

**EVALUATION OF SUPER-OXIDIZED WATER "MEDILOX" AS A  
STERILIZING AGENT**

**Report Reference Number: TMU-MI-TR-9202**

**Requested by:** Well Chain Science CO., LTD.

**Executed by:** Department of Microbiology and Immunology, Taipei  
Medical University

**Execution Coordinator:** Professor Yuan-Hsun Hsu

**Date of Execution:** 15/12/2001~31/1/2002

## **Evaluation of Super-oxidized Water "Medilox" as a Sterilizing Agent**

**Requested Topic of Experiment:** Evaluation of super-oxidized water, Medilox, as a sterilizing agent

**Requested by:** Well Chain Science CO., LTD.

**Executed by:** Department of Microbiology and Immunology, Taipei Medical University

**Execution Coordinator:** Professor Yuan-Hsun Hsu

**Date of Execution:** 15/12/2001~31/1/2002

**Address:** 250 Wu Hsing St., Taipei, Taiwan

**Phone Number:** 001-886-2-27361661 ext. 3170

**Email Address:** [microbes@tmu.edu.tw](mailto:microbes@tmu.edu.tw)

### **INTRODUCTION**

As Well Chain Science Co., Ltd. plans to import Medilox super-oxidized water made by a Korean manufacturer Soo San Enc Co., Ltd., as a sterilizing agent for use in hospitals around Taiwan, a request was made by the company to assess the efficacy of Medilox as a sterilizing agent, owing to health regulation demands of Taiwan. As such, the Department of Microbiology and Immunology of Taipei Medical University was chosen to carry out several experiments to assess the bactericidal efficacy of Medilox.

### **AIMS**

To assess the bactericidal efficacy of "Medilox" super-oxidized water, this project has been divided into three parts:

1. Assessing the efficacy of Medilox *in vitro*
2. Assessing the efficacy of Medilox *in vivo* on human body
3. Assessing the efficacy of Medilox on medical utensils

The organisms chosen for the purpose of the experiment has been based on the characteristics of Medilox as proven by the Korean manufacturer previously. These include: 1. those more commonly found in hospitals as source of nosocomial infection (i.e. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*) and 2. *Candida albicans* as the fungal specimen.

## **METHODS AND MATERIALS**

### **Test samples**

- Medilox super-oxidized water manufactured by Soo San Enc Co., Ltd. was the sample tested in this experiment
- Distilled de-ionized water was used as control for Medilox

### **Test organisms**

The organisms used in this experiment were all type strains and pure cultures, all stored at 4°C for no longer than 30 days. These organisms included:

1. *Escherichia coli* (ATCC No. 8739)
2. *Pseudomonas aeruginosa* (ATCC No. 9027)
3. *Staphylococcus aureus* (ATCC No. 6538)
4. *Klebsiella pneumoniae* (ATCC No. 4352)
5. *Candida albicans* (ATCC No. 10231)

### **Culture medium used**

Bacterial cultures were based on both the liquid form Nutrient Broth (NB) and the solid form Nutrient Agar (NA). Fungal cultures were based on both the liquid form Potato Dextrose Broth (PDB) and the solid form Potato Dextrose Agar (PDA).

The three parts of the project was performed as follows:

#### **1. Assessing the efficacy of Medilox *in vitro***

The methods were as follows:

- The organisms were cultured in liquid medium, and cultured by a rotatory shaker with a speed of 150rpm, at 37°C 18 hours for bacteria and 28°C 48 hours for fungus.
- The above cultures were further centrifuged at 12,000 rpm. followed by washing with sterile, distilled de-ionized water, after which an organism count was performed.
- These were then titrated into fluid containing  $2 \times 10^6$  And  $5 \times 10^4$  organisms/ml for bacteria and fungus respectively. 0.1ml was extracted from the titre and added to/mixed with 9.9mls of Medilox.
- Extracts of the mixture (50µl) was taken at 30 seconds, 1 minute, 3 minutes and 5 minutes and inoculated onto solid culture plates. The conditions of culture was 37°C 24 hours for bacteria, and 28°C 72 hours for fungus. Sterile distill, de-ionized water was used as control.
- Colony Forming Units (CFU) were then counted. The result being the average of the triplicated counts (without standard deviation SD).

## 2. Assessing the efficacy of Medilox *in vivo* on human body

- For this part of the experiment, *E. coli* and *Staphylococcus aureus* were used as the indicator bacteria, and were processed into bacterial broth containing  $2 \times 10^6$  organisms/ml.
- 30 $\mu$ l of the mixture was applied onto the middle fingers of both hands of the subject. the subject was then asked to rub the middle finger and the thumb together several times, after which both hands were wind dried with cold air.
- Medilox was sprayed onto the left middle finger and thumb, and equivalent amount of sterile, distill de-ionized water was sprayed onto the right middle finger and thumb as control. Both hands were wind dried with cold air after 90 seconds.
- Both fingers were imprinted onto NA and cultured at 37°C for 24 hours, at which time, Photo image scanning was used to calculate bacterial grwoing area, which was divided into 5 categories ("A" being surface area <5%, "B" being surface area between 5%~25%, "C" being surface area between 25%~50%, "D" being surface area between 50%~75%, E being surface area between 75%~100%).

