

A: Identification of the test laboratory	Cheshire Scientific Limited 7 Brunel Road Bromborough Wirral Merseyside CH62 3NY
B: Identification of the sample	187642
Name of the product	Sanichem-2 Alcohol Based Hand Sanitiser (contains 75% (v/v) Propan-2-ol) WHO-recommended hand-rub formulation.
Date of Manufacture	20/05/2020
Manufacturer	Midland Chemicals Ltd
Date of delivery	22.05.2020
Storage Conditions	Room Temperature and Darkness
Product Diluent recommended by the manufacturer for use	Neat
Active substance (s) and its (their) concentrations(s)	75% Propan-2-ol
C: Test method and its validation	
Method Based on	BS EN 1500:2013
Neutraliser	Lecithin 3g/l, polysorbate 80 30g/l, saponin 30g/l in 1 litre of water.
D: Experimental conditions	
Period of analysis	3 rd June 2020 – 5 th June 2020
Product diluent used during test	Tested Neat
Product test concentrations	Neat
Appearance product dilutions	Clear liquid with strong alcohol smell.
Contact Times	30 to 60 seconds
Test temperature	21.0 Deg C
Temperature of incubation	37 +/- 1 Deg C
Identification of bacterial strains used	Escherichia coli K12 NCTC 10538
Tested by	R.Jones
Report Authorised by	A.Griffiths
Report dated	10 th June 2020
Test Results – see subsequent test tables.	
Conclusion – The test hand sanitizer is not inferior to the reference hygienic hand rub. Therefore the test hand sanitizer is deemed suitable to be used as medical hygienic handrub.	

Neutralizer Control – verification of the absence of toxicity of the neutralizer.

This test was performed by adding 1 ml of validation suspension to 9mls of neutralizer. 10 seconds later, 0.5ml of this suspension was added to 4.5ml Neutralizer, mixed, and repeated. Then 10 seconds later all 3 were plated out and poured with TSA agar.

Neutralizer Validation

This test was performed by adding 8mls of neat test hand sanitizer to 2 mls TSB. Leave for 60 seconds, shake, then add 1ml of this suspension to 8mls neutralizer. Leave for 10 seconds, and then add 1ml of the validation suspension. Leave for 30 mins, then plate out.

Test Organism	Bacterial Suspension	Experimental Conditions	Neutralizer Toxicity Control	Neutralizer Validation
Escherichia coli K12 NCTC 10538	190,271	143,148	149,148	110,126
	2.31×10^8	1.46×10^4	1.49×10^4	1.18×10^4

Neutralizer Validation Conclusion

The Neutralizer successfully neutralized the antibacterial properties of the test hand sanitiser as evidenced by the results for the Neutralizer validation being very close to the level of bacteria added to the test procedure. If the neutralizer was toxic, then the neutralizer validation results would be much lower than they are. The results figures have been adjusted to accommodate any dilution factors that occurred during the validation procedures.

Table 1
Reference hygienic handrub – experimental results.

Product: "RP" (propan-2-ol 60% V/V)
Test organism: E.coli K12 NCTC 10538
Date of experiment: 3rd June 2020

Handwash procedure: rub in 3ml/30 s, repeat once
Number in contamination fluid (N): 2.31×10^8 cfu/ml

Volunteers		Number of cfu Per Plate From Dilution					
No	Hand L/R	Pre values			Post values		
		10^{-3}	10^{-4}	10^{-5}	10^0	10^{-1}	10^{-2}
1	L	>330	>330	41	34	0	0
	R	>330	>330	264	40	0	0
2	L	>330	>330	16	171	7	0
	R	>330	>330	244	71	3	0
3	L	>330	>330	12	>330	14	0
	R	>330	>330	48	>330	19	3
4	L	>330	>330	53	78	7	1
	R	>330	>330	43	120	7	0
5	L	>330	79	0	183	1	0
	R	>330	67	0	44	0	0
6	L	>330	53	0	29	1	0
	R	>330	85	0	79	1	0
7	L	>330	178	1	>330	38	4
	R	261	45	0	251	4	1
8	L	>330	131	0	16	0	0
	R	>330	14	0	26	0	0
9	L	>330	99	0	>330	176	31
	R	>330	255	0	>330	102	1
10	L	>330	230	0	34	0	0
	R	>330	153	0	61	2	0
11	L	>330	265	19	81	8	0
	R	>330	324	0	81	8	0
12	L	>330	267	15	>330	80	1
	R	>330	>330	35	161	21	2

Volunteers		Number of cfu Per Plate From Dilution					
No	Hand L/R	Pre values			Post values		
		10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁰	10 ⁻¹	10 ⁻²
13	L	>330	174	0	>330	54	9
	R	>330	14	0	>330	238	11
14	L	>330	170	18	171	17	2
	R	>330	258	30	18	1	0
15	L	>330	186	0	103	3	0
	R	>330	167	0	104	11	1
16	L	>330	>330	34	144	0	1
	R	>330	274	1	100	11	0
17	L	>330	199	12	201	11	0
	R	>330	184	9	222	13	0
18	L	>330	166	8	71	6	0
	R	>330	172	7	84	4	0

