Study Title:
Chemical disinfectants and antiseptics - Hygienic hand wash (Phase 2, Step 2)

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Report date: 20/05/2024
Issue number: 1

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Scope

This method simulates practical conditions for establishing whether a product for hygienic hand wash reduces the release of transient microbial flora on hands when rubbed onto the artificially contaminated hands of volunteers.

Outline of Test Method (Obligatory Test Conditions)

Hands of volunteers are artificially contaminated with test organisms. The number of test organisms released from their fingertips into sampling fluids is assessed before and after hygienic hand wash. The ratio of the two resulting values represents a measure for the antimicrobial activity of the product tested. The necessary precision is achieved by repeating the test on 12-15 volunteers. To compensate for extraneous influences, it is compared with the reduction obtained by a reference hand wash, which is performed with the same volunteers, on the same day and under comparable environmental conditions.

Prior to the test a suitable neutralizer is validated.

Acceptance Criteria

When tested in accordance with the standard, the mean reduction of the release of the test organism E. coli K12 achieved by the hygienic hand wash with the product under test shall be larger than that achieved by a reference hand wash.
Pass criteria - If the smaller sum of ranks is <9, the PP is significantly more effective than the RP, where n=12 at a level of significance p=0.01 (=12).

Test information		Deviation
Name of Product	Antibacterial Hand Soap	
Batch Number & Expiry Date		
Date of Delivery	08/04/2024	
Period of Analysis	10/05/2024	
Manufacturer / Supplier	Anglian Chemicals Ltd	
Storage Conditions	Ambient	
Appearance of the Product	Clear Gel	
Neutraliser	N1	
Neutralisation Method	Dilution	
Test Concentrations	Neat	
Test volume	2 x 3ml	
Test Temperature	20°C ± 1°C	
Temperature of Incubation	Bacteria – 37°C ± 1°C for 24hr to 48hrs	
Identification of the Bacterial Strains:	*Escherichia coli k12* NCTC 10538	
Contact Times	1 minute ± 10s	

Deviations from Standard Method

There were no deviations from the standard method

Test Result Summary

Pass criteria
If the smaller sum of ranks is <9, the PP is significantly more effective than the RP, where n=12 at a level of significance p=0.01.

Conclusion
When assessed using the one sided Wilcoxon matched-paired signed-rank test, the smaller sum of ranks was 0 and the product was seen to be significantly more effective that the reference hand wash, at level of significance p=0.01, when using 2 X 3ml of product and a contact time of 1 minute.

See page 2 for acceptance criteria and raw data tables below for complete test results.

Raw data

Subject	Control product								Test product							
	Pre Values				Post Values				Pre Values				Post Values			
Hand	Left		Right		Left		Right		Left		Right		Left		Right	
	Dilutio	Count	Dilutio	Count	Dilution	Count	Dilutio	Count	Dilutio	Count	Dilutio	Count	Dilutio	Count	Dilution	Count
1	5	30	5	22	1	25	1	52	5	22	5	16	1	14	1	14
2	4	136	5	18	1	81	1	20	4	126	5	14	1	20	0	54
3	3	54	3	30	1	27	1	19	3	97	3	86	0	33	1	20
4	5	31	5	57	1	129	1	121	4	195	4	136	1	22	1	45
5	5	14	4	72	1	18	1	18	4	111	4	88	0	51	0	17
6	5	17	5	19	1	24	1	35	5	17	5	15	1	16	1	18
7	4	94	4	98	1	37	1	90	4	111	5	14	1	32	1	15
8	5	15	5	28	1	80	1	71	4	121	5	19	1	15	1	15
9	5	25	5	25	2	59	3	16	5	17	5	22	2	43	2	36
10	4	66	4	82	1	40	1	37	4	73	4	32	1	18	1	16
11	5	60	5	104	2	37	2	34	5	41	5	35	1	30	1	21
12	5	46	5	34	1	43	1	51	5	39	5	34	1	42	1	36

