



# Abbott Analytical

Consulting Scientists to the Disinfectant Industry



## Certificate of Analysis

**Sample(s) :** One sample of Germ Free 24

**Received from:** Zoono Pty Ltd. Level 2, Carlton DFK Centre, 135 Broadway,  
Newmarket, Auckland 1149, New Zealand

**Date received:** 7 December 2009      **Date tested:** 13 December 2009 -  
14 December 2009

**Certificate no:** 09M.064.ZOO      **Certificate date:** 8 January 2010

**Sample ref:** 9M/064      **Page:** 1 of 8

**Analysis required:** *Adaptation of EN 12791, Chemical disinfectants and  
antiseptics - Surgical hand disinfection - Test method and  
requirements (phase 2, step 2)  
to determine residual effect of product on the hands after  
24 hours of normal activity post handrub with product*

**Product stored at:** Room temperature

**Active substance:** Not declared

**Volume of product used:** 6ml (2x 3ml)

**Total rubbing time with product:** 1 minute (2x 30 seconds)

**Identification of bacterial  
strain(s) used:** Normal skin flora      N/A

**Neutralising solution:** 30g/l polysorbate 80, 3g/l lecithin,  
1g/l histidine, 1g/l cysteine

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## **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 10 subjects (healthy adults) were chosen, comprising two teams of 5; each one carrying out the test procedure in precisely the same way as the others.

## **Experimental procedure:**

### **1) Pre-treatment values**

Each of the 5 subjects from each team 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 fingertips (including that of the thumb) were then rubbed for 1 minute on the base of a Petri dish containing 10ml of Tryptone Soy Broth (TSB) without neutraliser in order to assess the release of skin bacteria before treatment of hands (pre-treatment values). A separate dish is used for each hand. 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.

This process took place at 08:00 for the first team and at 16:00 on the same day for the second team.

### **2) Hand disinfection procedure with test product**

Each of the 5 subjects from each team had approximately 3ml of the test product sprayed onto one hand and rubbed vigorously for 30s onto the skin, up to the wrists in accordance with the standard handrub procedure. This was carried out by the author on day one at the times indicated in section 1. The treated hand was then allowed to air dry for 5 minutes before determination of the number of residual bacteria remaining on the skin as below.

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### **3) Post-treatment values:**

Immediately after drying the 5 subjects from each team were asked to rub the fingertips on the base of a Petri dish containing 10ml of Maximum Recovery Diluent (MRD) 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.

The subjects from the first group were then sent about their normal business for a 2-hour period and asked to wash their hands only the minimum number of times for personal hygiene reasons during that period. After a 2-hour period each subject from the group was re-tested as above. This was repeated for two further 2-hour periods to obtain counts at 2, 4 and 6 hours after initial treatment.

The same procedures were carried out on the second team of 5 subjects who were first retested at 08:00 on the following day and then at 2-hour intervals until 16:00, to obtain counts for 16 to 24 hours after initial treatment.

### **4) 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. The counts from treated and untreated hands of each subject were taken separately for pre-treatment values and post-treatment values. The mean count was calculated for all five subjects from each team for each of the time intervals, from which log reduction factors were calculated.

### **Results:**

See tables on following pages.

### **Conclusion:**

Germ Free 24 shows residual activity post-application giving an average log reduction of 2.16 in numbers of bacteria on the hands compared to untreated hands over a 24-hour period. It shows efficacy against bacteria even after contact with the environment on the subjects' hands during the period.