Table 2

Hygienic handrub procedure with product under test – experimental results

Product: “PP” (Sanichem-2)
Test organism: E.coli K12 NCTC 10538
Date of experiment: 3rd June 2020

Hand rub procedure: rub-in 3 mls/30s,
Number in contamination fluid (N): 2.31×10^8 cfu/ml

Volunteers		Number of cfu Per Plate From Dilution					
No	Hand L/R	Pre values			Post values		
		10^{-3}	10^{-4}	10^{-5}	10^0	10^{-1}	10^{-2}
1	L	>330	>330	38	86	3	0
	R	>330	>330	77	97	5	0
2	L	>330	>330	28	47	6	0
	R	>330	>330	101	55	5	0
3	L	>330	>330	38	>330	185	13
	R	>330	>330	39	>330	120	10
4	L	>330	65	0	100	5	0
	R	>330	21	0	270	20	0
5	L	>330	>330	22	51	3	0
	R	>330	>330	31	40	1	0
6	L	>330	>330	48	34	0	0
	R	>330	>330	40	156	29	2
7	L	>330	>330	41	67	2	0
	R	>330	>330	113	44	3	0
8	L	>330	>330	46	174	20	4
	R	>330	>330	101	208	17	3
9	L	>330	>330	55	>330	76	0
	R	>330	>330	41	>330	80	4
10	L	>330	>330	31	61	4	0
	R	>330	>330	38	48	4	0
11	L	>330	>330	110	40	1	0
	R	>330	>330	97	52	3	0
12	L	>330	>330	44	111	10	0
	R	>330	>330	58	104	9	1

Volunteers		Number of cfu Per Plate From Dilution					
No	Hand L/R	Pre values			Post values		
		10^{-3}	10^{-4}	10^{-5}	10^0	10^{-1}	10^{-2}
13	L	>330	66	5	103	8	0
	R	>330	37	3	108	11	1
14	L	>330	>330	66	>330	37	4
	R	>330	>330	34	211	18	0
15	L	>330	>330	44	66	4	0
	R	>330	>330	89	101	18	1
16	L	>330	>330	22	49	3	0
	R	>330	>330	30	54	4	0
17	L	>330	>330	49	71	6	0
	R	>330	>330	41	44	3	0
18	L	>330	>330	141	31	3	1
	R	>330	>330	159	222	20	1

Table 3
List Of Computed lg Values (Means of Left & Right Hand) And lg Reductions

Volunteers	Sequence	Reference handrub (Propan-2-ol 60% V/V) (RP)			Hand Wash With Product Under Test (PP)		
		Lg Pre Values	Lg Post Values	Lg R	Lg Pre Values	Lg Post Values	Lg R
1	RP>PP	7.18	1.57	5.62	6.76	1.96	4.80
2	PP>RP	7.11	2.08	5.03	6.81	1.71	5.10
3	RP>PP	6.48	2.22	4.26	6.59	2.99	3.59
4	RP>PP	6.68	2.00	4.69	5.63	2.26	3.37
5	PP>RP	5.86	2.05	3.81	6.42	1.66	4.77
6	PP>RP	5.84	1.73	4.11	6.64	1.98	4.66
7	PP>RP	6.01	2.50	3.51	6.89	1.74	5.14
8	PP>RP	5.86	1.32	4.54	6.87	2.28	4.59
9	PP>RP	6.25	3.16	3.09	6.68	2.89	3.79
10	PP>RP	6.28	1.68	4.61	6.54	1.74	4.80
11	PP>RP	6.46	1.91	4.56	7.01	1.66	5.35
12	PP>RP	6.48	2.68	3.80	6.71	2.03	4.68
13	PP>RP	5.97	3.16	2.81	5.71	2.02	3.69
14	RP>PP	6.34	1.98	4.36	6.70	2.46	4.24
15	PP>RP	6.25	2.01	4.23	6.82	1.92	4.90
16	PP>RP	6.49	2.09	4.40	6.41	1.71	4.70
17	PP>RP	6.28	2.33	3.96	6.65	1.76	4.89
18	PP>RP	6.23	1.89	4.34	7.18	2.10	5.08
X s NN	Overall	6.34	2.13	4.21	6.61	2.05	4.56
		0.38	0.49	0.66	0.39	0.15	0.32
		18	18	18	18	18	18
X s NN	RP>PP	6.67	1.94	4.73	6.39	2.41	4.00
		0.10	0.06	0.29	0.19	0.14	0.31
		4	4	4	4	4	4
X s NN	PP>RP	6.24	2.19	4.06	6.67	1.94	4.72
		0.11	0.26	0.35	0.11	0.10	0.20
		14	14	14	14	14	14
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 of values (= volunteers)					

