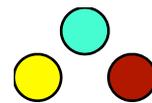


Abbott Analytical



Consulting Scientists to the Disinfectant Industry

8th September 2009

Certificate of Analysis

Samples: One sample of Germ Free 24 received from Zoono Ltd, 20
Royston Court, Lichfield Road, RICHMOND, Surrey. TW9 3EH
26th August 2009.

Certificate No: 09H.120h.Z00

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Sample Ref: 9h / 120

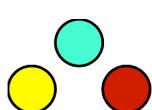
Analysis Required: EN 1500

Samples Tested: 3rd - 4th September 2009

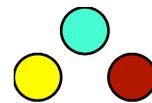
Principle of test:

The number of test organisms released from the fingertips of artificially contaminated hands is assessed before and after 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 handwash 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 **twelve (12)** 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 handwash 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 12 subjects was asked to wash their hands for 1 minute in soft soap to remove natural commensal organisms and dried 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 occurrence. The hands were immersed in the contamination fluid (containing an overnight culture of the test organism in this instance *E. coli* at a concentration of approximately 10^8 cfu per 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 12 subjects was asked to rub their fingertips, including the thumbs for 1 minute on the base of a petri dish, using a separate petridish 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^{-3} and 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..

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3) Hygienic Handrub procedure.

Each of the 12 subjects was asked to pour 3ml of 60% propan-2-ol into the cupped hands and rub vigorously for 30s 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. Repeat with a further 3ml propan-2-ol to give a total rubbing time of 60s. After 60 seconds the hands are rinsed under running tap water for 5 seconds, excess water is shaken off.

4) Handwash procedure with test product (P).

The above procedure was repeated exactly using the product *Germ Free 24* in place of propan-2-ol.

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5) Postvalues.

Immediately after wrinsing the 12 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's per 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 *Germ Free 24* to pass the criteria of EN 1500 the mean log reduction factor obtained must not be significantly smaller than that obtained for the alcohol rub. Test of significance of log reduction factors of P against R is carried out using the Wilcoxon matched pairs signed ranks test.

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Reference Handwash Procedure (R)

Handwash with Germ Free 24 (P)

Subject	Log x	Log y	Log z	Log x	Log y	Log z
1	6.37	1.47	4.90	6.45	1.30	5.15
2	6.29	1.54	4.75	6.40	1.18	5.22
3	6.38	1.30	5.08	6.40	1.48	4.92

4	6.44	1.18	5.26	6.38	1.30	5.08
5	6.40	0.70	5.70	6.44	0	6.44
6	6.33	1.40	4.93	6.37	1.18	5.19
7	6.44	1.54	4.90	6.45	1.18	5.27
8	6.36	1.54	4.82	6.44	1.40	5.04
9	6.43	1.78	4.65	6.47	1.60	4.87
10	6.51	1.30	5.21	6.36	1.00	5.36
11	6.32	0	6.32	6.43	0	6.43
12	6.41	0	6.41	6.44	1.00	5.44
X	6.39	1.14	5.25	6.42	1.05	5.37
N	12	12	12	12	12	12

Where $\text{Log } x = \log \text{ prevalue.}$

$\text{Log } y = \log \text{ post value}$

$\text{Log } z = \log \text{ reduction factor}$

X = overall mean value of $\log x$, $\log y$ and $\log z$.

N = number of subjects in each column.

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Log RF derived from Difference Rank of difference

Subject	R	P	R - P	Without sign	With sign
1	4.90	5.15	-0.25	6	-6
2	4.75	5.22	-0.47	3	-3
3	5.08	4.92	0.16	10	10

4	5.26	5.08	0.18	9	9
5	5.70	6.44	-0.74	2	-2
6	4.93	5.19	-0.26	5	-5
7	4.90	5.27	-0.37	4	-4
8	4.82	5.04	-0.22	7.5	-7.5
9	4.65	4.87	-0.22	7.5	-7.5
10	5.21	5.36	-0.15	11	-11
11	6.32	6.43	-0.11	12	-12
12	6.41	5.44	0.97	1	1
Sum of rank +					20
Sum of rank -					58

Comparison of matched pairs with Wilcoxon signed - rank test values shows that there is a significant difference in values at the 1% level ($p = 0.01$ level) sum of ranks for test being 38 compared to expected 12 for 12 subjects and *Germ Free 24*, when used neat as received can be considered a suitable hygienic handrub when tested under the procedures described above giving significantly better reduction in bacterial numbers.

D C Watson