ChemSil
AIR & WATER) SDN. BHD. (515400-V)
20A, Jalan Kota Raja E27/E, Taman Alam Megah, Seksyen 27, 40400 Shah Alam, Selangor Darul Ehsan, Malaysia
Tel: 03-5192 3500 Fax: 03-5192 4600 E-mail: chemsil@hotmail.com

CERTIFICATE OF ANALYSIS

Lab No. Company	CAW/M/0701/2720 (A)
Nature Of Sample	BACTAKLEEN ANTI BACTERIA SOLUTION
Received Date	2nd MARCH 2010
Analyzed Date	2nd MARCH - 6th APRIL 2010
Reported Date	6th APRIL 2010

TEST: CHALLENGE TEST ON BACTAKLEEN

ORGANISM USED TO CHALLENGE: *a) Escherichia coli*
b) Staphylococcus aureus
c) Candida albicans (Yeast)
d) Aspergillus niger (Mould)

Procedure:

1) Prepare culture of organism to be challenged from pure working culture by subculturing onto Tripytic Soy Agar slant for bacteria and Sabouraud Dextrose Agar for mould and yeast.

2) Incubate the cultures at 35°C for 24 hrs for all the cultures except mould at 25°C for 5 days.

3) Prepare bacterial and fungal suspension by washing the slant with 18ml 0.85 % NaCl solution and then compare the turbidity with Me Farland solution and dilute further to achieve similar turbidity with the standard.

4) This suspension is equivalent to 10 cfu/ml for bacterial and 10 cfu/ml for fungal. Perform serial dilution to obtain 10^3, $10^2$and 10^1 cfu/ml.

5) Use the above three dilution to know the actuall cfu/ml in the suspension prepared b}' plating 1ml from each dilution onto the petri dish and use pour plate method (Triptytic Soy Agar / TSA for bacteria and Sabouraud Dextrose Agar / SDA for fungal).

6) Spike each 1ml of 10 suspension prepared respective!}' for bacterial and 10^5 fungal into sample (Bactakleen).

ChemSil
(AIR & WATER) SDN. BHD. (**515400-V)**
20A, Jalan Kota Raja E27/E, Taman Alam Megah, Seksyen 27, 40400 Shah Alam, Selangor Darul Ehsan, Malaysia Tel: 03-5192 3500 Fax: 03-5192 4600 E-mail: chemsil@hotmail.com

CERTIFICATE OF ANALYSIS

7) Mix vigorously and leave for 5.15. 30 and 60 minute and at each time interval do serial dilution using 0.1% peptone water containing 0.5% lecithin plus 4.0% Polysorbate 20. This chemical properties act as neutralizing agent to inhibit the chemical reaction between the sample and bacteria at each time interval so actual efficacy of the sample can be studied. From each dilution transfer 1 ml diluents onto petri dish and then perform pour plate method as in step no.5.
Each plates are duplicated.

8) Let the agar settle after poured and then incubate the TSA at 35 C for 48 hrs and SDA at 25 C for 5 days.

9) After the incubation period the plates are removed from incubator and the colonies are counted.
The results are shown in the table below:

Table 1: Holding Time Between Sample and Cultures suspension is 5 minute

ORGANISM USED	INOCULUM USED (cfti/ml)	INOCULUM RECOVERED (cfu/ml)	*PRECENTAGE KILLED (%)
a) *Esherichia coli*	**2.0×10^6**	**1.2×10^6**	**40.00**
b) *Staphylococcus aureus*	**2.5×10^6**	**1.6×10^6**	**36.00**
c) *Candida al hi cans*	**1.2×10^6**	**1.9×10^5**	**84.17**
d) *Aspergillus niger*	**1.6×10^3**	**2.2×10^3**	**98.63**

Table 2: Holding Time Between Sample and Cultures suspension is 15 minute

ORGANISM USED	INOCULUM USED (cfu/ml)	INOCULUM RECOVERED (cfu/ml)	*PRECENTAGE KILLED (%)
a) *Esherichia coll*	**2.0×10^6**	**6.5×10^3**	**99.68**
b) *Staphylococcus aureus*	**2.5×10^6**	**8.8×10^4**	**96.48**
c) *Candida albicans*	**1.2×10^6**	**2.2×10^4**	**98.17**
d) *Aspergillus niger*	**1.6×10^5**	**1.4×10^2**	**99.91**

cfu- colony forming units

(AIR & WATER) SDN. BHD. (515400-V)
20A, Jalan Kota Raja E27/E, Taman Alam Megah, Seksyen 27, 40400 Shah Alam, Selangor Darul Ehsan, Malaysia
Tel: 03-5192 3500 Fax: 03-5192 4600 E-mail: chemsil@hotmail.com

CERTIFICATE OF ANALYSIS

Table 3: Holding Time Between Sample and Cultures suspension is 30 minute

ORGANISM USED	INOCULUM USED (cfu/ml)	INOCULUM RECOVERED (efu/ml)	*PRECENTAGE KILLED (%)
a) *Esherichia coli*	2.0×10^6	5.0×10^1	99.99
b) *Staphylococcus aurens*	2.5×10^6	6.2×10^4	97.52
c) *Candida albicans*	1.2×10^6	8.3×10^2	99.93
d) *Aspergillus niger*	1.6×10^5	NG (<10)	>99.99

Table 4: Holding Time Between Sample and Cultures suspension is 60 minute

ORGANISM USED	INOCULUM USED (cfu/ml)	INOCULUM RECOVERED (cfu/ml)	*PRECENTAGE KILLED (%)
a) *Esherichia coli*	2.0×10^6	NG (<10)	>99.9
b) *Staphvlococcus aurens*	2.5×10^6	2.8×10^4	98.88
c) *Candida albicans*	1.2×10^6	NG (<10)	>99.9
d) *Aspergillus niger*	1.6×10^5	NG (<10)	>99.9

$$\text{Precentage Killed} = \frac{\text{Inoculum used - Inoculum Recovered}}{\text{Inoculum Used}}$$

cfu- colony forming units
NG – No Growth

Verified By:

Ganesan Gunasagaran
Microbiologist
B.Sc.(Hons).