Difference of mean Rs (RP->PP): 4.73-4.00 = 0.73
 Difference of mean Rs (PP->RP): 4.06-4.72 = -0.66
 Absolute difference of differences: Abs [0.73-(-0.66)] = 0.07

Acceptance Criteria For Test Results:

- a). Complete set of results from 18 volunteers (minimum of 18).
- b). Mean of lg prevalues for RP = 6.34 and for PP = 6.61 (hence both greater than 5.00).
- c). Individual lg reductions less than 3.00: with RP = 1, with PP = 0 (hence each less than 3.00 reduction (R) values).
- d). For group with sequence RP->PP difference of lg R: 4.73-4.00 = 0.73; for group with sequence PP->RP difference of lg R: 4.06-4.72 = -0.66; absolute difference of mean differences : abs [0.73-(-0.66)] = 0.07 (hence = less than 2.00).
- e). Neutralizer validated.

All acceptance criteria are fulfilled.

Table 4
Computation of individual differences of lg Rs of RP-PP

Volunteer	Log R (= Reduction) Derived From		Difference RP-PP
	Reference procedure (RP)	Product procedure (PP)	
1	5.62	4.80	0.82
2	5.03	5.10	-0.07
3	4.26	3.59	0.67
4	4.69	3.37	1.32
5	3.81	4.77	-0.96
6	4.11	4.66	-0.55
7	3.51	5.14	-1.63
8	4.54	4.59	-0.05
9	3.09	3.79	-0.70
10	4.61	4.80	-0.19
11	4.56	5.35	-0.79
12	3.80	4.68	-0.88
13	2.81	3.69	-0.88
14	4.36	4.24	0.12
15	4.23	4.90	-0.67
16	4.40	4.70	-0.30
17	3.96	4.89	-0.93
18	4.34	5.08	-0.74

Table 5 – Sorting of individual differences and computation for Hodges-Lehmann 97.5% upper confidence limits.

Sorted Differences	Mean pairwise differences $(d_i + d_{ij})/2$									
	1.32	0.82	0.67	0.12	-0.05	-0.07	-0.19	-0.30	-0.55	-0.67
1 1.32	1.32 ¹									
2 0.82	1.07 ²	0.82 ⁴								
3 0.67	0.99 ³	0.75 ⁵	0.67 ⁷							
4 0.12	0.72 ⁶	0.47 ¹²	0.40 ¹³	0.12 ³²						
5 -0.05	0.64 ⁸	0.39 ¹⁴	0.31 ¹⁹	0.04 ³⁷	-0.05					
6 -0.07	0.63 ⁹	0.38 ¹⁶	0.30 ²¹	0.03 ³⁸	-0.06	-0.07				
7 -0.19	0.57 ¹⁰	0.32 ¹⁸	0.24 ²⁵	-0.04	-0.12	-0.13	-0.19			
8 -0.30	0.51 ¹¹	0.26 ²⁴	0.19 ²⁹	-0.09	-0.18	-0.19	-0.25	-0.30		
9 -0.55	0.39 ¹⁵	0.14 ³¹	0.06 ³⁴	-0.22	-0.30	-0.31	-0.37	-0.43	-0.55	
10 -0.67	0.33 ¹⁷	0.08 ³³	0.00 ⁴⁰	-0.28	-0.36	-0.37	-0.43	-0.49	-0.61	
11 -0.70	0.31 ²⁰	0.06 ³⁵	-0.02⁴¹	-0.29	-0.38	-0.39	-0.45	-0.50		
12 -0.74	0.29 ²²	0.04 ³⁶	-0.04	-0.31	-0.40	-0.41	-0.47			
13 -0.79	0.27 ²³	0.02 ³⁹	-0.06	-0.34	-0.42	-0.43				
14 -0.88	0.22 ²⁶	-0.03	-0.11	-0.38	-0.47					
15 -0.88	0.22 ²⁷	-0.03	-0.11	-0.38						
16 -0.93	0.20 ²⁸	-0.06	-0.13							
17 -0.96	0.18 ³⁰	-0.07								
18 -1.63	-0.16									

The differences of the individual lg Rs 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 9th and 10th value $[-0.55 + (-0.67)]/2 = -0.61$. The small exponents represent the ranks.

The mean pairwise differences that do not exceed the median (here: -0.61) are computed. From Table 6 of critical values for Wilcoxon's matched-pairs signed-ranks test the entry for $n=18$ and a 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 -0.02.** Hence the Hodges-Lehmann upper one-sided 97.5% confidence limit for the difference in lg Rs between RP and PP is -0.02, 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

N (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

Critical values of the lower of both sums of ranks (+) or (-) sign at the different significance levels used for calculation of the Hodges-Lehmann confidence limits. In this case, zero differences are include



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