



# Abbott Analytical



Consulting Scientists to the Disinfectant Industry

## Certificate of Analysis

**Sample(s):** One sample of Alcohol Free Hand Sanitizer

**Received from:** Cleenol Group Ltd. Beaumont Road, Banbury, OX16 1RB

**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):**  $2.30 \times 10^8$

**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 <sup>x</sup>				
Number	Hand	Prevalues		Postvalues		
		10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>0</sup>	10 <sup>-1</sup>	10 <sup>-2</sup>
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 <sup>x</sup>				
Number	Hand	Prevalues		Postvalues		
		10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>0</sup>	10 <sup>-1</sup>	10 <sup>-2</sup>
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

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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
<b><math>\bar{x}</math></b>	6.43	0.82	5.61	6.38	0.49	5.89
<b>s</b>	0.07	0.48	0.48	0.10	0.48	0.46
<b>N</b>	15	15	15	15	15	15

log x: log prevalue

log y: log postvalue

log z: log reduction factor

$\bar{x}$ : 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	<i>Escherichia coli</i> (K12)	
Validation Suspension (N <sub>v</sub> )	Vc1 214	Vc2 200
	$\bar{x} = 207$	
Neutraliser Control (B)	Vc1 192	Vc2 178
	$\bar{x} = 185 \geq 0.5N_{v_0}$	
Method Validation (C)	Vc1 184	Vc2 202
	$\bar{x} = 193 \geq 0.5N_{v_0}$	
Test Suspension	10 <sup>-6</sup> Vc1 208	Vc2 241
	10 <sup>-7</sup> Vc1 26	Vc2 31
(N)	$\bar{w} = 2.30 \times 10^8$	

Vc = plate count per ml  
 $\bar{x}$  = average of Vc1 and Vc2  
 $\bar{w}$  = weighted mean of  $\bar{x}$   
R = reduction ( $\lg R = \lg N_0 - \lg N_a$ )

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