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**Results table: Hand disinfection procedure with test product**

Count at:	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Mean
<b>T. (pre-treatment)</b>	1540	1600	940	1220	700	1200
<b>2 hours</b>	270	180	60	120	40	134
<b>4 hours</b>	180	220	210	320	80	202
<b>6 hours</b>	360	540	170	140	260	294
<b>16 hours</b>	530	270	310	380	510	400
<b>18 hours</b>	640	480	580	720	800	644
<b>20 hours</b>	1040	1700	860	940	1620	1232
<b>22 hours</b>	2580	1460	1380	2040	2760	2044
<b>24 hours</b>	3360	2120	1460	2960	4440	2868

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**Results table: Untreated hand (control)**

Count at:	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Mean
<b>T. (pre-treatment)</b>	12800	22800	16000	19000	32200	20560
<b>2 hours</b>	32400	58600	44000	51200	30800	43400
<b>4 hours</b>	57200	64400	43800	47800	51200	52880
<b>6 hours</b>	94200	51600	38200	74400	61600	64000
<b>16 hours</b>	127000	96000	74000	133000	48000	95600
<b>18 hours</b>	158000	76000	92000	184000	58000	113600
<b>20 hours</b>	198000	136000	152000	204000	214000	180800
<b>22 hours</b>	170000	224000	196000	308000	316000	242800
<b>24 hours</b>	227000	396000	274000	433000	548000	375600

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**Results table: Computed log values and log reduction factors of the mean counts and equivalent percentage reductions**

	Log x	Log y	Log z	As percentage
T. (pre-treatment)	4.31	3.08	1.23	
2 hours	4.64	2.13	2.51	99.69
4 hours	4.72	2.30	2.42	99.62
6 hours	4.81	2.47	2.34	99.54
16 hours	4.98	2.60	2.38	99.58
18 hours	5.05	2.81	2.24	99.43
20 hours	5.26	3.09	2.17	99.32
22 hours	5.38	3.31	2.07	99.16
24 hours	5.57	3.46	2.11	99.24
X	4.97	2.81	2.16	99.45
N	5	5	5	

Where:

Log x = log of the mean counts for the untreated hands

Log y = log of the mean counts for the treated hands

Log z = log reduction

X = Mean value of the column

N = number of subjects tested at each time interval

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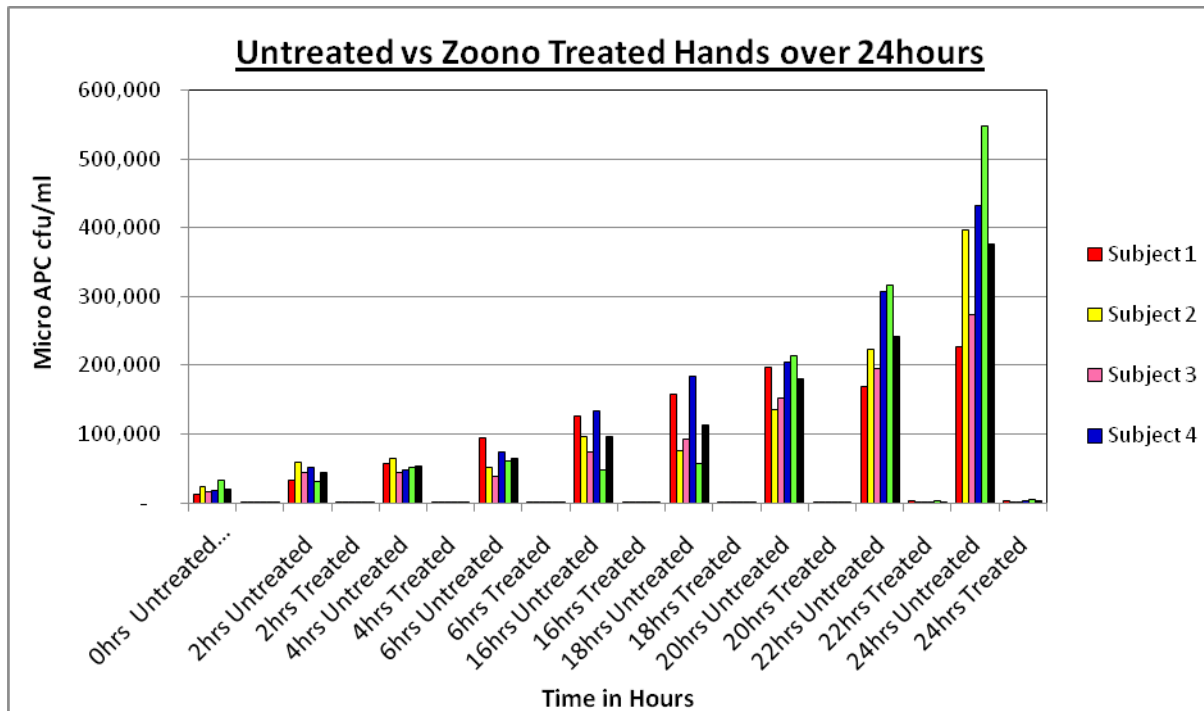


