$ (hence less than 2.00)
- 5) All quotients of weighted mean counts between 5 and 15 (results which were used for weighted mean counts in appendix 1 and appendix 2)

Acceptance criteria are fulfilled.

Appendix 4

Computation of individual differences of lg Rs of RP - PP

Volunteer	lg reduction (R)		Difference RP-PP
	Reference procedure (RP)	Product procedure (PP)	
1	3.47	4.20	-0.74
2	3.34	3.90	-0.56
3	3.32	3.74	-0.41
4	3.26	3.75	-0.49
5	3.25	3.52	-0.26
6	3.26	3.95	-0.69
7	3.17	3.92	-0.74
8	3.16	4.09	-0.93
9	3.43	3.96	-0.52
10	3.40	3.52	-0.12
11	3.28	3.87	-0.59
12	3.34	3.84	-0.50
13	3.04	3.51	-0.48
14	3.25	3.57	-0.32
15	3.34	3.71	-0.37
16	3.40	3.86	-0.46
17	3.49	3.77	-0.28
18	3.27	4.21	-0.94

Appendix 5

Sorting of individual differences and computation for Hodges-Lehmann 97,5% upper confidence limits

No	Sorted differences	Mean pairwise differences (di+dii)/2							
		-0.12	-0.26	-0.28	-0.32	-0.37	-0.41	-0.46	-0.48
1	-0.12	-0.12/1							
2	-0.26	-0.19/2	-0.26/6						
3	-0.28	-0.20/3	-0.27/7	-0.28/9					
4	-0.32	-0.22/4	-0.29/11	-0.30/12	-0.32/17				
5	-0.37	-0.24/5	-0.31/16	-0.32/18	-0.34/22	-0.37/30			
6	-0.41	-0.27/8	-0.34/21	-0.35/23	-0.37/29	-0.39/37	-0.41		
7	-0.46	-0.29/10	-0.36/24	-0.37/28	-0.39/36	-0.42	-0.44	-0.46	
8	-0.48	-0.30/13	-0.37/26	-0.38/31	-0.40/39	-0.42	-0.44	-0.47	-0.48
9	-0.49	-0.30/14	-0.37/27	-0.38/33	-0.40/40	-0.43	-0.45	-0.47	-0.48
10	-0.50	-0.31/15	-0.38/31	-0.39/35	-0.41	-0.44	-0.46	-0.48	-0.49
11	-0.52	-0.32/19	-0.39/34	-0.40/38	-0.42	-0.45	-0.47	-0.49	-0.50
12	-0.56	-0.34/20	-0.41	-0.42	-0.44	-0.46	-0.48	-0.51	
13	-0.59	-0.36/25	-0.43	-0.44	-0.46	-0.48	-0.50		
14	-0.69	-0.41/41	-0.48	-0.49	-0.51	-0.53			
15	-0.74	-0.43	-0.50	-0.51	-0.53				
16	-0.74	-0.43	-0.50	-0.51					
17	-0.93	-0.53	-0.60						
18	-0.94	-0.53							

The differences of the individual lg R's of RP-PP from table at the Appendix 4 are sorted in the second column and in the headline according to their size in descending order.

The median is between 9th and 10th value: $[-0,49+(-0,50)]/2 = (-0,495)$. The numbers behind the fracture line represent the ranks.

The mean pairwise differences that not exceed the median are computed. From the table E.5 (EVS-EN 1500:2013) of critical values for Wilcoxon's matched-pairs signed-ranks test the entry for n=18 and for one-sided 0,025 level of significance, the critical value of 40 is found. Hence $c = 40+1 = 41$. The pairwise differences are sorted in descending order. The 41st value is -0.41. Hence the Hodges-Lehmann upper one-sided 97,5% confidence limit for the difference in lg Rs between RP and PP is -0.41, 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.

Appendix 6

Interpretation

In the case of the reference substance the mean of the log of the pre-values (lg prevalues) is 5.93, which overrides the validation criterion ≥ 5 . Tested product CHEMISEPT MED (Batch no. 196101017) shows the mean of the log of the pre-values (lg prevalues) 6.07, which is higher than the validation rate ≥ 5 as well.

There were not any of the lg reductions less than 3,00. The validation criterion is not more than three individual lg reductions less than 3,00 in RP.

Conclusion

Therefore, it can be claimed that by the validation criteria, test results and following counting: lg values of reduction, Wilcoxon test and Hodges-Lehmann system: the tested product – CHEMISEPT MED - is accepted for usage in hygienic handrub disinfection procedures on the following application: rub 3 ml of the product onto the hands within 30 seconds.



.....
Diana Kaare, MSc

Head of laboratory, microbiologist