Pre values – Control

Pre Values		Control product								
Hand	Left				Right				Average	Average
Subject	Dilution	Count	total cfu	log10	Dilution	Count	cfu	log10	cfu	log10
1	5	30	3.00E+07	7.48	5	22	2.20E+07	7.34	3.E+07	7.41
2	4	136	1.36E+07	7.13	5	18	1.80E+07	7.26	2.E+07	7.20
3	3	54	5.40E+05	5.73	3	30	3.00E+05	5.48	4.E+05	5.62
4	5	31	3.10E+07	7.49	5	57	5.70E+07	7.76	4.E+07	7.64
5	5	14	1.40E+07	7.15	4	72	7.20E+06	6.86	1.E+07	7.03
6	5	17	1.70E+07	7.23	5	19	1.90E+07	7.28	2.E+07	7.26
7	4	94	9.40E+06	6.97	4	98	9.80E+06	6.99	1.E+07	6.98
8	5	15	1.50E+07	7.18	5	28	2.80E+07	7.45	2.E+07	7.33
9	5	25	2.50E+07	7.40	5	25	2.50E+07	7.40	3.E+07	7.40
10	4	66	6.60E+06	6.82	4	82	8.20E+06	6.91	7.E+06	6.87
11	5	60	6.00E+07	7.78	5	104	1.04E+08	8.02	8.E+07	7.91
12	5	46	4.60E+07	7.66	5	34	3.40E+07	7.53	4.E+07	7.60
							Log of Mean			7.40
							SD of mean			0.58

Post values and log reduction – Control

Post Values		Control product									
Hand	Left				Right				Average	Averag	Reduction
Subject	Dilution	Count	cfu	log10	Dilutio	Count	cfu	log10	cfu	log10	log
1	1	25	2.50E+03	3.40	1	52	5.20E+03	3.72	4.E+03	3.59	3.83
2	1	81	8.10E+03	3.91	1	20	2.00E+03	3.30	5.E+03	3.70	3.50
3	1	27	2.70E+03	3.43	1	19	1.90E+03	3.28	2.E+03	3.36	2.26
4	1	129	1.29E+04	4.11	1	121	1.21E+04	4.08	1.E+04	4.10	3.55
5	1	18	1.80E+03	3.26	1	18	1.80E+03	3.26	2.E+03	3.26	3.77
6	1	24	2.40E+03	3.38	1	35	3.50E+03	3.54	3.E+03	3.47	3.79
7	1	37	3.70E+03	3.57	1	90	9.00E+03	3.95	6.E+03	3.80	3.18
8	1	80	8.00E+03	3.90	1	71	7.10E+03	3.85	8.E+03	3.88	3.45
9	2	59	5.90E+04	4.77	3	16	1.60E+05	5.20	1.E+05	5.04	2.36
10	1	40	4.00E+03	3.60	1	37	3.70E+03	3.57	4.E+03	3.59	3.28
11	2	37	3.70E+04	4.57	2	34	3.40E+04	4.53	4.E+04	4.55	3.36
12	1	43	4.30E+03	3.63	1	51	5.10E+03	3.71	5.E+03	3.67	3.93
							Log of Mean			4.21	
							SD of mean			0.51	

Pre values – Test

Pre Values		Test product								
Hand	Left				Right				Average	Average
Subject	Dilution	Count	cfu	log10	Dilution	Count	cfu	log10	cfu	log10
1	5	22	22000000	7.3424	5	16	16000000	7.2041	2.E+07	7.28
2	4	126	12600000	7.1004	5	14	14000000	7.1461	1.E+07	7.12
3	3	97	970000	5.9868	3	86	860000	5.9345	9.E+05	5.96
4	4	195	19500000	7.29	4	136	13600000	7.1335	2.E+07	7.22
5	4	111	11100000	7.0453	4	88	8800000	6.9445	1.E+07	7.00
6	5	17	17000000	7.2304	5	15	15000000	7.1761	2.E+07	7.20
7	4	111	11100000	7.0453	5	14	14000000	7.1461	1.E+07	7.10
8	4	121	12100000	7.0828	5	19	19000000	7.2788	2.E+07	7.19
9	5	17	17000000	7.2304	5	22	22000000	7.3424	2.E+07	7.29
10	4	73	7300000	6.8633	4	32	3200000	6.5051	5.E+06	6.72
11	5	41	41000000	7.6128	5	35	35000000	7.5441	4.E+07	7.58
12	5	39	39000000	7.5911	5	34	34000000	7.5315	4.E+07	7.56
							Log of Mean			7.23
							SD of mean			0.43

