Abbott Analytical

Consulting Scientists to the Disinfectant Industry

Certificate of Analysis

Sample(s):	One sample of Alcohol Free Hand Sanitiser		
Received from:	Serenity Group, Kemp House, London, EC1V 2NX		
Date received:	10 December 2012	**Date tested:**	19 December 2012 & 2 January 2013
Certificate no:	12M.046HR.CLG	**Certificate date:**	4 January 2013
Sample ref:	12M/046	**Page:**	1 of 7
Analysis required:	EN 1500, Chemical disinfectants and antiseptics – Hygienic handrub - Test method and requirements (phase 2, step 2)		

Volume of product used:	6ml (2x 3ml)
Total rubbing time with product:	1 minute (2x 30 seconds)
Appearance of product (dilution):	Clear, colourless, viscous solution
Identification of bacterial strain(s) used:	*Escherichia coli* (K12) NCIMB 10083
Number in contamination fluid (N):	2.30×10^8
Neutralising solution:	3% Polysorbate 80, 3g/l Lecithin, 1g/l L-histidine, 1g/l L-cysteine

Principle of test:

The number of test organisms released from the fingertips of artificially contaminated hands is assessed before and after using the hygienic handrub. The ratio of the two resulting values is called the reduction factor; it represents a measure of antimicrobial activity of the hygienic handrub product tested.

In order to achieve the necessary precision a large number of subjects has to be used because of the possible variation in bacterial flora found on human skin. In this case a total of 15 subjects (healthy adults) were chosen, each one carrying out the test procedure in precisely the same way as the others. To compensate for extraneous influences the test sample reduction factor (P) is compared with the reduction factor obtained with a parallel reference handrub procedure (R) which is performed with the same subjects, on the same day and under comparable environmental conditions.

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Experimental procedure:

1) Application of the contamination fluid

Each of the 15 subjects was asked to wash their hands for 1 minute in soft soap to remove natural commensal organisms and then dry them thoroughly on a paper towel. The hands were then contaminated with very large numbers of bacteria well in excess of that experienced in normal everyday conditions. The hands were immersed in the contamination fluid (containing an overnight culture of the test organism, in this instance *E. coli* (K12), at a concentration of approximately 10^8 cfu/ml) in a suitable sized container for 5 seconds. The hands were removed from the contamination fluid and surplus liquid allowed to drain back into the container. This time the hands were allowed to air dry for approximately 3 minutes holding them horizontally with fingers spread out and rotating them to and fro to avoid the formation of droplets.

2) Prevalues

Immediately after drying, each of the 15 subjects was asked to rub their fingertips, including the thumbs, for 1 minute on the base of a petri dish (a separate dish for each hand) containing 10ml of maximum recovery diluent (MRD) without neutraliser, in order to assess the release of test organisms before treatment of the hands. Dilutions of these sample fluids were prepared to 10^{-4}. A 1ml aliquot of each dilution for each hand was placed in a separate petri dish, 10-15ml of Tryptone Soy Agar (sterilised and cooled to 45°C) added and mixed thoroughly. Plates were allowed to set and incubated at 37°C for 24 hours. Each plate was then examined for growth of the test organism.

3) Hygienic handrub procedure

Each of the 15 subjects was asked to pour 3ml of 60% propan-2-ol into cupped hands and rub vigorously for 30 seconds onto the skin, up to the wrists in accordance with the standard handrub procedure. This comprises five strokes backwards and forwards palm to palm, right palm over left dorsum and left palm over right dorsum, palm to palm with fingers interlaced, back of fingers to opposing palms with fingers interlocked, rotational rubbing of right thumb clasped in left palm and left thumb clasped in right palm, rotational rubbing with clasped fingers of right hand in palm of left hand and clasped fingers of left hand in right palm. Repeated with a further 3ml propan-2-ol to give a total rubbing time of 60 seconds.

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4) Handrub procedure with test product (P)

The above procedure was repeated exactly using the test product in place of propan-2-ol. The volume of test product used and the total rubbing time were as detailed in the table on the first page.

5) Postvalues

Immediately after rinsing the 15 subjects were asked to rub the fingertips on the base of a petri dish containing 10ml of MRD with neutraliser for 1 minute using a separate petri dish for each hand. Then 1ml of each of the undiluted sample fluids was placed in a petri dish and covered with 15ml of TSA mixed thoroughly and allowed to set. Plates were then incubated overnight at 37°C and examined for growth of the test organism.

6) Calculation

The number of colony forming units (cfu) per plate for each dilution was recorded and the number of cfu/ml of sample fluid calculated. For both reference and test procedure the log counts from right and left hands of each subject were averaged separately for prevalues and postvalues.

From the difference between this individual combined log prevalue and the log postvalue a log reduction factor is established for each subject. Then the two arithmetic means of all individual log reduction factors are calculated for both the reference and the test procedure.

For the test product to pass the criteria of EN 1500 the mean log reduction factor obtained must not be significantly smaller than that obtained for the propan-2-ol.

Results: (see tables on following page)

See tables on following pages.

Difference of mean log reduction factors: R-P = 5.61-5.89 = -0.28

Conclusion:

This batch of Alcohol Free Hand Sanitizer passes the requirements of EN 1500 for hygienic handrubs when tested under the procedures described above.

