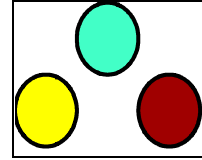


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14th December 2009

### **Certificate of Analysis**

**Samples:** One sample of Germ Free 24 received from Zoono Pty. Ltd, Level 3, 235 Broadway, Newmarket. New Zealand. 7th December 2009

**Certificate No:** 09M.064.Zoo

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**Sample ref:** 9m / 064

**Analysis Required:** Adaptation of EN 12791 to determine residual effect of Germ Free 24 on the hands after 24 hours normal usage post handrub with product.

**Samples Tested:** 13th - 14th December 2009

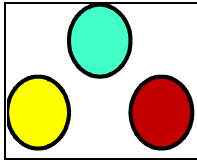
#### Principle of test:

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. A number of subjects were used because of the possible variation in bacterial flora found on human skin. In this case a total of **ten (10)** healthy adults were chosen comprising of two teams of 5, each one carrying out the test procedure in precisely the same way as the others.

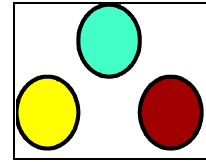
#### 1) Hygienic Handrub procedure.

The volunteers were asked to wash their hands in soft soap to remove extraneous bacteria from the skin and to dry each hand with a paper towel. Each of 5 subjects was asked to spray approximately 3ml of **Germ Free 24** into the cupped hands and have it rubbed vigorously for 1 minute onto the skin up to the wrists in accordance with the standard handrub procedure. This was carried out on one hand for each of the volunteers by the author at 8 am on day 1. The treated hand was allowed to air dry for 5 minutes before determination of the number of residual bacteria remaining on the skin. The process was also carried out for a second batch of 5 volunteers at 4 pm the same day. After treatment of one hand for each of the volunteers they were asked to go about their normal daily business but avoiding washing of the hands wherever possible.

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## 2) Postvalues .

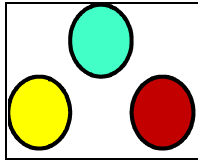
Each of the ten subjects were asked to go about their normal work. The first group of five was recalled after 2 hours to test for residual activity by rubbing the fingertips of each hand separately on the base of a petri dish containing 10ml of Maximum Recovery Diluent (MRD) for 1 minute using a separate petri dish for each hand. Then 1ml of each of the sample fluids, diluted as shown in the table, 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. This was repeated for each of the first group of 5 over the 8 hour working day to get counts at 2, 4, and 6 hours after treatment. This was repeated with the second group of 5 at 8am the following morning and again at 2 hour intervals until 4pm to obtain counts at 16 - 24 hours post treatment.

## 3) Calculation.

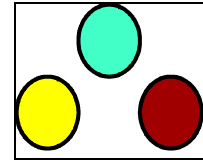
The number of colony forming units (CFU) per plate for each dilution was recorded and the number of cfu's per ml of sample fluid calculated.

From the difference between the 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. In this test we are only interested in demonstrating the presence or absence of a residual effect on the ability of **Germ Free 24** to kill bacteria after given time periods after application to the skin. The numerical reduction rates are recorded below.

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## 5) 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.

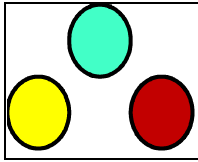
### Handrub with Germ Free 24

Count at	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Mean count
Time 0 (pretreatment)	1540	1600	940	1220	700	1200
2 hours	270	180	60	120	40	134
4 hours	180	220	210	320	80	202
6 hours	360	540	170	140	260	294
16 hours	530	270	310	380	510	400
18 hours	640	480	580	720	800	644
20 hours	1040	1700	860	940	1620	1232
22 hours	2580	1460	1380	2040	2760	2044
24 hours	3360	2120	1460	2960	4440	2868