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## Appendix 1:



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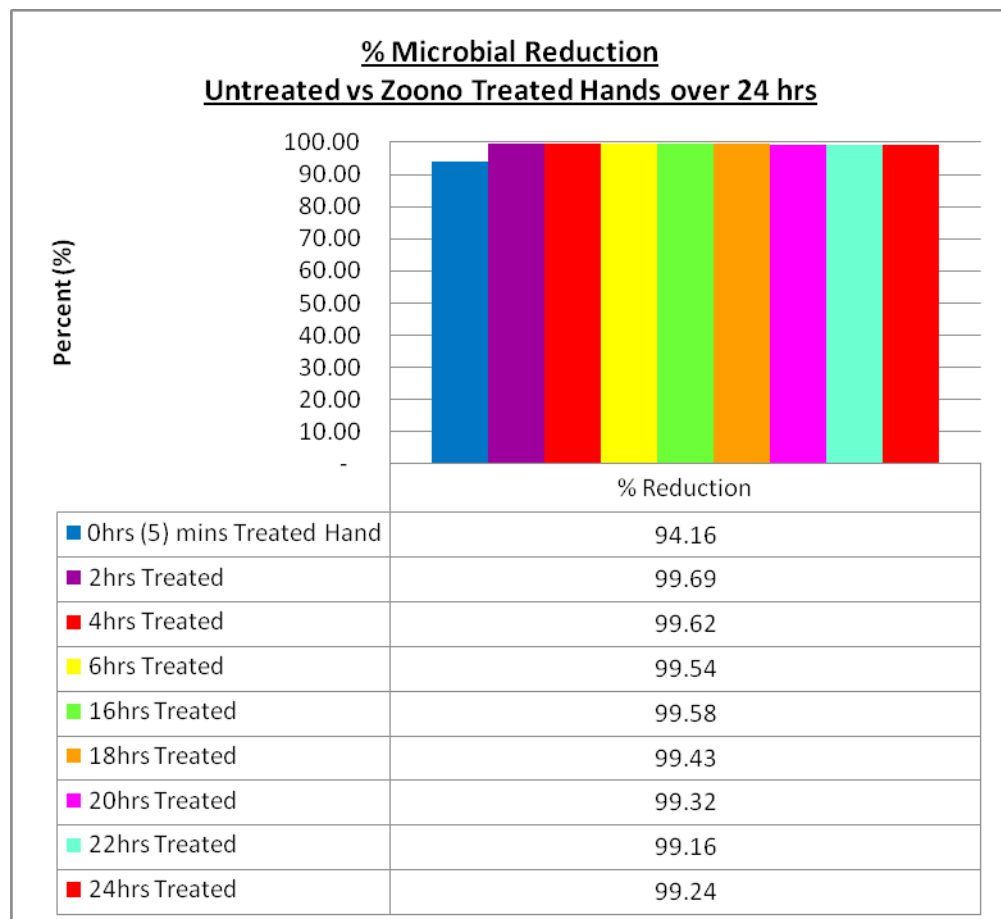


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## **Appendix 1:**



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