## 3. Assessing the efficacy of Medilox for medical utensils

- A stainless steel medical utensil was immersed in bacterial broth containing  $2 \times 10^6$  *Escherichia coli* /ml.
- After wind drying, Medilox was mist-sprayed onto the plate and wind-dried after 90 seconds.
- A wet sterile cotton wool bud was used to sample the utensil surface over a surface area of 5 $\times$  5cm<sup>2</sup>.
- The cotton wool bud was then spread over NA and cultured at 37°C for 24 hours, after which time, the number of colony forming units were counted.
- Once again, spraying the plate with sterile distill de-ionized water was used as control.

**RESULTS**

**1. Assessing the efficacy of Medilox *in vitro***

*In vitro* evaluation of the antimicrobial activity to Medilox

Test organism	Predisinfection count (cfu/ml)	Remaining count (cfu/ml) per ml after exposure to Medilox for X min, X* =			
		0.5 min	1 min	3 min	5 min
<i>Escherichia coli</i> (ATCC No. 8739)	$2.0 \times 10^6$	0	0	0	0
<i>Pseudomonas aeruginosa</i> (ATCC No. 9027)	$2.0 \times 10^6$	1	0	0	0
<i>Staphylococcus aureus</i> (ATCC No. 6538)	$2.0 \times 10^6$	0	0	0	0
<i>Klebsiella pneumoniae</i> (ATCC No. 4352)	$2.0 \times 10^6$	1	1	0	0
<i>Candida albicans</i> (ATCC No. 10231)	$5.0 \times 10^4$	0	0	0	0

  

Test organism	Predisinfection count (cfu/ml)	Remaining count (cfu/ml) per ml after exposure to sterilized water for X min, X* =			
		0.5 min	1 min	3 min	5 min
<i>Escherichia coli</i> (ATCC No. 8739)	$2.0 \times 10^6$	$1.7 \times 10^5$	$1.0 \times 10^6$	$1.4 \times 10^6$	$5.8 \times 10^5$
<i>Pseudomonas aeruginosa</i> (ATCC No. 9027)	$2.0 \times 10^6$	$1.1 \times 10^6$	$7.1 \times 10^5$	$2.2 \times 10^5$	$8.5 \times 10^4$
<i>Staphylococcus aureus</i> (ATCC No. 6538)	$2.0 \times 10^6$	$1.3 \times 10^6$	$1.0 \times 10^6$	$5.6 \times 10^5$	$3.0 \times 10^5$
<i>Klebsiella pneumoniae</i> (ATCC No. 4352)	$2.0 \times 10^6$	$7.0 \times 10^5$	$8.5 \times 10^5$	$6.7 \times 10^5$	$4.6 \times 10^5$
<i>Candida albicans</i> (ATCC No. 10231)	$5.0 \times 10^4$	$2.9 \times 10^4$	$6.0 \times 10^3$	$4.7 \times 10^3$	$2.0 \times 10^3$

※ All results are expressed as mean of three demonstrations (SD omitted).

**2. Assessing the efficacy of Medilox *in vivo* on human body**

Efficacy of Medilox against *Escherichia coli* and *Staphylococcus aureus* in the skin disinfection test

Test organism	Grade of growing area			
	Medilox		Sterilized water	
	Middle finger	Thumb	Middle finger	Thumb
<i>Escherichia coli</i> (ATCC No. 8739)	A	A	C	C
<i>Staphylococcus aureus</i> (ATCC No. 6538)	A	A	D	E

※ A: Growing area < 5%; B: 5% < Growing area < 25%; C: 25% < Growing area < 50%; D: 50% < Growing area < 75%; E: 75% < Growing area < 100%

**3. Assessing the efficacy of Medilox for medical utensils**

Disinfection effect of Medilox to medical utensils

Test organism	Viable count after exposure to	
	Medilox	Sterilized water
<i>Escherichia coli</i> (ATCC No. 8739)	< 10	> 100
<i>Staphylococcus aureus</i> (ATCC No. 6538)	< 10	> 100

## **DISCUSSION**

This series of experiments has proven that Medilox super-oxidized water has obvious bactericidal activity against common, clinically significant organisms (i.e. *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Candida albicans*). However, this effect is limited to the potency of a disinfectant but not an antimicrobial agent.

## **REFERENCE**

The methods of antimicrobial activity evaluation in this project has been performed according to the guidelines of Microbiological tests, as deemed appropriate by ASTM, AATCC and USP.