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Results table: Reference handrub procedure

Subject		Number of cfu per plate from dilution 10^X				
		Prevalues		Postvalues		
Number	Hand	10^{-4}	10^{-5}	10^{0}	10^{-1}	10^{-2}
1	Left	>300	**32**	**15**	2	0
	Right	>300	**37**	**23**	2	0
2	Left	**294**	**32**	**7**	0	0
	Right	**260**	**27**	**11**	0	0
3	Left	**288**	**30**	**25**	3	0
	Right	>300	**35**	**18**	3	0
4	Left	**252**	**26**	**23**	3	0
	Right	**214**	**25**	**11**	1	0
5	Left	**266**	**31**	**8**	0	0
	Right	>300	**33**	**5**	0	0
6	Left	>300	**33**	**0**	0	0
	Right	**272**	**29**	**0**	0	0
7	Left	**300**	**35**	**0**	0	0
	Right	**244**	**29**	**0**	0	0
8	Left	**262**	**29**	**0**	0	0
	Right	**298**	**33**	**0**	0	0
9	Left	**204**	**21**	**7**	0	0
	Right	**276**	**30**	**11**	0	0
10	Left	**233**	**25**	**8**	0	0
	Right	**285**	**33**	**3**	0	0
11	Left	>300	**37**	**15**	2	0
	Right	**294**	**32**	**21**	3	0
12	Left	**284**	**35**	**20**	3	0
	Right	**296**	**38**	**14**	3	0
13	Left	**234**	**29**	**25**	4	0
	Right	**192**	**19**	**11**	3	0
14	Left	**206**	**25**	**7**	1	0
	Right	**274**	**33**	**8**	1	0
15	Left	**211**	**25**	**5**	0	0
	Right	**185**	**27**	**3**	0	0

Numbers in **blue** used for further computation

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Results table: Handrub procedure with test product

Subject		Number of cfu per plate from dilution 10^{X}				
		Prevalues		Postvalues		
Number	Hand	10^{-4}	10^{-5}	10^{0}	10^{-1}	10^{-2}
1	Left	**284**	**33**	**5**	0	0
	Right	>300	**37**	**3**	0	0
2	Left	**255**	**24**	**0**	0	0
	Right	**222**	**24**	**0**	0	0
3	Left	**218**	**23**	**11**	3	0
	Right	**272**	**29**	**7**	0	0
4	Left	**294**	**33**	**19**	3	0
	Right	>300	**37**	**25**	3	0
5	Left	>300	**35**	**0**	0	0
	Right	**255**	**28**	**0**	0	0
6	Left	**262**	**29**	**0**	0	0
	Right	**218**	**23**	**0**	0	0
7	Left	**256**	**18**	**0**	0	0
	Right	**230**	**25**	**0**	0	0
8	Left	**224**	**27**	**5**	0	0
	Right	**250**	**33**	**7**	0	0
9	Left	**178**	**16**	**2**	0	0
	Right	**154**	**16**	**2**	0	0
10	Left	**162**	**20**	**5**	0	0
	Right	**200**	**25**	**6**	0	0
11	Left	**270**	**32**	**3**	0	0
	Right	**238**	**25**	**8**	0	0
12	Left	**296**	**35**	**12**	2	0
	Right	>300	**38**	**5**	1	0
13	Left	**204**	**20**	**12**	3	0
	Right	**186**	**20**	**17**	1	0
14	Left	**216**	**22**	**0**	0	0
	Right	**255**	**29**	**0**	0	0
15	Left	**200**	**23**	**0**	0	0
	Right	**158**	**18**	**0**	0	0

Numbers in **blue** used for further computation

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Results table: Computed log values (means of left and right hand) and log reduction factors

Subject	Reference disinfection procedure (R)			Disinfection procedure with test product (P)		
	log x	log y	log z	log x	log y	log z
1	6.54	1.27	5.27	6.51	0.59	5.92
2	6.44	0.94	5.50	6.38	0.00	6.38
3	6.50	1.33	5.17	6.39	0.94	5.45
4	6.37	1.20	5.17	6.52	1.34	5.18
5	6.47	0.80	5.67	6.48	0.00	6.48
6	6.48	0.00	6.48	6.38	0.00	6.38
7	6.44	0.00	6.44	6.38	0.00	6.38
8	6.45	0.00	6.45	6.38	0.77	5.61
9	6.38	0.94	5.44	6.22	0.30	5.92
10	6.42	0.69	5.73	6.26	0.74	5.52
11	6.52	1.25	5.27	6.41	0.69	5.72
12	6.47	1.22	5.25	6.53	0.89	5.64
13	6.33	1.22	5.11	6.29	1.15	5.14
14	6.38	0.87	5.51	6.37	0.00	6.37
15	6.31	0.59	5.72	6.26	0.00	6.26
ẍ	6.43	0.82	5.61	6.38	0.49	5.89
s	0.07	0.48	0.48	0.10	0.48	0.46
N	15	15	15	15	15	15

log x: log prevalue
log y: log postvalue
log z: log reduction factor

ẍ: overall mean of log x, log y, log z
s: standard deviation
N: number of values (subjects) in each column

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Neutralization & toxicity validation

Test Organism		***Escherichia coli (K12)***	
Validation Suspension		Vc1 214	Vc2 200
(Nv_o)		$\ddot{x}$ = 207	
Neutraliser Control		Vc1 192	Vc2 178
(B)		$\ddot{x}$ = 185	≥ $0.5Nv_o$
Method Validation		Vc1 184	Vc2 202
(C)		$\ddot{x}$ = 193	≥ $0.5Nv_o$
Test Suspension	10^{-6}	Vc1 208	Vc2 241
	10^{-7}	Vc1 26	Vc2 31
(N)		$\ddot{w}$ = 2.30×10^8	

Vc = plate count per ml
$\ddot{x}$ = average of Vc1 and Vc2
$\ddot{w}$ = weighted mean of $\ddot{x}$
R = reduction ($\lg R = \lg N_o - \lg Na$)

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