Post values and log reduction – Test

Post Values		Test product									
Hand	Left				Right				Average	Average	Reduction
Subject	Dilution	Count	cfu	log10	Dilution	Count	cfu	log10	cfu	log10	log
1	1	14	1400	3.14612804	1	14	1400	3.146	1.E+03	3.15	4.13
2	1	20	2000	3.30103	0	54	540	2.732	1.E+03	3.10	4.02
3	0	33	330	2.51851394	1	20	2000	3.301	1.E+03	3.07	2.90
4	1	22	2200	3.34242268	1	45	4500	3.653	3.E+03	3.53	3.69
5	0	51	510	2.70757018	0	17	170	2.23	3.E+02	2.53	4.47
6	1	16	1600	3.20411998	1	18	1800	3.255	2.E+03	3.23	3.97
7	1	32	3200	3.50514998	1	15	1500	3.176	2.E+03	3.37	3.73
8	1	15	1500	3.17609126	1	15	1500	3.176	2.E+03	3.18	4.02
9	2	43	43000	4.63346846	2	36	36000	4.556	4.E+04	4.60	2.69
10	1	18	1800	3.25527251	1	16	1600	3.204	2.E+03	3.23	3.49
11	1	30	3000	3.47712125	1	21	2100	3.322	3.E+03	3.41	4.17
12	1	42	4200	3.62324929	1	36	3600	3.556	4.E+03	3.59	3.97
							Log of Mean			3.70	
							SD of mean			0.48	

Wilcoxon signed rank

Group		Control	Test	Difference	Result to be used in statistics	Difference	Absolute	Rank	Signed		
	Subject	log reduction	log reduction	Control - Test			difference		rank	W+	W-
1	1	3.83	4.13	-0.30	Y	-0.30	0.30	5	-5		5
1	2	3.50	4.02	-0.52	Y	-0.52	0.52	7	-7		7
1	3	2.26	2.90	-0.63	Y	-0.63	0.63	10	-10		10
1	4	3.55	3.69	-0.15	Y	-0.15	0.15	2	-2		2
1	5	3.77	4.47	-0.70	Y	-0.70	0.70	11	-11		11
1	6	3.79	3.97	-0.19	Y	-0.19	0.19	3	-3		3
2	7	3.18	3.73	-0.55	Y	-0.55	0.55	8	-8		8
2	8	3.45	4.02	-0.56	Y	-0.56	0.56	9	-9		9
2	9	2.36	2.69	-0.33	Y	-0.33	0.33	6	-6		6
2	10	3.28	3.49	-0.21	Y	-0.21	0.21	4	-4		4
2	11	3.36	4.17	-0.81	Y	-0.81	0.81	12	-12		12
2	12	3.93	3.97	-0.04	Y	-0.04	0.04	1	-1		1
Average		3.35	3.77								

Summary	
n	12
W+	0
W-	78
W value for P = 0.05	
14	

Inoculum

Participants	Test Organism	Suspension N
1-13	*Escherichia*	10^6 >330 ; >330
	coli	10^7 36 ; 40
	NCTC 10538	N_0 : 8.58 Valid

Neutraliser validation

Validation suspension (Nv_0)				Validation suspension (NvB)				Neutraliser or Filtration Control (B)				Method Validation (C)			
			$\bar{x}$ =				$\bar{x}$ =				$\bar{x}$ =				$\bar{x}$ =
Vc1	Ec.	60	Ec. 58	Vc1	Ec.	74000	Ec. 72000	Vc1	Ec.	50	Ec. 61	Vc1	Ec.	80	Ec. 75
Vc2	Ec.	56		Vc2	Ec.	70000		Vc2	Ec.	71		Vc2	Ec.	69	
$30 \leq \bar{x}$ of $Nv_0 \leq 160$?				$30000 \leq \bar{x}$ of $Nv_0 \leq 160000$?				$\bar{x}$ of $B \geq 0.0005$ NvB				$\bar{x}$ of $C \geq 0.5$ Nv0			
Yes				Yes				Yes				Yes			

Fungal Test Results

KEY