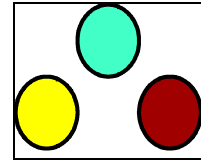
### Untreated control hand

Count at	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Mean count
Time 0 (pretreatment)	12800	22800	16000	19000	32200	20560
2 hours	32400	58600	44000	51200	30800	43400
4 hours	57200	64400	43800	47800	51200	52880
6 hours	94200	51600	38200	74400	61600	64000
16 hours	127000	96000	74000	133000	48000	95600
18 hours	158000	76000	92000	184000	58000	113600
20 hours	198000	136000	152000	204000	214000	180800
22 hours	170000	224000	196000	308000	316000	242800
24 hours	227000	396000	274000	433000	548000	375600

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Mean log reduction and percentage reduction in numbers over 24 hours for all subjects.

	Log x	log y	Log z	Percent reduction
To	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
24hours	5.57	3.46	2.11	99.24
X			2.16	99.44
N	5	5	5	

Where  $\text{Log } x = \text{Log count of Untreated hand.}$

$\text{Log } y = \text{log count of treated hand}$

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

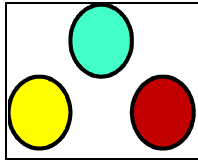
X = overall mean value of column.

N = number of subjects tested.

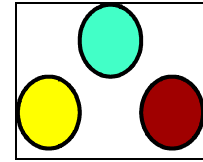
Conclusion.

Germ Free 24 shows residual activity post application with the contaminating organism giving an average of a 2.16 log reduction in numbers over the untreated hands during the 24 hour test period showing efficacy against bacteria even after contact with the environment on volunteers hands during the period from infection to final examination.

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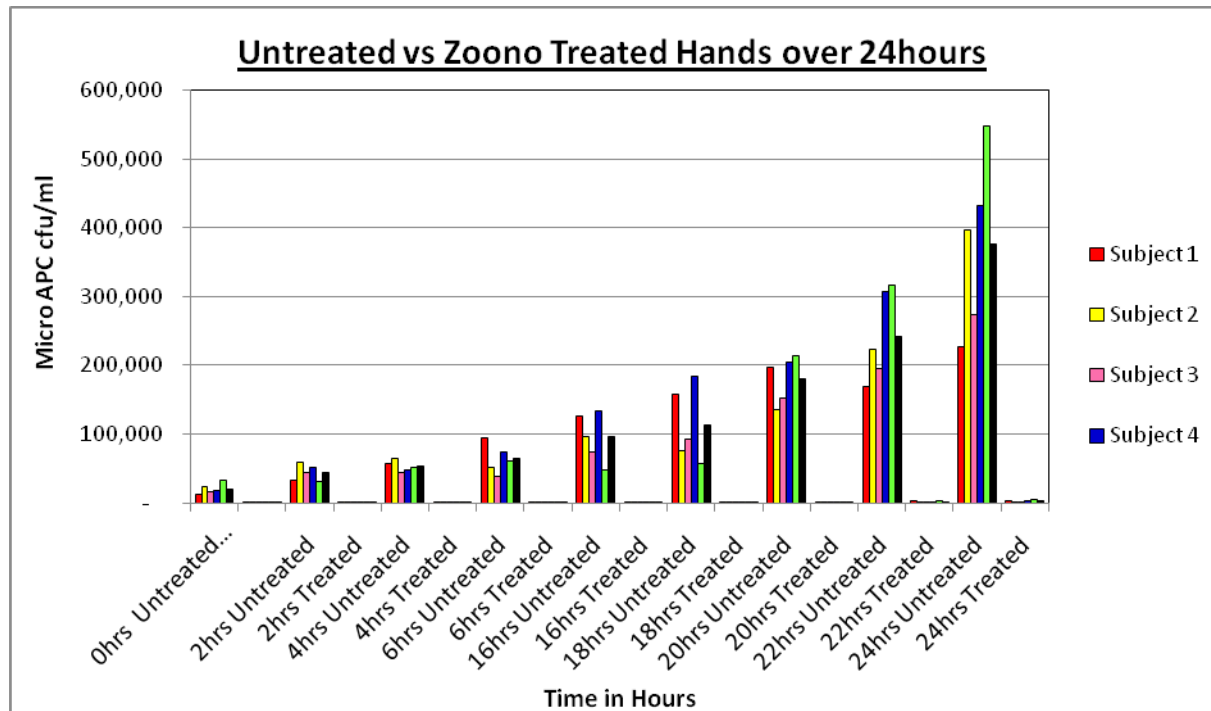
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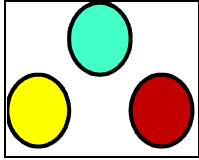
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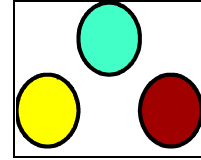
## Appendix 1



