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**Dato' Marcus K.F. Kam** B.E. (Hons.), M.Sc., C.Chem., FRACI, MRSC, MBA, AMICHEM.

Executive Director:

**Datin P.K. Wong** Dip. NIOA, FRSH, FCMI, FEI

**CERTIFICATE OF ANALYSIS**

Company : Mr. Mark Tools (Malaysia) Sdn Bhd  
No. 4, Jalan Anggerik Mokara 31/48,  
Kota Kemuning, Section 31,  
40460 Shah Alam, Selangor Darul Ehsan.

Lab No : PJ-L/MS/2805A/11

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Sample Description : One (1) sample of Water Product  
Sample Marking : Cashido Superoanion Multifunctional Water, Model OH-6800+  
Water Flow Level : 4.5 Superoanion Density Level : 150 mg/hr (5<sup>th</sup> level)  
Date Sample Received : 5<sup>th</sup> September 2011  
Date Analyzed : 6<sup>th</sup> September 2011 – 12<sup>th</sup> September 2011  
Date Reported : 12<sup>th</sup> September 2011


**BACTERIOLOGICAL ANALYSIS :**

**Challenge Test**

**Procedure :**

**Positive control**

1. Prepare an inoculum suspension about 10<sup>5</sup> cfu/ml of each microorganism required.
  - a. *Escherichia coli* ATCC 8739
  - b. *Staphylococcus aureus* ATCC 6538
  - c. *Pseudomonas aeruginosa* ATCC 9027
  - d. *Salmonella choleraesuis* ATCC 10708
  - e. *Candida albicans* ATCC 10231
  - f. *Aspergillus niger* ATCC 16404
2. Perform the reverse serial dilution until 10<sup>1</sup> using 0.85% sterile saline water.
3. From each dilution, directly pipette 2.5 ml and add into Petri dish containing TSA agar (incubate at 35°C/48 hours) and SDA agar (incubate at 25°C/120 hours).
4. Count the colony expressed from each dilution (initial count).
5. Determine the average plate count.

  
**Mahizan Mohd Hamzah**  
Microbiologist

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**BACTERIOLOGICAL ANALYSIS :**

**Challenge Test**

**Collecting samples**

1. Prepare sterile Schott Duran bottle 250 ml.
2. Start test with introduce 2.5 ml of the inoculums at concentration 10<sup>5</sup> cfu/ml into the empty Schott Duran (250 ml).
3. After complete the setting and connection of Cashido unit to the tap water, start to open the tap water valve.
4. Set the water flow to 4.5 liters per minute and ensure the blue light is on.
5. Start to collect the water using sterile Schott Duran bottle 250 ml containing inoculums.
6. Collect water until the neck of the bottle.
7. After 10 seconds contact time of ozone water with the inoculums, perform the reverse serial dilution until 10<sup>1</sup> using 0.85% sterile saline water. (Do 10 seconds test and 20 seconds test)
8. From each dilution, directly pipette 1 ml and add into Petri dish containing TSA agar (incubate at 35°C/48 hours) and SDA agar (incubate at 25°C/120 hours)
9. Count the colony expressed from each dilution (survive count).
10. Determine the average plate count.
11. Repeat the testing procedure for 20 seconds contact time.
12. Repeat the test procedures for the other microorganisms required.

  
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Microbiologist

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**BACTERIOLOGICAL ANALYSIS :**

**Challenge Test**


**For Calculate the percentage of kill, follow the following calculation**

$$= \frac{\text{Initial count of microorganisms} - \text{survive count of microorganisms}}{\text{Initial count of microorganisms}} \times 100\%$$

**RESULTS :**

Organism Tested	Initial Count, cfu/ml (A)	Count of survive organism, cfu/ml (B)		Percentage of kill, (A) - (B) x 100% (A)	
		10 seconds	20 seconds	10 seconds	20 seconds
a) <i>Escherichia coli</i> ATCC 8739	5.2 x 10 <sup>5</sup>	No Growth (<10)	No Growth (<10)	> 99.99 %	> 99.99 %
b) <i>Staphylococcus aureus</i> ATCC 6538	3.8 x 10 <sup>5</sup>	No Growth (<10)	No Growth (<10)	> 99.99 %	> 99.99 %
c) <i>Pseudomonas aeruginosa</i> ATCC 9027	2.5 x 10 <sup>5</sup>	No Growth (<10)	No Growth (<10)	> 99.99 %	> 99.99 %
d) <i>Salmonella choleraesuis</i> ATCC 10708	8.5 x 10 <sup>5</sup>	No Growth (<10)	No Growth (<10)	> 99.99 %	> 99.99 %
e) <i>Candida albicans</i> ATCC 10231	1.5 x 10 <sup>6</sup>	2.0 x 10 <sup>5</sup>	No Growth (<10)	86.67 %	> 99.99 %
f) <i>Aspergillus niger</i> ATCC 16404	2.5 x 10 <sup>6</sup>	1.9 x 10 <sup>5</sup>	2.0 x 10 <sup>1</sup>	92.40 %	99.99 %

Test Method : In House Based on United States Pharmacopoeia 28

  
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