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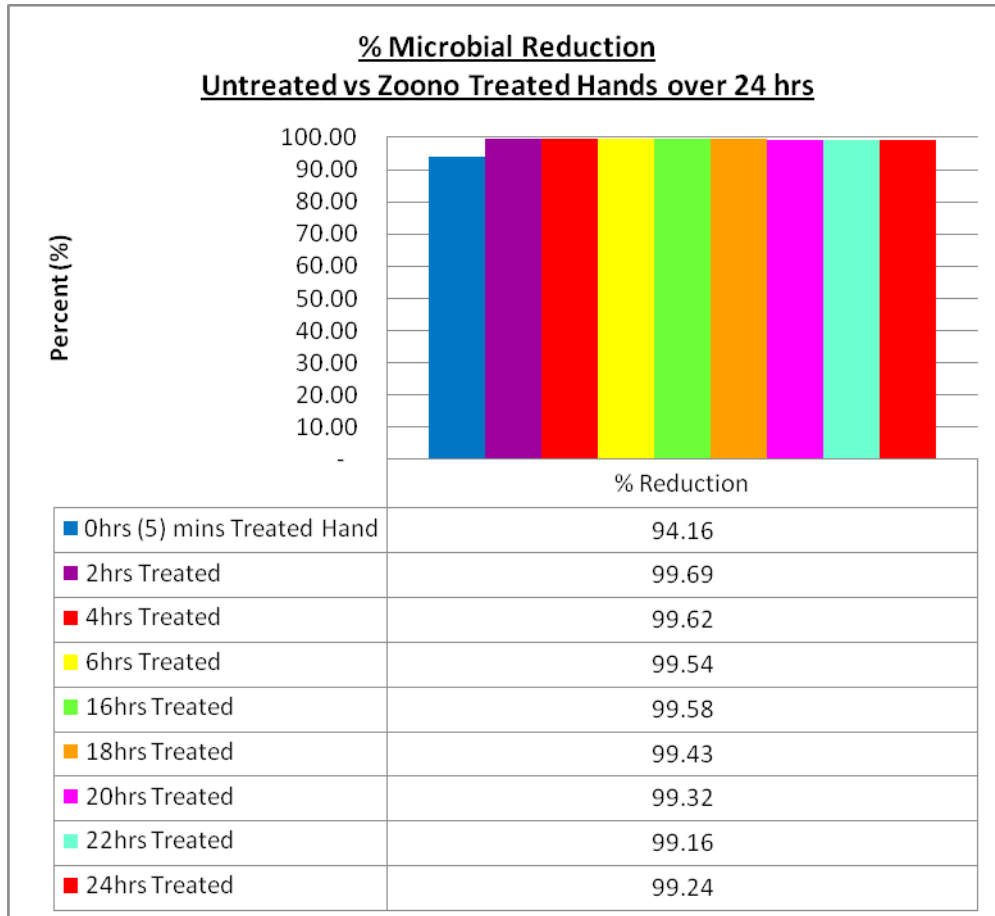
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## Appendix 2



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